PONTIFICIAE ACADEMIAE SCIENTIARVM SCRIPTA VARIA

40

SEMAINE D'ETUDE

SUR LE THÈME

MEMBRANES BIOLOGIQUES ET ARTIFICIELLES ET LA DESALINISATION DE L'EAU

14-19 avril 1975

ÉDITÉ PAR ROBERTO PASSINO

PONTIFICIA
ACADEMIA
SCIENTIARVM

EX AEDIBVS ACADEMICIS IN CIVITATE VATICANA

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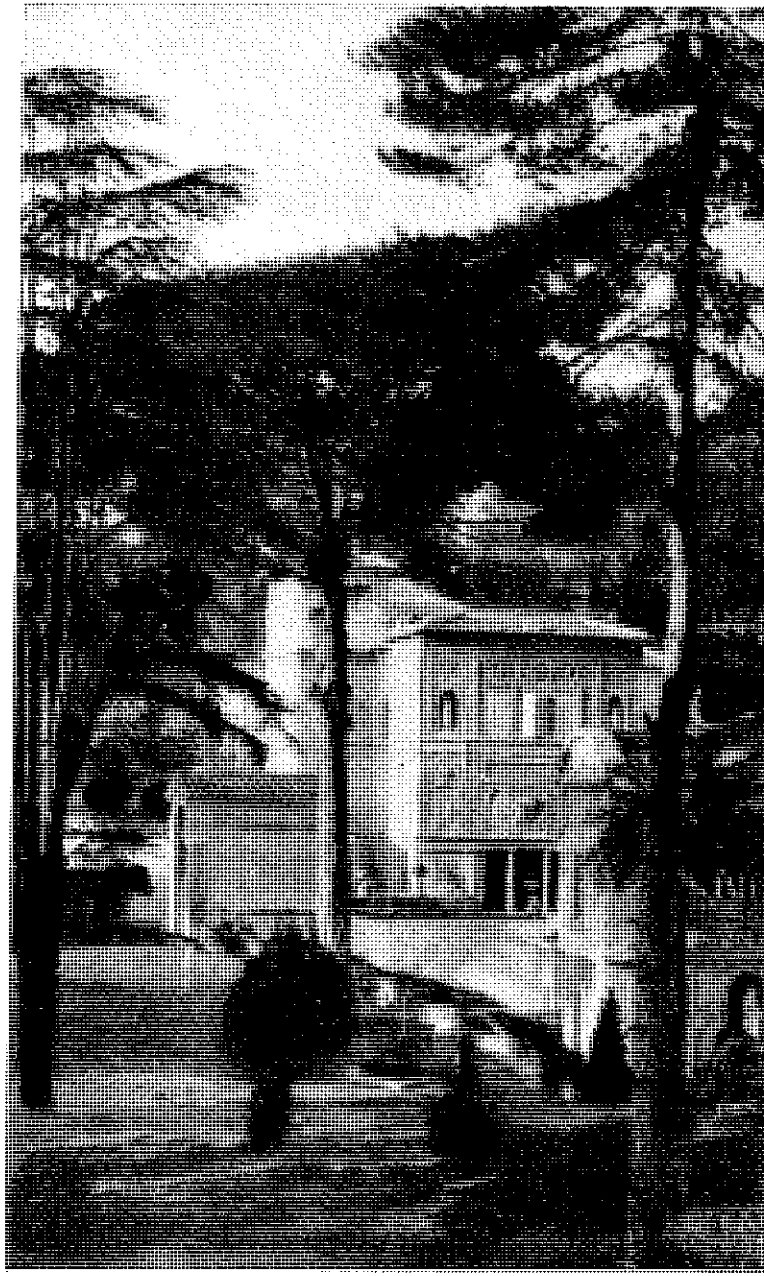

PONTIFICIAE ACADEMIAE SCIENTIARVM SCRIPTA VARIA

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STUDY WEEK

ON

BIOLOGICAL AND ARTIFICIAL MEMBRANES AND DESALINATION OF WATER

April 14-19, 1975

EDITED BY ROBERTO PASSINO

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INTRODUCTION

L'eau est une des plus importantes ressources naturelles, étant un élément irremplaçable pour la vie.

On peut admettre que l'eau représente à peu près 90 % de la matière vivante et encore qu'un taux de 50 % indique un tissu mort, une vie mise en demeure, ou une structure biologique qui joue un rôle purement mécanique.

Il est hors de doute, de même, que les mécanismes homéostatiques qui assurent la vie de l'homme sont en étroite dépendance des variations du taux de l'eau de l'organisme. Dans l'organisme humain, l'eau occupe un pourcentage dont la moyenne est de 67 %.

En plus, le flux hydrique se lie dans le monde végétal à la fonction chlorophylienne, sans laquelle la vie humaine serait impossible. Une perte trop grande de l'eau par transpiration végétale fait paraître une diminution de la photosynthèse, due à une réaction de défense de la plante par retrécissement des « stomata », de même que la baisse d'humidité au sol produit l'impossibilité d'absorption de l'eau par la racine et nuit à la vie végétale, ce qui montre l'importance de l'humidité du sol pour la vie végétale et indique le rôle essentiel de l'irrigation pour la productivité agricole.

D'autre part, le développement social et, de même, l'industrie, dépendent intrinsèquement des disponibilités de l'eau

existante, dont la diminution peut avoir des résultats les plus funestes.

A première vue, il semble que les réserves hydriques existantes sont suffisantes pour les besoins présents et futurs de l'humanité, surtout si l'on considère les charactéristiques particulières de l'eau, ressource naturelle qui possède un cycle, par lequel l'eau souterraine passe aux surfaces marines et terrestres pour gagner l'état gazeux par évaporation et transpiration, et de là, à sa retombée et à sa réintégration aux dépôts souterrains et à l'eau des surfaces terrestres et maritimes, en formant un flux constant. A l'exception d'une fraction minimale, non récupérable ou très onéreusement, dans certains procédés industriels, le système est fermé.

Les réserves hydriques de la Terre ont une valeur approximative de $1{,}34 \times 10^9$ km^3 (kilomètres cubes), dont 97% se trouvent dans les océans et les lacs salés, et $38{,}3 \times 10^6$ km^3 sous forme d'eau douce.

Une grande partie de cette eau se trouve dans les glaciers et les calottes polaires (ap. 77 %), inutilisables jusqu'à présent, $8{,}6 \times 10^6$ km^3 dans les dépôts souterrains, ce qui donne approximativement 1250 km^3 d'eau douce sur la surface terrestre. La valeur dynamique correspondant à l'apport annuel du cycle à la surface est de l'ordre de 20 à 30 fois la valeur statique moyenne donnée, soit de l'ordre de 35000 km^3.

Si l'on admet la valeur de 500-1000 mètres cubes par an et par habitant de la Terre comme suffisante, la demande pour une population de 3,5 à 10×10^9 habitants serait de l'ordre de l'eau de surface existant actuellement.

Le problème doit se poser néanmoins différemment, d'abord parce que l'eau qui existe à la surface est distribuée très irrégulièrement dans l'ensemble, et ensuite, parce que l'accroissement de son usage industriel ou des besoins d'irrigation supposent une augmentation de l'utilisation de l'eau que nous sommes loin de prévoir.

Il faut souligner encore l'existence de zones arides, qui présentent de grandes difficultés ou presque l'impossibilité de

développement agricole et industriel et qui ne font qu'augmenter, surtout à cause du déboisement et de la mauvaise utilisation agricole. De ce fait, le manque d'eau est présent ou menace une grande partie de la population terrestre.

D'autre part, les conditions de notre évolution sociale rendent le besoin d'un accroissement de l'utilisation de l'eau chaque jour plus pressant. Ce sont l'augmentation de la population, qui entraîne le besoin d'irrigation nécessaire à l'amélioration de la production alimentaire, l'accroissement du niveau de vie et de l'éducation sanitaire, et l'industrialisation, qui montrent, entre d'autres causes, et d'une façon précise, la nécessité d'un accroissement de la disponibilité de ressources hydriques. Il est prévu que ce besoin va se dédoubler dans les prochaines décennies, alors que les volumes excédentaires vont s'amenuiser l'une façon dangereuse, même dans les régions qui nous paraissent maintenant abondamment favorisées.

En raison de l'aggravation du problème hydrique, l'UNESCO a organisé une Décennie Internationale de l'Hydrologie (1965-1975), qui a bien étudié la situation actuelle et sa perspective, et les Nations-Unies organisent une « Conférence de l'Eau », qui aura lieu du 7-18 mars 1977 à Mar del Plata en Argentine.

Les problèmes posés par la consommation croissante de l'eau exigent la création de règles pour son utilisation rationnelle, soit dans le cadre national, soit au niveau régional ou international, et, dans le domaine de la recherche scientifique et technique, l'étude de méthodes industrielles de conservation de l'eau, de celles capables de diminuer l'évaporation de l'eau des divers réservoirs, comme les grands barrages et les lacs artificiels, celles d'obtention du récyclage de l'eau utilisée, et encore des techniques de dessalement, dont l'étude se poursuit depuis longtemps, afin d'obtenir de l'eau pure soit à partir de l'eau de la mer, soit des eaux marâtres. Quelques-unes de ces techniques commencent à être appliquées en mesure considérable, mais leur rendement laisse encore beaucoup à désirer.

D'une façon générale, on peut diviser les techniques de dessalement en deux groupes. Celles où l'eau purifiée obtenue

se maintient liquide au cours de l'opération, et celles où elle est soumise à changements de phase.

La première catégorie comprend les échangeurs ioniques, l'utilisation de précipitants de sels, l'extraction des solutés par des solvants, l'électrodialyse. On peut placer encore dans cette catégorie les diverses formes d'osmose et la méthode qui utilise l'absorption biologique sélective. Dans la deuxième catégorie se placent la distillation et la congélation.

Certaines de ces méthodes peuvent produire de l'eau extrêmement pure — ayant une conductivité électrique très basse. C'est le cas des échangeurs d'ions. Ces techniques, néanmoins, ne sont utilisables en pratique qu'à faible échelle et pour des eaux à concentration saline relativement basse.

On peut admettre que les méthodes qui présentent actuellement les meilleures perspectives d'utilisation industrielle sont la distillation et l'électrodialyse à membrane sélective.

Le problème du dessalement présente un aspect énergétique important. Il correspond au besoin de fournir l'énergie nécessaire à la séparation de sels dissous de leur solvant.

Pour diminuer la concentration saline de 90% à partir de l'eau de la mer (contenant à peu près 35 kg par m^3), il est nécessaire d'employer au moins 2 kwh par m^3. Le besoin d'énergie est en pratique de 20 à 80 fois plus élevé à cause de l'irreversibilité des processus. D'où l'intérêt de diminuer le coût énergétique des processus de distillation en utilisant comme source d'énergie à bon marché l'énergie solaire, ou encore, la chaleur dissipée dans les centrales thermiques et les processus industriels. Dans les procédés d'électrodialyse le rendement énergétique doit être possiblement amélioré avec le perfectionnement de la sélectivité des membranes utilisées pour la séparation des solutés.

Un grand effort est fait actuellement dans l'étude du perfectionnement de ces membranes, lesquelles possèdent une des propriétés les plus importantes des membranes biologiques indispensables au maintient des cellules animales et végétales, c'est-à-dire, leur sélectivité.

L'étude de cette propriété, soit chez les membranes naturelles, soit chez les membranes artificielles, est multidisciplinaire et exige la participation étroite de physiciens, biochimistes, physico-chimistes, biophysiciens, chimistes, morphologistes, physiologistes, botanistes, biologistes, ingénieurs, étc. Son importance ne peut être suffisamment soulignée. Ainsi, une grande partie des travaux faits en Biologie moderne se penche sur les propriétés des membranes, parce que c'est à leur niveau que se joue une grande partie des actions qui déterminent l'activité cellulaire.

De même, en vue de leur importance pour la production du dessalement, l'attention de nombreux groupes de travail se porte activement sur l'étude des membranes sélectives utilisées à cette fin. On espère à chaque moment des résultats positifs, nécessaires au perfectionnement d'une méthode qui pourra être très utile pour la solution d'un des problèmes qui préoccupent beaucoup les pays en voie de développement, où se trouvent la majeure partie des zones arides: l'obtention de l'eau pour la vie de l'homme, pour l'agriculture et pour l'industrie.

Il a paru ainsi d'intérêt au Conseil de l'Académie Pontificale des Sciences de réunir dans une « Semaine d'Etude » des grands spécialistes des membranes naturelles et artificielles, en vue d'un échange d'idées, d'informations scientifiques et de techniques capables d'apporter des progrès aux méthodes utilisées pour le dessalement, d'une part, et d'autre part, de donner à ceux qui s'occupent des propriétés de membranes biologiques des nouvelles perspectives de travail et la connaissance des techniques qui auraient pû passer inaperçues.

L'intérêt démontré par tous les participants, la qualité des mémoires présentées, les débats qui les ont suivies, la chaleureuse ambiance qui a persévéré au cours des cinq jours de travail indiquent sans aucun doute que l'événement a eu des résultats positifs, dont le présent recueil de textes et de discussions est un témoignage.

Au nom de l'Académie je désire renouveller aux participants de la « Semaine d'Etude » ma profonde gratitude

pour le zèle, la dédication et le dévouement avec lesquels ils se sont donnés à leur tâche. J'aimerais aussi signaler avec toute ma gratitude la collaboration donnée à l'organisation de la « Semaine d'Etude » par M. le professeur Roberto Passino, responsable aussi pour l'édition de ces comptes-rendus.

Mes remerciements vont aussi, avec réjouissance, au Directeur de la Chancellerie de l'Académie le Révérend Père Enrico di Rovasenda, aidé par Mme. Michèle Porcelli et Mr. Filippo Colelli, ainsi qu'au service de secrétariat dirigé par Melle Lucas, aidée par ses auxiliaires, et encore à Mme. Nerina Anglesio Cibrario, qui s'est occupée d'une partie du service d'interprétation technique.

CARLOS CHAGAS

Président de l'Académie Pontificale des Sciences
et de la Semaine d'étude

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L'organisation de la Semaine d'Etude était confiée au Directeur de la Chancellerie de l'Académie Pontificale des Sciences, Père ENRICO DI ROVASENDA, en collaboration avec Madame MICHÈLE PORCELLI-STUDER, Mr. FILIPPO COLELLI, Mademoiselle JOSEPHINE LUCAS et Madame NERINA ANGLESIO.

Les visites des dames à Rome et Castelgandolfo, où leur offrit une réception le Dr. CARLO PONTI, Directeur des Villas Pontificales, furent organisées par Madame PAOLA GATTI.

En même temps que la Semaine d'Etude, l'Académie a tenu sa Séance Plénière, à laquelle ont participé les Académiciens Pontificaux suivants: CARLOS CHAGAS, GEORGES CHAUDRON, ANTONIO DE ALMEIDA, CHRISTIAN DE DUVE, PAUL A. DIRAC, PERCY C. GARNHAM, WOLFGANG GENTNER, Rév. MARTINO GIUSTI, GERHARD HERZBERG, SVEN HÖRSTADIUS, Rév. JOSEF JUNKES, THOMAS A. LAMBO, JEAN LECOMTE, JÉRÔME LEJEUNE, LOUIS LEPRINCE-RINGUET, MANUEL LORA-TAMAYO, GIOVANNI BATTISTA MARINI-BETTÒLO, SANICHIRO MIZUSHIMA, SEVERO OCHOA, Rév. DANIEL P. O'CONNELL, JAN H. OORT, MAURO PICONE, GEORGE PORTER, MARCEL ROCHE, MANUEL SANDOVAL-VALLARTA, Rév. ALFONS STICKLER, Rév. PATRICK TREANOR, HANS TUPPY, ALFRED R. UBBELOHDE.

Au cours de la Semaine d'Etude, les Conférences suivantes ont été données:

Prof. SEVERO OCHOA, Roche Institute of Molecular Biology, Nutley, New Jersey (USA): *The Molecular Basis of Heredity and Evolution.*

Prof. GIAMPIETRO PUPPI, Istituto di Fisica dell'Università di Bologna (Italia): *Gli aspetti scientifici e tecnici della salvaguardia di Venezia.*

En outre, la Médaille d'or Pie XI, Dr. STEPHEN WILLIAM HAWKING de l'Université de Cambridge (Angleterre) a illustré au cours d'une conférence ses recherches concernant les *Trous Noirs.*

L'AUDIENCE

ET

LE DISCOURS DU SAINT-PERE

Le 19 avril à 12 heures le Saint-Père accorda dans la Salle du Concistoire du Palais Vatican une Audience solennelle aux Membres de l'Académie Pontificale des Sciences, aux Participants à la Semaine d'Etude et à la Médaille d'or Pie XI, Dr. STEPHEN WILLIAM HAWKING.

Le Groupe était guidé par le Président de l'Académie Prof. CARLOS CHAGAS, le Président émérite Rév. P. DANIEL JOSEPH KELLY O'CONNELL et le Directeur de la Chancellerie Rév. P. ENRICO DI ROVASENDA.

A l'Audience étaient présents en outre les Epouses des Académiciens et des Participants à la Semaine d'Etude, les parents du Dr. HAWKING, les Membres de la Chancellerie de l'Académie et du Secrétariat de la Semaine d'Etude.

L'Assemblée était honorée par la participation de quatorze Eminentissimes Cardinaux, de Leurs Excellences Mons. GIOVANNI BENELLI, Substitut à la Secrétairerie d'Etat et Mons. AGOSTINO CASAROLI, Secrétaire du Conseil pour les Affaires Publiques de l'Eglise et du Corps Diplomatique accrédité près du Saint-Siège.

Après avoir gagné le trône, le Saint-Père donna son assentiment au Président de l'Académie le Prof. CARLOS CHAGAS, qui s'adressa au Souverain Pontife en ces termes:

Sainteté,

Au terme de la Séance Plénière qui vient d'avoir lieu et d'une laborieuse Semaine d'Etude, l'Académie Pontificale des

Sciences, à la présence des éminents Cardinaux, du Corps diplomatique et de toute cette assemblée d'élite, Vous rend son hommage le plus profond et désire Vous exprimer sa gratitude pour l'appui que constamment Vous lui avez accordé.

En outre, elle Vous dit très humblement sa profonde admiration pour l'intérêt que Vous portez au rôle que la science doit jouer dans l'humanisation de notre civilisation présente et pour l'amélioration de la condition de l'homme sur la terre.

Au cours des deux premiers jours de la Séance Plénière, les Académiciens Pontificaux ont présenté de nombreux mémoires scientifiques représentatifs de leur activité inépuisable et féconde.

L'Académie reçoit de cette façon des informations précieuses au sujet des travaux qui se déroulent dans les laboratoires situés dans les diverses nations d'où sont originaires les Académiciens Pontificaux et réaffirme ainsi le caractère international qui lui est propre.

Pendant la Séance Plénière trois conférences ont été prononcées. La première par le Prof. Giampietro Puppi a traité « les aspects scientifiques et techniques de la sauvegarde de Venise ». La question surpasse de beaucoup son aspect purement esthétique, parce que Venise est en fait ce qu'on appelle un « laboratoire naturel ».

La seconde conférence, tenue par l'Académicien Pontifical Prof. Severo Ochoa, a eu comme objet « les bases moléculaires de l'hérédité et de l'évolution ». Peu de sujets ont une telle signification pour l'avenir de l'homme et reçoivent une attention aussi grande.

La troisième conférence a consisté dans la présentation, selon la coutume, des travaux du lauréat de la Médaille d'or Pie XI, le Dr. Stephen William Hawking.

Les Académiciens Pontificaux ont pris aussi connaissance, au cours de notre Séance Plénière, des initiatives prises par l'Académie depuis que Votre Sainteté a bien voulu m'honorer en me confiant la charge d'en assurer la présidence.

Dans l'activité future de notre Académie, l'organisation des Groupes de travail, tel que celui qui a eu lieu en 1974, doit

prendre sans doute, avec Votre bienveillance, un rôle toujours plus significatif.

Les Semaines d'Etude que l'Académie Pontificale des Sciences organise s'insèrent dans un cadre qui rend les travaux de l'Académie favorablement connus dans toute la comunauté scientifique.

Cette année, un groupe d'éminents spécialistes a essayé de rassembler des données scientifiques et méthodologiques sur les membranes naturelles et artificielles, au but d'intégrer leurs connaissances dans un ensemble qui permettrait d'offrir une contribution utile aux problèmes du déssalement, dont le besoin s'accentue au fur et à mesure que s'accroissent l'urbanisation et le déboisement, et s'aggrave le problème de l'irrigation. Les travaux réalisés ont été extrêmement fructueux.

Il se peut que le progrès de chaque discipline scientifique fasse que sa compréhension soit plus hasardeuse, ce qui donne aux réunions multi-disciplinaires, comme celles qui ont eu lieu, une grande importance. Elles se transforment dans un lieu d'échange d'informations, de compréhension et d'incitation à l'ouverture de nouveaux sentiers de recherches.

En effet, sans cet échange d'informations on ne peut attendre que très peu de l'indispensable contribution que la science doit donner au progrès social, dont chaque aspect réclame l'apport simultané de diverses disciplines scientifiques. Il incombe aux Académies scientifiques de réaliser cet échange d'informations. Votre Académie le remplit d'une façon vigoureuse et le fera davantage encore.

Il est vrai toutefois, que quelquefois les solutions scientifiques ne se transforment pas en succès d'application pratique, parce qu'elles se trouvent immobilisées par la puissance économique de quelques groupes ou de certaines nations.

Ceci ne doit pas décourager la poursuite de la connaissance scientifique par les savants qui doivent être profondément réconfortés dans des moments comme celui-ci, quand ils se sentent appuyés par Votre Sainteté.

La sérénité qui guide Vos paroles et Vos actions, malgré

l'inquiétude et l'angoisse qui bouleversent le monde, doit être un espoir pour tous ceux d'entre nous, très nombreux, qui se demandent si l'essor de leurs découvertes et l'application de la connaissance qu'ils ont fait progresser, seront utilisés au profit de l'Humanité et pour l'apaisement des esprits. Votre réception et l'attention que Vous accordez aux travaux, nous assurent que le résultat obtenu au cours de nos réunions portera des fruits.

Très Saint-Père, je me permets de Vous exprimer à nouveau la gratitude des Académiciens Pontificaux et de tous les Participants à la Semaine d'Etude. Je dois aussi Vous exprimer ma reconnaissance filiale pour la générosité avec laquelle Vous avez soutenu la charge très honorable que Vous m'avez confiée.

Je me réjouis de poter à Votre connaissance que la Médaille d'or Pie XI a été assignée cette année au Dr. Stephen William Hawking de l'Université de Cambridge. Très jeune il a acquis une juste notoriété par ses travaux réalisés grâce aux méthodes mathématiques qu'il a développées. Ces travaux portent sur la relativité et le champ gravitationel, sur les « trous noirs » et sur l'univers en expansion, et se placent ainsi dans la même lignée de recherches de notre regretté Président Monseigneur Georges Lemaître. Par l'apport considérable qu'il a donné à la science moderne, son jeune auteur est devenu un scientifique de renommée internationale.

Veuillez, Très Saint-Père, bénir les travaux de Votre Académie des Sciences, ainsi que ceux entrepris par les Participants à la Semaine d'Etude. Je suis sûr qu'ils contribueront tous à l'avancement de la science et au progrès de la condition humaine.

Sainteté, je Vous prie encore de remettre, dans Votre paternelle bienveillance, au Dr. Stephen William Hawking la Médaille Pie XI, qu'il a si bien méritée.

Le Saint-Père daigna répondre par le Discours suivant:

Messieurs les Cardinaux, Excellences,

A l'issue de votre Semaine d'Etudes, Nous sommes heureux de vous renouveler l'expression de notre profonde estime et nos encouragements chaleureux à apporter au progrès scientifique la contribution de haute qualité dont l'Académie Pontificale des Sciences est capable.

Si le Saint-Siège se réjouit de cette contribution, et en partage avec vous la fierté, c'est en raison du service notable que vous pouvez rendre à l'humanité pour une connaissance approfondie de la nature et l'amélioration des conditions de vie. L'Eglise est encore plus directement concernée lorsqu'il s'agit

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Your Eminences, Excellencies

At the end of your Study Week, we are happy to renew to you the expression of our deep esteem and our warm encouragement to make to scientific progress the high quality contribution of which the Pontifical Academy of Sciences is capable.

If the Holy See rejoices at this contribution, and is proud of it together with you, it is on account of the considerable service you can render mankind for a more thorough knowledge of nature and the improvement of living conditions. The Church is even more directly concerned when it is a question of fields in which science, ethics and faith are involved concurrently, sectors in which your testimony as believers together with your scientific competence is particularly appreciated.

During the year 1974, the activities of the Pontifical Academy of Sciences were continued, under the great drive of its President,

de domaine où sont impliquées en même temps la science, l'éthique et la foi, secteurs où votre témoignage de croyants joint à votre compétence scientifique est particulièrement apprécié.

Durant l'année 1974, les activités de l'Académie pontificale des Sciences n'ont pas manqué, sous l'impulsion vigoureuse de son Président auquel Nous tenons à rendre hommage. Travaux et confrontations d'experts, publications scientifiques, manifestations culturelles, interventions en relation avec le Synode des Evêques ont manifesté avec éclat la vitalité de votre institution, qui va bientôt fêter ses quarante ans. Nous gardons un souvenir particulier de la Commémoration de Guglielmo Marconi, due à votre initiative.

Actuellement vous venez d'étudier le problème hautement spécialisé des membranes biologiques et artificielles capables de procurer le déssalage des eaux. Nous n'entrerons point, vous le pensez bien, dans la complexité de cette question technique, ni dans ses possibilités d'application qui seraient sans doute encore prématurées. Mais Nous savons qu'il s'agit là d'une sorte de métabolisme important que l'humanité a intérêt

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to whom we wish to pay tribute. Work and meetings of experts, scientific publications, cultural events, interventions in connection with the Synod of Bishops manifested brilliantly the vitality of your institution, which will soon celebrate its fortieth anniversary. We remember particularly the Commemoration of Guglielmo Marconi, due to your initiative.

At present you have just studied the highly specialized problem of the biological and artificial membranes capable of bringing about the desalination of water. As you can imagine, we will not go into the technical question, or into its possibilities of application, which would probably still be premature. But we know that it is a question of a kind of important metabolism, which it is in the interest of mankind to determine, since the shortage of its reserves of fresh water threatens to hinder its development.

Let us just emphasize, in the more general field of scientific research, two attitudes which, it seems to us, should characterize

à bien cerner, alors que la pénurie de ses réserves d'eau douce risque d'entraver son développement.

Soulignons seulement, dans le domaine plus général de la recherche scientifique, deux attitudes qui Nous semblent devoir caractériser le savant, et spécialement le savant chrétien. D'une part, il doit se poser loyalement la question de l'avenir terrestre de l'humanité et, en homme responsable, concourir à le préparer, à le préserver, à éliminer les risques; Nous pensons que cette solidarité avec les générations futures est une forme de charité à laquelle beaucoup d'hommes sont d'ailleurs sensibles aujourd'hui, dans le cadre de l'écologie. Mais en même temps, le savant doit être animé de cette confiance que la nature réserve des possibilités secrètes qu'il revient à l'intelligence de découvrir et de mettre en oeuvre, pour parvenir au développement qui est dans le dessein du Créateur. Cette espérance bien comprise dans l'Auteur de la nature et de l'esprit humain est capable de donner une énergie nouvelle et sereine au chercheur croyant.

Dans cet esprit, Nous vous encourageons à poursuivre vos

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the scientist, and especially the scientist who is a Christian. On the one hand, he must loyally consider the question of the earthly future of mankind and, as a responsible person, contribute to prepare it, preserve it and eliminate risks; we think that this solidarity with future generations is a form of charity to which a great many men are sensitive today, in the framework of ecology. But at the same time, the scientist must be animated by the confidence that nature has in store secret possibilities which it is up to intelligence to discover and make use of, in order to reach the development which is in the Creator's plan. This hope in the Author of nature and of the human spirit, rightly understood, is capable of giving new and serene energy to the researcher who is a believer.

In this spirit, we encourage you to continue with your work and to carry out, according to the financial means, alas limited, of the Academy, the happy initiatives that do it credit.

We have the joy of presenting now the Pius XI Medal to

travaux et à réaliser, selon les moyens pécuniers, hélas limités, de l'Académie, les heureuses initiatives qui lui font honneur. Nous avons la joie de remettre maintenant la Médaille Pie XI à Monsieur Stephen William Hawking, dont les études, entre autres, sur les « Black Holes » lui ont mérité à juste titre une réputation internationale. Toutes nos félicitations, cher Professeur, et à vous tous, chers Messieurs, nos voeux les meilleurs pour vos activités et celles de l'Académie. Nous y joignons, en gage de notre sollicitude pour votre vie spirituelle et celle de vos proches, notre Bénédiction Apostolique.

Mr. Stephen William Hawking whose studies, among others, on "Black Holes" have rightly won him an international reputation. All our congratulations, dear Professor, and our best wishes for all of you, Gentlemen, for your activities and those of the Academy. We add our Apostolic Blessing, as a token of our solicitude for your spiritual life.

Achevé son Discours et reçu l'hommage des Eminentissimes Cardinaux, le Pape s'entretint ensuite avec le Président de l'Académie, le Président Emérite et le Directeur de la Chancellerie, avec les Académiciens et les Participants à la Semaine d'Etude. D'une façon émouvante et paternelle le Saint-Père s'attarda avec la Médaille d'or Pie XI, le Dr. STEPHEN WILLIAM HAWKING et ses parents.

Avant de quitter Rome, les Participants à la Semaine d'Etude envoyèrent le télégramme suivant pour exprimer au Saint-Père leur profonde gratitude:

Les scientifiques réunis dans la Cité du Vatican pour présenter leurs travaux et échanger leurs points de vue sur les structures et les propriétés des membranes qui peuvent être utilisées pour la désalinisation de l'eau en prenant comme modèle les membranes biologiques naturelles adressent à Votre Sainteté leurs plus chaleureux remerciements pour les avoir appelés par l'intermédiaire de l'Académie pontificale des sciences à échanger leurs expériences et leurs idées dans un climat de sérénité et d'entière liberté pour la recherche du vrai. Le souvenir de ces journées, de l'esprit qui les a guidées, de l'accueil reçu et enfin le bienveillant intérêt, accordé par Votre Sainteté, à nos études dirigées vers le bien de l'humanité, représentent la récompense à notre intense travail et engagement. Daigner recevoir, Sainteté, nos plus chaleureux et humbles remerciements pour l'audience solennelle accordée et pour l'enseignement précieux que nous avons reçu.

C. CHAGAS, G. ALBERTI, G. ASTARITA, P. F. BAKER, M. BÂTISSE, P. D. BOYER, A. P. DE CARVALHO, C. DE DUVE, I. DE MEIS, W. HASSELBACH, O. KEDEM, R. D. KEYNES, L. LEPRINCE-RINGUET, A. M. LIQUORI, E. A. C. MACROBBIE, P. MEARES, A. M. MONNIER, P. MUELLER, G. PALADE, R. PAOLETTI, R. PASSINO, R. PATERSON, R. L. POST, I. M. RITCHIE, C. L. SLAYMAN, K. SOLLNER, A. K. SOLOMON, K. S. SPIEGLER, A. I. STAVERMAN, W. STOECKENIUS, T. TEORELL, S. WEIDMANN.

TRAVAUX SCIENTIFIQUES
ET
DISCUSSIONS

N. B. Les épreuves ont été corrigées par les Auteurs.

I

WATER NEEDS AND THE IMPORTANCE OF DESALINATION

BALANCING NEEDS AND RESOURCES IN THE USE OF WATER

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The history of man has always been marked by a special relationship with water. Indeed, the history of man could be written in terms of the struggle to find solutions — always provisional — to the increasingly complex problems of the management of our planet's most valuable renewable resource. Yet while it is a truism to state that water is the basis of all life and an absolute necessity for all our varied domestic, agricultural and industrial activities for which no substitute can be devised, we are seldom fully aware of the implications of this truism and the countless direct and indirect ways in which we depend on water.

This complex man-water relationship occurs in an infinite variety of geographic, economic, social and cultural situations and gives rise to problems which are themselves of a great diversity all over the world. But since our times are favourable to global approaches and to anxiety about the future, there is no dearth of literature in which these infinitely varied problems are loosely lumped together and referred to as the world-wide " water crisis ". This simplistic terminology is employed in connexion with many other prohlems (the " food crisis ", the " population crisis ", the " energy crisis ", etc.) and has, of

course, the advantage of highlighting their global dimensions; but it always tends to blur the fact that situations vary considerably from place to place. Indeed, in the case of water, the very nature of the problems makes the use of such terminology, as we shall see, virtually meaningless.

Broadly speaking, it could be said that there is a water problem when demand is not adequately balanced by supply. This can occur not only when there is an absolute *scarcity* of water, as in the case of arid areas, but also when there is an *excess* of water, as in the case of floods. The *use and development* of water resources may be *insufficient,* as in the case of inadequate domestic water supply; it may be *inappropriate,* leading for instance to wasteful consumption; finally, the water may be of *poor quality,* from natural causes or through human action. In most cases, several of these types of problems interact in a particular area, combining to produce complexes of water problems. Floods can occur in very arid areas; or a poorly drained irrigation scheme could result in waterlogging of the fields and salinization of the soils and this might occur in combination with an inadequate domestic supply to the farmers. It would not be correct to assume, therefore, that problems stem simply from insufficiency in the quantity or deterioration in the quality of water. In reality, quantity, quality and management methods are intrinsically linked. Furthermore, most water problems are themselves directly connected with changes occurring in the human environment, such as urbanization, industrialization or agricultural development. Water problems cannot, therefore, be reduced to the problem of the manipulation of a natural product or raw material, since water itself is a fundamental factor in the shaping of the environmental system in which it is used.

Remembering its very special nature and role in our total environment, water has to be managed rationally as a " renewable " natural resource in the best interest of man. In this respect, the solution of water problems in a particular area, and in the world in general, will depend on our managerial

capacity to balance needs and supplies, in other words, to be able to provide the amounts of water required, in the quality required, where and when they are considered to be needed. We have therefore firstly to examine the demand and needs, secondly to analyze the resources, and finally to see how the balance can be achieved.

THE DEMAND FOR WATER

Water is used for many purposes by man: drinking, cooking, washing, irrigation, livestock breeding, industrial cooling and processing, hydropower generation, transportation, fishing, boating, swimming, fire control, etc. Conservation of certain water bodies and wetland areas is also a form of use. Water demands, however, are usually grouped for statistical purposes under three major headings: *domestic* or *municipal* use, *agricultural* use, and *industrial* use. This subdivision is not a rigid one, since water drawn by industry from public water supply systems, for instance, is often included under municipal use. On the other hand, it does not take into account a number of uses such as transportation, fishing, recreation or conservation. These latter uses however do not result in withdrawal of water from the water bodies and do not produce significant pollution (although they may restrict the possibility of other uses). They can, therefore, be set aside when discussing the main demands for water, although this does not mean that they should in any way be neglected in the overall management of water resources.

Some fundamental distinctions must be made concerning the nature of water use, be it for municipal, agricultural or industrial purposes. Some industrial uses do not imply *withdrawal* of water: this is the case of hydropower production which is called an in-stream use. Most of the water used for municipal or industrial purposes is actually withdrawn but returns to the river or to the ground water system and can possibly be re-used once or several times as long as it is not

too severely polluted. Conversely some water is *consumed* in the industrial process — for instance it may enter the product or go back as vapour to the atmosphere via a cooling installation.

The important point to stress here is that in agricultural uses, i.e. principally irrigation, most of the water which is withdrawn is also consumed through evaporation and transpiration. The same applies to water which is evaporated from reservoirs or fresh-water bodies. This water is in fact lost to that particular area. It cannot be re-used on the river basin concerned, although it will eventually fall again as rain somewhere.

a) *Domestic and municipal demand*

The water requirements of human beings depend in part on climate and physical activity but mainly on what is known as the standard of living. These requirements vary, therefore, from some 2 litres per day as a minimum in non-equipped rural areas to some 400 litres per day in high-standard urban areas. For the present population of the world an average daily consumption of 200 litres per capita would amount to a yearly total of less than 300 Km^3. In fact only about half this amount is actually used at present. This is any way a very small amount compared to dependable resources; for instance the average yearly discharge of the Mississippi alone is about 600 Km^3. Of course, human settlements have not been located only on the basis of availability of water and in fact have spread around older cities without much consideration for urban planning so that in many of them water supply raises serious technical problems. However, domestic and municipal needs, which only represent a few percent of total water demands, generally speaking, could easily be met from available water resources in practically all parts of the world. The problems lie elsewhere, and are mainly of a financial and organizational nature.

[1] I, 1 - *Bâtisse* - p. 4

Water for human use must be of good quality. It should normally not contain more than 500 ppm of dissolved salts, although higher amounts are tolerable, particularly in arid regions. It should be bacteriologically safe. Experience shows that there is a direct correlation between health standards and the regression of many endemic diseases on the one hand and adequate domestic water supply on the other.

Unfortunately, according to recent WHO studies [1], the present situation and the immediate future appear rather gloomy for a large part of the population of the world. Everywhere the spread of urbanization over-taxes water supply systems, which cannot cope with rapidly increasing demands, and favours the development of slums, bidonvilles, barrios or favellas where distribution systems are inexistant. However there is usually an expressed will in these settlements to benefit from adequate water supply and the problems of improving the situation could usually be solved, although this will not be easy. The situation is far more difficult in rural areas where the costs of supplying water to scattered dwellings are much higher, and in particular much higher per capita. WHO has calculated that to meet the modest goal of having 25 % of the rural population of developing countries benefiting from minimal safe water supply in 1980 would require an expenditure of more than 13 billion US Dollars. In 1970 only 12 % of this rural population had an improved water supply.

Two sets of questions might be raised here. First of all, in these rural areas, should not simple improvements be promoted, by inducing initiatives from the populations themselves, rather than by attempting to achieve directly the relatively high levels of safety and quality which are considered essential elsewhere? This approach is taken in China and some other countries and is in fact gradually becoming internationally accepted.

As regards urban areas, should not the double supply system, which exists partly in some large cities, be promoted,

[1] I, 1 - *Bâtisse* - p. 5

to avoid the use of high quality water for general industrial purposes, fire control or gardening?

In other words, if domestic water supply of sufficient quality is to be progressively recognized as a right, should not the techniques of management be geared towards a feasible and progressive satisfaction of this right? [2]

b) *Agricultural demand*

In temperate and humid regions, water is supplied to agriculture mainly by rainfall. Land use practices affect soil moisture, run-off and percolation, but water does not have to be supplied to the crops from other sources than precipitation. In arid and semi-arid regions, irrigation is indispensable and calls for an external source of water from a river or from ground water.

The difficulty with irrigation is that it requires considerable amounts of water, of which most is consumed through evaporation and transpiration, and this precisely in areas where water is normally scarce. On average, between 10,000 and 15,000 m^3 of water are required to irrigate one hectare per year. Over 2,000 Km^3 per year are used at present for irrigation in the world, but only some 30 % of this amount returns to rivers or ground aquifers. On top of this, water is lost through evaporation from the reservoirs created by irrigation dams: for instance, one litre out of eight from the Nile discharge is evaporated at Aswan. And again there are considerable losses through seepage from irrigation canals. Irrigation is a formidable water-waster.

Yet, at the same time irrigated land is far more productive than most other agricultural lands and the world of hunger in which we are bound to live in the foreseeable future requires an intensification of irrigation wherever this is feasible. According to FAO, in order to meet the growing demand, the irrigated area of the developing countries (excluding China) should be

[1] I, 1 - *Bâtisse* - p. 6

increased from 72 million ha in 1965 to 107 million ha by 1985. This would require an investment of some 36 billions US Dollars [3]. For the world as a whole, recent estimates indicate an expected increase of irrigated land from 230 million ha in 1970 to 310 million in 1985 and 420 in 2000.

These global figures do not necessarily provide a good appreciation of the magnitude of the problem. India alone has some 38 million ha under irrigation, with an increase of nearly 1 million ha per year, mainly through the use of tube wells. Its present water use is of the order of 370 Km^3 per year, some 95 % of which is devoted to irrigation. The country could irrigate more than 100 million ha, but under present technological conditions would not have sufficient water resources to do so. In Israel, where available water resources are almost entirely used, the share given to irrigation, still of the order of 75 %, has been reduced to satisfy domestic and industrial needs. This reduction had to be compensated for by improved irrigation efficiency and sewage water reclamation. In the USA the rate of increase in irrigation water demand is expected to diminish slowly as a result of improved efficiency in the use and transmission of the water but the amounts withdrawn as well as the amounts actually consumed will still be increasing after 1980 even under the most favourable hypothesis. (Annual withdrawal is expected to grow from 153 Km^3 in 1965 to 187 Km^3 in 1980 and to some 208 Km^3 in 2000 while annual consumption would grow from 90 Km^3 in 1965 to 112 Km^3 in 1980 and 125 Km^3 in 2000).

Water for large-scale irrigation must be very cheap, not exceeding a maximum of 3 cents per m^3, if it is not to exceed the economic value of the crops. Water for industrial uses can provide a return one hundred times greater. Yet irrigation cannot be regarded from the water use point of view only and will continue to demand large quantities of low-cost water. The quality of this water has also to be reasonably good. Although experiments such as those conducted by Unesco in Tunisia [4] show that, with adequate drainage, waters of

[1] I, 1 - *Bâtisse* - p. 7

4,000 ppm of dissolved salts and even more can be utilized successfully, it is generally estimated that irrigation water should not contain more than 1,000 ppm, depending on crop, soil and water characteristics.

c) *Industrial demand*

Industry uses water in many ways. These are usually grouped as follows: cooling water, processing water (entering the product or used in its processing), boiler water (for generating steam or for manufacturing processes), and water for miscellaneous purposes. These various groups vary greatly both as regards actual consumption of water and as regards pollution. Cooling, particularly in power plants, requires considerable amounts of water, and may account for more than 60 % of industrial water use in some countries. But fortunately most of the water is returned to the stream and its pollution is essentially limited to an increase in temperature. At the same time, water of poor quality can be used for cooling purposes and re-cycling is possible. It is therefore necessary, when planning industrial uses, to determine not only the gross water demand, but at the same time, the acceptable quality, the consumptive use foreseen, and the waste load it is anticipated will be discarged in the final effluent (*). Naturally these parameters are themselves affected by the amount of re-cycling or of waste water treatment taking place within the particular industry considered.

Because industrialization is rapidly developing everywhere, industrial use of water is also growing rapidly and may reach

(*) Waste load is often expressed in weight of biochemical oxygen demand (BOD) necessary for its degradation. However, this relates mainly to organic pollution and does not take into account other growing forms of industrial pollution due to toxic chemicals, salts, heavy metals, etc.... A combined index for measuring pollution levels is required none as yet has been internationally agreed upon.

some 80 % of total water withdrawal in the technologically advanced countries of Northern Europe where irrigation is not a necessity. In countries which are at the same time industrialized and have an important demand for agriculture, such as the United States, USSR, Hungary or France, the percentage of industrial withdrawals is only of the order of 40 or 50 %. It is virtually negligeable in Asia in comparison to irrigation withdrawals.

In any case, two essential factors are to be considered in industrial water demand. The first is the possible reduction of consumptive use. This depends on the industry concerned (for instance, food processing may consume five times more water per ton of product than the production of steel or chemicals) and can be achieved through re-cycling or technological improvements. The second factor is the reduction in waste load. This can be achieved through various methods such as in-plant treatment, underground disposal or modified production technology, since ultimately the greatest problem arising from industrial use is obviously stream pollution.

It is clearly not possible to discuss the problem of industrial water pollution in general terms, but it is necessary to stress that the drastic reduction of direct harmful industrial effluent discharge into streams or aquifers as well as of discharge of treated or untreated sewage from human settlements to levels at which self-purification can actually take place must be considered a priority objective for all communities and nations.

In this respect, it should be noted that, in the case of most industries, water is not an important element in total production costs or in the value of industrial products. This may have a negative effect since there is no incentive for industry to make rational and economical use of water. On the other hand it has the positive advantage that industry — unlike cities and agriculture — could achieve efficient water use and pollution control, particularly by adequate utilization of water re-cycling and purification techniques, unhampered by an excessive cost burden.

WATER RESOURCES

The amount of water in the hydrosphere is enormous. However, about 97.5 % of this is salt water contained mainly in the oceans and seas. Only 2.5 % consists of fresh water. But here again about 70 % of this fresh water is in the form of ice in the polar regions and in glaciers, representing some 24 million Km^3 [5]. Most of the remainder — approximately 30 % — is in the form of ground water. In fact, this evaluation of quantities of ground water is extremely approximate in most parts of the world and the statement that there are some 10 million Km^3 in easy-to-reach aquifers and in deep ground water aquifers is little more than an educated guess. The amount of water present as moisture in the soil (above the water table) is roughly estimated at 70,000 Km^3, while freshwater lakes contain some 90,000 Km^3. At any given moment, the amount present in the atmosphere is of the order of 13,000 Km^3 and the amount in all river channels of the world is only about 2,000 Km^3. Thus the figure for rivers, from where most of the water we need comes, looks dangerously small. However, these figures have little meaning in an actual evaluation of available water resources, because they are merely static figures. It is the dynamics of water systems, and particularly the rate at which rivers are continuously replenished which really matters here. With the exception of some waters which may be considered as " fossil " and are " mined " from very deep aquifers, all the water we use is derived from the hydrologic cycle whereby fresh water is precipitated on the surface of the earth in the form of rain or snow, percolates as ground water, evaporates from soil and plants, or runs off in rivers and lakes, until it reaches the sea from where it evaporates again.

In dynamic terms, the study of the world water balance in the hydrologic cycle shows that, whereas it would take several thousand years to renew the amounts of water stored in the oceans and in aquifers, it takes only about 10 days to

[1] I, 1 - *Bâtisse* - p. 10

renew the rivers and the atmosphere. According to the latest estimates, the total annual surface run-off to the world oceans is about 45,000 Km^3, to which could be added a direct discharge of ground water into the oceans of some 2,000 Km^3. It can be noted here that the annual run-off of rivers is more than 20 times higher than the amount of water stored in the river channels at a given moment.

In reality, these global figures for the world water balance have little meaning for water resources development in concrete cases. The phenomena which take place within the hydrologic cycle are much more complex in any given basin or geographic area, and their quantitative analysis and prediction forms part of the science of hydrology. The full understanding of regional and local water balances, which constitute subsystems of the global cycle, is essential for any specific resource assessment and management, because the use of water by man amounts always in fact to modifying and short-circuiting the natural functioning of these sub-systems. The aquisition of this understanding was one of the basic objectives of the International Hydrological Decade, which ended in 1974 [6].

In any detailed study, the interplay between streamflow, ground water flow, soil moisture transfer, evaporation from land and reservoirs and transpiration from plants has to be analyzed so that the possibilities and effects of human interventions can be assessed. We can, for instance, no longer ignore the influence of watershed management on the flood regime, or the interactions between streamflow and watertable aquifer exploitation, as was the case only a few years ago.

Modern tools and methods, in particular experimental river basins, representative basins, nuclear techniques, mathematical models and analog models, allow for understanding of hydrological systems and for studying their response to alternative possible utilization schemes. Such studies can be conducted for comparatively small areas, but are particularly useful for large areas. For instance, models have recently been established for the entire Chad basin, showing the remarkable features

which allow for Lake Chad to be a freshwater lake, for the deep artesian aquifers of the Northern Sahara, or for the huge sponge constituted by the Pantanal region in the Upper Paraguay river basin in Brazil. In all these cases, as in many others, available resources can be evaluated, and land and water management hypotheses can be tested.

It must be stressed here that the natural distribution of water resources is highly variable on the planet both in place and time, so that huge quantities of high quality water are not utilized and will not be utilized for a long time. The water of the Amazon cannot irrigate the Sahara, nor even the dry areas of Brazil. On the other hand, regulation of stream flow through the building of dams can easily be limited by the law of diminishing returns. For this latter reason, namely the irregularity of river flow, the dependable water supply is far less than the total runoff. Dependable streamflow in all rivers of the world under natural conditions can be evaluated at 12,000 Km^3 per year — not much more than a quarter of the total streamflow. To this figure should be added approximately 2,000 Km^3 representing the comparatively very large amount of water which is regulated by reservoirs. These are again global figures, but in the case of certain rivers the dependable runoff may constitute only a few per cent of the average runoff.

The above discussion may give the impression that only streamflow is taken into account in resource evaluation. This is in fact the case in many countries. Is it justifiable, given the growing use made of ground water resources? One should here make a distinction between three main types of ground water occurrence. The first is the water table aquifer, which furnishes directly the baseflow of rivers in alluvial plains. It would not be correct to consider this as a totally separate resource since there is constant interchange between the river and the aquifer. But this is not the case for the two other types — one where the ground water aquifer is not connected with any river flow and takes part in the water cycle through

[1] I, 1 - *Bâtisse* - p. 12

direct evaporation from the soil, a case very common in arid areas; and the other one where ground water occurs in deep artesian aquifers, where water recharge takes place only at a very slow rate or even not at all (fossil water). Because the two latter types are usually not well understood, they are often neglected in resource assessments. However, any correct assessment of resources should take fully into account the various types of ground water potentials. This is all the more important in that ground water has great advantages. It is better protected from pollution and from evaporation losses. It can often be found close to the area where it is needed. It can be developed rapidly and in progressive stages without the large scale investments required by surface storage. For these reasons, the utilization of ground water has seen a sharp increase in recent decades. In France for instance it is estimated that some 60 % of the water used comes from aquifers, where it is free from pollution, while only 40 % comes directly from rivers. This proportion is even higher in some northern European countries, and of course much higher in arid areas. The fact is that much still remains to be done to achieve a full evaluation of ground water resources.

There is no such thing as pure water in nature, and any assessment of water resources should refer to chemical composition. Ground water in particular tends to be more or less mineralized. Water with less than 1,000 ppm of dissolved salts is usable for a large number of purposes. However, as we have seen, a maximum of 500 ppm is normally preferable for domestic and agricultural uses and there are many specific uses in industry demanding still lower mineralization. From the point of view of water desalination, and particularly desalination with membranes, it is important to know the availability of brackish water, containing between 1,000 ppm and 10,000 ppm of dissolved salts. Comparatively little is known of the occurrence and amounts of ground water of this nature. In the United States, maps have been prepared by the U.S. Geological Survey giving the location, depth and quality of brackish waters

having respectively between 1,000 and 3,000 ppm (which can be used as such by industry or diluted with fresh water) and between 3,000 ppm and 10,000 ppm, which could be used after desalination [7]. The amounts available appear to be considerable. In Israel, however, detailed studies indicate that amounts of brackish ground water do not exceed 200 million m^3 per year [8], of which some 150 million m^3 are already being used either directly or diluted with fresh water.

How can water needs be met?

What conclusions can be drawn from the foregoing discussion of water demands and resources? A most reassuring conclusion would be that we are not, in fact, faced with a serious *global* water shortage, since the quantities of water of fair quality on which we can reliahly count are well in excess of present and foreseeable aggregate needs. These needs, which have been estimated at about 3,000 Km^3 per year in 1975, are expected to be of the order of 4,000 Km^3 in 1985 and 6,000 Km^3 in the year 2000, although predictions of this kind are of little scientific value. In the year 2000, dependable river flow will still amount to some 12,000 Km^3 per year. To this must be added the water stored in reservoirs, whose total capacity will probably be much greater than it is today, as well as additional water coming from the water table, from artesian aquifers and from non-conventional sources. The margin appears comfortable.

At the same time it must be emphasized that water can be, and in fact often is used more than once. The figures of dependable supply thus give only a very crude indication on the low side of what can actually be done with that supply. This means that with a reasonable amount of re-use we should still be well off at the turn of the century.

In practical terms, however, the picture is rather different and the water situation will have to be taken very seriously

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in many parts of the world, particularly in the industrialized and the arid countries.

The *global* figures which have been given should not hide the fact that present water demand is largely concentrated in a number of relatively small but densely populated areas of the world, usually not located close to the largest sources of supply which are the tropical rivers and some sub-arctic rivers. For this reason, the aggregate dependable streamflow *per capita* is, for instance, much smaller for Europe and Asia where it is of the order of 2,000 m^3 per year than for South America where it is over 20,000 m^3 per year. Again, within South America itself, this average figure takes no account of the local climatic and demographic conditions.

All this points to the need for projections of demands compared to existing and potential water resources carried out in specific conditions, not at the global or continental level, but at the level where interactions between needs and supplies in given quantities and qualities can be analyzed and managed technically and economically, which is usually the level of the river basin. In some cases, for instance in a small country with only one major river, this level might be the country level. In other cases, to ensure a co-operative approach involving adjacent basins or adjacent countries might be required. Elaborate methodologies are available for such projections the object of which is to provide a basis for actual planning of water resources development. Such methods usually involve the formulation of alternative scenarios for future developments, based on economic and social forecasts and goals, but they usually neglect possible changes in technology which are, of course, even harder to predict than economic and social variables. Although many of our present environmental difficulties can be traced back to the fact that in the past sectoral approaches have been taken in water resources development, single-purpose water use planning is still often the basis on which development decisions are made. But the tendency is more and more towards multipurpose regional planning where

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the different uses of water are considered simultaneously. In such planning, the respective advantages of various water uses — agricultural, industrial or domestic — as well as their disadvantages, such as consumptive use and pollution should be taken into account.

In practical terms, for economic planners, a kind of water-needs model should be established for balancing demand and supply in a particular basin. The method normally consists in evaluating and locating all water withdrawals for the various uses in the basin and at the same time determining for each waste disposal site the amount of water required to provide an acceptable level of dilution or to ensure self-purification. More or less sophisticated modelling approaches can be used. For instance the " loss-flow model " is centred on the maintenance of a given water quality, measured in dissolved oxygen content, at the same time as a given availability of flow in the river basin considered [9]. The comparison of the water " requirements " of the basin (water actually lost through various and successive uses plus water needed for dilution flow) with the water " supply " at various levels of regulation will show the situation under various hypotheses of economic, social and demographic development. Such models are far from perfect, in particular since they tend to ignore ground water potentialities, technological improvements, or changes in demand resulting from changes in water costs, but they constitute useful tools for planning and have the merit of considering the actual possibilities of re-use of water after sufficient pollution abatement.

Two points emerge from the preceding remarks in which it has been possible to touch only briefly upon the subject of water resources development planning. The first is that modern planning methods must now be carefully applied to all new water supply and development schemes, which can no longer be undertaken on the assumption that there is plenty of water and that anything can he dumped downstream [10]. The second is that control of pollution and maintenance of water

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quality is becoming progressively of greater importance than water quantity, and this not only in industrialized countries but equally in densely populated tropical areas as well as in arid areas. In other words it could be said that there should be no serious water supply problem for most parts of the world, thanks to the possibilities of re-use of water for industrial and municipal purposes, as long as a sufficient quality level can be maintained in streams and also in ground water aquifers. Nevertheless, if pollution control is not drastically improved, many industrialized areas where water supply is already becoming difficult, could soon be in serious trouble.

The question naturally comes to mind of the possibility of increasing the amount of water supply, or of improving its quality, through unconventional means. The potentialities offered by ground water, particularly from deep aquifers, have been mentioned, but they are often limited by high drilling and pumping costs. Similarly the transportation of water over long distances, from water-rich areas to water-short areas, using, for instance, pipelines where water is neither lost nor polluted, is attractive in some cases such as for municipal use. Such transportation, which can be combined with underground storage, is also rapidly limited by cost considerations. Water is still so cheap and is required in such large amounts that it cannot simply be treated like many other commodities. The towing and melting of icebergs remains for the moment in the realm of futurology. More significant — although much less spectacular — is the improvement in amount, regularity and quality of supply which can result from relatively simple methods of watershed management, which have of course to be considered as part of compresensive regional planning and land use policy.

There remain the possibilities offered by desalination. These are difficult to assess in view not only of the increasing cost of energy but also of the wide variety of situations in which the desalination process might be applicable. Specialized industries, tourist resorts or the inhabitants of small islands and

of arid coastal regions might consider it worthwhile paying the high price demanded for the relatively small amounts of water the desalination process can supply. But it seems obvious that in the foreseeable future desalination can offer no help for irrigation, that is for irrigation producing commercial crops on a market scale. It appears equally true that for certain industrial and municipal uses, provided present costs are seriously reduced through scientific and technological advances, desalination of brackish or saline water can play a significant role in the future. This seems all the more likely in that, as shown previously, the treatment of polluted water for re-use is any way a growing necessity and that there will be situations where choices will have to be made between these other forms of treatment and the use of desalinated water.

Ultimately, therefore, the problem of balancing water needs and resources appears in general to be more an economic and managerial problem than a problem of availability. Whichever way one looks at it, the development of water resources, at the scale and at the pace required by expanding populations and increasing demands, will call for enormous investments all over the world amounting to several billions of dollars per year, whether these investments are directed towards reservoir and irrigation channel construction, sewage treatment, pollution control, desalination, or any other appropriate activities. It would of course be ridiculous to consider investment on this scale without providing for a substantial amount of research and development in order to elucidate the many questions which are still unsettled, for instance in groundwater occurrence, or to improve technologies and practices in water use. There is no reason why precious irrigation water should continue to be lost through seepage and evaporation more or less in the way it was lost hundreds of years ago when water was plentiful [11]. Generally speaking, it is essential to develop the scientific and technological basis which will provide us with improved tools for rational management of water resources. This is the main objective of the new International

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Hydrological Programme, the Co-ordinating Council of which held its first session in Paris in April 1975.

It would be equally fallacious to engage in costly water resources development programmes without mastering their engineering-economic aspects. This implies that we give ourselves the kind of water management institutions which correspond to modern hydrological knowledge and to the dimensions of current and forthcoming programmes. Such institutions require interdisciplinary capability, intersectoral authority, and sufficient specialized manpower. This is not to be found in many places at the moment and indeed runs against the traditional *ad hoc* institutional arrangements which have evolved in the past. Yet, although no recipe valid for all cases exists, considerable improvement has been recently witnessed in many countries in this field and the forthcoming UN Water Conference to be held in 1977 could stimulate countries to put their water management house in order.

Does this mean that money, management, research and training will be sufficient? Probably not. For water problems are problems of people. And people have a wrong attitude towards water — at least those people whom we all know, the urban dwellers in industrialized countries who take it for granted that water will flow when they turn on the tap and who are also unware that most people in the world have no taps to turn. If it is considered that every human being has the right to an adequate supply of safe water for his domestic needs, it should also be understood that every-one has the duty to regard water as a precious resource which should not be wasted. The reduction of losses, and the maintenance or restoration of water quality can only be achieved if this end becomes the collective will of society, which itself implies an important change in the thinking of each individual. This can only be achieved through a generalized process of environmental education at all levels and adapted to the particular conditions prevailing in each country. Perhaps the real answer to water problems is for the human race to learn — or to re-learn — the value of water.

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DISCUSSION

Chairman: Prof. C. CHAGAS

KEDEM

You haven't at all mentioned artificial rain. Was that by chance or do you think that this has no serious chance of to be value whatsoever?

BÂTISSE

There are lots of things that I have not mentioned in trying to deal with such a vast subject as water, in this period of time. Artificial rain, it seems to me, comes quite low on the list of water resources. Pouring silver iodide on clouds, utilizing aeroplanes and that sort of things involves some environmental problems. On the other hand the effects of these techniques are not very convincing except in mountain areas or very localized areas. And there is also an international problem: what would happen if rain were increased in one place and diminished in another? There are however, serious studies made on artificial rain, including studies on these questions. But at the moment I don't think it is going to be of much practical value for increasing water resources.

MEARES

In cases where new land is brought into agricultural use, which

as you say must be done on a vast scale in the next decades, will there be any beneficial or deleterious effects resulting from land coming under agricultural use producing by itself local climatic changes which may enhance or reduce the rainfall in the area where the crops have been introduced?

BÂTISSE

The effect of vegetation, whether artificial, that is to say new cultivation, or natural vegetation on climate and rainfall are not clearly understood and this is one of the areas where much more research is needed. However, it doesn't seem that the replacement of natural vegetation by agriculture is leading to drastic changes. There has been a lot of talk recently about what would happen if the entire Amazon Basin were put under cultivation, assuming that this were possible, and many of the statements that have been made are not based on any relevant scientific evidence.

A similar reasoning has been made in suggesting that it would be a good thing to have the sea invading the lower parts of the Sahara, such as the Quattara depression in Egypt. This would supposedly change the climate. It would indeed change the micro climate locally, but it would not change the overall climate and the rainfall. I think there is a simple way of showing this. If, for instance, you consider the Red Sea which is a large body of water, you can see that it doesn't affect very much the climate of the shores of that sea. The same would apply to changes in land use. This does affect the hydrology of the watershed, as I have stressed, but I think the dangers or benefits of bringing new land under cultivation upon the climate or the rainfall are generally very much exaggerated.

SOLLNER

I have been interested in water problems for a long time. As a matter of fact at the beginning of World War II I started

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a few projects to provide lifesaving equipment for ship-wrecked people and persons in similar situations, for example in desert warfare. These projects were soon taken over by the U.S. government. Not much came of these efforts but there were other projects, some rather ingenious. As far as I know all these reports are now declassified and some of them describe equipment which might be developed for use on isolated farms, for humans and livestock, with a limited need for water. Anyone interested in this type of problem should look at these now declassified reports which still must be somewhere in Washington.

Second, I feel that our speaker was very circumspect in pointing towards the root of the problem of clean uncontaminated water. It is largely a political problem, primarily a political problem. If you want to prevent industry from dumping waste material into our great lakes, you have to make a law, that is all there is to it. Industry must be regulated and regulated on an international scale. This could be done, for instance in the following manner: if certain companies do not adhere to internationally agreed regulations, their products should automatically be banned by all other countries.

A third problem is the use of rivers and streams as industrial sewers. Would it be possible to have parallel with each stream a pipeline that disposes of the saline waste into the sea? Take the Colorado River into which the highly saline water coming, from large scale irrigation, is dumped. The Colorado River is by now saline to such a degree that the Mexicans who, if I remember correctly, are entitled to half the Colorado River water can hardly use it any more for irrigation. Such things are primarily political problems.

BÂTISSE

Well, I think everything can be described as a political problem, particularly in the case of water management. On the first point I agree that a considerable amount of information exists

on various methods and on the economics of desalinization for various uses. But I don't think this is classified any more. I think it is available and there has been very good work carried out in this respect by the Office of Saline Water of the U.S. Department of the Interior. They have done quite a lot in publishing bibliographies and monographs on this matter, which includes the experience which has been acquired in the past. As regards the second point, the question of legislation and regulation, I believe legislation itself must be based on the will of the people, at least I hope it has to be that way. The people therefore and their legislators must have a clear understanding of what is at stake but usually they do not. And also, as in the case of all legislation, certain choices have to be made.

In the particular case of water, these choices are not easy to make: therefore, as I tried to say, the whole legislation process ultimately must be based on a clearer awareness of all the people of the value of water. Then better legislation can be enacted; whilst at the moment there is reluctance, there is resistance to such a legislation. The main problem is that it is very difficult to balance the costs of water improvement programmes with the returns to society of these programmes. This balancing of costs and returns involved questions of what values you choose. It is very difficult to say what is the value of a clean lake, for instance, until 20 years ago, almost nobody would pay much attention to the cleanliness of a lake. Now people want their lakes to be clean. This change in values affects legislation now, but could not affect it 20 years ago. Then there is another point about this, which is the need for governments and public authorities to have the appropriate institutions to look after water problems in a balanced manner, taking all the pros and cons of the various possible options. This is something that does not exist in most cases, because as I tried to say, water use has been looked at usually from a single-purpose point of view, and not from the multi-purpose approach. Yet this integrated approach to the development of water resources is the basis on which sound legislation can be introduced. Finally

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you mentioned the question of dumping sewage into the sea, but I think this is mainly replacing one problem by another one without really solving anything. This is done, of course, in coastal areas, but when we pollute the sea instead of polluting the rivers, we are still faced with the dangers this sea pollution creates. Here again we have to be sure that the self purification process is taking place and the location of dumping and the amounts which the sea can tolerate have to be evaluated carefully before this can be done.

SOLLNER

The sea can tolerate an infinite amount of salt.

BÂTISSE

Yes, if we talk of salt, but sewage is not only salt. In some cases, such as the Mediterranean, sewage dumping is already a major problem.

KEYNES

Since we have an energy conservation problem as well as a water conservation problem, presumably one should be seeking for solutions to the water problem which are beneficial in energy terms. One of the things I can think of which I am sure has occurred to many other people, where you might get best returns for your money, would be development by genetic engineering of plants which can tolerate water in irrigation systems in a much less good quality than one has to supply at present. This I am sure is an area in which work is being done, but I would like to ask how important it is likely to be in quantitative terms.

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BÂTISSE

Yes, this is important, but it is in itself a complicated problem. First we don't now exactly what the plants can tolerate. It seems that they are more susceptible to the ratio between, for instance, potassium and sodium, than to the absolute content of sodium, but we don't know really this. It seems also that they are particularly susceptible to specific elements. For instance, borum is very toxic but borum is perhaps less toxic if it is associated with something else. This is an area of plant physiology where probably not enough is known. Another consideration is the tolerance of plant to the total salt content. As indicated briefly, we have made experiments on this, particularly in Tunisia. Experiments have been made also in other countries. In Tunisia we found that provided the drainage conditions were good, provided the soils were right, we could go in some cases, up to 6,000 p.p.m. for irrigating date palms. For other crops up to 4,000 p.p.m. which would be considered, for instance, by American irrigation engineers as absolutely impossible. Water of up to 3,000 p.p.m. is currently used now in good soils with good drainage in a number of arid countries. I don't think, however, we can go much further in this direction. Whichever way we may look at this there will be a need in the future for very considerable amounts of water for irrigation to feed the world, and perhaps the main thing we can do about irrigation is not so much to try to modify the quality of irrigation water, which has to be good, but to avoid the losses in irrigation water which are absolutely fantastic, particularly through seepage in the canals. Seepage losses can be of the order of fifty percent in some irrigation schemes. Tbis is water which is really lost in economic terms and the energy which has been used to store and distribute it is also lost.

DE DUVE

As you pointed out, we have an important quantitative problem for irrigation water to produce proper food. Every time you have

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industrial use of water and return it to the river or lake or sea, the water comes out modified either grossly in chemical terms or at least in physical terms such as temperature for instance, and some studies are coming out showing that this modifies plankton and the quantity and type of the fish that live in that water. I am wondering if this type of physical modification and even chemical modification of water as it is returned to lakes and streams and the sea can be used in an intelligent way to augment the supply of food, in terms of fish which is an easy souce of protein for many countries.

BÂTISSE

I do not think we have much information on this but I am told that salmon are coming back in some British waters as a result of thermal pollution. I do not know whether this can become important or not in food production terms. It seems that the thermal pollution of the sea has no real disadvantage — the sea can tolerate this kind of pollution easily. But there is quite a controversy going on about the effects which building of power plants, of nuclear plants in particular, will have through the heating of rivers, which would affect their whole ecology. It may be that in some cases it could be used in a positive sense for fishculture. It may be also possible to combine intelligently fish culture with drainage water in some irrigation schemes.

CHAGAS

I should like to thank Dr. BÂTISSE for his presentation. I should like to make two points. One, he stressed extremely well the problem of people knowing exactly the value of water, but I think this is a problem of environmental education and, as he said, of the greatest importance, and if we are not able to secure

this knowledge, certainly the problem will be even more dangerous than it is now. Secondly I would like to stress something he said « en passant ». During a certain period a group of people interested in development and social justice thought a much better indicator for development would be the amount of fresh water per capita rather than the gross national product.

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NUCLEAR ENERGY AND WATER DESALINATION

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The problem of sea or brackish waters desalination is extremely important and is becoming more and more up-to-date, since fresh water consumptions are constantly growing throughout the world.

Presently, only one thousand factories yield one million and a half cubic meters of fresh water per day, throughout the world. It is a small amount, if compared, for instance, to the fresh water consumption in the United States: it is only a 2% of this figure.

Presently, fresh water consumptions vary quite a lot from one country to another; while most of the underdevelopped countries consume from 20 to 50 liters per day, per person, France consumes 1 cubic meter and 1/2, that is 1,500 liters, and the United States over three times that figure.

Such a figure covers everything, that is feeding of factories, irrigation, feeding of towns. We certainly waste a great amount of fresh water. We could certainly reduce our consumptions in the western countries, but these are growing and growing.

The problem of obtaining fresh water from sea water was felt at first in the ships: sea water was distilled; then, during the first years of 20th Century, a thermic plant boiler was

constructed in BAKOU, to distil the Caspian sea water. The Caspian sea is an internal sea, the salinity degree of which is 1/3 of that of the oceans.

When one wants to desalinate by distillation, at industrial level, one realizes how difficult it is, because of corrosion and trartrum, and besides that, costs are rather high.

We may state that, presently, the average growth rate is 15% a year, as to the world desalination capacity. We may also give another figure: a desalination unit yields roughly 25,000 cubic meters per day, which corresponds, for a big town, such as Hong Kong, to a tenth of its present needs.

Research and procedures

A lot of research is being carried out almost everywhere especially in the United States, through the Office of Saline Water and Oakridge Center for Nuclear Studies, and also through large private corporations, such as Dupont de Nemour.

In Great Britain, it is partly the commission for nuclear energy which carries out such studies. In Japan, since 1970, large distillation plants are under study. In URSS, the program is focused on distillation and also on the electrodyalisis, combined with atomic energy.

In France, research is carried out especially since 1966, through the managing bodies for scientific and technical research, the "Commissariat à l'Energie Atomique"; it deals with distillation procedures, with electrodyalisis and reverse osmosis, with special reference to the study on semi-permeable walls. Among the various procedures, we may distinguish those using distillation and the others. The first ones, are the most used and many of them are realized either by successive process or by multi-effects, with long vertical pipes, or by the solar energy.

The method using freezing procedures, which is quite interesting, from the thermodynamic standpoint, was abandoned because of technical reasons, and high costs too. In particular

there always is some salt in the ice, which we want to extract.

On the other hand, the solar energy is only used in a very limited proportion. With the exception of distillation, to which we shall come back later, there are two procedures using membranes: one is the electrodyalisis, the importance of which is still limited but growing, and the other is the reverse osmosis, which is presently little employed, but rather promising, in certain cases.

We are now going to say something on each of these procedures.

Distillation procedures

We said they were the most employed. Some salted water is boiled, then the steam is pressed or condensed to yield heat which enables to recover the vaporization latent heat. This heat is then used as a fuel to warm sea water. Many systems were realized. Here is a good example: cold sea water is introduced in the device, and progressively heated up, in successive chambers. The first one is at a low temperature, while the other ones are at increasing temperatures; the pressure is kept at such a level to prevent the sea water from boiling, and this because of trartrum.

Finally, the sea water gets to a final chamber, still without boiling, at 120°, and then flows out again, from chamber to chamber, with self-vaporization, at gradually smaller temperatures and pressures.

The vaporization latent heat is then passed on to the sea water entering the chamber and has a part in its warming-up. I am not going to insist on the various facilities, but we may state that all the problems are due to corrosion and trartrum, to the precipitation of calcium salts and magnesium, in presence of CL-ions. The major deposits are calcium carbonate, magnesium hydroxyde and calcium solfate. We solve this problem by means of a pre-treatment.

On the other hand, corrosion resulting from sea water makes it necessary to use expensive materials, which enourmously increases the cost of a plant.

We may state that a desalination plant by distillation is quite expensive and very flexible for use. This last point is to be taken into account, with respect to the use of nuclear energy.

Other procedures

A few words, first of all, on the ion exchangers: the chlorine negative ions and the sodium positive ions may be transformed by ion-exchanger resins into OH^- and H^+ ions; however, resins should he regenerated by means of acids or reactive agents, which is very expensive and limits the use of the ion exchangers.

This method is only interesting for treating slightly saline waters. The electrodyalisis procedure is much more interesting: some membranes are piled up, of which a few are permeable to cathions only, while the others to anions only. A certain amount of parallel membranes are put in place and an electric field is set up, dragging the ions.

We may then demineralize water. The membranes consist of a nylon pattern, on which the substance made of sulphonic or ammoniacal radical polymers is induced.

These membranes are close to the ion-exchanger resins. We may get 1 m^2 surfaces. They are used for brackish waters, which is quite interesting, for instance, for Tunisia, Lybia, Algeria; they have in fact about 3 gr per liter of salt in their sea waters, corresponding to one tenth of oceans' salinity.

France realized a plant, in Port Crau island, with two electrodyalisis units, enabling to obtain 50 m^3 per day (covering the island's needs) either from brackish or from sea waters.

Reverse osmosis

In the reverse osmosis, water crosses the membrane under the action of a pressure, while in the electrodyalisis process, the membrane is crossed by salts. Reverse osmosis was the object of many research studies. All sorts of polymers were tried. Cellulose acetate is the most used under the form of collodion solved in a solvent. The big American Corporation, Dupont de Nemour produces a kind of polyamide which is as good, and consists of a great number of micro-pipes, similar to curled hair. In Florida, a reverse osmosis plant is operating for 6,000 cubic meters per day of brackish water, at 2 gr per liter.

Agricultural, industrial and nuclear units

Desalined water is expensive. It costs more than 1 French frank per cubic meter. It is too expensive for a current use in agriculture; prices are extremely high for normal irrigation, but we can organize in certain regions agricultural enterprises using irrigation methods aimed at saving water.

On the other band, we could choose tillings at high market prices requiring a minimum amount of water. Finally, we shall be interested in setting up big plants yielding a part of their heat to distil water and a part to electric power, to feed on the spot industries.

In this direction, were oriented the studies of Oakridge Nuclear Center. Some units could also be conceived for sunny sea resorts, where desert becomes fertile, once it is irrigated. Such places are to be found in Egypt, Israel, Chile, India, Pakistan, California. We may plan important nuclear units along the sea, coast, to enable their cooling, with a power of beyond 1000 thermic megawatts. These will be mixed plants, dividing their power into electric energy and steam for desalination. The industries we could think of would contribute to

cultivation: manufacturing of fertilizers, manures, insecticides, etcetera.

Thus, the study of Oakridge Nuclear Center concludes that 600 liters of water per day are enough to feed a person with cereals, proteins, etc. A reactor of 2,200 thermic megawatts and 25,000 hectars may feed a million people. We must not forget that every six days a million children is born.

Investments are quite huge, and they account for billions of dollars. Running water, according to Oakridge study, would cost at least 25 cents per cubic meter, however, this figure is certainly underestimated.

An accurate study was carried out for Porto-Rico with the gradual construction of six 3000 thermic megawatts reactors, on the whole. This project will yield 100,000 cubic meters of water per day to cultivate exotic fruit and 550 electric megawatts for petrol-chemistry, oil refineries, aluminium production, etcetera.

Americans started ten years ago a plant in San Diego, with two nuclear reactors, capable of yielding 600,000 cubic meters of water per day, and 1,800 electric megawatts. However, the initial cost of 444 million dollars, after revision, passed to 765 millions, which killed the project.

Realisations in Soviet Union

The only reactor for water desalination constructed at present is that of Chevtchenko, in Soviet Union. It is a rapid neutrons reactor. It is planned to yield 350 electric megawatts and to desalinate the Caspian sea water.

Considerations on the use of nuclear reactors

When a normal, high power, nuclear reactor is used, heat is produced at a temperature of ahout 350 to 400°. This heat

cannot be directly used to desalinate water, since the corresponding steam temperature is much too high. Desalination plants cannot bear a steam at a temperature of over 125°, because of corrosion and trartrum.

Therefore, the overheated steam temperature should be decreased from 350° to 125°. This decrease in temperature will result in an electric power output, after which steam at 125° shall be used in the desalination plant.

Thus, the greatest part of power will yield electricity while a smaller part, about 10% of it, shall be used for desalination. We may state that a 100 electric megawatts plant will yield about 200,000 cubic meters of water per day, to feed a large town, plus 900 megawatts of electricity.

Nuclear energy is presently much more interesting since the oil price is much higher. However, it still is to be excluded, as far as irrigation is concern ed. On the other hand, it is not escluded to obtain drinking water in large agglomerations. There are, of course, some difficulties, due to the limited availability of desalination plants. It must be constantly fed by the same steam flow.

If a nuclear plant yields electricity power in a discontinuous way, with piks and drops of tension, something should be provided for, in order to avoid these variations. Thence, the idea of a double plant, for the large nuclear units; a part of the energy will only be addressed towards producing electricity through a first circuit, just as for normal nuclear units.

And this will be submitted to variations. The weakest one shall be addressed to another circuit in which the above mentioned decrease in temperature from 350 to 120° will produce a continuous flow of electricity, while steam at 120° will constantly feed the desalination plant.

We may also think of setting up smaller nuclear plants not intended to yield electricity but heat only. This is presently under study, especially for town heating needs. We could think of reservoir reactors, producing heat at 110°. It is a new concept which has some possibilities of being realised in

the future and which could give good units for desalination, if the starting costs are rather low.

Conclusions

Presently, the largest plant for sea water desalination is located in Kuwait. It yields 200,000 cubic meters per day operating on a gas boiler. The main unit built by Thomson yields 112,000 cubic meters per day. Kuwait will bring its production to 500,000 cubic meters per day.

The most impressive one, after that of Kuwait, is the Hong Kong plant, operating on fuel, which is almost completed. It shall yield 180,000 cubic meters per day; the price of water, however, is of 2 franks per cubic meter, which is rather high. This enterprise produces electricity only for pumping needs.

Many gigantic plants are being planned, using membranes: one yielding 380,000 cubic meters per day for the brackish waters finishing in the Colorado river; and another one of 115,000 m^3 in Saudi Arabia.

Among the most important realisations, we also find that of Rotterdam, yielding 30,000 cubic meters per day, which is associated to the biggest incineration factory for waste products and industrial residues.

Nuclear energy was already used in the Caspian sea; other possibilities with large mixed plants yielding both steam and electricity to desalinate were taken into account over the last years, but are not yet under construction, because of their costs.

It is very likely that, if the price of nuclear energy, decreases and if the price of fuel remains still very high, we shall address ourselves towards the setting up of nuclear energy projects; anyway, clear water thus produced will be too expensive for normal use and it should therefore be confined to special uses, which we dealt with before.

DISCUSSION

Chairman: Professor LEPRINCE-RINGUET

CHAGAS

The paper of Prof. LEPRINCE-RINGUET is under discussion. Any questions?

BÂTISSE

Quand on cite les chiffres de 1 franc par m^3, ou pour Hong Kong de 2 francs par m^3, est-ce que ces chiffres tiennent compte des problèmes de l'évacuation des quantités de sel qui sont produites par le déssalement lui-même? Et quand on parle de chiffres pour l'utilisation par irrigation, est-ce qu'on tient bien compte dans les chiffres qui sont cités du prix de délivrance de l'eau à la ferme même?

LEPRINCE-RINGUET

J'ai un dossier sur les prix que je pourrais vous montrer. Je puis vous donner pour l'usine de Hong Kong, qui est en cours d'achèvement actuellement, la valeur de la consommation d'énergie et de vapeur: on consomme 760 tonnes par heure de vapeur saturé à 127 °C, qui exige une consommation de fuel de 1320 tonnes par jour; on consomme ainsi en vapeur à moyenne pression,

15 tonnes à l'heure à 20 bars. La puissance électrique pour le pompage et les auxiliaires est faible; 12 mégawatts électriques, la plupart des grosses pompes étant actionnées par turbo-moteur utilisant la vapeur vive sortant des chaudières à 34 bars. Voilà le genre d'information que je puis donner. En tout cas, il n'est pas plus facile de savoir quel est le prix de l'eau que de savoir quel est le prix du courant produit par un réacteur nucleaire. C'est un problème quasi insoluble.

CHAGAS

I would like to ask Prof. LEPRINCE-RINGUET a question, but before that I would like to tell a story. I have just come from the Cleveland Clinic where I met Dr. HEFFLER, coming back from Saudi Arabia. He told me the extraordinary fact that in Ryiadh a ton of water costs the double of a barrel of oil. So there desalination is a real problem. Now the question I would like to put to Prof. LEPRINCE-RINGUET is about the use of solar energy for desalination in some places; for instance in the north-east of Brazil, where we have 3.000 hours of sunshine per year.

LEPRINCE-RINGUET

On parle beaucoup de l'énergie solaire comme énergie de remplacement. Il faut dire que les photons du soleil ont différentes qualités. Le spectre continu solaire contient des photons d'énergie notable (ultraviolet, violet, bleu,..) dont les plus durs correspondent à la température de la zone émettrice, environ 7.000 °C, et une très grande proportion de photons plus mous, jusqu'à l'infrarouge, correspondent à quelques dizaines de degrés seulement.

On peut utiliser le rayonnement dans son ensemble, pour chauffer de l'eau à environ 80 °C. Il est même possible de la faire bouillir en ajoutant quelques réflections. Ainsi on peut utiliser le rayonnement solaire pour les usages de chauffage d'une

maison et pour les besoins ménagers (cuisine, lessive.). Il n'est pas impossible d'envisager des installations de déssalement d'eau de mer. Cela a déjà fait l'objet de quelques réalisations de faible importance. Avec la partie dure du spectre, on peut provoquer un effet de photoconductivité qui met en mouvement les électrons d'un semiconducteur. D'où les piles solaires qui sont utilisées pour l'alimentation des satellites. Le prix de ces piles est cinquante à cent fois trop élevé actuellement, mais on pense qu'il va se réduire rapidement. L'utilisation de grandes surfaces de ces piles dans les pays très ensoleillés est une éventualité prometteuse: le courant électrique ainsi obtenu directement pourra être utilisé pour le déssalement. Ce sont les considérations économiques, très variables au cours des années prochaines, qui orienteront les réalisations.

DE DUVE

My question is directed either to Mr. LEPRINCE-RINGUET or to Mr. BÂTISSE. Some projetcs — I know there is one in the Caribbean — aim at combining the development of mariculture with the exploitation of temperature differences on the surface and in the depth of the sea for energy production, following the old idea of Georges Claude. What is your opinion of these schemes?

BÂTISSE

I think they constitute a very promising area for further research and development. One of the major difficulties encountered by Claude in the twenties was the fragility of the very long pipe needed to obtain the cold deep sea water. Much progress has been made since in metallurgy and plastics, and the cost of energy is going up, so that there could be a significant future for the Claude process, for production of power and/or fresh water. Since the deep water brought up to the surface is rich in nutrients,

it can be used in mariculture — particularly shellfish and crustaceous — and this is being experimented with in the Virgin Islands. However, the combination of this type of mariculture with the Claude process is not yet economical and does not seem to have been tried out yet on a prototype scale.

[2] I, 2 - *Leprince-Ringuet* - p. 12

II

STRUCTURE OF BIOLOGICAL MEMBRANES AND METHODS OF MEMBRANE STUDY

ENZYMATIC PROPERTIES OF RAT-LIVER CYTOMEMBRANES

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INTRODUCTION

Thanks to the work of many laboratories, every main type of cytomembrane that can be recognized in rat-liver cells by means of the electron microscope has now been isolated in purified form and analyzed biochemically.

A key conclusion emerging from these investigations is that distinct membrane systems have distinct enzymatic compositions. The results obtained do not, however, permit to decide whether these distinctions are clearcut and sharply defined, or whether transitional overlaps exist between different membrane systems, in particular between those that are closely related morphologically and functionally, such as for instance the endoplasmic reticulum and the Golgi apparatus.

The reasons for this uncertainty lie in the inevitable drawbacks of the preparative approach, which usually falls considerably short of its ideal goal of complete purification with quantitative yield. The problem of inadequate purification is complicated by the difficulty of assessing the degree of purity of a membrane preparation by morphological means. Low yield, when it concerns a population of unknown heterogeneity, carries the obvious danger of sampling bias.

TABLE 1 — *Marker Enzymes in Microsome Fraction.*

Enzyme	Typical location	% (M ± SD) in microsomes
Glucose. 6-phosphatase	Endoplasmic reticulum	75.5 ± 8.4
NADH cytochrome *c* reductase	80% endoplasmic reticulum 20% outer mitochondrial membrane	58.4 ± 6.4
Monoamine oxidase	Outer mitochondrial membrane	19.9 ± 4.3
Cytochrome oxidase	Inner mitochondrial membrane	4.2 ± 2.0
5'-Nucleotidase	Plasma membrane	49.9 ± 7.2
Galactosyltransferase	Golgi membranes	72.9 ± 4.0

These difficulties are illustrated in Table 1 by some findings concerning the enzyme content of rat-liver microsomal fractions. Ever since the pioneering work of PALADE and SIEKEVITZ [14], it has been known that this fraction is made up principally of vesicles originating from the endoplasmic reticulum. In the case of glucose 6-phosphatase, there is direct cytochemical evidence that this enzyme is indeed a true constituent of the endoplasmic reticulum [12, 13]. But similar evidence is lacking for the other enzymes listed in Table 1, even for the microsomal NADH cytochrome *c* reductase, which is generally taken to belong to the endoplasmic reticulum, but without absolute proof. As for typical marker enzymes of other types of cytomembranes, the question obviously comes up to what extent their presence in the microsomal fraction reflects their true association with endoplasmic reticulum, or, alternatively, the occurrence of other membranes in the fraction.

This problem is a general one. Almost every purified membrane fraction isolated from rat liver contains, in addition to its own typical marker, variable amounts of the other

enzymes listed in Table 1. The significance of such findings has been variously interpreted, on the basis of arguments that are sometimes more subjective than objective.

In our laboratory, such problems have traditionally been approached in an analytical fashion. This approach has been described in detail in several theoretical and experimental publications [6 - 9]. Rather than focusing on the biochemical composition of a single fraction believed to contain a representative sample of a given subcellular component in a satisfactory state of purity, which characterizes the preparative approach, the analytical approach looks at the complete distribution of given biochemical constituents between all the fractions isolated by quantitative separation methods. Each approach has its merits; the two obviously complement each other.

The analytical approach was first developed and refined in experiments that led to the resolution of the mitochondrial fraction into a major component, the mitochondria proper, and two minor components, the lysosomes and the peroxisomes [7]. When this work was largely completed in the late 1950's, we decided to apply the same approach to the microsomal fraction, which was known to have as main component vesicles derived from the endoplasmic reticulum [14], but could conceivably include one or more unidentified minor components. Early experiments were undertaken by Mrs. Rahman-Li, who performed a number of isopycnic centrifugation experiments on microsomal fractions. The material was initially incorporated homogeneously in a sucrose gradient, and then centrifuged for some 18 hours in the SW-39 rotor of the Spinco preparative centrifuge. This long-drawn procedure resulted in poor biochemical and morphological preservation of the preparations, and we were not satisfied with the results. These were never published, except for the observation, already clearly made at that time, that the microsomal

monoamine oxidase equilibrates at a distinctly lower density than glucose 6-phosphatase and the bulk of the protein [10].

An improved technique was deemed necessary, and this prompted Beaufay to design a new rotor which, in addition to complete automation and freedom from the usual artifacts that affect centrifugation in swinging-bucket rotors, showed a several-fold increased efficiency, thanks to its ring-shaped cell with large starting radius and short path-length [3]. The development of this rotor turned out to be a major undertaking, and it became available only in 1966. It has since been used extensively for the analysis of microsomal fractions [1, 2, 4, 5, 17]. The present paper summarizes very briefly the basic principles that have been followed in this work and the main results that have been obtained. Details are to be found in the original publications.

It is my privilege to present this paper at this meeting. But I wish to make it clear that I am here only as a spokesman of a large group of investigators, which includes, in alphabetical order: Alain Amar-Costesec, Henri Beaufay, Jacques Berthet, Ernest Feytmans, Stanley Fowler, Danièle Godelaine, José Remacle, Mariette Robbi, Denise Thinès-Sempoux, André Trouet, Maurice Wibo, and a number of dedicated research assistants.

Experimental approach

Microsomal fractions, containing some 40 mg protein per g liver and about 75% of the total glucose 6-phosphatase activity of the tissue, were prepared from rat liver under careful biochemical monitoring of contamination by larger particles. The fractions were then subdivided into 15 to 20 subfractions by density gradient centrifugation. Various procedures were used for this purpose: isopycnic centrifugation in

linear aqueous sucrose gradients, or occasionally in other gradients; partial sedimentation in a stabilizing sucrose gradient for the purpose of sedimentation boundary analysis. All experiments were performed in a Beaufay rotor, at a temperature close to 0°C, and in media buffered to pH 7.4 by the inclusion of 3 mM imidazole-HCl buffer. The microsomes and their subfractions, as well as the other cell fractions and the original homogenate, were then analyzed for up to 27 distinct biochemical constituents and enzymes, in such a way as to allow quantitative recoveries to be calculated. This point is important, since results were considered acceptable only if recoveries were satisfactory.

These experiments were performed on normal microsomal fractions, and on fractions subjected to various treatments, which included exposure to an amount of digitonin (6.35 mg per g original fresh liver) approximately stoichiometric (1.2/1.0) with the cholesterol content, to 15 mM sodium pyrophosphate pH 8.2, to 50 mM EDTA pH 7.4, or successively to EDTA and digitonin. Digitonin was without influence on the chemical or enzymatic composition of the microsomes and did not, at the concentration used, affect the structural latency of nucleoside diphosphatase or of galactosyltransferase. The main effect of inorganic pyrophosphate or EDTA was to detach an important part of the RNA (ribosomal subunits).

In parallel experiments, purified preparations of plasma membranes, Golgi membranes, and outer mitochondrial membranes were made by recognized procedures under the same careful biochemical monitoring, and were then subjected to density gradient analysis as were the microsomes.

In the interpretation of the results, major importance was given to tbe shape of the overall distribution patterns of enzymes and other biochemical constituents, rather than to the composition of any individual fraction.

Results

General Survey. On the basis of their behavior upon isopycnic centrifugation in sucrose gradients, the various constituents of the microsomal fraction could be classified into four distinct groups of increasing median density, as shown in Table 2. The characteristic behavior patterns are illustrated in Fig. 1 for one enzymatic and one non-enzymatic representative of each group.

The significance of the observed difference could be further elucidated by additional experiments performed by other centrifugation procedures or on microsomal fractions pretreated in various ways, by parallel tests on purified membrane fractions, and by special morphological procedures. The main conclusions are summarized below.

Group d. We start with this group because it is largely artifactual. There is good evidence that the enzymes in group *d* are attached to ribosomes by weak electrostatic forces which are easily broken by a moderate increase in ionic strength or by detachment or hydrolysis of the ribosomal RNA. We believe their association with the microsomal fraction to be due to an adsorption artifact and to be of little biological significance.

One important point concerns the distribution of RNA itself, which is seen to be increasingly concentrated with respect to protein as the equilibrium density of the fractions rises (Fig. 1). This observation raises two types of questions: 1) Is the microsomal RNA entirely associated with ribosomes, or is part of it incorporated in the membranes as was claimed some years ago? 2) Does the increase of the RNA to protein ratio with increasing fraction density reflect an increase in the ratio of rough to smooth vesicles or an increase in the ribosome load of all vesicles? The work of Wibo *et al.* [17] has provided clear answers to these two questions: 1) Essentially all the microsomal RNA appears to be ribosomal. 2) On an average, all vesicles in a given fraction have approximately the same number

TABLE 2 — *Classification of Microsomal Constituents.*

Group	*Enzyme or biochemical constituent*
a1	Monoamine oxidase
a2	5'-Nucleotidase Alkaline phosphodiesterase I Alkaline phosphatase Cholesterol
a3	Galactosyltransferase N-Acetylglucosaminyltransferase Sialyltransferase
b	NADH cytochrome *c* reductase NADPH cytochrome *c* reductase Aminopyrine demethylase Cytochrome b_5 Cytochrome P-450 Phospholipid (*)
c	Glucose 6-phosphatase Esterase Nucleoside diphosphatase Glucuronyltransferase β-Glucuronidase Protein (*)
d	Fumarase Aldolase Glutamine synthetase RNA Ribosomes

(*) Assignment to group is based on shape of distribution pattern (See Fig. 1) and obviously does not imply exclusive location (See Table 4 for protein partition).

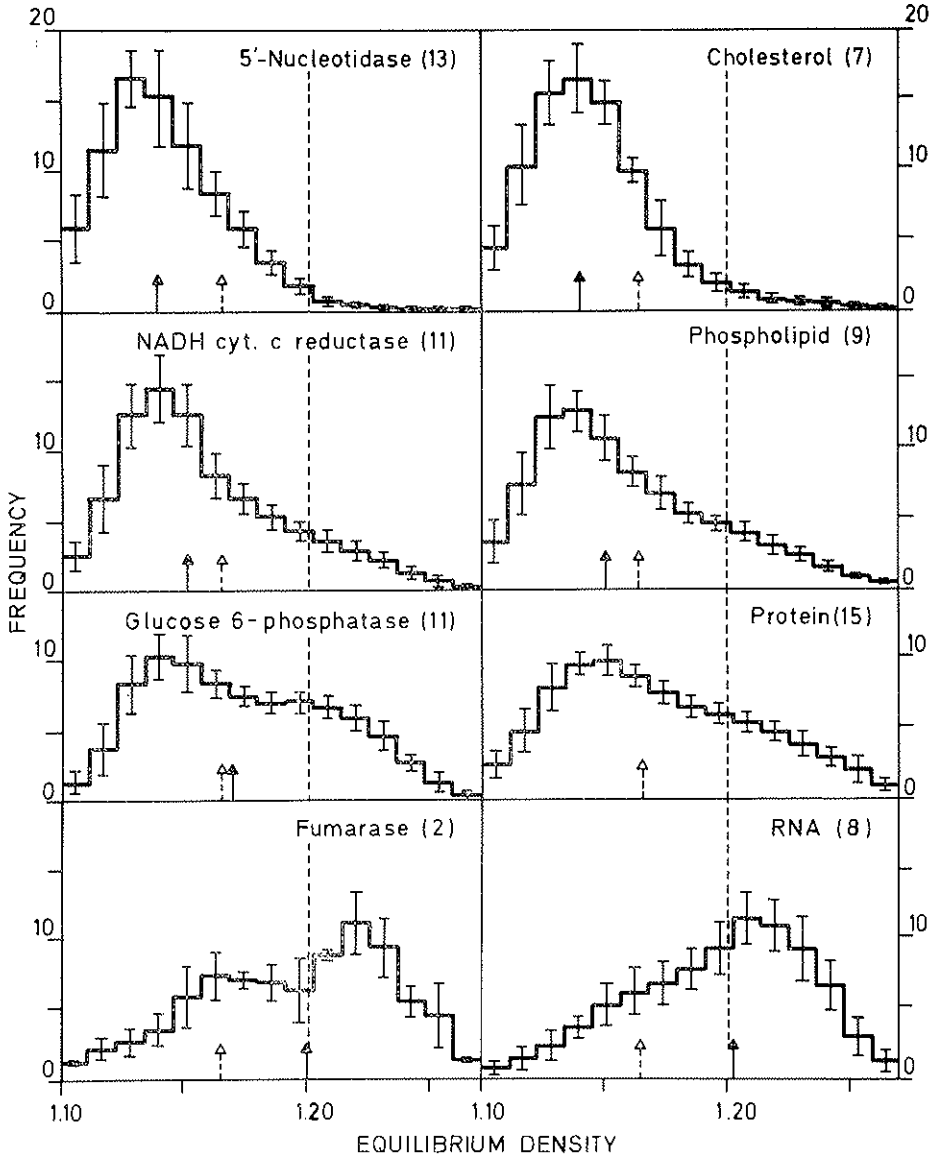

FIG. 1 — Density distribution of some characteristic constituents after isopycnic equilibration of microsomes in sucrose-H_2O gradient. Frequency histograms are normalized and averaged. The represented portion of histograms, divided into 15 normalized fractions of identical density increment, extends from 1.10 to 1.27, and corresponds to more than 95% of constituents. Vertical lines through histogram bars represent standard deviations. Numbers in parentheses refer to number of experiments. Black arrows indicate the median density of constituent; white arrows, repeated on all graphs, indicate the median density of protein. Demarcation line at density 1.20 allows easier comparison of distribution profiles.

From BEAUFAY and coll. [5], with permission of the authors and of The Rockefeller University Press.

of attached ribosomes per unit surface area of membrane, and this load increases with increasing density. Most probably, as will be pointed out below, the ribosome load plays an important role in determining the equilibrium density of individual vesicles. This is understandable, in view of the high density of ribosomal particles.

These findings have demonstrated that the distinction between « rough » and « smooth » microsomes, as separated by published procedures, is to some extent arbitrary, and certainly not as sharp as is sometimes assumed. In reality, there is a continuous spectrum from truly smooth vesicles, with no attached ribosomes at all, to vesicles heavily loaded, and perhaps saturated, with ribosomes.

Group a. The enzymes in this group, which share a low modal and median equilibrium density, also have in common that their density distribution patterns are little affected by treatment of the microsomes with inorganic pyrophosphate or EDTA. As will be pointed out below, the latter property indicates strongly that there are no attached ribosomes on the membranes with which the enzymes of group *a* are associated.

Other experiments, involving sedimentation boundary analysis, or isopycnic centrifugation of microsomes pretreated with digitonin, revealed that group *a* is actually heterogeneous, consisting in fact of three distinct groups. As shown in Table 3, group *a1*, of which monoamine oxidase is the only known representative, sediments more slowly than the average microsomal proteins and is unaffected in its density distribution by digitonin treatment. In contrast, the enzymes of group *a2*, which include 5'-nucleotidase, alkaline phosphodiesterase I and alkaline phosphatase, behave very differently: they sediment distinctly faster thant the average microsomal proteins, and their density distribution is shifted markedly towards higher density values by digitonin treatment. The microsomal cholesterol behaves in a very similar fashion. Finally, galactosyl and other glycosyltransferases, which make up group *a3*, sediment

TABLE 3 — *Main Criteria for Classification of Microsomal Constituents.*

Group	Sedimentation rate relative to protein	Equilibrium density in sucrose gradient		
		Untreated microsomes (median)	Effect of treatment of microsomes with	
			Digitonin	PP_i or EDTA
a1	Slower	~ 1.13	No effect	Slight decrease
a2	Faster	~ 1.14	Marked increase	Slight decrease
a3	Slower	~ 1.13	Moderate increase	Slight decrease
b	Similar or slightly slower	~ 1.15	No effect	Marked decrease, distribution more symmetrical
c	Similar or slightly slower	~ 1.17	No effect	Marked decrease, distribution more symmetrical
d	—	~ 1.20	No effect	Enzymes detached

slowly like monoamine oxidase, but show a distinct increase in density after digitonin treatment. This shift is however of smaller magnitude than that of group *a2*. Characteristically, the enzymes of groups *b* and *c* show no detectable change in density distribution after digitonin treatment.

In our opinion, these results indicate clearly that the bulk of the enzymes of group *a* are not associated with the endoplasmic reticulum proper, but belong to contaminants. It is highly suggestive that the three *a* groups correspond to three distinct types of cytomembranes that have been characterized previously. As shown in Table 1, monoamine oxidase is a known constituent of the outer mitochondrial membrane;

5'-nucleotidase is a typical constituent of the plasma membrane, and so are alkaline phosphodiesterase I and alkaline phosphatase; galactosyltransferase is an established Golgi marker. These 3 types of membranes were purified by recognized procedures and found to react to digitonin treatment in the same way as do their counterparts in the microsomal fraction: plasma membranes show a large digitonin shift, Golgi membranes a moderate one, and outer mitochondrial membranes none at all, in full agreement with our interpretation. It is interesting that in each preparation, marker enzymes for other cytomembranes showed the behavior of their main site of location. For instance, the alkaline phosphodiesterase I activity of mitochondrial outer membranes showed a large digitonin shift, whereas the NADH cytochrome *c* reductase of Golgi preparations was not shifted by digitonin, thus contrasting with the main component in each preparation.

The digitonin shift is itself an interesting phenomenon, which is most likely due to the binding of digitonin to cholesterol molecules incorporated within the membranes. Plasma membranes have a high cholesterol content, consistent with their marked digitonin shift. Golgi elements also contain cholesterol, but less than the plasma membranes, which accounts for their smaller displacement by digitonin. The fact that the other membranes of the microsomal fraction are not affected by digitonin indicates that they contain little or no cholesterol. This conclusion is further supported by the density distribution of cholesterol itself (Fig. 1), and by its marked digitonin shift.

Groups b *and* c. The enzymes in these two groups follow fairly closely the distributions of protein and phospholipid, both in isopycnic centrifugation and in differential sedimentation experiments. In addition, they have in common a drastic change in density distribution following treatment of the microsomes with inorganic pyrophosphate or EDTA. As a result of this treatment, which detaches much of the ribosomal RNA from « rough » vesicles, the density distribution of group *b* and *c*

enzymes is shifted towards lower densities and loses its characteristic skewness, to become almost symmetrical. These properties indicate that the two groups must be associated, at least for a good part, with « rough » vesicles belonging to the endoplasmic reticulum, the main component of the microsomal fraction.

Such being the case, the existence of two groups is puzzling, especially since there seems to be a fair degree of homogeneity within each group, apparently correlated with some functional properties. Electron transport enzymes go together in group *b*, hydrolases, with glucuronyltransferase, in group *c*.

One possibility that would be consistent with the « postulate of biochemical homogeneity » [6 - 9] is that groups *b* and *c* belong to separate parts of the endoplasmic reticulum. If this were true, the enzymes of group *b* should occur only in a small proportion of the total microsomal vesicles, since it is known from cytochemical observations that glucose 6-phosphatase is widely distributed [12, 13]. This question has been investigated experimentally by REMACLE *et al.* [11, 15, 16], with the help of a hybrid antibody against cytochrome b_5 and against ferritin. Using this tool, they found that most microsomal vesicles bind ferritin in the presence of the hybrid antibody, thus demonstrating the widespread occurrence of cytochrome b_5.

What is true of cytochrome b_5 is most likely true also of the other members of group *b*. Therefore, we cannot escape the conclusion that groups *b* and *c* occur together on the same membranes, but in a non-uniform fashion. As the ribosome load of vesicles increases, their relative content in group *c* enzymes increases and that in group *b* decreases. There are two possible interpretations to this heterogeneity. It could exist within each individual cell and reflect the existence of enzyme gradients, correlated with ribosome load, along the membranes. Or it could be a consequence of differences between different cells, depending for instance, on their position within the lobule. In the latter event, which appears somewhat more

likely on « a priori » grounds, the endoplasmic reticulum of each individual cell could be biochemically homogeneous.

Composition of the microsomal fractions. If it is assumed that marker enzymes have the same specific activity in microsomal contaminants as they have in purified preparations of their characteristic host membranes, the contribution of each contaminant to the whole microsomal protein can be calculated. The results of these calculations are shown in Table 4, which includes also evaluations of contamination by larger particles. It is seen that less than 80% of the microsomal material appears to originate from the endoplasmic reticulum proper. Up to 15% of the total microsomal protein belongs to « smooth » membranes that are quite distinct from the endoplasmic reticulum. This point is of obvious importance in relation to the results obtained on « smooth microsomes ».

TABLE 4 — *Composition of Microsomal Fraction from Rat Liver.*

Component	Marker group	Contribution to total microsomal protein
Plasma membranes and related structures	*a2*	7-8 %
Golgi membranes	*a3*	4-5 %
Mitochondrial outer membranes	*a1*	3 %
Mitochondria	Cytochrome oxidase	6 %
Lysosomes	Acid hydrolases	1 %
Peroxisomes	Catalase	1 %
Endoplasmic reticulum (by difference)	*b* and *c*	≈ 77 %

Discussion

Purification experiments have disclosed considerable differences in the enzymatic composition of different cytomembranes. But owing to the limitations of the preparative approach, they could not decide whether the differences are qualitative or only quantitative. In the work summarized here, this question has been investigated by an analytical approach. The results obtained argue in support of the differences being truly qualitative. Admittedly, the resolution of the techniques is not such that the existence of minor overlaps can be excluded, but it is certainly striking that every technical improvement has served only to sharpen the observed distinctions. Clearly, the limit conclusion towards which we seem to be moving is that each type of membrane complex which morphological observation has identified as a distinct system or organelle has its own characteristic enzyme composition. It appears further that each membranous domain may be rather homogeneous enzymatically.

These findings extend further the limits of applicability of the two postulates, of « single location » and of « biochemical homogeneity », that provide the main conceptual support of the analytical approach [6 - 9]. They also illustrate exceptions to these postulates. For instance, cytochrome b_5 and the related NADH cytochrome *c* reductase occur both in the endoplasmic reticulum and in the mitochondrial outer membrane. On the other hand, the endoplasmic reticulum could provide an exception to the postulate of biochemical homogeneity. Here, however, as already discussed, an alternative interpretation compatible with biochemical homogeneity can be considered. Furthermore, if there is heterogeneity, it is of a strangely restricted kind, since it concerns the relative abundance of two groups, each of which seems to be internally homogeneous.

Appealing as they may appear through their simplicity, such clearcut distinctions as we are led to assume between different membranous domains are not readily reconciled with the observation that contents of one domain may be transferred to

another, or mixed with the contents of another, by means of connections which necessarily involve fusion between two different types of membranes. It could be argued that the degree of heterogeneity due to these connections may be small and therefore compatible with our results, or that the boundaries between two connected domains represent points of greater fragility, which are preferentially broken upon homogenization. Such explanations do not, however, fit very well with current concepts of membrane fluidity and free lateral diffusion of constituents within the plane of the membrane. This is particularly true for the endoplasmic reticulum and the Golgi apparatus, which many authors believe to be permanently connected by multiple tubular joints. One may well wonder how the two domains can maintain their biochemical and enzymatic individuality under such conditions. Even if the connections are transient and are established by vesicles which detach from one domain and fuse with another, one would still expect the recipient domain to have a fairly high content of constituents of the donor domain, especially since the turnover of membrane proteins is relatively slow. If the vesicles operate a shuttle, cross-mingling of the two domains should occur, unless lateral diffusion between the fused membranes cannot take place.

These are key problems which remain for future investigations to solve.

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DISCUSSION

Chairman: Prof. C. De Duve

Boyer

I noted in that very interesting slide on the ferritin fixation that cytochrome b_5 appeared always to be on the outside of all the mitochondria. Does the method serve to localise the enzymes not only to the outer membrane but to one surface of the outer membrane?

De Duve

The data are consistent but they do not prove such localization. This is because we can't get to the other side with our ferritin-labeled hybrid antibody.

Boyer

Can you temporarily open up the membrane to allow ferritin penetration, perhaps by use of digitonin?

De Duve

We have made some attempts to do this, but without success.

HASSELBACH

Can you give me any explanation for the formation of the tubular structures in the presence of digitonin. If you take the vesicles and you add digitonin to them, do you get the tubular structures on the grid or can you observe these structures also in pelleted material?

DE DUVE

Digitonin binds cholesterol to the membrane. We do not know how this binding causes the structural changes that are observed.

HASSELBACH

The same thing happens with the vesicles of the sarcoplasmic reticulum in the presence of lysolecithin.

LIQUORI

Do you think that these density changes can be explained in terms of filling holes or making holes in compact membrane structures or is it difficult to give interpretations of this kind?

DE DUVE

At the concentration used, digitonin does not seem to make holes in the membranes. At least it does not disrupt membrane structure sufficiently to affect the latency of masked enzymes such as nucleoside diphosphatase or galactosyl transferase. As to filling holes, there is no evidence, and it is in fact very unlikely, that the membrane may have holes of sufficient size to accomodate molecules of digitonin. In our opinion, the increase in density is explained simply by the addition to the membrane of digitonin, which is a glycoside of high density.

STRUCTURE OF BIOLOGICAL MEMBRANES; BACTERIORHODOPSIN AND THE PURPLE MEMBRANE

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Biological membranes may be considered as consisting of a general permeability barrier into which functional units such as specific transport systems and signal or energy transducers are inserted. These specific functional sites may be simple carrier molecules and ion specific channels or very complex units such as the electron transport chains of respiration and photosynthesis. The lipid bilayer constitutes the general permeability barrier and determines the main structural features of membranes. The structure and specificity of the functional complexes is mainly determined by the protein components. The properties of the lipid bilayer, some carriers and ion-conducting channels have been extensively studied in simple model systems. The more complex functional sites of natural membranes can be studied in essentially unifunctional membranes or must be isolated from multifunctional membranes. The composition and structure of the isolated units may then be further analyzed or they may be reincorporated into model

systems consisting of lipid bilayers for an analysis of their function in an asymmetric environment similar to that of the cells from which they have been obtained. Ideally such a system should allow one to change and monitor the ionic composition on both sides of the membrane and to record membrane potential and other parameters simultaneously. Examples of this approach are the isolation and reconstitution of the Ca^{++} - and the Na^+, K^+ - pumps from the sarcoplasmic reticulum and the cell membrane of eukaryotic cells, the electron transport chain of mitochondria and the light-driven proton pump of halobacteria. In all cases proteins that span the membrane appear to be involved; they are embedded in the lipid layer, penetrate the hydrophobic core and are accessible simultaneously on botb surfaces. The analysis in the best studied systems has reached a point where testable molecular models are proposed which are based on a combination of functional and structural data. We shall here examine more closely one of these systems, the purple membrane of halobacteria.

Halobacteria require high NaCl concentrations at or near saturation for maintenance of structure and function not only of their cells but also most of their cell components. The purple membrane is an exception to this general rule. It is stable even in distilled water and very insensitive to large cbanges in salt concentration. This facilitates its isolation. Extensive disaggregation and solubilization of most cell components occurs wben *Halobacterium halobium* cells are exposed to distilled water. The purple membrane remains intact and can easily be separated from the other cell constituents by differential and density gradient centrifugation. The preparation so obtained contains only one protein which constitutes $\sim 75\%$ of the mass of the membrane; the remainder is lipid, mainly phospholipid. Contamination with other cell constituents amounts to less than 0.5% [1, 2].

Tbe protein, bacteriorhodopsin, has a molecular weight of approximately 26,000 and contains one mole of retinal per mole of protein. The retinal is bound as a Schiff base to a

lysine residue of the protein. One would expect such a retinylidene protein to show an absorption maximum near 370 nm; however, we find that similar to the visual pigments of animals the absorption maximum is red shifted to $\sim$ 570 nm. This indicates complexation of the retinal chromophore with other amino acid residues in the protein. Indeed, the retinal is found to be rather inaccessible to aqueous reagents such as borohydride and hydroxylamine. The absorption band at 570 nm has a molar extinction coefficient of $\sim$ 63,000 (moles dm^{-3} cm^{-1}) and imparts a deep purple color to the membrane [3].

The protein forms distinct patches in the surface membrane of the cell, from which the other membrane proteins are excluded. Every patch is a single two-dimensional crystal of rhodopsin and lipid. The lattice is hexagonal, plane group P_3, with the centers of the hexagons 63 Å apart. The planar crystals show a high degree of order, giving X-ray reflections out to about 3 Å [4, 5]. The patches can clearly be recognized in freeze-fracture electron micrographs, because the lattice is preserved and imparts a texture to the fracture faces quite different from the irregular particle distribution seen on the remainder of the cell membrane (Figure 1). The patches are typically 0.5 µm in diameter and elongate when they become larger. The two fracture faces are quite different in appearance; the outer leaflet is smooth and shows indications of the hexagonal lattice only in optimally resolved replicas. The inner fracture face adjacent to the cytoplasm shows a homogenous population of rather closely packed particles. When they are well resolved, it can be concluded from their center-to-center distance that they must represent 3 bacteriorhodopsin molecules clustered around threefold axes of the lattice. The difference in the appearance of the two fracture faces implies that the purple membrane has a highly asymmetric crosssection. Freeze-etch preparations also show that the fracture plane lies closer to the outer than the inner membrane surface (Figure 2). The asymmetry of the membrane becomes obvious also when

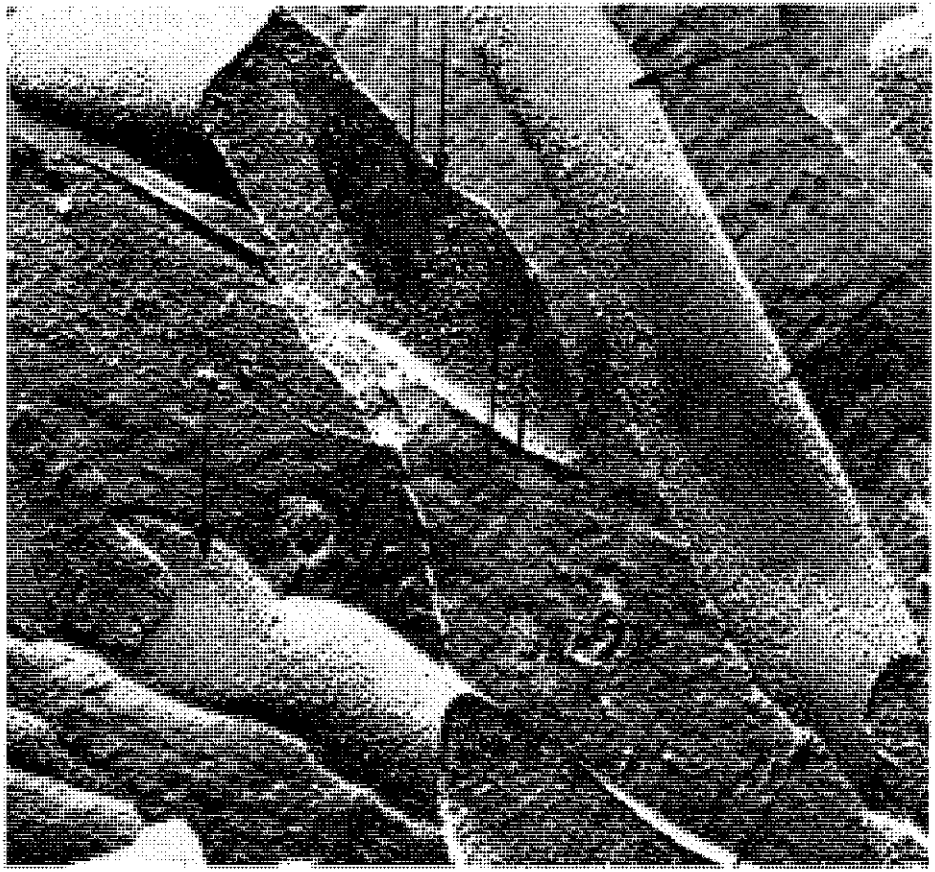

FIG. 1 — Freeze-fracture preparation of *Halobacterium halobium* cells containing large amounts of purple membrane. The purple membrane patches on the inner (convex) fracture face of the cell membrane are marked by single arrows, on the outer (concave) fracture face by double arrows. The inner fracture face of the purple membrane is characterized by an orderly array of small particles; the outer fracture face appears smooth. The few large particles seen in the smooth patches of the outer fracture face are probably artifacts. Magnification: 61,440 X.

FIG. 2 — Freeze-etch preparation of isolated purple membrane. The inner fracture face with the particles is indicated by a single arrow, the smooth outer fracture face by double arrows. The adjacent outer and inner surfaces of the membrane are revealed by etching. Note that the step from the inner fracture face to the outer surface of the membrane is too low to be resolved. The step from the outer fracture face to the inner surface appears nearly as high as the membrane is thick. This indicates that the fracture occurs close to the outer surface of the membrane. Magnification: 76,800 X.

shadowed preparations of the isolated membrane are viewed in the electron microscope. One surface is smooth within in the limits of even the best resolved electron micrographs and the other shows small very shallow depressions and deeper narrow cracks which follow the lattice planes. These apparently develop when the membrane sheets dry prior to shadowing.

Electron microscopy and X-ray diffraction results show that the purple membrane is only $\sim$ 50 Å thick. The electron density profile has prominent peaks close to the two surfaces and a central electron density considerably higher than usually found in biological membranes and in the rest of tbe surface membrane of *Halobacterium halobium* [4]. HENDERSON has recently shown by X-ray diffraction that the protein has a high α-helix content, that the helices are oriented preferentially at right angle to the plane of the membrane and that they are at least 30 Å long [5]. The high α-helix content is also borne out by CD spectra (Y.-W. TSENG and W. STOECKENIUS, unpublished).

We can conclude from these observations that the purple membrane patches contain only one protein, bacteriorhodopsin, which is arranged in a hexagonal lattice in the plane of the membrane and spans the membrane. All protein molecules are oriented in the same direction across the membrane. As we shall see, the function of the protein also is consistent with such an arrangement. The lipid apparently fills the space between the protein molecules in the form of a bilayer with its hydrophilic groups oriented towards both surface. During freezefracturing a perforted monolayer of lipid separates from the protein and the lipid which faces the cytoplasmic side of the membrane. The structure of the purple membrane differs therefore substantially from the general structure of cell membranes. Its unique protein composition implies that is constitutes a functional site. Deviations from the general membrane structure are expected to occur in functional sites because their different specific functions require different structures. The

purple membrane may nevertheless be considered as a variation of the general membrane structure. The high degree of crystallinity is unparalleled in any other known membrane or functional site. This crystallinity is not an artifact of isolation because it can be demonstrated to exist also in whole cells.

The similarity of bacteriorhodopsin to animal visual pigments may suggest a photoreceptor function for bacteriorhodopsin. A phototactic response has indeed been observed (our unpublished observations; see also 6). However, *Halobacterium halobium* forms large amounts of purple membrane when it is grown under light and at low oxygen concentrations. Under optimal conditions the purple membrane may constitute more than half of the total cell membrane [7]. The large amount of pigment present and the fact that the cells actively move towards an environment with higher radiation density at the wavelength absobed by bacteriorhodopsin and avoid shorter wavelength light, make it unlikely that the phototactic response is the sole function of bacteriorhodopsin. These observations rather suggested to us that the cells might use the light energy absorbed by the pigment for metabolic reactions.

It had been reported previously that *Halobacterium halobium* has no glycolytic pathway and depends entirely on oxidative phosphorylation for its energy supply [8]. We therefore measured the ATP content in anaerobic cells and found it to be low in the dark, but as high as in aerobic cells when the anaerobic cells were illuminated [9]. Only purple membrane containing cells showed this property and only light absorbed by the purple membrane was effective. We could further show that light reversibly inhibits respiration and that the cells eject protons against an electrochemical gradient when exposed to light [7]. These observations suggest that the light energy absorbed by the purple membrane is used to generate an electrochemical proton gradient across the cell membrane, which in turn can be used to synthesize ATP, presumably by the chemiosmotic mechanism proposed by MITCHELL [10]. An ATP-ase that could serve in this function had been

demonstrated earlier in *Halobacterium halobium* cells [11]. We have since shown that all the observed effects of light on intact cells show action spectra that correspond to the absorption spectrum of the purple membrane and that the quantum efficiency of the proton ejection is close to one [12]. We are, therefore, apparently dealing with a new light energy transduction mechanism and the purple membrane appears to function as a light-driven proton pump.

There are other possible explanations for the apparent light-driven proton ejection through the purple membrane in whole cells. It appeared therefore desirable to use a less complex system where this proposed funtion could be demonstrated unequivocally. When additional lipid is incorporated into the purple membrane the sheets close to form predominantly single-walled vesicles and these were found to constitute a suitable model system. The vesicles take up protons in the light and release them in the dark. This suggests that the purple membrane is preferentially oriented in the reverse direction as compared to the cell and this conclusion is confirmed by electron microscopy (Figure 3; S.-B. HWANG and W. STOECKENIUS, unpublished). We were further able to incorporate also mitochondrial ATP-ase into these vesicles and demonstrate a light-driven ATP synthesis in this model system [13].

We have further studied the mechanism of the proton pump mainly through flash and low temperature spectroscopy in our laboratory [14] and through resonance RAMAN spectroscopy in collaboration with A. LEWIS [15]. When bacteriorhodopsin absorbs a photon it undergoes a cyclic photoreaction. At least four, possibly five, spectroscopically distinct intermediates have been identified in this cycle. It is complete in approximately 6 msec at physiological temperatures and it involves a deprotonation and reprotonation of the Schiff base linkage between retinal and the lysine residue of the protein. During the cycle one proton is released from the membrane and subsequently one proton is taken up [14]. The photoreaction

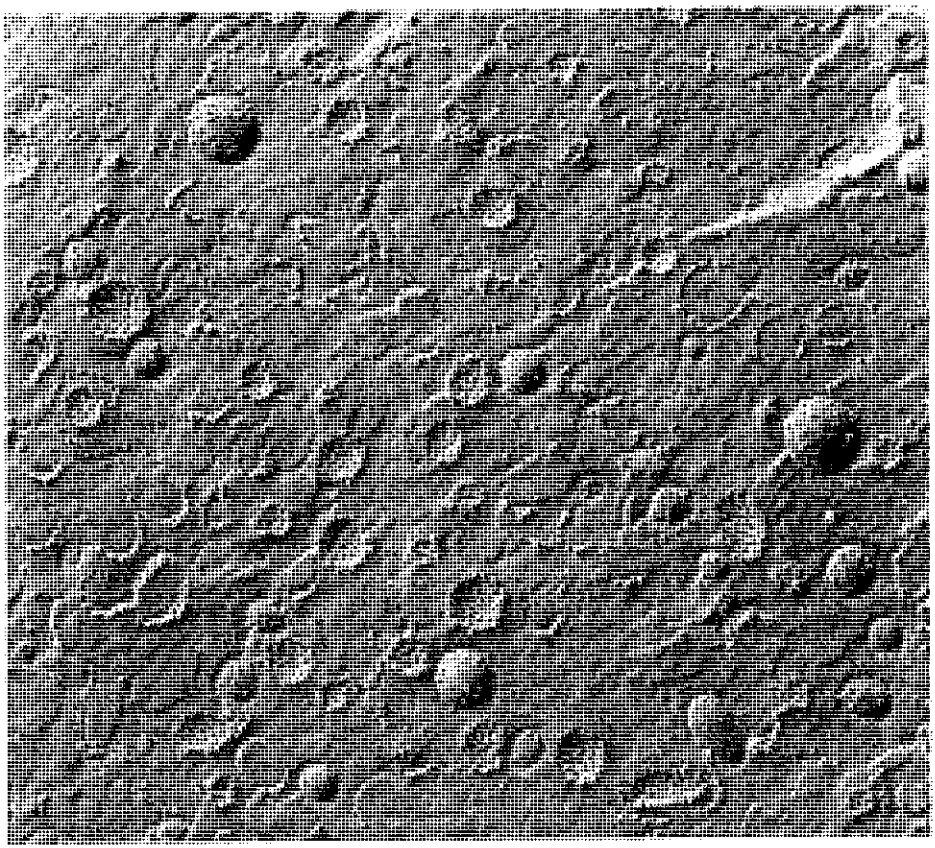

FIG. 3 — Freeze-fracture preparation of vesicles formed from isolated purple membrane and added phospholipids. The arrays of particles are seen nearly exclusively on the concave fracture faces of the vesicles, indicating that the orientation of bacteriorhodopsin is the inverse of that found in intact cells. Magnification: 132,300 X.

cycle has mainly been studied in suspensions of isolated purple membrane, but the main spectroscopic changes have also been detected in intact cells and the reaction times are essentially the same. We assume that in intact cells the proton is released on the outer surface of the membrane and taken up on the inner surface. A mechanism must therefore exist which translocates protons from the inner to the outer membrane surface during the reaction cycle. We further assume that this occurs via a chain of proton exchanging groups in or on the protein and that the Schiff base is one link in this chain of groups. A large light-induced reversible pK shift of one of the groups could drive the proton translocation provided that back reactions are prevented so that this group can donate protons only to the following group in the chain and accept them from the preceding group. Small conformational changes in the protein during the reaction cycle could impart this vectorial character to the reaction.

The chemical similarity of bacteriorhodopsin to the visual pigments of animals also extends to its light reactions. The spectral changes are similar; the main difference appears to be that bacteriorhodopsin completes the photoreaction cycle after absorption of only one photon, whereas the animal pigments need additional energy input to complete the reaction. This raises the question of possible isomerizations of the retinal during tbe reaction cycle. So far there is no evidence that these occur. The chromophore apparently is all-trans retinal in both the initial complex and the one intermediate of the light reaction cycle that has been investigated. In addition to the " light-adapted " retinal which we have heen discussing so far, there exists, however, a " dark-adapted " complex. It arises with a half-time of $\sim$ 30 minutes, when purple membrane is kept in the dark. The dark-adapted complex, when illuminated, shows a photoreaction cycle similar to that of light-adapted bacteriorhodopsin. A small amount of the cycling pigment is converted to the light-adapted complex during each cycle. At saturating light intensities the conversion is complete in a few

seconds [14]. The dark-adapted complex contains 13-cis retinal [16, 17].

We have here described a new light energy transducing system as well as a simple biological ion pump which functions apparently as efficiently in isolation as it does in intact cells. It can easily be isolated in relatively large amounts, 400 to 500 mg from a 10-liter culture. The flow of protons which it provides can be coupled to the flow of other ions in the reconstituted model systems. In principle, it should therefore be possible to construct a model system that uses light energy for the desalination of water. It will be interesting to see how its efficiency compares to other desalination methods.

ACKNOWLEDGMENT

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DISCUSSION

Chairman: Prof. C. DE DUVE

KEYNES

I think DENNIS CHAPMAN has done experiments similar to CONE's on this type of bacteria, in which he found that your protein rotated very much more slowly than rhodopsin. Are these findings compatible with your structure? Is the protein fixed altogether in the membrane, or would you expect it to be able to rotate? Is it each of the patches that rotates, or what?

STOECKENIUS

DENNIS CHAPMAN has tried to measure rotational relaxation times of bacteriorhodopsin — in the purple membrane — and found that is was essentially immobile. We have done similar experiments observing the decay of induced dichroism in collaboration with RICHARD CONE and spin-labelling experiments with WAYNE HUBBELL, with essentially the same results. The purple membrane is an unusually rigid structure compared to other membranes. I also should point out that the model for the function of bacteriorhodopsin, as I have proposed it here, does not require mobility of protein in the membrane. Small conformational change in the protein to prevent a back reaction after the SCHIFF base has been deprotonated would be sufficient.

KEYNES

But I think that the rotations that CHAPMAN and CONE observed would have been in a different plane, that is, in the plane at right angles to the one you're talking about.

STOECKENIUS

That is correct. A rotation of the molecule around an axis normal to the plane of the membrane would be the most likely cause of a rapid decay of the induced dichroism in view of RICHARD CONE's results on the rotational relaxation of rhodopsin in rod outer segment membrane. The decay time for the induced dichroism is compatible with the assumption that it is due to the tumbling of the purple membrane pieces.

LIQUORI

According to your scheme, you suppose that when you split the membrane the lipids come off and leave some space empty which according to the electron microscope has a low electron density. Now would you say that this space is hydrophobic and is not replaced by water?

STOECKENIUS

Yes. The membrane is fractured in the frozen state and the replica is prepared while the preparation is still at −100°C. We see the arrangement of the protein after the lipid has been fractured away because it leaves empty spaces between the protein particles.

LIQUORI

Now the second question: — the kind of transition you think of seems to be what I like to call a « non catastrophic transition »

(nothing to do with helix-coil transitions). It is like a second order transition that leaves the overall structure almost unchanged. Therefore I think your model is very reasonable.

STOECKENIUS

That is exactly what I had in mind. The model so far is not entirely satisfactory. It is mainly of heuristic value and will eventually, I hope, lead to the correct model. Its main value is that it can be tested experimentally and can be modified as required by the experimental results.

MONNIER

This reminds me of a classical H^+ion pump, the gastric mucosa. Would there be a way to photoactivate the gastric mucosa? More precisely, is the linkage between rhodopsin or rhodopsin-like pigment and the pump quite specific? Could another pigment with a different absorption band activate a pump of that kind?

STOECKENIUS

I don't know about a pigment in the gastric mucosa that could be activated by light. If you want to speculate you could imagine an experimental system where you incorporate purple membrane into the apical cell membrane of the HCl — segreting cell and with light generate a pH gradient across this cell membrane which would result in acid secretion if the membrane were also permeable to chloride.

DE DUVE

Is the cycle reversible?

[4] II, 2 - *Stoeckenius* - p. 17

STOECKENIUS

We do not know. So far all we know is that the first step in the reaction cycle is photoreversible but not thermally reversible.

MUELLER

What is the orientation of the chromophore with respect to the membrane plane?

STOECKENIUS

The chromophore is oriented with its long axis in the plane of the membrane. The same orientation is found for the rhodopsin chromophore in the rod outer segment discs.

MUELLER

Is there a cis-trans transition?

STOECKENIUS

No. In the lower cycle shown on the slide which is the photoreaction cycle of the light-adapted pigment the retinal is in the all-trans configuration in the bR_{570} complex and in the M_{412} complex. In the dark-adapted pigment the retinal is in the 13-cis configuration.

MUELLER

Dark-adapted?

STOECKENIUS

The isomerization from all-trans to the 13-cis configuration is a slow thermal reaction with a half-time of about 30 minutes at room temperature.

MUELLER

So the cycle itself does not involve necessarily a cis-trans transition?

STOECKENIUS

There is no evidence for an isomerization of C=C bonds in the light-adapted pigment during the cycle.

BOYER

On your interesting model of a proton pump you suggested a number of groups x, x_2m, x_3 etc., that were participating successively in the proton transport. This is somewhat like a « bucket-brigade ». This would appear to require the presence of a cluster of hydrophilic groups in the protein interior. This seems less likely to me than the penetration of water channels from both sides of the membrane too close to the key group undergoing protonation and deprotonation. Is there a reason why you preferred the aligned group model to the water channel model?

STOECKENIUS

We think that the most logical candidate for the group that actively changes its pK is the Schiff base. We know that the Schiff base is not directly accessible from the outside. It does not

react with $NaBH_4$ or hydroxylamine in the dark. It will react in the light but only slowly even at high light intensities and the reaction is complete only after several hours.

BOYER

Lack of borohydride reduction needs not complete inaccessibility to water. Hindrance of approach of the borohydride to the Schiff base carbon would suffice. The nitrogen might be exposed to water for protonation.

INTERACTIONS AMONG CELLULAR MEMBRANES

Problems and Perspectives

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Eukaryotic cells are highly compartmented systems in which large amounts of membranes of different types are used to construct distinct compartments. Irrespective of their type, all these membranes appear to be essentially bimolecular leaflets of polar lipids modified by interactions with proteins and glycoproteins and functioning as diffusion barriers with permeability characteristics different from one compartment to another.

Cellular membranes are endowed with *spatial continuity* in the sense that they form closed compartments by being in continuity with themselves. They show no free margins and no resolvable discontinuities at the level of microscopic resolution currently attained (i.e., $\leq$ 10 Å). In the case of the plasmalemma, the " closed system " is the cell itself, while in the case of intracellular membranes (endoplasmic reticulum, Golgi complex, mitochondria, chloroplasts, etc.) the closed compartments appear as vesicles, tubules and cisternae interconnected in 3 dimensions to varied degrees of complexity.

Cellular membranes are also endowed with *temporal continuity,* in the sense that each cell inherits from its mother cell

its plasmalemma as well as a complete set of intracellular compartments bounded by their corresponding membranes. Irrespective of what the cell is doing, as long as it stays alive, no type of membrane is lost and no discontinuities develop in any membrane type. From its inception (or very close to it) life has lived behind membranes.

Temporal and spatial continuity can be looked upon as visible expression of the *functional continuity* of the cellular membrane systems: they continue to function as diffusion barriers, which create and maintain chemical and electrochemical gradients, irrespective of the position of the cell in the cell cycle and irrespective of events that affect them directly, such as membrane growth and turnover of membrane components.

As far as we know, there is no *de novo* membrane formation in any cell, eukaryotic or prokaryotic. In keeping with the different aspects of continuity mentioned above, membrane growth is in fact membrane expansion achieved by the insertion of new components into the preexisting membrane structure. The general process is reasonably well established, but the nature of the inserted " units " is still unknown: they could be individual molecules, or molecular assemblies, or patches of already organized membrane. If the last alternative were to apply, it would follow that a precursor → product relationship exists among certain cellular membranes, and among some intracellular membranes and the plasmalemma.

The number of membrane types so far studied in appropriate detail is too small to permit any generalization. We have only a few disparate pieces of evidence and we can not decide at present whether they represent special cases or reflect general processes. Evidently more information is needed bearing on a variety of mebranes from a variety of cell types. The asynchronous character of the response of the components of the membrane of the endoplasmic reticulum (ER) of rat hepatocytes to cell differentiation and to induction by xenobiotics [1, 2], the asynchrony ohserved in the turnover of the

proteins of the plasmalemma [3] and the ER membrane [4, 5] in the same cell type, and the asynchrony of incorporation of new components into the thylakoid membranes of the green alga *Chlamydomonas reinhardtii* (wild type) during the cell cycle [6-8] are all favoring membrane modulation or membrane expansion by insertion of individual molecules into preexisting membranes. So far, there is no evidence indicating that the inserted units are molecular assemblies. But there are extensive and well documented observations which show that organized membrane coming from the boundary of one compartment as a distinct vesicle can be incorporated into the preexisting membrane of another compartment or into the plasmalemma. The difficulty is that in all these cases neither the functional role, nor the ultimate fate of the incoming membrane is fully and unambiguously understood.

At this point, it becomes necessary to take a second look at the spatial continuity of cellular membranes and to consider it in relation to the membrane interactions involved in the intracellular transport of secretory macromolecules in eukaryotic cells. The membranous partitions that separate the various intracellular compartments are handled by the cell according to a consistent and characteristic pattern. Certain partitions are never interrupted: those which bound the cytoplasmic matrix belong to this group. Others are only occasionally removed to establish continuity between the extracellular medium and the cisternal space of the nuclear envelope [9] or the external mitochondrial space. Such instances are well documented, but their rarity and their restriction to a few species in a single order (Hepaticae, so far) make them appear as exceptions which, for the moment at least, deserve less attention than the next group. Finally, the membranes of the compartments that together from the secretory pathway (i.e., the endoplasmic reticulum, the Golgi complex, the secretion vacuoles and the plasmalemma) are regularly and frequently involved in fusion to one another, followed by fission within the area of fusion, followed in turn by continuity established

between the membranes and contents of the interacting compartments. At the terminus of the pathway this process is recognized as exocytosis. The above sequence of operations is reversible: continuity is discontinued and the compartments regain their separateness. The coupling and uncoupling are intermittent events which recur at relatively high frequency.

A number of features characterize these recurrent membrane interactions along the secretory pathway. Membrane fusion occurs in series between pairs of compartments (i.e., endoplasmic reticulum → Golgi complex and secretion vacuoles → → plasmalemma). It involves a high degree of specificity: the ER membrane interacts only with Golgi membranes and secretion vacuole membranes only with the plasmalemma (often only with a restricted domain of the plasmalemma), although many other membranes belonging to other different compartments are present at the same time and at equal distances within the cytoplasmic region where the fusion takes place. The transport is vectorial in the direction ER → plasmalemma, and certain steps along the pathway require energy. The anatomy of the connections is variable, but in some places separate membrane containers (vesicles or vacuoles) are involved so that membrane is bodily removed from the donor compartment and relocated into the boundary of the receiving compartment. The clearest example of this type is found at the level of the interaction between secretion vacuoles and the plasmalemma (in the process called exocytosis), but a similar situation is encountered — at least in certain cell types — between the ER and the Golgi complex. Finally, in all these operations, the partitions along the secretory pathway are removed and the corresponding compartments are connected sequentially and in series, while the diffusion barriers between these compartments and the cytoplasmic matrix remain apparently untouched. As far as the cell is concerned, the important point seems to be the prevention of any sudden leakage from, or into, this central compartment which is trated as the most vital part (the " vitalia vitalium " of the entire compartmented system. With

these considerations in mind, the principle of spatial continuity can be redefined: it applies strictly to the membranes limiting the cytoplasmic matrix and as a rule to those of the mitochondrial and chloroplastic compartments; it applies only in part to the membranes of the secretory pathway since the cell can couple and uncouple the corresponding compartments as required by the different steps involved in intracellular transport.

Since the secretory products can be labeled cytochemically, radiochemically or immunochemically, there is no doubt about their nature and final destination: they are proteins, glycoproteins or polysaccharides which are eventually discharged into the external medium of the cell by exocytosis. It is clear, however, that tre transport operation also involves membrane containers which come from a compartment and are relocated in the membrane of its distal partner. The cell transports concomitantly membrane and products down the secretory pathway; but, in contradistinction to the content, the nature and the ultimate fate of the membrane containers are still uncertain. The membrane of the containers may have no other function but to package the secretion products; concomitantly it could be — in part or in toto — a precursor membrane on its way to its final relocation in the Golgi complex or the plasmalemma. In other words, a patch of organized membrane coming for insertion at the station of its final function. Images of this type have, in fact, been used as suggestive evidence for the postulate that membrane grows by insertion of patches of organized membrane (vesicular membranes), and for the hypothesis that the membranes of the secretory pathway are biogenetically related to one another, the ER membrane being the precursor of the Golgi membrane, which in turn is the precursor of the plasmalemma [12].

In the steady state condition, the compartments involved in the secretory pathway retain their relative volume and membrane areas while transport and discharge go on. Rapid and sustained discharge (by continuous stimulation) leads to unequal distribution of membranes (i.e., enlargement of the luminal

plasmalemma by relocated membranes of secretion vacuoles [10, 11]; but even in this situation the original membrane distribution is quantitatively reestablished in about 1 hour.

To control membrane distribution, the cell must be able to remove membrane from the receiving compartment and add membrane to the donor compartment. This could be accomplished either by recovery and reutilization of membrane (an operation which could be called recycling of containers), or by removal and degradation of the membrane at the receiving end coupled with compensatory synthesis at the donor end.

In principle, reutilization is possible in the few cases so far studied, i.e., the acinar cells of the pancreas [13] and the parotid [14], since the rate of tynthesis of membrane proteins is generally lower than the rate of synthesis of secretory proteins [see, however, 15]. Recovery of membrane from the enlarged cell surface after the discharge of secretory products has been demonstrated in a few secretory systems [16-19] by introducing a cytochemically — detectable marker into the extracellular medium: excess membrane is recovered as closed vesicles whose content is labeled by the marker.

The type of removal actually practised by the cell is expected to affect critically the chemistry and function of the membranes of the secretory pathway, plasmalemma included. Specific chemical composition and specific functions for each of the interacting membranes could be maintained if removal were non-random. Chemical and functional equivalence, as opposed to specificity, would exist if random removal were in operation.

Questions related to membrane interactions along the secretory pathway, including type of removal of excess membrane, can be answered in principle in the case of the mammalian hepatocyte, since cell fractions representative of the plasmalemma and of the main compartments of the pathway are available. A careful comparison of their components and activities should indicate clearly whether their membranes are endowed, or not, with chemical and functional specificity, and

accordingly whether random or non-random removal applies. The results should also help in defining the boundaries within which biogenetic relations among the membranes under inquiry should be considered. If all these membranes were equivalent, the precedent established for the rough ER-smooth ER relationship [20, 21] would apply for the entire secretory pathway. The proteins of their membranes would be synthesized and inserted by polysomes in the rough part of the ER and would spread in time (by lateral diffusion or by membrane transport) down the pathway. If the membranes of each compartment were endowed with specificity, then the amount of " precursor membrane " carried out by membranous containers from compartment to compartment would represent a small fraction of the total membrane of the carriers. In the case of the terminus of the pathway, for instance, the ratio (cell surface membrane: secretion granule membranes) is low in most cells and the turnover rate of the components of the plasmalemma is also low. Most of the container membrane would be, therefore, membrane of the donor compartment which remains to be removed from the receiving compartment.

Sufficient information has been available in the last 15 years to indicate convincingly that the ER membrane and the plasmalemma are different, biochemically and functionally. Only 6 years ago, however, procedures started to be devised for the isolation of cell fractions representing the Golgi complex [22, 23], until then the missing link in the series of cell fractions representing the secretory pathway. Three years ago a combination of Golgi fractions of reasonable homogeneity was obtained in high yield by modifying the density of Golgi elements by overloading them *in vivo* with a secretion product, namely very low density lipoproteins, the overloading being brought about by ethanol treatment [24]. With Golgi fractions available, it is finally possible to open a meaningful inquiry into the membrane interactions of the secretory pathway.

The investigation was focused at the beginning on two compartments, the ER and the Golgi complex, but the latter

was subfractionated into 3 fractions of which the lighter can be considered as the equivalent of secretion granules in other cell types [24].

The data so far obtained in a number of laboratories indicate that there are qualitative or quantitative differences between ER — and Golgi membranes. On the one hand, the activities of many typical " microsomal marker enzymes " (i.e., ER membrane enzymes) like glucose-6-phosphatase, cytochrome P 450 and NADPH-cyt. *c* reductase are present in low concentrations [22, 25] in most Golgi fractions or absent [26] from the best fractions available at present; the same applies for the activity of the series of enzymes involved in the synthesis of triacylglycerols and glycerophosphatides [27]. On the other hand, galactosyl transferase activity is present in high concentration [22, 25, 28] and can be fully recovered [26] in Golgi fractions; apparently it is not present in ER membranes. Incomplete evidence suggests that the same obtains for fucosyl — and sialyl transferase activities [29]. Other enzyme activities, i.e., 5'-nucleotidase, cytochrome b_5, NADH-cyt b_5 reductase, are shared but appear to occur in different concentrations in the two cell fractions under discussion. Quantitative differences have also been recorded between rough microsomal and smooth microsomal fractions isolated from the guinea pig pancreas [30]. In this particular case, the smooth microsomes are derived primarily from Golgi elements.

Finally, significant quantitative differences in lipid composition have been recorded between ER and Golgi fractions obtained from rat liver [31] or guinea pig prancreas [32]. In both cases, the concentration of sphingomyelin and cholesterol is higher in Golgi fractions (presumably in Golgi membranes) than in ER fractions. In the liver, the acyl groups of the phosphatides are generally longer and more saturated in Golgi than in ER membranes, a finding which suggest that the latter are more fluid and more permeable than the former.

For the moment, comparative data on enzyme activities in secretion granule membranes and in the plasmalemma are

limited. Besides most of them are not directly useful, since in the case of the plasmalemma they refer to the whole membrane, rather than to the restricted domain with which secretion granule membranes interact. Whatever data are available suggest the existence of quantitative differences between the membranes discussed [26]. Lipid composition is this time nearly identical [31, 32].

The data so far collected indicate quite clearly that the membranes of the first pair of compartments of tbe secretory pathway, i.e., the ER and the Golgi complex are different in all respects covered by the investigations: there are qualitative and quantitative differences in enzyme activities, presumably in enzyme concentrations, and there are quantitative differences in lipid composition. On the strength of this evidence it can be concluded that no mixing of membrane components occurs during the recurrent membrane fusion-fission process connected with intracellular transport, althougb the membranes in question are in continuity, at least intermittently. It can also be concluded that removal of transported membrane from the receiving compartment must be non-random.

The situation at the level of the last pair of interacting membranes (i.e., secretion vacuole membrane - plasmalemma) is much more difficult to asses because of the scarcity of data, but tbe same general findings seem to apply: the interacting membranes appear to be different.

The data summarized above concern a limited number of activities ascribable to membrane proteins: about a dozen for the pair ER — Golgi complex, and only a few for the pair secretion vacuole membrane — plasmalemma. This is only a fraction, and most prohably a small fraction of the total number of proteins anticipated in these membranes. A comprehensive view of tbeir proteins would be — of course — preferable since, in principle, it could indicate much more reliably than the approach used so far how extensive (or conversely how restricted) is the degree of compositional overlap between the different pairs of membranes of the secretory pathway. A com-

prehensive view of the polypeptides of these membranes could be easily obtained by electrophoresis of solubilized membranes in the presence of denaturing detergents (sodium dodecyl sulfate) [cf. 33]. But for the moment at least, the technology of polypeptide separation is ahead of the technology needed for separating homogeneous cell fractions and especially acceptable membrane subfractions. Membrane preparations so far obtained proved to be extensively contaminated primarily by proteins adsorbed (or incompletely removed) from the cavities of the secretory pathway and from the cytoplasmic matrix. Such preparations must be thoroughly cleaned of adsorbed molecular contaminants before undertaking any meaningful comparative studies. Most of the containants are secretory proteins [34]; hence, they are expected to be the same, or nearly the same in each compartment of the secretory pathway. Progress along this line is slow, but the results begin to be encouraging. For years, what was seen in the SDS gel electropherograms of the membranes under discussion were primarily the contaminant secretory polypeptides. Now, finally, membrane polypeptides begin to be detected. For the moment no firm data are available and they will be slow to come since the evidence needed for definitive conclusions must go beyond the recognition of bands of similar or identical electrophoretic mobility.

It should be clear from the preceding comments that the picture is still rather hazy. The evidence for membrane specificity is becoming stronger; it is in general agreement with what we know about modification by sequential glycosylation of secretory proteins [29]; and it is also in good agreement with autoradiographic observations on the sites of incorporation of distal residues in the oligosaccharide chains of glycoproteins [35]. But it is a view taken through a narrow slit at a very large problem. What we have seen through the slit is still compatible witb extensive compositional overlap. Hence, only a comprehensive view at the problem can provide a full and reliable answer.

What makes the situation intriguing and unusually attrac-

tive are the many contradictions between findings and prevalent concepts that beset the picture.

The membranes of the various compartments of the secretory pathway are endowed with specificity evident even at the incomplete level of analysis available at present. This specificity is retained notwithstanding the fact that these membranes become continuous at least intermittently, and notwithstanding current evidence on membrane fluidity and lateral diffusion of various components in the plane of the membranes [36]. Differences in lipid composition are particularly difficult to rationalize in the current conceptual framework.

Concomitant transport of membrane and product is clearly established but the bulk of the membrane seems to be removable container rather than precursor membrane of the receiving compartment.

The immediate question is: where are the proteins of the Golgi membranes and plasmalemma synthesized and how are they transported from their sites of synthesis to their sites of assembly. Relying on analogies, it could be assumed that they are synthesized by the free polysomes of the cytoplasmic matrix. These polysomes are generally considered as the source for most mitochondrial and chloroplastic membrane proteins. Yet many membrane proteins, especially many plasmalemma proteins, are glycoproteins, and as far as we know at present glycosylation (terminal glycosylation) occurs only in the Golgi complex. But moving proteins across membranes (to modify them) is a highly unlikely operation in the framework of our current ideas. The list of contradictions could be extended, yet the situation is not really discouraging. Behind any apparent contradiction there must be an interesting answer.

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DISCUSSION

Chairman: Prof. C. DE DUVE

TEORELL

Later this week I have to discuss the feedback hetween biologists and memhrane bio-physicists. Dr. PALADE's beautiful presentation, as well as Prof. DE DUVE's this morning, may have caused a great deal of concern among those people who deal with the physical chemistry of membranes. The biologists can depict and can photograph, they can make everything very simple, if they have talent and imagination and present suggestive schemes. Yet the bio-physicists may ask the question: what is the nature of the invisible physical forces driving Dr. PALADE's vacuoles along? Are they electrical, chemical forces and/or some kind of transformed surface forces?

PALADE

What a cell biologist like myself should do is to define the structural framework in which certain events take place and to establish as well as he can the structural modulations and biochemical changes that occur in connection with these events. Others — biophysicists among them — are better qualified to work out the forces and mechanisms involved in these processes. The challenge sbould concern the biophysicists (even more than the cell biologists) since the analysis of forces involved in transport operations in general belongs to their field of direct interest.

In secretory cells of the type described (hepatocytes, pancreatic exocrine cells), transport of products along the secretory pathway is well established, but the forces involved in vesicle or secretion vacuole movements are at present entirely unknown.

In the cells of the vascular endothelium which possess a large population of small (diam. ≃ 700 Å) plasmalemmal vesicles involved in the transport of macromolecules from the blood plasma to the interstitial fluid (and back), it has been calculated that the slow diffusion of the vesicles (or their Brownian movement) in the cytoplasmic matrix can account for the effective crossing of the endothelium (given a series of assumed values for the viscosity of the cytoplasm). These calculations are based essentially on the rates of transport of macromolecules across the capillary wall and on the geometry and dimensions of the endothelium and its plasmalemmal vesicles. What remains difficult to estimate is the time taken by the formation of vesicles on one side of the endothelial cells and their discharge (by membrane fusion-fission) on the other side. In other words the time involved in the membrane interactions considered separately from movement across the cytoplasm.

Membrane recognition, membrane fusion-fission and membrane removal are major problems in cell biology, about which we know practically nothing in terms of chemical reactions and physical forces involved. In the case of the pancreatic exocrine cell we know that energy is required at two different steps (to reach the Golgi complex, and to discharge), but the endergonic reactions involved at these steps are unknown.

TEORELL

Thank you very much, Dr. PALADE. I wish to remind my colleagues here that irreversible thermodynamics people have started to attack the problems of "structure formation", i.e. the forces behind organized structures.

CHAGAS

Speaking about fusion of membranes, could you give us some information about the establishment of channels between cells? This is becoming a biologically very important phenomenon.

PALADE

We should realize first that these phenomena, i.e. membrane fusion-fission, are widespread and critically involved in a long list of important biological processes: fecundation, cell division, secretation, phagocytosis, lysosome formation, to name a few. I have the impression that Dr. CHAGAS' question concerns another type of membrane interaction whose structural expression is the « gap junction ». At the level of these junctions (which involve two adjacent cells) the apposed cell membranes are differentiated « in phase » and adhere strongly to one another. The differentiation takes the form of a planar aggregate or lattice of particles ($\sim$ 80 Å diameter). Each particle seems to consist of a number of subunits arranged around a central channel of $\sim$ 20 Å diameter. Such structures are present in areas of low resistance coupling between cells. The transmembrane channels required by this type of coupling might be formed by the alignment of the central channels in the particles of the two interacting lattices. The lattices are maintained by strong protein-protein interactions within the plane of the lattice; the coupling may be secured and maintained by similar interactions this time between the proteins of the two lattices. Low resistance coupling must involve, in addition, more subtle interactions, since in uncoupled cells the structure of the gap junctions is not detectably changed.

RITCHIE

One of the things that puzzle me about the membrane recycling concept is that it appears to imply that somewhere in the system,

maybe in the endoplasmic reticulum there is a piece of prototypic membrane that possesses all the properties that we know the individual membranes have. However, if one looks even at a single cell, like a nerve cell, and we look at just one of its membranes, namely, the plasmolemma, we know that certain parts of this membrane have some properties and others have not. For example, parts of the membrane are electrically excitable and chemically inexcitable, whereas other parts of the same plasmo-membrane are chemically excitable and electrically inexcitable. Where is the traffic controller that decides where each type of membrane is to be laid down; that selects the appropriate properties from the prototypic membrane for local incorporation into the different areas of plasmolemma?

PALADE

In the cases I discussed, recycling of membrane containers is postulated between the endoplasmic reticulum and the Golgi complex and between the Golgi complex and the plasmalemma. Since the cell has in the corresponding regions a relatively large number of containers, and since the rate of synthesis of membrane proteins (in the few cases where it is known) is much slower than the rate of synthesis of secretory proteins (~20 times slower, in the pancreatic exocrine cell), the cell is not obliged to mobilize membrane reserves from somewhere else in the cytoplasm. The large number of containers found « where the action is » might represent the « reserve ».

The second part of the question is more difficult to answer. It relates to the existance of functionally differentiated domains in the plasmalemma (chemically vs. electrically excitable) which — in contradistinction with the membrane patches discussed by Dr. STOECKENIUS (purple membrane) or brought into discussion by Dr. CHAGAS (gap junctions) — do not depend for their existence on delectable protein lattices. In this case, we are dealing with specialized regions in a presumably fluid membrane. The situation

is similar, in fact, to that discussed in my presentation: retention of functional specificity in membranes which become continuous as a result of fusion-fission processes. We do not know whether a traffic controller is necessary within the cytoplasm to ensure directed movement (vs. random movement) of membrane containers from one comparment to another, but we believe that specific recognition is necessary in membrane-membrane interactions. The traffic controllor may be, in fact, that specific recognition.

BAKER

What your elegant analysis implies surely is that the container is yet another type of organelle in the cell whose job it is to go between one compartment and another in a specific way.

PALADE

This is a very tempting hypothesis which — if true — would explain away many of our current difficulties. But, to validate it, we must isolate and characterize biochemically the membrane of the containers and prove that they are different from the membranes at the two terminals. We are, in fact, working at present on the isolation and characterization of the membranes of secretion granules.

DE CARVALHO

Do you think it is possible that one of the « traffic controllers » — to use Dr. RITCHIE'S words, could be the environment which the membrane is facing? For instance, in the neuromuscular junction, the membrane would be exposed to certain types of transmitters which would not be present in the rest of the surface of the muscle cells and for this reason « receptor » molecules

would be directed towards this area; or if you have kidney tubular cells, one side of the membrane is exposed to a given environment and the other side is exposed to an entirely different environment and this makes the plasma membrane of the same cell do different things in the two surfaces. I am wondering whether this difference in exposure might determine in some way which specific membrane proteins should be incorporated in each side of the cell. The same question can be extended to other instances, such as the formation of specialized membrane patches at cell junctions.

PALADE

Yes, the immediate environment may play a role in membrane recognition. There is, moreover, ample morphological and biochemical information indicating that specific domains, differentiated structurally, chemically and functionally, exist in the plasmalemma and are related to the different environments the cell faces. Typical examples are found in the intestinal and nephron epithelium. Again, membrane interactions are specific and in the case of the plasmalemma the specificity applies to differentiated domains and governs interactions during transport of secretory products and undoubtedly during membrane growth. I should point out that this specificity has limits: upon stimulation with optimal doses of the appropriate neurotransmitter or hormone, the pancreatic exocrine cell for instance, discharges its secretory products only on the luminal domain of the plasmalemma, but upon stimulation with supraoptimal doses (supraoptimal by an order of magnitude or more), it begins discharging also on the lateral and basal domains of the plasmalemma. More concisely, the immediate environment per se may play a role (the local differentiations of the plasmalemma are related to it), but the most important factor in « traffic regulation » appears to be specific membrane recognition in the interactions of all types of membrane, plasmalemma included.

PATERSON

I wonder if as a non-biologist you could tell me how this membrane transfer process will affect for example the half-life of the cell, the growth of the cell.

PALADE

The data considered in my presentation concern cells in the stationary phase of growth. The time interval between mitoses is known in the case of the liver cell. It is of ~150 to 200 days. The turnover rates of membrane proteins have been established for the membrane of the endoplasmic reticulum and for the plasmalemma. There are no corresponding data for Golgi membranes. The average half life is in both known cases of about 2 days, but the turnover is asynchronous even for proteins involved in a common chain of enzymatic reactions. In the multienzyme system involved in fatty acid desaturation cytochrome b_5 has a half life longer than 2 days; for the corresponding reductase (NADH-cyt b_5 reductase) the half life is shorter than 2 days, and for the associated fatty acid desaturase only a few hours. This type of data suggests that membrane proteins are inserted *individually* into preexisting membrane. Now, you asked something about metabolism. Would you be so kind as to repeat it?

PATERSON

I wondered if such transport concerned the nutritional requirements or excretion from the cells, since you are taking vacuoles from one situation to another as a surplus.

PALADE

So far our experiments have been carried out under standardized nutritional conditions; hence, we do not know whether the

events described would be affected or not by variations in the metabolic condition of the cell. We know only that metabolic energy is necessary for intracellular transport and discharge.

The secretory products of these cells are proteins, most of them enzymes, highly valuable for the rest of the organism. They can not be considered an excretory product (in the usual sense of « waste product »). In a very general way, they can be considered « surplus » by the cell. If they accumulate in large amounts during intracellular storage, prior to discharge, they may cause an increase in the size of the cells, which will last until the next discharge.

As far as we know, the phenomena described are not part of either the growth of the cell or of the turnover of its components. We are looking at the functioning of a fully differentiated cell that has stopped growing and we cannot detect structural expressions of turnover. We are recording changes in position (distribution) of subcellular components. Simplistically, it is like looking at the movements of the parts of a working engine.

DE DUVE

As Drs. PALADE and STOCKENIUS have mentioned, membranes are believed to have a fluid structure which allows diffusion of constituents within the plane of the membrane except in such cases where this movement is prevented by some rigid structure or lattice. On the other hand there is increasing suspicion that a number of membrane proteins may be synthesized by free ribosomes, from which they somehow find their way into the appropriate membrane. Conceivably, therefore, exchanges could take place continuously between membranes and cytosol. In this event, when a piece of membrane A fuses with membrane B, is it not possible that its proteins start diffusing into membrane B from which they would then be ejected into the cytosol due to lack of appropriate anchoring points? From the cytosol they would eventually come back to membrane A.

PALADE

Well, since this is a complex and difficult question I'll try to answer it in instalments. To begin with, it has been established beyond reasonable doubt, in a number of cases, that there is lateral diffusion of proteins in the membrane plane. Perhaps the most relevant results (for our discussion) are those publisbed by STRITTMATTER which show that exogenous excess protein (cytochrome b_5) can be « inserted » or incorporated in microsomal membranes *in vitro* and that the added protein is functional and — as a corollary — mobile in the plane of the membrane (all cytochrome molecules have access to the indigenous reductase molecules of the membrane). These results show therefore that there are relatively large areas of unoccupied lipid bilayer in microsomal membranes at least, after isolation. If we assume that the same situation applies in the intact cell, it becomes difficult to rationalise the rejection of « invading » molecules presumably because they do not fit the « slots » available in the membrane. So much free bilayer surface seems to be available that the « invaders » should have no difficulty in populating it — irrespective of fit or no fit with the proteins already in residence in the membrane. Since mixing of components is not detected between interacting membranes (notwithstanding the fluidity and incomplete occupancy of the lipid bilayer), the simplest way out of this contradictory situation is to postulate the existence of a « frozen » domain between the two membranes. This domain (« frozen » by strong interactions among its proteins) could be represented by the membrane containers plying between the compartments mentioned. Dr. BAKER suggested earlier in the discussion a similar solution for this problem.

Coming now to possible interactions among cell sol proteins and membrane proteins, the idea of an equilibrium between a soluble and an insoluble form for membrane proteins was advanced a few years ago to explain the asynchrony of turnover. At present we are interested, however, in another — at least in part different — type of interaction. We are obliged to postulate the existence

of soluble precursors for the proteins of those membranes which do not have attached polysomes. Moreover, there is recent evidence obtained by LODISH and his collaborators, which indicates that such precursors are synthetised in reticulocytes by free polysomes, and are present in soluble form in the cell sol before appearing in the membrane. For one of these proteins the soluble precursor is longer (by about 30 amino acid residues) than the corresponding membrane protein. Partial proteolysis is apparently concomittant with insertion in the membrane. The removed polypeptide segment could be the « signal » or « code » for insertion in a specific membrane and proteolysis could convert a soluble component into an insoluble membrane bound molecule. In this situation, the simple equilibrium mentioned would no longer apply. Perhaps every protein destined for a certain membrane carries its « address » in a removable segment. Perhaps its receptor in the membrane is the protein (enzyme) responsible for its partial proteolysis and insolubilization. If this were the case then recognition would precede insertion and may no longer apply after the latter. Hence, the idea of resident proteins recognizing and rejecting « foreign » proteins may not explain the situation.

RITCHIE

How many protein molecules are there in the membrane in relation to the number of phospholiped molecules; and what is their absolute number expressed per square micron of membrane?

PALADE

Yes, there are data available in literature covering this point, but I couldn't quote them right now. If I remember correctly, there are in the red blooc cell membrane about 700 phospholipid

molecules per protein molecule. Density of protein molecules per unit area could be calculated from available data, but they should be interpreted with two main consideration in mind: 1) multiplicity of protein species in the membrane and; 2) asymmetry of their distribution.

III

METHODS OF MEMBRANE STUDY

ELECTRICAL METHODS IN THE STUDY OF BIOLOGICAL MEMBRANES

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Since I can probably claim to have processed with my own fair hands a greater number of samples of radioactive sodium and potassium than anyone else present here, you may be surprised to find me in the role of advocate for electrical methods. However, one must not forget in studying the ionic permeability of membranes that the driving force for the movement of ions derives both from the electrical potential difference across the membrane and from the chemical activity gradient, so that a knowledge of the magnitude of the transmembrane potentials is essential even in tissues not generally considered to be electrically "active". When, moreover, ionic fluxes have to be measured, electrical methods often offer considerable advantages over either flame photometry or radioactive tracer techniques. In the experiments on gating currents that I shall discuss in a moment, there is no great difficulty in measuring not only the absolute magnitude of a transfer of 3×10^{-9} Coulombs across the membrane but also the details of its time course. This quantity of charge would be carried by 3×10^{-14} moles of Na^+, and so would represent less than one hundredth of the net entry of Na^+ during the passage of a single impulse along 4 cm of a squid giant axon. It would

not be easy to make direct measurements of the ion movements in even 100 impulses, let alone to learn anything about the time course of each. Both in absolute sensitivity and in time resolution, electrical methods are generally superior to chemical ones by several orders of magnitude. The one disadvantage of electrical methods is their lack of specificity, for there are sometimes several candidates for the honour of transporting the measured current. In conducting experiments on nerve membranes, the difficulty can be circumvented with the aid of high-affinity blocking agents like tetrodotoxin, but in working with secretory epithelia, measurements of shortcircuit current can sometimes be dangerously ambiguous.

By far the most powerful method for studying the electrical properties of cell membranes is the voltage-clamp technique, first applied by HODGKIN, HUXLEY and KATZ (1952) in their classical analysis of the current-voltage relations in the squid giant axon. It consists in measuring the flow of current through a prescribed area of membrane when the potential is changed to a new level, usually in a stepwise fashion, a feedback amplifier being used to regulate the membrane current so as to clamp the membrane potential at precisely the commanded level or sequence of levels. Conversely, though less usefully, the membrane current can be clamped to pursue a pre-determined time course, and the corresponding changes in membrane potential can be recorded. From their voltage-clamp records, HODGKIN and HUXLEY (1952) arrived at their well known description of the changes in the sodium conductance of the nerve membrane during excitation in terms of three equations relating g_{Na} to time and voltage, which are

$$g_{Na} = \bar{g}_{Na} m^3 h, \tag{1}$$

$$\frac{dm}{dt} = \alpha_m (1 - m) - \beta_m m, \tag{2}$$

$$\frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h, \tag{3}$$

where $\bar{g}_{Na}$ is a constant representing the peak sodium conductance per unit area, m and h are dimensionless and vary between 0 and 1, and the α's and β's are transfer rate constants which vary with voltage but not with time. Before considering the implications of these equations for the mechanism by which the sodium conductance of excitable membranes is controlled, I should point out that the application of the voltage-clamp technique has not heen confined exclusively to nerve and muscle, since USSING and ZERAHN'S (1951) method of measuring the short-circuit current in frog skin and equating it with the net flux of Na^+, which has since been applied to many other secretory epithelia, is in effect a procedure in which the trans-epithelial potential is simply clamped at zero. I feel, indeed, that voltage-clamping could profitably be applied more extensively than it has been so far to examine the electrical properties of such epithelia, especially if, as seems possible from the recent work of HILL (1975), the coupling between salt and water movements turns out to be electro-osmotic at membrane level.

The HODGKIN-HUXLEY equations are simply a mathematical description of the time and voltage-dependence of the sodium and potassium conductances, and as such do not throw direct light on the nature of the physical processes involved. So many different mechanisms can, in fact, be described by two first order equations like (2) and (3) that there are an embarrassingly large number of models that might display HODGKIN-HUXLEY kinetics. One implication is, however, common to all of them. The existence of a voltage-dependent gating mechanism means that the opening of the gate must involve the passage of electric current, whose flow necessarily precedes the movement of ions through the open channel. Early attempts by HODGKIN and HUXLEY (1952) and CHANDLER and MEVES (1965) to measure the " gating current ", as it has come to be called, were unsuccessful, but with the availability of tetrodotoxin (TTX) to block the sodium current, it has now been recorded in the squid giant axon, first by ARMSTRONG

and BEZANILLA (1973) and shortly afterwards by KEYNES and ROJAS (1973), and also at the node of Ranvier (NONNER, ROJAS and STÄMPFLI, 1975).

The basic phenomenon is illustrated in Fig. 1, which shows single-sweep records of the displacement current flowing on application of ± 120 mV voltage-clamp pulses to a squid axon in which the sodium current was abolished by treatment with TTX and removal of external sodium, and the potassium current was blocked by perfusion with caesium. At the start of the hyperpolarizing pulse, the capacity transient is seen decaying with a time constant of the order of 10 μsec to a small but constant inward leakage current. When, however, a depolarizing pulse of the same size is applied, the trace is not a symmetrical mirror-image, but has an obvious slow tail coming after the fast transient. Graphical addition of the two records to eliminate the symmetrical capacity current reveals that the asymmetrical component pursues an exponential time course with a single time constant of about 250 μsec. This asymmetrical displacement current, rising sharply and declining exponentially, is the gating current. When the elimination of the capacity transient was carried out with the aid of a signal averager, we obtained families of records like that seen in Fig. 2, which shows how the gating current time constant varied with pulse size, decreasing progressively as the potential during the pulse became more positive. In this axon, as was often the case, there was some rectification of the leakage current during the pulse, which resulted in the superimposition of a rectangular pedestal on the exponential tails.

There is no doubt that for short pulses the asymmetrical component of the displacement current originates from the movement of mobile charged particles or dipoles that constitute an integral part of the membrane, because (1) the total transfers of charge in each direction at the start and finish of the pulses are equal, that is $Q_{on} = Q_{off}$; (2) the charge displacement reaches a definite ceiling (Q_∞) for large pulses; (3) Q_∞ is the same at all temperatures, although the time constants

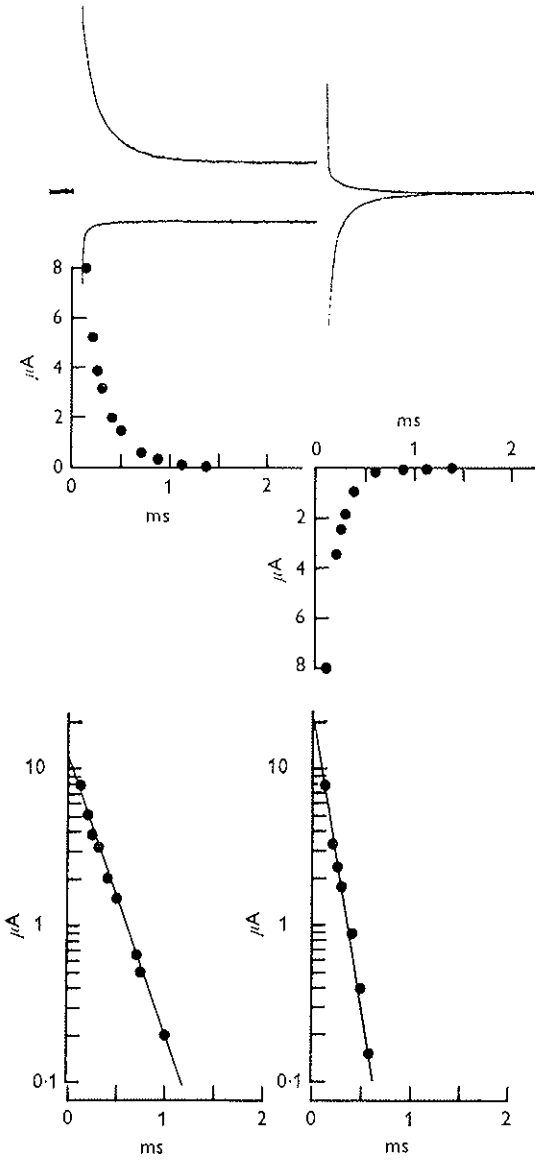

FIG. 1 — Asymmetry of the displacement current on application of equal and apposite voltage-clamp pulses to a squid axon perfused with 300mM-CsF and bathed in Na-and K-free saline containing 1000nM-tetrodotoxin. The top traces are single-sweep records of the membrane current for ±120 mV pulses. The difference between them is plotted beneath on linear and logarithmic scales. (Fig. 4*B* from KEYNES and ROJAS 1974).

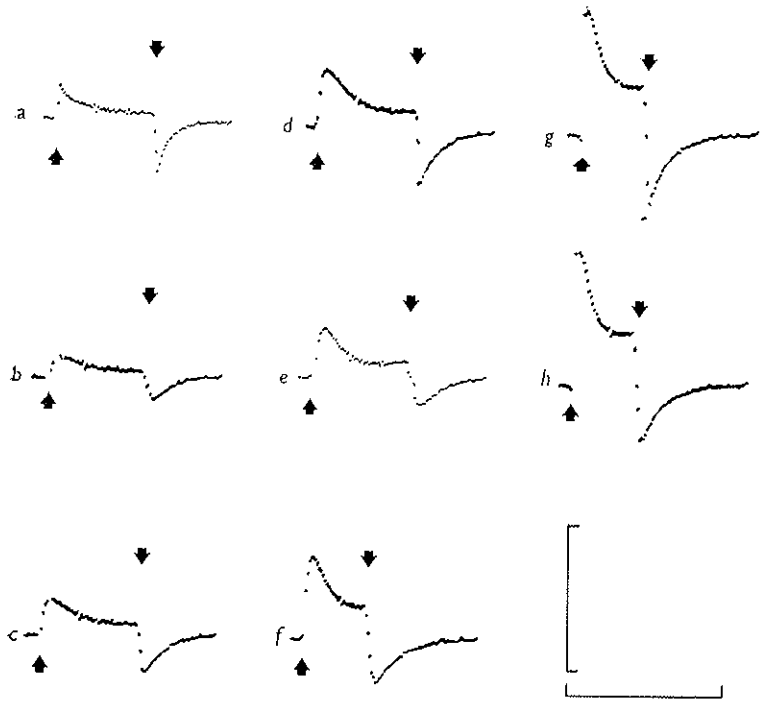

FIG. 2 — A family of gating current records obtained by summation with a signal averager of the membrane currents for 60 positive and 60 negative pulses which started and finished at the arrows. The axon was perfused with 55mM-CsF, and bathed in Na-and K-free saline containing 300nM-saxitoxin: pulse amplitude, *a-h*, 40-110 mV; holding potential —70 mV; vertical bar 5.56 μA, horizontal bar 2500 μs; membrane area 0,06 cm²; temperature 7 °C (Fig. 6 from KEYNES and ROJAS 1974).

have a Q_{10} of 3 (KEYNES and ROJAS, 1974). When the pulse duration is increased, there are indications that some of the mobile charges may undergo conformational changes that appreciably increase their relaxation time, giving rise to an apparent reduction in the ratio Q_{off}/Q_{on}. Such changes could possibly be related to the inactivation of the sodium channels, and to the complications that arise when the holding potential is lowered, but the experimental evidence on this point is still fragmentary.

There also seems to me to be rather little doubt that, at least with the membrane potential held at − 80 mV or beyond, the charged particles whose mobility is responsible for generating the asymmetrical displacement current can be positively identified with the previously hypothetical particles that control the opening and closing of the sodium channels. For when the gating current time constant τ_{Ig} is determined as a function of membrane potential, it is found that the values lie on a bell-shaped curve with a maximum of a little under 500 μsec at 6°C that falls at a potential of − 36 mV (KEYNES and ROJAS, 1976). Independent measurements of the sodium conductance time constant τ_m, which equals $\frac{1}{\alpha_m + \beta_m}$ where α_m and β_m are the rate constants of eqn. (2), yield values that lie on an almost identical curve. If there were no connexion between the two parameters, it would be rather unlikely that they would fortuitously have precisely the same absolute magnitude and voltage dependence. Again, the midpoints of the curves for the steady-state distribution of the mobile charges and for m_∞ (calculated according to eqn. (1) as $\sqrt[3]{g_{Na}/\bar{g}_{Na}}$) as a function of potential are within a few mV of one another (KEYNES and ROJAS, 1976). Fresh and independent support for the concept that there are 3 gating particles for each channel, all of which have to be in the active configuration before the channel can open, which rested originally on the observation

of HODGKIN and HUXLEY (1952) that the time course of activation of the sodium conductance fitted the expression

$$I = I_\infty \ (1 - e^{-t/\tau_m})^a \qquad (4)$$

better with a = 3 than 2 or 4, now comes from the fact that an e-fold change in the charge distribution is brought about by 19 mV shift in the membrane potential, whereas an e-fold change in the sodium conductance needs a shift exactly one third as great (KEYNES and ROJAS, 1976). We have shown (KEYNES and ROJAS, 1974; 1976; ROJAS and KEYNES, 1975) that the steady-state charge distribution curve fits well with a simple relation derived from Boltzmann's Law for particles with an effective valency of 1·3 distributed between two alternative energy states. Since the total quantity of mobile charge Q_∞ is about $3 \times 10^{8-}$ C/cm^2 membrane, the assumption that there are 3 such particles per channel leads to an estimate of the density of channels as 470 μm^{-2}, in good agreement with measurements of the number of TTX binding sites (LEVINSON and MEVES, 1975; KEYNES, BEZANILLA, ROJAS and TAYLOR, 1975).

Although this quantitative examination of the data thus far supports the identification of the mobile charges as the sodium gating particles, there is one respect in which my argument needs slight amplification. If eqn. (1) is strictly obeyed, then the time constant for turning off the sodium conductance at the end of a brief depolarizing pulse, $\tau_{Na,\,off}$, should be exactly one third of τ_m and hence also of $\tau_{Ig,\,off}$. It has, however, been reported both by ARMSTRONG and BEZANILLA (1974) and ourselves (KEYNES and ROJAS, 1974) that this expectation does not hold good, the conductance apparently being appreciably slower to turn off than it should be. A further difficulty in this connexion is to account for the tendency noted by KEYNES and ROJAS (1974) and MEVES (1974) for $\tau_{Ig,\,off}$ to increase with pulse size, instead of being determined purely by the holding potential. Both problems have been resolved,

for it has been demonstrated (KEYNES and ROJAS, 1976) that provided special care is taken to avoid errors from incomplete compensation for the Schwann cell series resistance, $\tau_{Na,\,off}$ is actually close to $\frac{1}{3}\,\tau_{Ig,\,off}$ after a large pulse. The dependence of $\tau_{Ig,\,off}$ on pulse size can, moreover, be explained if there are two successive steps in the opening of a sodium channel, the first being the movement of the gating particles in the electric field from the resting to the active state, and the second being an interaction between them that creates the pathway for the passage of Na^+ ions. On the plausible supposition that this interaction involves a conformational change within the particles that increases the relaxation time for their restoration to the resting state on repolarization of the membrane, it follows that after a large pulse leading to an interaction of the majority of the particles $\tau_{Ig,off}$ will be greater than after a small pulse during which few of the channels have been opened. It further follows that $\tau_{Na,off}$ should be independent of pulse size at a given holding potential, as in fact it is observed to be (KEYNES and ROJAS, 1976).

This relatively straightforward interpretation of the behaviour of the asymmetrical displacement current in the squid giant axon applies only to experiments conducted with the membrane held at a large negative potential. When the holding potential is lowered, there appear to be alterations in the properties of the gating particles that are not yet well enough characterized experimentally to justify detailed discussion. The most obvious change is a shift of up to 20 mV towards a more negative potential of the midpoint of the steady-state charge distribution curve which results in the apparent inversion of the gating current records at a holding potential of about − 50 mV that has been reported by KEYNES, ROJAS and RUDY (1974) and MEVES (1974). There are also slow changes in the total amount of charge transferred (MEVES, 1974), which may be caused by immobilization of a certain proportion of the

particles. It is my belief that these effects are related somehow to the processes involved in the inactivation of the sodium channels, that is to say in their inexorable closure after they have been opened by depolarization of the membrane. This is an important area for further investigation.

It may seem from what I have said so far that the gating current studies have done little more than confirm what was already known from HODGKIN and HUXLEY's (1952) analysis of ionic conductance kinetics. This would be a wrong impression, for in fact these new results enable a choice to be made on lines not previously open to us between different classes of molecular model for the ionic channels. In discussing their implications for the molecular structure of the sodium channels, a limitation of the evidence must first be emphasized. As far as the individual gating particles are concerned, we know that their effective valency is 1·3, this being the product of the actual charge that they carry and the fraction of the total electric field through which they move; but there are many ways in which it might be achieved. Thus a globular dipole carrying two charges and rotating through 180° at 45° to the plane of the membrane would have an effective valency of $2 \sin 45 = 1 \cdot 3$. Equally, a helical structure carrying, say, 10 singly charged groups disposed appropriately along its length that each moved through about 1/8 of the field would have the same effective valency. Such a structure might be envisaged as operating concertina-fashion to open and close the gate, or it might twist along its own track. It is at present a matter of taste, and perhaps of a knowledge of the kinds of conformational change that are most likely to take place within a protein, to decide between these alternatives. Nevertheless, we can now say with some confidence that each channel in the squid giant axon must incorporate just three identical gating particles, or two at the node of Ranvier. Possibly the most important conclusion from our experiments is that it is unnecessary to postulate the existence of a separate and independent "h" particle. It can readily be calculated from

HODGKIN and HUXLEY's (1952) data for the voltage-dependence of τ_h and h_∞ that if inactivation involved the movement of another type of charged particle, the corresponding " h " gating current, although smaller and slower than the " m " current, should be readily detectable. In fact, no component of the gating current displaying the kinetics of the " h " process has yet been seen. This suggests rather strongly that inactivation involves a time- but not voltage-dependent conformational change in the gating particle triads, rather than the delayed movement of a blocking particle in the electric field. It has been shown by GOLDMAN (1975) that there is no incompatibility between Hodgkin-Huxley kinetics and the behaviour of a three-state system of this general kind. The proposition that the sequence: — channel resting/closed — channel conducting — channel inactivated/blocked, arises from the transition of three gating particles through a succession of different states poses some awkward questions over the voltage-dependence of the final step, as does the complicated behaviour of the system when the holding potential is lowered. The chief importance of being able to record the gating currents is, however, precisely that they provide a considerable restraint on model-building, and it is the very existence of these difficulties that offers hope that when they are understood, we shall also understand much better how the sodium channel operates.

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DISCUSSION

Chairman: Prof. A. M. MONNIER

RITCHIE

If you have the *m* and *h* particles arranged in the way shown on your second last slide, I can see that one would get displacement currents when their position is altered. If, however, they were rotating at right angles one would not expect a displacement current. And if the *h* particle was rotating at right angles to the axis of rotation of the *m* particles, it would still do its gating but would not be associated with a displacement current. Could this be an explanation for the lack of an *h* gating current?

KEYNES

No. While it is true that charges moving laterally in the membrane could affect the gate, such a model would not display the observed voltage sensitivity. One cannot evade the fact that the operation of a potential-dependent system must necessarily give rise to a gating current.

HASSELBACH

Can you make any estimate about the size of the charged particles, assuming that they rotate in the membrane?

KEYNES

If the rotating dipole model were correct, the particles would have the same diameter as the width of the low dialectric part of the membrane, that is about 25 Å. There is also some evidence as to the molecular weight of the whole structure because LEVINSON and ELLORY have recently been doing radiation inactivation analysis using tetrodotoxin binding as a criterion of the intactness of the particles, and their answer is 230,000. Whether that represents the molecular weight of the whole sodium channel it is a bit hard to say, because since, as we have seen, the filter part of the mechanism is functionally to some degree independent of the gating part of the channel, one cannot be sure that knocking out the gating part of the molecule would actually affect the TTX binding. The interpretation of this 230,000 is therefore slightly in doubt.

HASSELBACH

What is the evidence for assuming that the tetrodotoxin binding particle is different from the channel particle?

KEYNES

We normally conduct all the experiments in the presence of tetrodotoxin, but you can, when you know what you are looking for, exploit the delays in the initial rises of the ionic currents to make measurements of the gating currents without having first stopped up the channels with tetrodotoxin. And it turns out that the gating currents in the absence of tetrodotoxin are very similar to the gating currents in the presence of tetrodotoxin.

PALADE

What is known about the ionic properties of the particles that

are generating the gating current? What is the ionic species that carries the gating current?

KEYNES

That is the 64,000 dollar question. It is preumably a protein, and as I have said it has a molecular weight which could be 230,000. It has got a highly complicated internal structure, because it has the three voltage-sensitive subunits, in addition to the selectivity filter. I imagine that in due course it will be possible to solubilise the sodium channels as has been done by HENDERSON and others, and it might then be possible to determine the aminoacid sequence. If you found three regions which had the same sequence, they might be the three subunits. But we are a very long way from being able to do that yet.

KEDEM

What is your reaction to the current work on the acetyl-choline receptor and its role in the mechanism of conductance? These lines seem to run in parallel without touching. What is your view in the enzymatic destruction of acetyl-choline as the mode of inactivation?

KEYNES

Well there is actually an enzyme which destroys the inactivation, for it was shown by ROJAS and ARMSTRONG that pronase injected into or perfused into a squid axon knocks out the inactivation process without affecting activation. It does not have an obvious effect on the gating currents, but I am sure it must have some effect, analysis of which would be extremely interesting. I think that Prof. ROJAS feels that the pronase affect provides a strong argument

in favour of the existence of an independent *h* particle, since one could assume that the pronase acted by breaking up the *h* particle but not the *m* particles. But you can equally well say that what the pronase is doing is chopping off some side groups in the proteins, and so simply blocking the step from B* to C in my multi-state mechanism. I think, in connection with your other comments about parallels between field jump effects and gating currents, that we are probably going to hear some more about analogies with ionophores from Dr. MUELLER later on.

BOYER

I would like to return to the question of the motion of the protein particles. Does your model necessarily mean that the particles must move? There are other alternatives arising from knowledge of protein chemistry that might be considered. Would your observations be satisfied if the pK of key groups changed to control the gate? As you know, for example, a change in protein conformation could change the pK of lysine residues so that they would accept protons from water and give fixed changes where they did not previously exist. This would not demand the rotation of the proton or similar large movement. Do your physical observations mean that you must have charge movement or just appearance of a charge?

KEYNES

You must have a transfer of charge, but I would have thought that a shift in pK could meet the requirement. There must be an effective transfer of charge across the membrane, by whatever means.

BOYER

Such events would not demand as much motion as that of a subunit rotating around.

KEYNES

Yes. I do not personally like the model which involves proteins completely rotating.

BOYER

Then it might be possible that the gate current results from conformationally linked changes in pK values for key groups, with the small movement required to expose the groups to an appropriate aqueous channel.

KEYNES

I think that kind of mechanism could fit.

LIQUORI

I suppose that your helix needs not be an α-helix, it might be a very short helix made of globular particles, like polyactin or tobacco mosaic virus.

KEYNES

Yes, I suppose so.

LIQUORI

This seems realistic and in line with what one thinks about the reversal of orientation which might correspond to a reversal of screw sense.

KEYNES

I am sure that there are all sorts of possibilities along the lines that you are suggesting, and I don't think we can discriminate between them as yet. I think I am going to find it very difficult to introduce a model that will satisfy everybody, for everyone seems to have different ideas as to what is plausible. In any case I can't draw any of them.

HASSELBACH

Is there any influence of aconitine on the relaxation times?

KEYNES

We haven't really got very far with studying any drug effects, although what seems certain to come out of these studies is more information about the action of local anaesthetics. We have made some preliminary observations on procaine, but there are all sorts of other drugs which should have very interesting effects that we haven't yet had a chance to look at.

RITCHIE

I wonder if you would be prepared to modify slightly your opening statement that electrical measurements have the edge over chemical measurements all the time? It would certainly be true with squid giant fibres, as you said. But small fibres, like the pike or the garfish olfactory non-myelinated fibres, because they have a much larger area of axonal membrane, are exceptionally suitable for TTX binding studies, for example, whereas electrical experiments in these fibres are extremely difficult to do. In garfish olfactory

fibres, for example, it has been easy to measure the potassium fluxes in a single impulse (RITCHIE and STRAUB, 1975).

KEYNES

Yes, I would accept that emendation, for as Professor VON MURALT has shown, you can actually get better action potential records in a pike olfactory nerve by using an optical recording technique than you can with electrical equipment.

A PRELIMINARY REPORT ON THE EFFECT OF CURARE AND CURARE LIKE AGENTS ON THE DIFFRACTION OF A COHERENT BEAM OF VISIBLE LIGHT BY THE ISOLATED ELECTROPLATE

CARLOS CHAGAS (*) - MARIA APPARECIDA ESQUIBEL (*)
MAURICE FRANÇON (**)

Introduction

Due to phenomena of diffraction information can be gathered when under appropriate conditions a beam of light passes through a translucid object.

One may consider two types of diffraction phenomenona: Fresnel and Fraunhofer diffractions. Fresnel diffraction describes the case when light distribution is observed on a screer at finite distance. A relation between the object structure and the amplitude distribution on the screen is given by *Fresnel Transform.* Fraunhofer diffraction describes the case when light distribution is observed at infinite distance (i.e. in the focal plane of a lens, also called Fourier plane). A relation be-

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tween the object structure and the amplitude distribution in the lens focal plane is given by a *Fourier transform.*

We will now consider only the second type. Figure 1 shows schematic setup for Fraunhofer diffraction observation. A beam of parallel rays of monochromatic light, goes through a D diaphragm that limits the useful region of the object. This one is represented by the shaded region. The O lens forms in its focal plane H, the diffraction phenomenon of the object, limitated by diaphragm D. Let us suppose now that there is no object. One may then observe in the H plane, the diffraction phenomenon of the diaphragm D. When D is a circular aperture, one knows that the diffraction pattern is a very bright central circular spot surrounded by slightly bright ring whose intensity decrease when going away from the center. Those bright rings are separated each other by dark ones. If A_0 is the radius of the diaphragm (D) aperture and λ the light wavelenght, the angular radius V of the first dark ring is:

$$V = \frac{1{,}22\ \lambda}{2\ A_0}$$

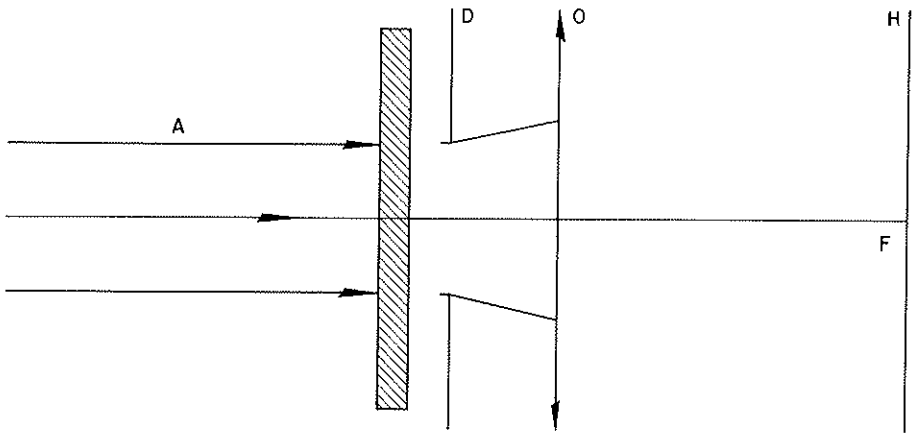

FIG. 1

This radius is called the central diffraction spot radius. Most of the energy is concentrated into this central spot.

Let us now place a transparent object before diaphragm D. This object may be caracterised by thickness variations, index variations or both of them. Those variation act as small elements diffracting incident light. When those elements have very small lateral size, the formula shows they diffract within wide angles: the diffracted light beam spreads in the H plane. When diffusion by the object is not too important, one may ohserve in F a bright central spots that practically represents the diffraction pattern of diaphragm D. Light diffracted by the object spreads around that narrow central spot.

When the lateral size of the elements increases, diffracted light illuminates a narrower region around focus F. As a matter of fact, the diffracting elements may have quite various shapes, and diffraction rings will vanish. One may only see a more or less wide spot, whose variations will anyway lead to interesting information on structure variations of the object.

Let us consider now an object constituted by randomly distributed fibers which are contracted under any way. In this case the diffracted light is spread out in the plane H as in the case of a lattice the mesh of which is restrained.

With these considerations in mind, this paper presents some preliminary results of the study of the diffraction of a coherent beam of light by the active surface of the electroplate of the Electrophorus electricus. This surface as shown by R. D. KEYNES and H. MARTINS-FERREIRA [1] is the one whose activation produced by stimulation of the nerves is responsible for the unit action potential. A rather complicated mechanism commanded by the central nervous system of the eel synchronizes the discharge of the extremely numerous unit electrogenic elements, the electroplate, thus allowing the appreciably high voltage discharge produced by the fish.

When stained for the detection of acetylcholinesterase and observed from its rear, the electroplate is seen as a spotty

surface where the elements containing the enzymes seem to cover a surface at least equal to the one occupied by nerve endings.

Experimental procedures

The isolated electroplate was obtained by the already classic technique first described by E. SCHOFFENIELS [2]. The structure was placed in a physiological solution of known composition [3] in an appropriate specially built translucid cell into which the drugs used (decamethonium and d-tubocurarine) were placed. A helium-neon laser of one milliwatt power was used. The optical system was constituted by a specially built lens whose main parameters are seen in Figure 2 (measures in mm.). The final arrangement is shown in Figure 3. C_1 is a quartz cell divided in two compartments by a teflon (D_1) membrane, in whose central part a small rectangular slit of 6mm × 0,5mm is cut out. Over this opening is fixed the electroplate thus rending the two compartments impervious. The beam of light is directed in such a way that it passes through the slit after traversing the electroplate and enters the diaphragm (D_2) of the optical system. In practical terms Fourier's plane corresponds to the surface of the film placed in the focal plane of the optical system used.

The diffraction images were analysed by a microdensimeter in a standard way in order that the results might be comparable.

For the experiments both compartments were filled with physiological solution to which were added in compartment C_2 amounts of d-tubocurarine chloride or decamethonium iodide, necessary to obtain concentrations known to produce complete block of the nervous transmission in the first instance and depolarisation in the second one.

The experiments were made placing the electroplate in such a way that the innervated membrane (posterior surface of

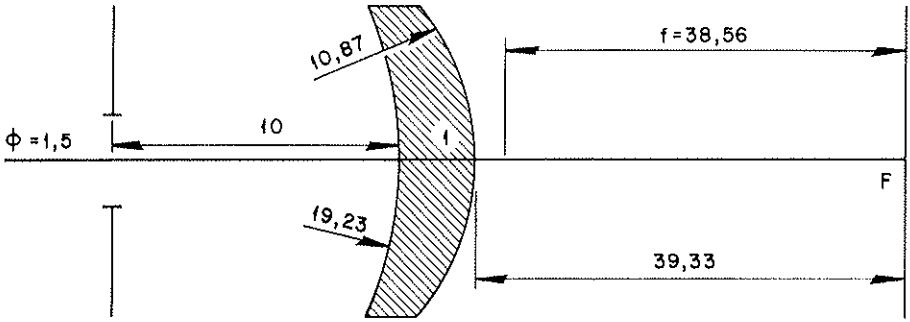

FIG. 2

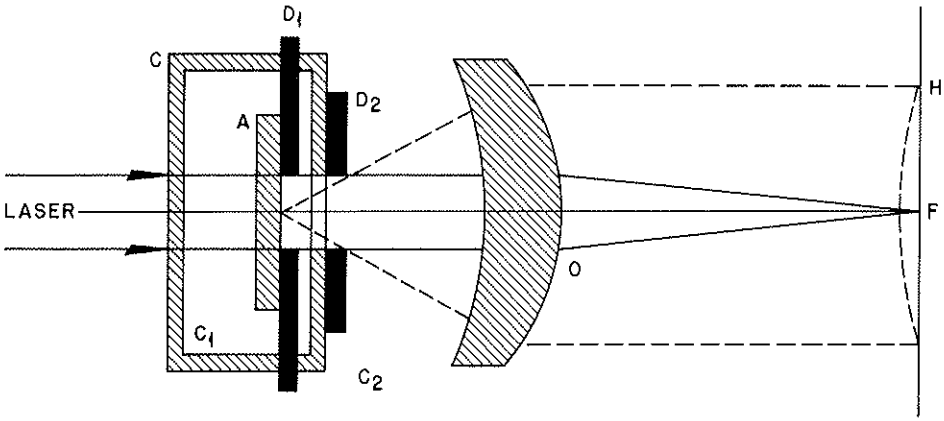

FIG. 3

the electroplate) faced the iris-diaphragm, or in the inverse position, wherein the interior surface was in its place. The connective tissue of the tissue hives where the electroplates are placed "in vivo", was also examined.

Results

The results of the action of decamethonium are presented in Figure 3. Each curve presents the average of a number of experiments, in ordinates is shown the intensity of light and in abscissae the distance to the center of the diffraction spot. The actual curves are not smooth but show continuous oscillations around the filled one. One of the diffraction pattern measured is seen in Figure 4. Curve N° 1 was obtained before the addition of decamethonium; curve N° 2 after 1 minute of the presence of the drug; curve N° 3, 15 minutes thereafter. These curves show that decamethonium reduces clearly the diffraction pattern thus showing an elargement of an hypothetical diffraction mesh.

This fact can be seen directly when one compares Figure 5 and Figure 6. The first photograph was taken after the action of decamethonium and the second before it. The elongation of the diffraction pattern of each granular component is due to the form of the slit, through which the laser beam passes. No changes were observed when the anterior surface of the electroplate was studied under the same time constants. When the DMDT was used, the opposite was observed. Results obtained are presented in Figure 7. Curve N° 1 corresponds to time zero of the experiment. N° 2 was taken from diffraction pattern obtained after 5 minutes of the introduction of the drug in compartment C_2. Curve N° 3, 10 minutes after the beginning of the experiment. Here again the curves are the average of numerous experiments.

One sees clearly that in this condition the diffraction spot spreads and thus the corresponding densitometric curve is

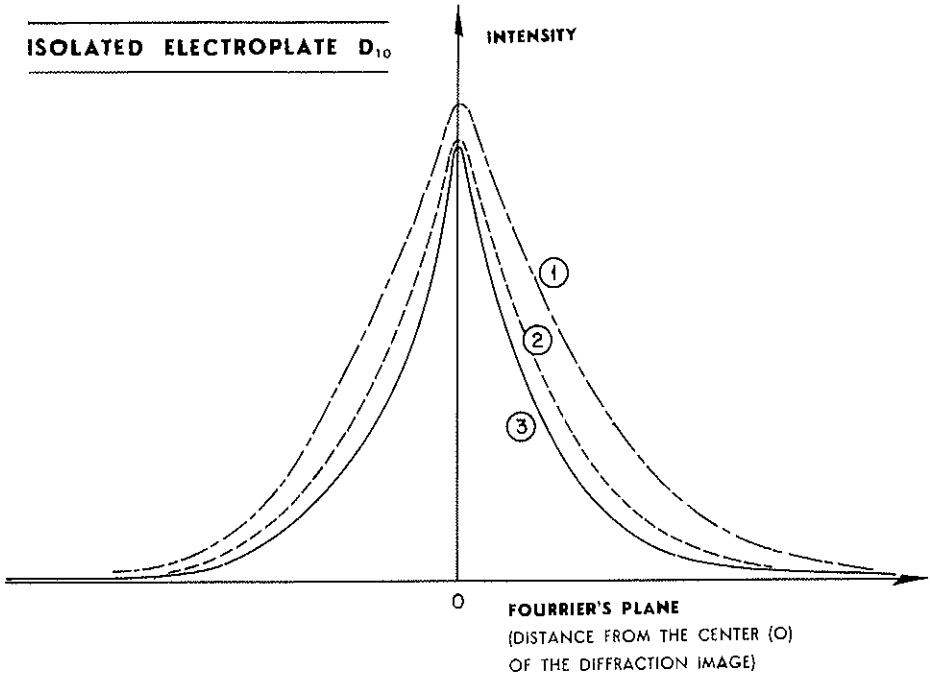

FIG. 4

enlarged, showing that the unknown diffraction mesh has been tightened by the drug.

No effect was detected when the connective tissue substituted for the electroplate.

Discussion

One may assume that the observed images are the result of purely diffraction phenomena as the absorption of light is negligible as determined by the surface measurements made.

The volume of tissue involved is very small. It must be of the order of less than 0.45 mm^3. This is a datum that needs

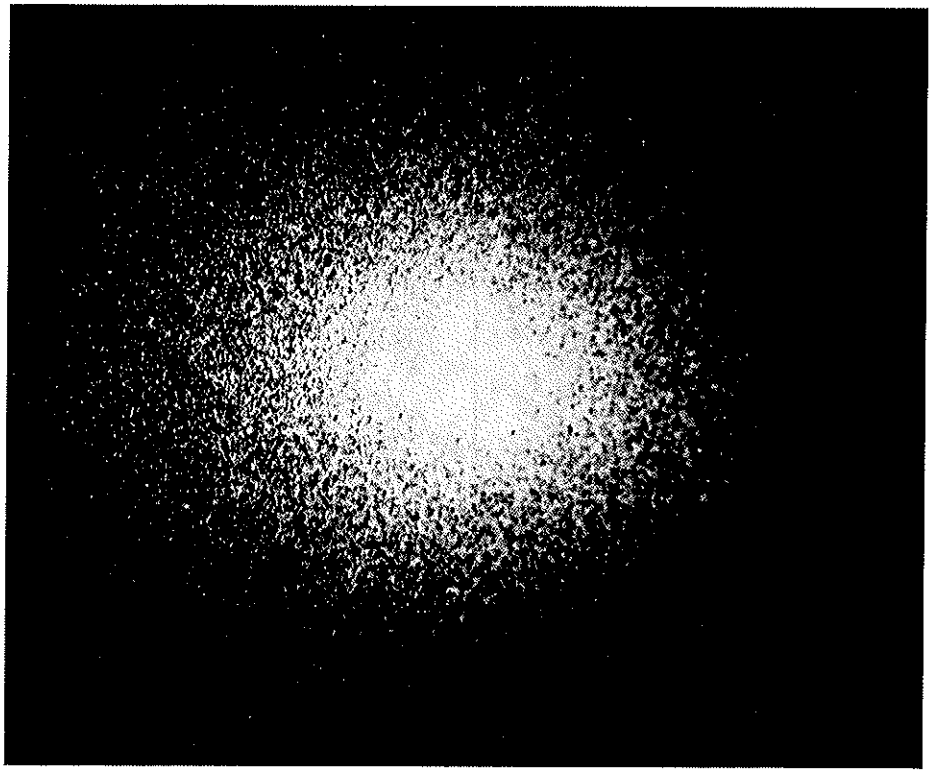

FIG. 5

further investigation. It must be pointed out, however, that the action of curare occurs only at the surface. Iodide of decamethonium acts primarily at the surface, but may diffuse to the interior to the electroplate. As a matter of fact it can pass through the electroplate as seen with radioactive samples, but with a rather low time constant. It is interesting to note that no effect was observed when the nonactive surface of the electroplate was studied. As a matter of fact this surface is covered hy a sheath of intercellular substance.

The electroplate posterior surface, is constituted by very

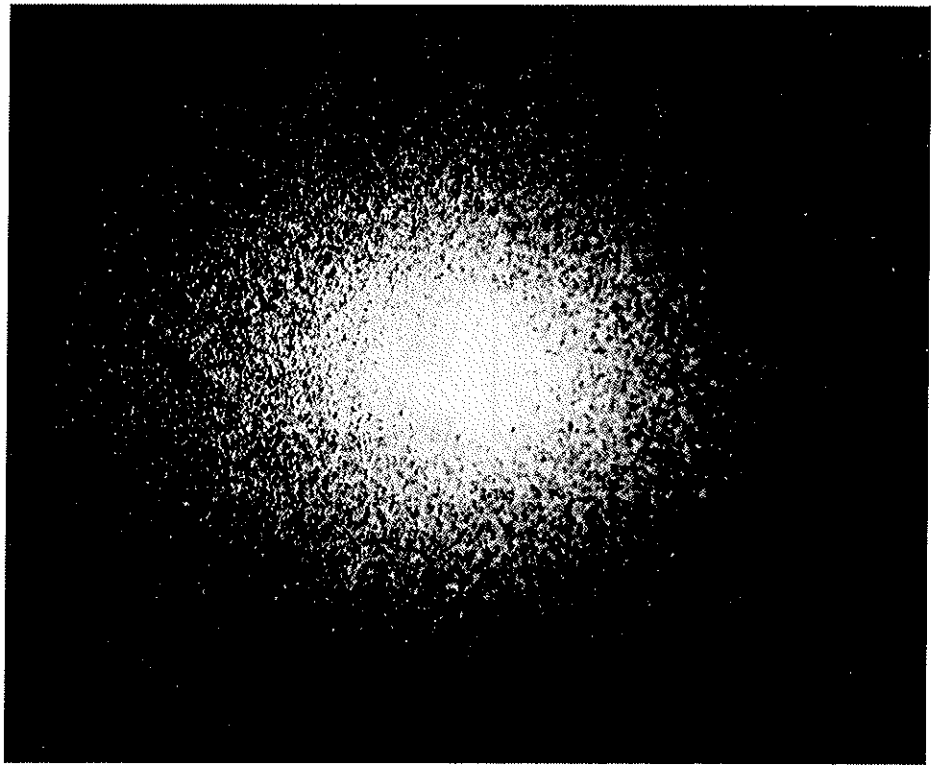

FIG. 6

abondant cholinergic synapes [4]. When viewed appropriately from the posterior-anterior direction after appropriate histochemical detection of acetyl-cholinesterase it presents a great number of dark speckles which correspond to the same synapses (Fig. 8 and Fig. 9). The surface is covered also by fine nerve fibers whose terminal endings are present at the synapses. The surface is not plane as it may be seen in a transversal sections. Its villosities are however less pronounced than the ones seen on the anterior surface. The presence of synapses is probably responsible for the granular aspect and some linear

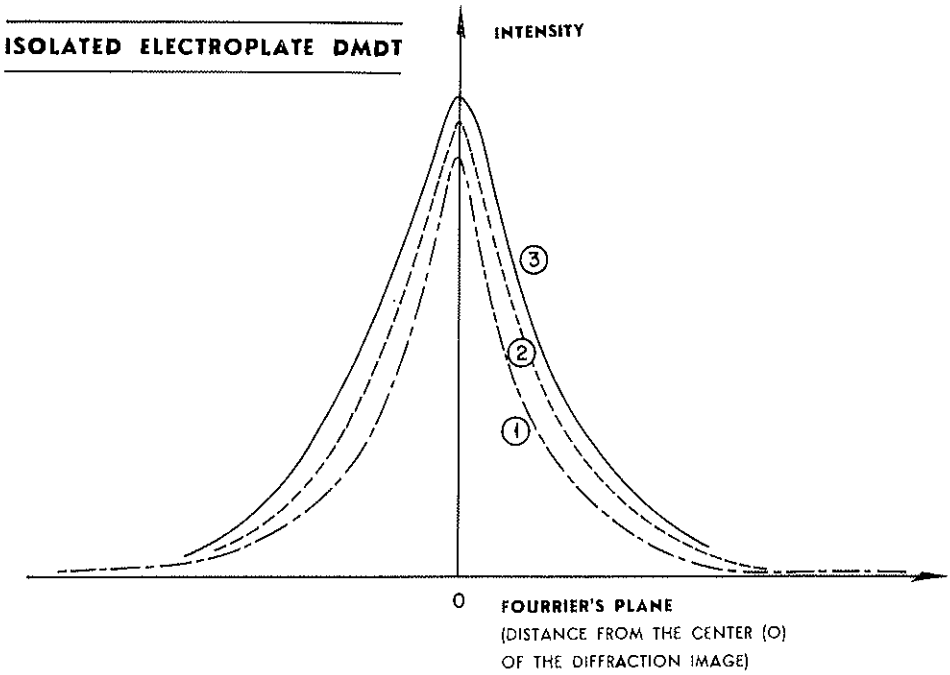

FIG. 7

arrangements of the emulsion grains of the diffraction pattern observed on the Fourier's plane.

It does not invalide the results herein presented because the curves are reproductible and represent average data from a population distributed at random.

The mentionned irregularities make it impossible, however, at the present time to speculate on the dimensions of the unit of diffraction mesh. The phenomena are however so net that they give rise to some conjectures. As a matter of fact we are observing in the visible microscopic level a projection of a phenomenon which occurs on the submicroscopical one.

One admits with some reason that the action of DMDT is a more stable one as consequence of more lasting complex

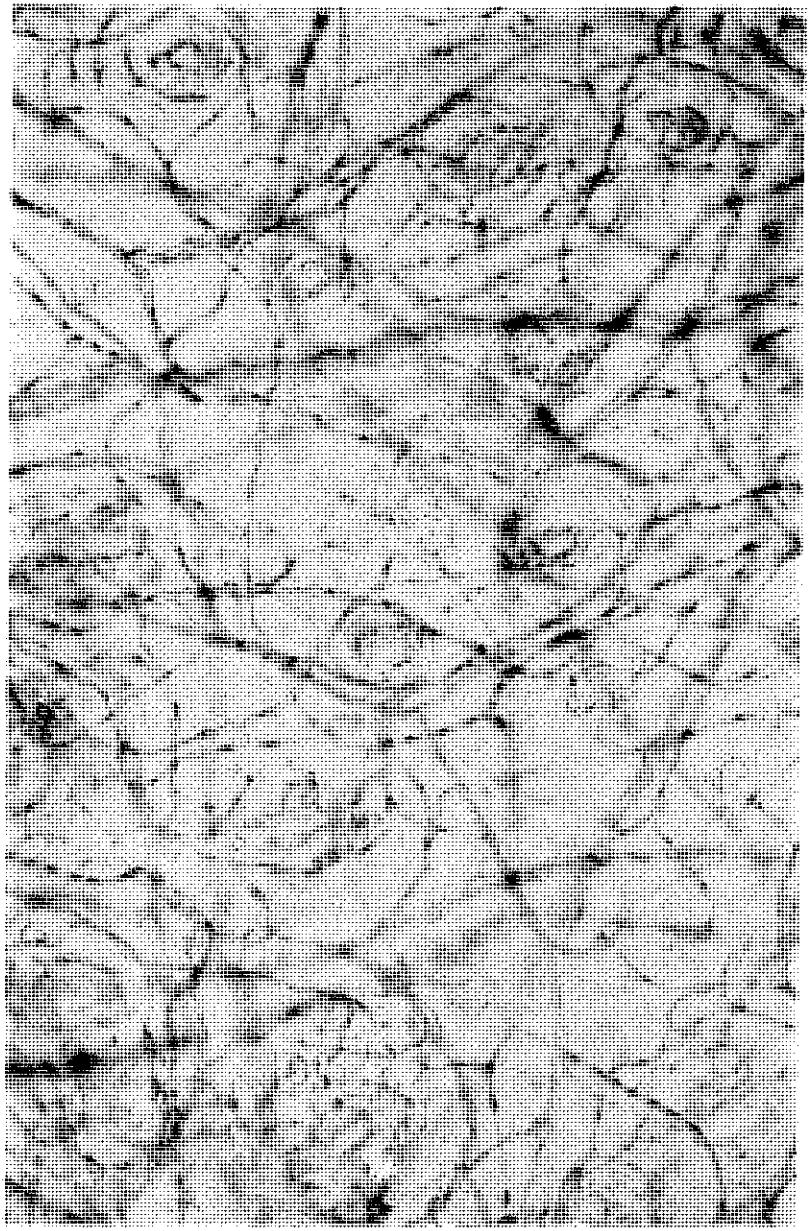

FIG. 8 — Isolated posterior face of the electroplate. Stained with methylene blue (≃ 1500 x). Few vessels. Numerous nerve fibers.

FIG. 9 — Isolated posterior face of the electroplate. Same region. Terminal ending of nerve fibers focused (≃ 1500 x).

formation between the drug and the cholinergic receptor and drug acceptors. For the decamethonium the mechanism of each action may be similar to the hit-and-run type, by which a molecular transconformation occurs. As a consequence the ionic flow in and out of the cell is facilitated. An increase of permeability is thus obtained.

Our results show first that light passes with less diffraction through the membrane when it is more permeable. This seems reasonable to admit. We are however far from admitting that we are observing an increase of the diameter of the "ionophores" but only a gross modification of molecular agglomerates, responsible for the permeability change.

On the other hand the spread of the diffraction pattern produced by DMDT implies a tightening of the hypothetical diffraction mesh. This may be considered an interesting finding, even if we are unable up to now, to give it an interpretation.

ACKNOWLEDGMENTS

This work was supported by W.H.O. (contract R/00436); National Bank for Economical Development (contract FUNTEC-241) and National Research Council, Brasil; Research Council of the Federal University of Rio de Janeiro; and Scientific Cooperation, Ministry of Foreign Affairs, France.

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DISCUSSION

Chairman: Prof. A. M. MONNIER

KEYNES

It wasn't quite clear whether you found that these changes were restricted to the innervated membranes?

CHAGAS

They are restricted only to the active membranes and they are not observed on the inactive membrances and on the collective tissue.

TEORELL

It was most interesting to hear about your revival of the diffraction method. As you mentioned in your presentation, it was used once for determination of changes of the red blood cells. I used it myself and I found that it basically showed volume changes. Now my question is, can possibly volume changes, swelling or shrinkage, or changes of hydration be involved? This leads, of course, to the thought of model experiments with swelling and shrinkage of, for example, gelatine with the use of the modern lasers.

CHAGAS

The technical improvement which made diffraction methods much easier was the use of lasers. Now one of the hypothesis which would explain the effect of curare, for instance, would be to imagine that its action is followed by dehydration and this would explain very easily what we are finding. I think the method used must be quite good for the observation of changes in volume and swellings and dehydration.

RITCHIE

In the skeletal muscle where decamethonium produces a depolarization block, this depolarization disappears with time (in the continued presence of the drug) but the block remains thougb the type of block is changed. So my question is: how stable are these patterns that you see? Is there any suggestion that tbe narrow decamethonium pattern turns after a time into the broader pattern characteristic of the curareform response?

CHAGAS

Well, if we wash the preparation and it comes back to its normal state of response, it assumes the normal pattern. It never goes to the other pattern.

RITCHIE

No, my question was: if you kept the decamethonium there (the condition in which the character of the block in skeletal muscle changes), is there any suggestion that the character of your response changes?

CHAGAS

No, we do not have this evidence.

OCHOA

May I ask a question even if I am not a member of the study group? I have seen the light diffraction method used on electron micrographs, for example, in the study of the structure of viruses, and I am wondering if this procedure would be applicable in studies like the one you have been doing?

CHAGAS

The technique involved becomes extremely more difficult, what we have is a very simple device which I hope, when we use the new shorter wave laser, will give much better information.

MONNIER

Your method would be very useful in the study of tissues, well suited for optical studies. The olfactory nerve of the pike, with one million of transparent fibres, might thus show optical changes during the precipitation phase.

CHAGAS

There must be an aggregation of elements on the submicroscopial level to produce something which is seen on a microscopial level but we are far from knowing what it is. The fact is that the observation of the phenomenon is quite clear but its interpretation and the creation of a model of it needs imagination.

PHASE-PLANE ANALYSIS OF PROPAGATED ELECTRICAL ACTIVITY IN MUSCLE CELLS (1)

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EDUARDO A. C. GARCIA (3) - TEOBALDO A. SALDEÑA (4)
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The study of ionic channel kinetics in excitable membranes can be performed with elegance and accuracy through voltage-clamp experiments, as shown by HODGKIN and HUXLEY (1952 a, b, c, d) fo rthe squid nerve. However, geometry characteristics of the cell under study often render voltage-clamp techniques difficult to apply. This is the case, for instance, of voltage-clamp studies in heart muscle, where perfect spatial control of membrane voltage at the begining of large depolarizations has been so far impossible to achieve in close-to-physiological experimental conditions (JOHNSON and LIEBERMAN, 1971; TRAUTWEIN, 1973; WEIDMANN, 1974).

JENERICK (1963, 1964) has shown that the phase plane display of membrane voltage (dV/dt versus V) during a pro-

(1) This work was made possible through Research Grants from the Research Council of the Federal University of Rio de Janeiro (CEPG/UFRJ), National Research Council of Brasil (CNPq) and National Development Bank of Brasil (BNDE/FUNTEC 241).

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pagated action potential can yield much valuable information on the active membrane currents in simple cable-like cells. We have extended Jenerick's original treatment and applied it to heart muscle in the hope of obtaining current-voltage relationships during the upstroke of the action potential, a time during which strong sodium currents are flowing across the membrane. In the process of pursuing this goal we came to realize that the membrane voltage of skeletal and cardiac muscle membranes does not behave in accordance to expectation when a simple parallel resistive-capacitive model is adopted. This discrepancy is clearly shown during the subthreshold foot of the action potential and its transition to a fully active membrane (PAES DE CARVALHO et al, 1969; GARCIA et al, 1973; PAES DE CARVALHO, 1975).

METHODS

Mammalian cardiac muscle from rabbits (right atrium) and dogs (ventricular Purkinje strands) as well as frog's skeletal muscle (m. gracilis and semitendinosus, *Leptodactilus ocellatus*) were used in this study. Heart preparations were kept under a constant flow of Tyrode solution at 35°C equilibrated with a 0_2 (95%)/CO_2 gas mixture (PAES DE CARVALHO et al, 1969). Frog skeletal muscle was kept in Ringer's solution (ADRIAN, 1956) at ambient temperature.

Transmembrane recordings were obtained through 3M KCl-filled glass capillary microelectrodes (tip diameter around 0.1 micron, tip resistance 20-30 megohms). Suitable capacity-compensated pre-amplifiers and an electronic differentiator allowed simultaneous determination of membrane voltage (V) and its first time-derivative ($\dot{V}$), both of which could be displayed in an oscilloscope either as a function of time or in the phase plane ($\dot{V}$ in ordinates, V in abcissae; see Fig. 1). Optimal capacity compensation and differentiator frequency response were checked by feeding a constant velocity ramp pulse

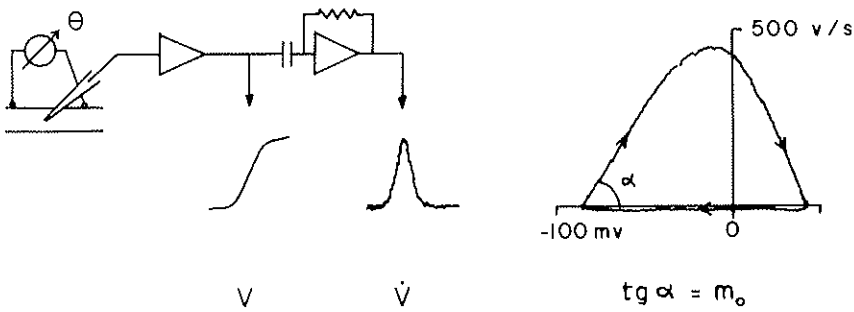

FIG. 1 — *Left,* Recording diagram for phase-plane analysis of the propagated action potential. Membrane potential (V) is measured by a single microelectrode. The first time derivative (V) is obtained through electronic differentiation. Conduction velocity (θ) is determined from simultaneous surface recordings. *Right,* cardiac action potential (non-pacemaker) displayed on the phase-plane. Ordinates, V in volts/sec. Abcissae, V in milivolts. Arrows point direction of inscription of the loop, which starts from resting potential (extreme left of loop) and courses clockwise to the peak of potential (extreme right of loop) and back to rest. The slope of the phase-plane is generally termed m; m_o is the initial slope and corresponds to the exponential foot of the action potential.

as calibration for both $\dot{V}$ and V. The phase-plane image of the ramp allowed control of capacity compensation and of distortion introduced by unwanted time lag between V and $\dot{V}$ (see PAES DE CARVALHO et al, 1969). Record analysis was performed either manually or with the aid of a digital computer (DEC-PDP 12, 8K memory).

Modified solutions, when used, have their composition specified in Results.

MATHEMATICAL BASIS OF IONIC CURRENT DETERMINATION THROUGH PHASE PLANE ANALYSIS IN A CYLINDRICAL CELL

HODGKIN and HUXLEY (1952d) in their analysis of the propagated action potential of the squid giant axon used the

then familiar parallel resistive-capacitive membrane model to state that membrane ionic current (I_i) equals the difference between total membrane current (I_m) and capacitive current (I_c) in any given patch of membrane at any time, as for instance at any instant during a propagated action potential. Assuming a propagated action potential of constant shape and propagation velocity, the traveling wave equation and simple cable considerations permitted these authors to state that:

$$I_i = \frac{\ddot{V}}{(r_i + r_e)\,\Theta^2} - c\dot{V}$$

where $\dot{V}$ and $\ddot{V}$ are the first and second time-derivatives of membrane voltage at any given time, and c, r_i and r_e are the values for membrane capacitance and longitudinal resistances inside the cell and external to it, all taken *per unit length of cable*. Conduction velocity is represented by Θ. This can be rewritten as follows:

$$I_i/c = (\ddot{V}/k) - \dot{V} \qquad (1)$$

where k is the propagation constant and equals $(r_i + r_e)\,c\Theta^2$. Note that k has the dimensions of time $^{-1}$.

JENERICK (1963, 1964) has shown that it is possible to integrate equation 1 to obtain:

$$I_i/c = \dot{V}\left(\frac{m}{k}\right) - 1 \qquad (2)$$

where $m = d\dot{V}/dV$, that is, the *slope* of the phase-plane at any given instant during a propagated action potential. As the constant k is not directly measurable, Jenerick estimated its value by using in its place the value for the initial slope m_o of the phase plane trajectory of the propagated action potential in skeletal muscle. This approximation implies that membrane ionic current should be practically zero during the subthreshold foot of the action potential.

In fact, differences of the order of 10 % can be observed between the values of k and m_o (the first is always smaller).

This difference is important if one wishes to study the subthreshold behaviour of the membrane and determine its threshold voltage. Threshold is defined as the voltage at which membrane ionic current becomes zero as it changes from outward to inward. This happens as m, decreasing from its initial value m_o, becomes momentarily equal to k.

From eq. 2 and the above considerations it becomes apparent that Jenerick's approximation for k underestimates both the threshold potential and the intensity of the peak inward current when an ideal Hodgkin-Huxley action potential is considered (Fig. 2). This inaccuracy is difficult to correct due to the proximity of the values of k and m_o and to the difficulty of experimental determination of k with a sufficient degree of precision.

We have attempted to determine k by studying one same cell under conditions which yielded variations in m_o and k as a result of changes in θ only (r_i, r_e and c constant). From the definition of k it is easy to show that:

$$\frac{k}{k^*} = \frac{\theta^2}{\theta^{*2}} = Z$$

where the asterisk designates a test situation.

Once a relative value Z is obtained for k, a determination of absolute value in normal conditions can be achieved from the following considerations. At the beginning of the exponential foot of the action potential the membrane is still at rest and its time constant τ can be described by:

$$\tau = \frac{1}{m_o \left(\frac{m_o}{k} - 1 \right)} \qquad (3)$$

This follows directly from the definition of τ, from eq. (2) and from the fact that during the passive foot of the action potential

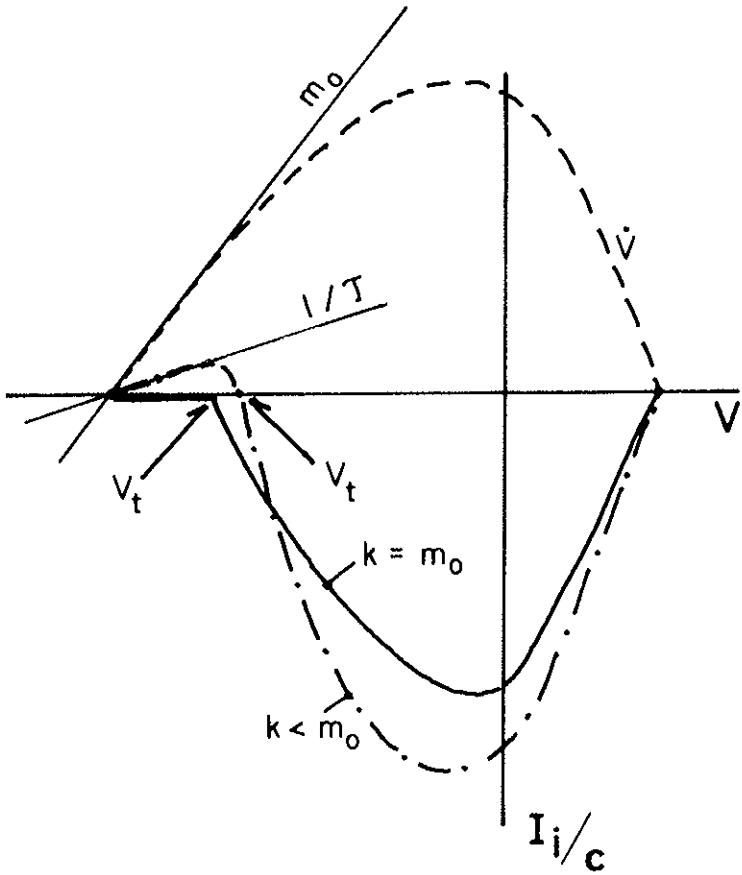

FIG. 2 — Theoretical diagram showing the upstroke of an action potential in the phase-plane (V, dashed line) and current-voltage curves calculated assuming $k = m_o$ and $k < m_o$. Note discrepancy in threshold voltage (V_t) and peak inward current. The initial slope of the current curve when $k < m_o$ equals the inverse of the resting time constant τ. Current curves not drawn to scale.

the membrane voltage and its first time-derivative are related as $V = m_o \dot{V}$. Now, if only θ changes in the test solution (i.e., resting values for r_m, r_i, r_e and c remain constant), τ is unchanged and one can write:

$$k = \frac{m_o^2 - Zm_o^{*2}}{m_o - m_o^*} \tag{4}$$

If resting r_m also changes, a value for k can still be obtained:

$$k = \frac{m_o^2 - bZm_o^{*2}}{m_o - bm_o^*}$$

where b is the ratio r_m^*/r_m.

Once k is known, the time constant of the resting membrane (tested by an action potential) can be obtained from eq. (3). The determination of τ allows determination of the resting space constant λ:

$$\lambda = \frac{\theta}{m_o} \sqrt{1 + m_o \tau} \tag{5}$$

Note that λ is related to the space constant of the foot of the propagating action potential, which equals θ/m_o.

The knowledge of the exact value of k should then yield: *a*) a more precise current-voltage plot during the action potential as pointed out in the beginning of this section; and *b*) estimates of membrane time constant and tissue space constant, all from a single propagated action potential.

Results

Attempts at determining the constant k in heart and skeletal muscle

The mathematical treatment outlined above for the ideal propagated action potential in a cable-like cell can be extended

to cardiac muscle if one assumes that: *a*) excitation procedes at the same rate in cells of an individual bundle; *b*) action potential shape remains constant; and *c*) the excitatory wave front is flat enough to allow for the assumption that there is no difference in membrane potential between cells located at a given cross section along the propagation pathway.

Phase-plane displays of action potentials recorded from rabbit atrial muscle and dog Purkinje fibers were studied in Tyrode solution and compared with values obtained for the same cells in a low sodium medium (25 % reduction, choline chloride substitution in presence of a 16 μg/ml acetylcholine background for both normal and modified solutions). Values for 0 in both solutions were simultaneously determined through a close bipolar surface record. The values of r_m, r_i, r_e and c were taken as constant throughout the experiment, and eq. (4), (3) and (5) were used to calculate k, τ and λ for every cell. It was noted that only a minority of the cells studied yielded values for τ and λ in keeping with those obtained by other investigators using difference techniques. As pointed out before (PAES DE CARVALHO, 1975) the discrepancy is explained by an error in the determination of k. The wide scatter of data made us conclude that the simple cable model for the propagated action potential was not applicable to cardiac muscle, possibly due to failure of the assumptions outlined above. However, a similar experimental study carried out on single frog skeletal muscle fibers also yielded widely discrepant results, due to uncertainty in the determination of *k*. These findings led us to look deeper into the question.

Phase-plane display of the Hodgkin-Huxley action potential

The mathematical model for the propagated action potential in squid nerve was computed in a DEC PDP-12 digital computer (8K memory) employing 23 bit precision arithmetics developed by MOLNAR (1968). The same procedure and parameters put forth by HODGKIN and HUXLEY (1952d) were

used. No special precautions were taken to overcome the instability which FITZHUGH and ANTOSIEWICZ (1959) pointed out to exist during the repolarization phase. It could be shown that action potentials which tended towards $+\infty$ or $-\infty$ during repolarization presented similar depolarization phases, provided the adopted value for k remained within one bit of the value needed to balance the equation.

Fig. 3 shows a computed action potential which at some

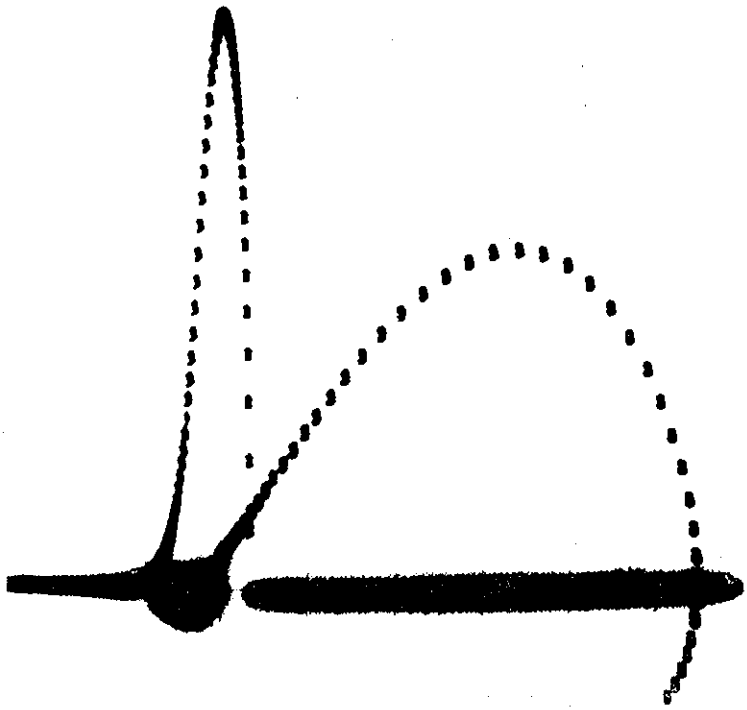

FIG. 3 — Theoretical squid nerve propagated action potential calculated in a PDP-12 (8k) computer using HODGKIN and HUXLEY'S (1952d) equations and parameters. Calculations depart from expected behaviour during repolarization and the action potential tends to $-\infty$ (see text). Also shown is the phase-plane display of the upstroke of the action potential. Note that there is no positive slope larger than m_o during the upstroke (SALDEÑA T. A., unpublished).

point during repolarization tends to $-\infty$. It also shows the simultaneous phase-plane display of the upstroke of the action potential, plotted in the same way as in Fig. 1. Note that m_o is the largest positive slope during the upstroke of the action potential. The initial linear portion of the phase display is rather short, and the curve deviates from linearity so that m becomes smaller than m_o. Using Hodgkin and Huxley's parameters one obtains an action potential where $m_o = 11.33$ $msec^{-1}$ and $k = 10.48$ $msec^{-1}$. The k adopted in Fig. 3 was only one bit removed from the value that would make the action potential tend to $+\infty$. If k is changed one further significant bit in 23 away from its true value, the result is that the instability point moves earlier in the action potential.

Comparison of computed action potential data with data from cardiac and skeletal muscle

PAES DE CARVALHO et al (1969) have shown that in most cardiac cells the initial slope m_o is not the largest positive slope during the upstroke of an action potential. The ascending limb of the phase-plane display (from resting potential to the voltage in which the highest rate of rise, $\dot{V}_{max}$ is observed) often shows one or more inflections, instead of the smooth appearance observed in the theoretical computed action potential. It is possible to show that these inflections are due to two classes of phenomena:

a) a simple inflection at or near threshold voltage;

b) one or more inflections in the subthreshold region due to local retardation or acceleration of the initial exponential foot.

Figure 4 illustrates the first type of inflection. It shows two phase-plane loops *recorded from the same cell* in the rabbit atrium. The only difference in experimental conditions was the site of stimulation of the preparation. The shape of the

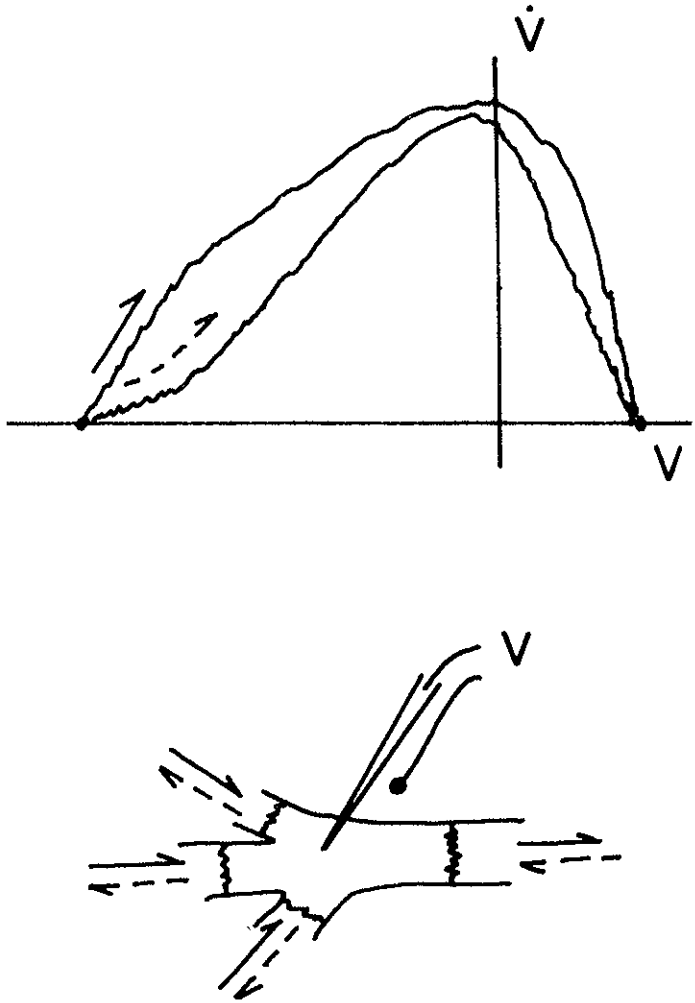

FIG. 4 — *Above,* diagramatic tracings of two phase-plane plots of the upstroke of the same atrial fiber, each corresponding to one direction of propagation. *Below,* diagram suggesting a cell geometry, interpretation for the above findings. Arrows relate direction of propagation to the records above. See text.

phase loop could be predictably controled by varying the site of stimulation. The lower portion of the illustration shows a diagram which attempts to explain the differences in terms of geometry of the cell input and output and its alteration when direction of invasion is changed. If a cell is invaded through a single junction, there will be less depolarizing subthreshold current to fire it than if it is invaded through two or more junctions. The initial slope m_o will be steeper in the latter instance than in the first. On the other hand, once the cell is fired, the $\dot{V}_{max}$ will be larger in the second case, because there will be less shunt of ionic currents through the single exit junction.

Inflections of the type *b*) described above seem to occur if the inputs of a cell are not synchronized. This situation yields two or more slopes or humps in the subthreshold region of the ascending limb of the phase-plane display.

Inflections of the type *b*) depend on geometry considerations and are observed only in cardiac. On the other hand, inflections of type *a*) are not only observed in cardiac muscle (Fig. 5B). It is then clear that if they can be enhanced by geometrical peculiarities ,they persist even in a single cable arrangement.

Inflections of this type seem to represent an important departure from the behaviour predicted for the Hodgkin-Huxley action potential in propagation along a simple, uniform cable-like cell. The source of this discrepancy is so far unknown and is discussed later in this article.

Several procedures which depress $\dot{V}_{max}$ through interference with the fast sodium channel tend to decrease the slope of the segment beyond threshold, making it closer to, equal and eventually smaller than m_o. Figure 6 shows the influence of increased extracellular potassium on frog skeletal muscle. Loss of resting potential progressively decreases $\dot{V}_{max}$ and the slope of the post-threshold segment of the phase loop, which tends then towards the shape expected from the theoretical prop-

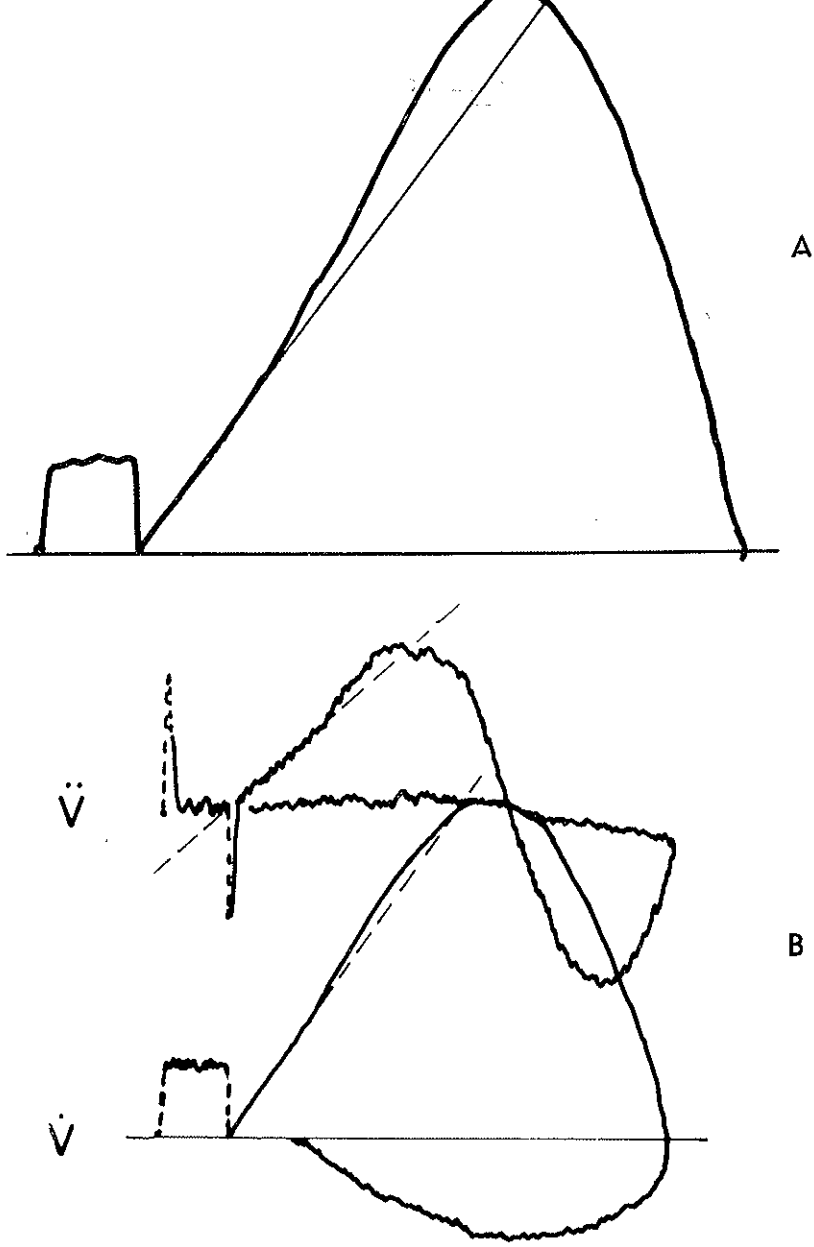

FIG. 5 — *A*, tracing of phase-plane record of upstroke in a Purkinje fiber, showing a threshold inflection of its ascending limb, followed by a slope larger than m_o (GARCIA E.A.C., unpublished). *B*, same finding in a frog skeletal muscle cell, showing also the second time derivative ($\ddot{V}$) as a function of voltage (abcissae) (SALDEÑA T.A., unpublished). Calibration at extreme left of phase-plane displays represent V = 100V/sec and V = 20mV both in *A* and *B*.

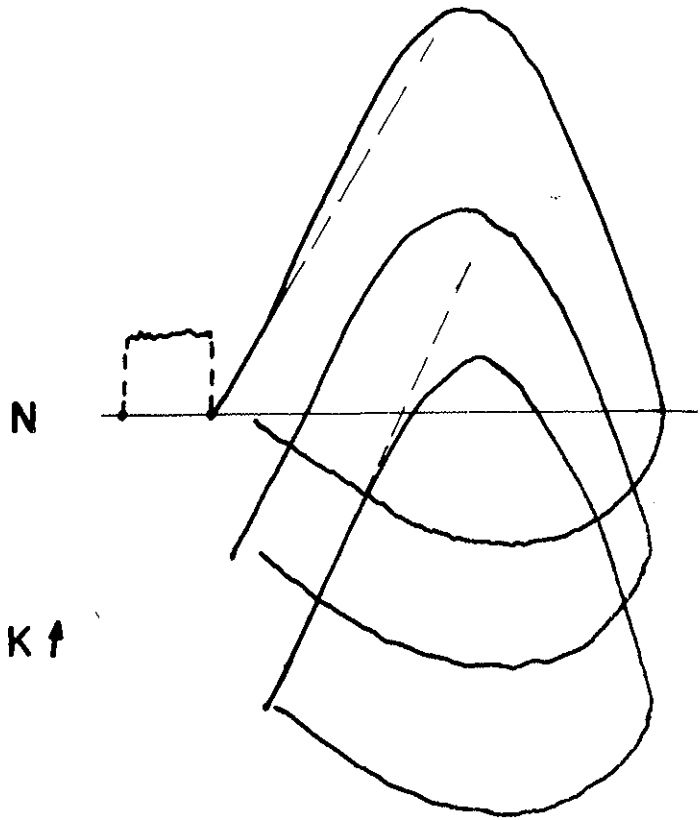

FIG. 6 — Effect of increased extracellular K^+ concentration on propagated action potentials in frog skeletal muscle. High K^+ solution applied topically through a micropippette onto the recording site. Upper record, normal Tyrode (N); calibration, 100 V/sec and 20 mV. Lower tracings, successive moments during onset of K^+ effect. Note depression of the slope of the suprathreshold part of the ascending limb of phase-plane display. Note also depolarization and decrease in V_{max} (SALDEÑA T. A., unpublished).

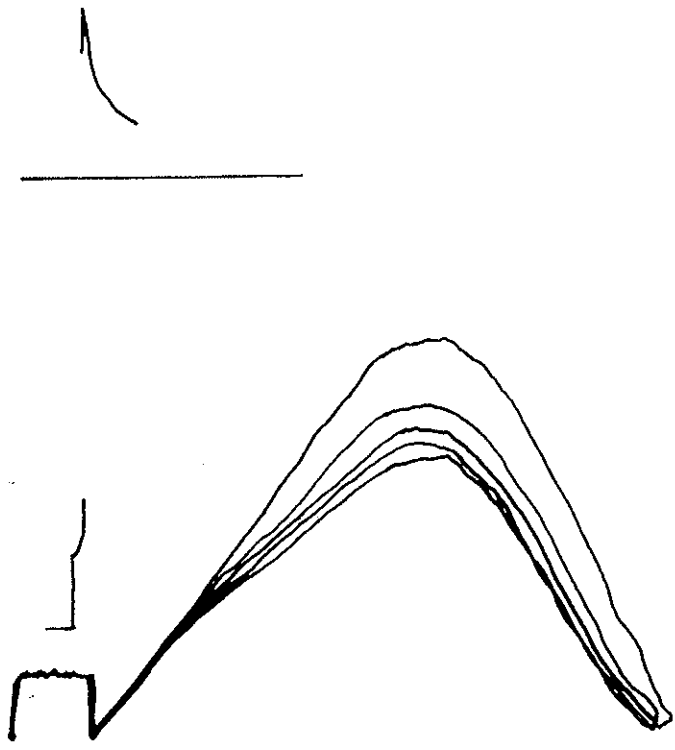

FIG. 7 — Effect of topic application of tetrodotoxin to a cardiac Purkinje fiber. Note depresion of the suprathreshold part of the ascending limb of the phase-plane display. Calibration: 100 V/sec and 20 mV. See text for details (GARCIA E.A.C., unpublished).

agated action potential. The same can be observed in cardiac muscle.

Figure 7 illustrates the statement made above to the effect that type *a*) inflection effectively occurs at or near the passage from the resting state to the excited state of the fast sodium channel. The data was obtained from dog Purkinje fibers. The experiment consisted in the local application of Tyrode solution containing tetrodotoxin (TTX) onto the site of re-

cording. The applying pippette (100 micra tip diameter approximately) was positioned in such way relative to the direction of flow of the bathing solution that a stream of TTX containing Tyrode flowed at right angles to the fiber and the direction of propagation. The microelectrode impalement was located near the proximal border of the affected area (relative to propagation). Successive phase loops recorded during the onset of TTX action appear superposed in the illustration. It can be seen that the foot of the action potential is not altered. But the slope of the segment beyond the threshold inflection is depressed, as $\dot{V}_{max}$ is also depressed. This is construed to mean that local contribution to excitatory current starts at or near the inflection we associate with threshold.

Discussion

Phase-plane analysis as a method of study of membrane ionic currents

The present study set out to perfect a technique that would enable one to obtain a current-voltage relationship during the action potential. This method would constitute an easy, accurate and physiological way of studying the kinetics of the fast sodium channel in cardiac and skeletal muscle. On the side, it would allow determination of τ and λ. It turned out that attempts at determining the constant k where futile for the simple reason that cardiac and skeletal muscle action potentials cannot be described by the mathematical model set forth by Hodgkin and Huxley (1952 d) for squid nerve. It can therefore be concluded that Jenerick's approximation ($k = m_o$) yields results as reliable as any for inward current during the upstroke, provided one bears in mind that current-voltage plots obtained in such a fashion understimate the value of peak inward current by about 5 %. Tberefore, Jenerick's approximation still yields a useful picture of the current voltage relation-

ship of the fast Na channel in a quasi-instantaneous condition and may be a useful substitute of voltage-clamping in this particular case.

On the other hand, it seems obvious that the phase-plane method can tell us nothing about subthreshold behaviour until understanding of the findings of subthreshold and threshold inflections can be better understood and mathematically described. Threshold determination obtained from phase-plane current-voltage plots is totally innacurate due to the presence of slopes larger than m_0 in the ascending limb of the phase display of the upstroke (suprathreshold region). Accordingly, our results with depression of the fast sodium channel suggest that threshold lies at a much lower potential than previously estimated by JENERICK (1963, 1964). It is also obvious that any attempt at determination of τ or λ, from the time course of the action potential foot should also meet with important problems of accuracy.

Phase-plane analysis as a method of study of propagation and cell coupling in the heart

If the phase-plane has so far failed to provide an easy answer to the study membrane currents, it has on the other band allowed us to look deeper into the process of propagation in heart and skeletal muscle. First, it bas become clear that the invasion of a cardiac cell by an action potential is not a simple process, but may involve fleeting "hesitations" and accelerations at points of crytical geometry. This fact had already been surmised from theoretical studies (HEPPNER and PLONSEY, 1970) and observations in cells at the borders of the atrioventricular node (HOFFMAN et al, 1959; CRANEFIELD et al, 1959). The phase-plane method seems to be an easy way to observe in greater detail such phenomena in bundles of common and specialized muscles.

Second, the phase-plane method has brought out the fact that even in absence of complicating geometry, propagation in

cardiac muscle bundles and in skeletal muscle fibers do not conform to the mathematical description of the propagated action potential in a uniform cylindrical cell (HODGKIN and HUXLEY, 1952 d) insofar as over 70 % of the records in either tissue show an inflection at threshold voltage followed by a segment of slope steeper than m_o. Unfortunately, no data on the phase-plane display of propagated action potentials recorded from squid nerve are yet available to see whether this discrepancy is peculiar to striated muscle cells. The problem may simply lie on the type of equivalent circuit chosen for the membrane. At any rate, it should be pointed out that added tubular capacitance in presence of an excitable tubular membrane is not an unlikely candidate for a suitable alteration of the equivalent circuit, and mathematical descriptions that take this factor into account (ADRIAN and PEACHEY, 1973) should be tested in the phase-plane.

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DISCUSSION

Chairman: Prof. A. M. MONNIER

MONNIER

Are there some questions? This phase plane presentation offers an alternative, which may be extremely useful. When a mathematical formulation gives a generalized expression for a body of facts, of course it is always very stimulating to present this expression under an entirely new aspect. This you did quite remarkably. I was very interested in this zone of instability which you show so clearly on the phase plane.

KEYNES

Could I ask if you have looked in the squid at records of both a propagated and a membrane action potential?

DE CARVALHO

No. We have so far conducted no experiments on squid giant axons. But there is some indication that even there the propagated action potential may show an upward inflection at threshold voltage when displayed in the phase plane. This suggestion is based on a graphical integration of dV/dt — time records published by HODGKIN and KATZ (see J. Physiol., 1949, *108*:37-77, figure 9).

The phase plane display of action potentials reconstituted from these records shows an inflection towards a more positive slope (upward) some 15-17 mV above resting potential. It would thus seem that the discrepancy between theory and experiment pointed out in my paper may well appear in structures other than cardiac and skeletal muscle.

MONNIER

As privately mentioned to you, the first encounter of these action potential problems of EC code were the early computers. For the last fellow it was the huge computer of the U. S. Navy in Washington D.C., and through erratic programming the results were gradually accumulating to an instability zone covering the entire diagram.

DE CARVALHO

I am well aware of this danger. Our computer results, however, are identical to those of FITZHUGH and ANTOSIEWICZ (1959) and the instability area we encountered is well beyond the peak of the action potential. It should not affect the phase plane record of the upstroke.

CELL COUPLING IN CARDIAC MUSCLE

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Department of Physiology, Bühlplatz 5
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Up to 1954 there was little doubt that cardiac muscle could be considered as a syncytium. This view was based on functional properties such as all-or-nothing excitability and conduction without decrement. A short communication by SJÖSTRAND and ANDERSSON (1954) came as a rather unpleasant surprise. Using high resolution electron microscopy these authors clearly demonstrated the existance of morphologically distinct cells. There were two major possibilities to reconcile morphology and function: either to postulate cell-to-cell transmission by a chemical mediator (see for instance SPERELAKIS, 1963), or to assume that adjoining cell membranes may have specialized regions of low resistance. The evidence, which has accumulated over the past 20 years, seems to favour the second alternative.

Electrical data

If current is injected into a single cardiac cell by means of a micropipette there is a change of membrane potential. This so-called electrotonic potential is not restricted to the surface membrane of the " injected " cell but spreads to its neighbours. One of the essential properties of a " biological

cable " is its so-called space constant (λ), i.e. the distance over which the amplitude of an electrotonic potential drops by a factor of e. This has been found in all parts of the heart to be many times the length of a single cell, indicating that there is tight electrical coupling between cells. The lowest values for λ are those for the sinus node of the rabbit heart (BONKE, 1973; 0.5 mm), the highest those for Purkinje fibres from ungulate ventricles (WEIDMANN, 1952; 2.0 mm).

Having measured the space constant λ and the so-called input resistance of a biological cable it is possible to calculate a value for the internal resistance. In the case of heart muscles, this is the resistance of cellular cytoplasm and cell-to-cell pathways as coupled in series. For current flowing in the longitudinal direction R_i — expressed as a specific resistance — works out to 100-200 Ω cm for Pukinje fibres and to about 400 Ω cm for trabeculae of the right ventricle of ungulate hearts (WEIDMANN, 1952, 1970). The important point about these values is this: bathing solution (Tyrode at 37°C) has a specific resistance of 51 Ω cm; R_i of cytoplasm must be somewhat higher. Depending on the ahundance of mitochondria and myofibrils and taking into account the ionic composition, a reasonable guess is 100-200 Ω cm. This indicates that the resistance between two adjoining cells is low and comparable to the cytoplasmic resistance of a single cell.

Morphological correlates

While most of the membrane of cardiac cells is in contact with extracellular space, as can be shown by incubating the preparations with ferritine or horse raddish peroxydase prior to preparation for electron microscopy (e.g. FORSSMANN and GIRARDIER, 1970), there are specialized regions, where the membranes of contiguous cells are in extremely close apposition; and extracellular space markers are not found within the interspace. These specialized regions are known as gap junc-

tions or nexuses, and it has long been speculated that they might be the site of low resistance between cells. By morphometric methods it has been found in rat ventricle that 10-13% of the structure known as " intercalated disk " consists of nexus-type junctions, the rest of the membrane facing the extracellular space (PAGE and MCCALLISTER, 1973). New methods in electron microscopy such as freeze etching have revealed a regular pattern in nexus-type membranes which makes it likely that there are aqueous pores connecting the interior of adjoining cells (for a review, see MCNUTT and WEINSTEIN, 1973). The diameter of these pores is estimated to be of the order of 10-15 Å, their length about 150 Å. From the number of holes per cm^2 and on the assumption that K^+ ions are present within the pores at a concentration of 150 mM it has been calculated by MATTER (1973) that 1 cm^2 of intercalated disk would represent an electrical resistance well below 1 Ohm, a value that would be in full agreement with the electrical data.

Cell-to-cell diffusion of tracers

If a bundle of fibres is placed in a two-compartment chamber, one compartment being perfused with ^{42}K Tyrode solution, the other with normal Tyrode solution, the intracellular radioactivity will eventually reach a steady state diffusion. From geometrical data, the resting membrane potential, and the distribution pattern of ^{42}K it will then be possible to calculate an approximate ratio of permeability of the disk membrane to the surface membrane (WEIDMANN, 1966). This ratio works out to be about 5000:1.

Studies on many different types of cells have shown that while small molecules can easily move to neighbouring cells, they can only with difficulty penetrate the surface membrane. The preparation, which for its morphological simplicity has been most studied is the (epithelial) salivary gland of chyronimus (for a review, see SOCOLAR, 1973). Fluoresceine^{2-} (mo-

lecular weight 330), when injected by iontophoresis into a given epithelial cell, has been shown to migrate to neighbouring cells, the results showing a clear-cut drop of concentration at the site of the cell boundaries. Analogous results using fluoresceine have been reported for heart muscle by POLLACK and HUNTMAN (1974).

A different method of introducing tracer molecules has proofed to be useful. It is based on an observation reported by DÉLÈZE (1970). An injured part of cardiac muscle does not heal over when kept in Ca^{2+}-free solution, but promptly seals when Ca^{2+} is readmitted. This makes it possible to introduce into the intracellular compartment such substances that would not readily penetrate through the surface membrane. A trabecula of ventricular fibres is placed in a chamber divided by a closely fitting rubber membrane. The left-hand compartment is perfused with Ca^{2+}-free, TEA-containing solution. The preparation is then cut at $x = 0$. TEA is allowed to enter the injured part until the latter is sealed by applying a Ca^{2+}-containing (TEA-free) Tyrode solution. Subsequently, both compartments are washed with normal Tyrode solution for several hours. The preparation is then removed, frozen in liquid air and cut into slices of equal length by a block of uniformly spaced razor blades. The pieces, 0.5 mm in length are (in the case of [^{14}C]-TEA) counted for radioactivity, and the results plotted as a function of distance. Using diffusion equations for the non-steady state (CRANK, 1956; WEINGART, 1974) an apparent diffusion coefficient can be calculated which applies for myoplasm and disks as coupled in series.

Using this cut-and-seal method, diffusion studies have so far been done for [^{14}C]-tetraethylammonium^{+} (mol.wt. 130; WEINGART, 1974), cyclic [^{14}C]-AMP (mol.wt. 328; TSIEN and WEINGART, 1974), and the dye Procion Yellow^{3-} (mol. wt. 697; IMANAGA, 1974). If the "apparent diffusion coefficient" in the longitudinal direction ($D_{apparent}$) is compared to that in free solution (D_{H_2O}) the ratios are 3:1 for K^{+}, 4:1 for [^{14}C]-TEA^{+}, but as high as 30:1 for Procion Yellow^{3-}. The largest

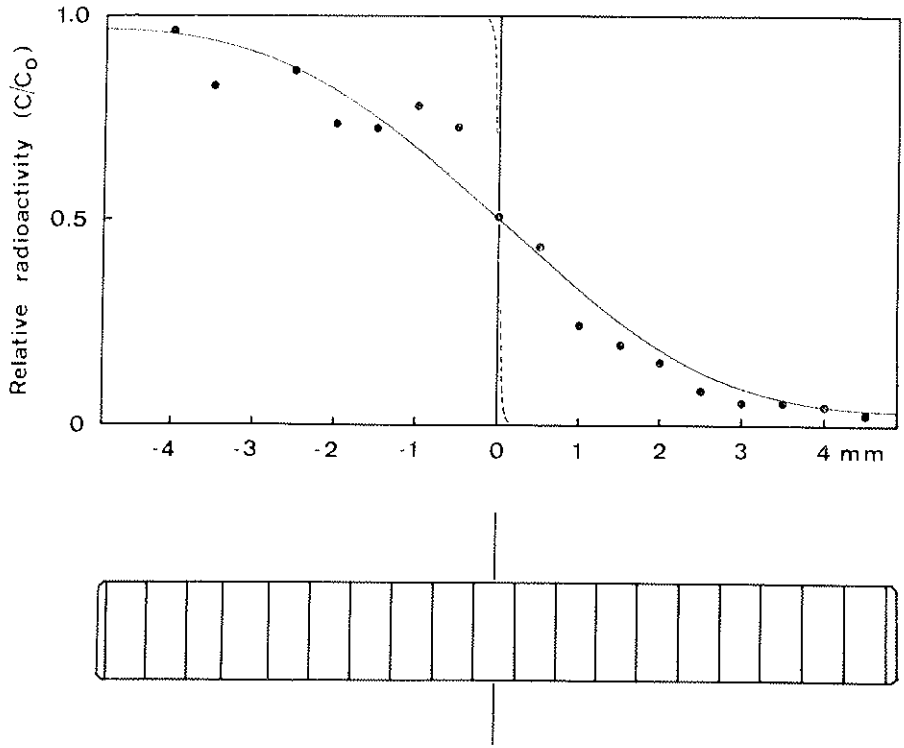

FIG. 1 — Distribution of [^{14}C] TEA within a trabecula of myocardial fibres. The preparation was exposed to TEA-containing solution on the left hand side, and washed by TEA-free solution on the right hand side of a partition wall. At the end of 4 hours it was cut into slices as indicated below. Evaluation of the TEA distribution (in non-steady state diffusion) gave a diffusion coefficient of 1.75×10^{-6} cm^2/sec. From WEINGART (1974).

particle which has been shown to migrate from cell to cell. Procion Yellow is a chainlike molecule which can be packed into a space of $5 \times 10 \times 27$ Å (WEINGART, 1974). It is conceivable that a pore diameter of 10-15 Å (MCNUTT and WEINSTEIN, 1973) would signify a marked but not a complete barrier to diffusion for this particle. The results obtained so far make it likely that many of the essential metobolites can pass from one cardiac cell to its neighbours. However, a larger series of different molecules, having different configurations and different electrical charges ought to be tested in order to specify more precisely the requirements for cell to cell diffusion.

Experiments on de-coupling

ENGELMANN (1877), discussing cardiac cell damage and its reversibility commented as follows: " Herzmuskelzellen leben zusammen und sterben einzeln ".

It has been shown by electrical measurements that de-coupling is produced by hypertonic solution (BARR, DEWEY and BERGER, 1965); the easiest way to account for the result is to assume shrinkage of the cells and breaking up of some of the nexus-type cell connections.

Halothane, a widely used narcotic, has been reported to decouple cardiac Purkinje fibres, fortunately at high concentrations (1-2%, HAUSWIRTH, 1968). Isotonic sucrose solution slowly uncouples the cells of myocardial trabeculae (KLÉBER, 1973). In the case of halothane and ion-free solution the uncoupling mechanism is not well understood.

On the basis of the hypothesis that an elevated Ca^{2+} concentration may be the reason for de-coupling (see below) DE MELLO (1974) ejected Ca^{2+} from the tip of a micropipette into a single cell of a Purkinje fibre by iontophoresis. Pulses of electrical current were made to flow into that same cell while the electrotonic potential was measured from a neighbouring cell. With rising intracellular Ca^{2+} concentration there

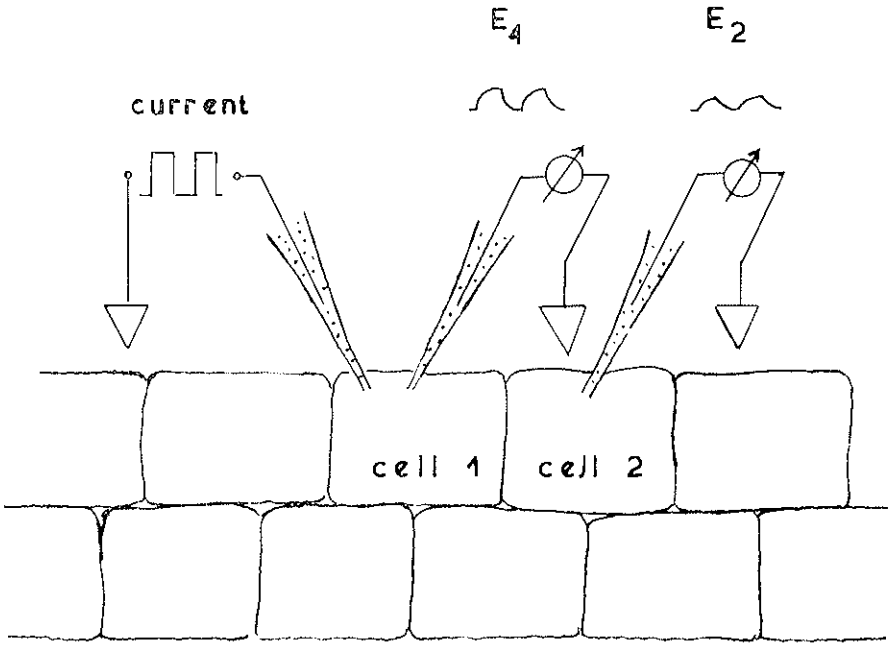

FIG. 2 — Method used by DE MELLO (1975) to measure electrical coupling between cells of cardiac Purkinje fibres. Pulses of constant current are injected into cell 1. Changes of membrane potential are recorded across the surface membrane of cell 1 and across the surface membrane of cell 2. A low resistance of the common cell boundary is indicated by the amplitude of E_2 being close to that of E_1. Electrophoresis of Ca^{2+} by the current electrode decreases the "coupling ratio" (E_2/E_1), indicating "sealing" of the common boundary.

was progressive de-coupling. When ejection of Ca^{2+} was stopped, allowing the cell to reduce its elevated Ca^{2+}, coupling was restored.

Likewise, treatment by ouabain tends to de-couple. If " therapeutic " concentrations are used the effect of increasing the internal longitudinal resistance of Purkinje fibres is moderate and reversible (WEINGART, 1975). With toxic concentrations the increase is marked and persists upon the removal of ouabain. As pointed out by LIEBERMAN et al. (1973), an increase of internal resistance would slow down conduction without markedly changing the shape of the action potential. It is known that cardiac glycosides increase the intracellular Ca^{2+} concentration, and it may well be that the nexus-type junction has a low resistance only if the Ca^{2+} concentration on both sides of its pores is low.

The formation of low-resistance pathways

Cardiac cells may be broken up by the action of enzymes plus mechanical treatment. Such cells may take up contact again and form tissue cultures, with all member-cells beating synchronously. Evidently, there is a mechanism for low-resistance pathways to be formed even in vitro. Contact formation has been followed in some detail with embryonic cells of the newt *triturus*. When similar cells from the macromeroblast are brought into close contact hy micromanipulations (ITO, SATO and LOEWENSTEIN, 1974 a, b) the forming of electrical contacts can be shown to be complete after about 30 minutes. This is demonstrated by injecting pulses of current into one of the cells, and by recording from the same cell and its neighbour: Coupling is signalled by an increase in amplitude of the signal from the second cell together with a decrease of the signal recorded across the surface of the injected cell. The size of *triturus* embryonic cells (300 μ in diameter) makes them especially suitable for this kind of experiment.

The " healing-over " of damaged cardiac muscle

The phenomenon of " sealing " is an important process in the demarcation between injured and non-injured parts of the heart, allowing the undamaged tissue to survive. By using scanning microscopy to examine the region between damaged and surviving tissue BALDWIN (1970) showed in a convincing way that healing-over occurs along the preexisting membranes rather than by new membranes being formed. DÉLÈZE (1970) has explored the conditions for " sealing ". If Purkinje fibres are damaged locally in a Ca^{2+} free solution there is no "sealing". Upon addition of Ca^{2+} to the bath healing-over is promptly observed. This can be judged by the rise of membrane resting potential near the damaged cells, as well as by the resistance offered to current flow through the site of cell damage. The common reason for low-resistance membranes to turn into high-resistance membranes thus seems to be a moderately high calcium concentration at one side (or both) of the nexus structure.

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DISCUSSION

Chairman: Prof. A. M. MONNIER

SLAYMAN

You talked about de-coupling in cardiac tissue by solutions of low ionic strength. Is the absence of any particular ions critical, or is it simply the difference of ionic strength that matters?

WEIDMANN

Well, I can give you a bit of additional information which, however, doesn't add much to an understanding of the mechanism. By adding calcium, 10-100 μM, you can actually make the de-coupling much slower. But what does it mean? Has anybody an idea?

POST

Is there any possible relationship between this phenomenon and the mechanism of cardiac fibrillation?

WEIDMANN

Possibly. You tend to facilitate fibrillation if by any means conduction velocity is decreased. To this you would all agree. Now

if you increase internal longitudinal resistance you certainly decrease conduction velocity. The question: is it of practical relevance? Internal resistance enters the equation for conduction velocity under a square root. Thus, you need quite a lot of increase of internal resistance to get a sizeable decrease of conduction velocity. Still I believe that in certain cases the effect might be sufficient to facilitate fibrillation. We have recently done some experiments with ouabain which in fact de-couples, probably because there is more calcium inside the cells.

CHAGAS

It is clear that the increase of resistance happens only in calcium-rich environment. The increase, for instance in the first LOEWENSTEIN experiments, was quite clearly shown by a resistance to diffusion of fluoresceine, and this is interesting because fluoresceine is also a rather large molecule which diffuses quite easily. It would happen only if there was calcium. This, in my opinion, raises a problem in membranes structure because without calcium there is an increase in electrical membrane conductance of all membranes which have been studied. In calcium-containing solution you have a sort of protection. Behaviour of cells in regard to aggregation is quite different when they are in a calcium-rich as against calcium-poor solutions. It really seems important to stress the significance of calcium in the formation of low resistance in contact areas.

WEIDMANN

This is a valuable comment that requires hardly any answer.

SLAYMAN

We are still in need of an explanation for de-coupling in low ionic strength.

WEIDMANN

Indeed. You have to realize that things are not in a steady state. For instance cells are losing potassium all the time. We have actually measured potassium efflux into an isotonic saccharose solution. At the end of four hours the tissue has lost about half its potassium. And this is a situation which can lead to all sorts of misbehaviour.

HASSELBACH

Is there any possibility to isolate intercalated membrane?

WEIDMANN

Yes, it would be nice to have isolated discs to see what is attached to them. We haven't heard of any such results.

I will readily confess that when I started to do experiments with ^{42}K I measured very short space constants until I was extremely careful in setting up the preparations. I am almost sure that it is stretching that damages them, in the sense that disk resistances increase.

PALADE

Gap junctions have been isolated from a number of sources and their protein components have been characterised partially. A number of laboratories have been involved in this work, including the laboratory of Dr. STOECKENIUS. Perhaps Dr. STOECKENIUS could tell us what is the present situation in this line of work.

STOECKENIUS

DAN GOODENOUGH in my laboratory has isolated the gap junction from mouse liver. He is now continuing his work in collaboration

with DON CASPAR. We have tried to analyse the chemical composition of the gap junction and to characterise the protein and to get additional structural information through x-ray diffraction from oriented samples of the isolated gap junction. In a recent grant application I have proposed to continue this work with an attempt to isolate the gap junction from Purkinjie fibers and incorporate the isolated gap junction into lipid vesicles and/or planar lipid films and study their permeability. I was told in no uncertain terms to stick to my purple membranes and forget about the gap junction.

I have one other remark concerning Dr. WEIDMANN'S presentation. The particle which is seen in freeze-fracture preparations of the gap junction apparently consists of six identical sub-units. They are probably arranged around a central channel. If the six sub-units in membranes of the two cells forming the gap junction are aligned across the gap they could form a continuous channel connecting the two cells. The channel should have a diameter of less than 50Å, because 50Å would be within the limits of resolution of our technique and we should clearly see it. This, however, is not the case and the indications of a central channel, which occasionally has been observed, are probably due to decoration effects and do not represent true resolution of the structure. I would guess that 20Å for the diameter of this channel would come closer to the real value of this structural feature.

IV

IONIC PERMEABILITY AND TRANSPORT IN BIOLOGICAL AND ARTIFICIAL MEMBRANES. I

THERMODYNAMIC ASPECTS OF NONELECTROLYTE PERMEATION OF LIPID BILAYERS

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The first great generality governing the permeation of biological membranes was OVERTON'S rule [1] which linked the permeation process to lipid solubility. Now, three quarters of a century later, his rule still provides one of the basic principles which govern the passage of nonelectrolyte solutes across the lipids of cell membranes. Two other primary rate limiting steps must also be considered, the first being passage across the interface that separates the membrane from its bathing solution, and the second being the resistance to diffusion through the membrane hydrocarbon.

These three factors have been linked simply to the permeation coefficient, P_d, (cm sec^{-1}) in an equation given by ZWOLINSKI *et al.* [2]

$$1/P_d = 2/k_{sm}\lambda + \delta/k_m\lambda^2 K \qquad (1)$$

The first term on the right contains the rate constant for crossing the interface, k_{sm}, and λ, the jump distance of the permeating solute from one position to the next. λ, which may be

computed from the partial molar volume, $\bar{V}$, and N_{Av}, AVOGADRO's number, as $(\bar{V}/N_{Av})^{1/3}$, lies between 4 Å and 6 Å for the solutes we will consider. The second term on the right relates to diffusion in the membrane hydrocarbon. It contains the rate constant for membrane diffusion, k_m, δ, membrane thickness, and K, the water:membrane partition coefficient. K equals k_{sm}/k_{ms}; k_{ms} is the rate constant for passing from the membrane to the bathing solution. ZWOLINSKI *et al.* point out that diffusion coefficients are related to rate constants by $D_i = k_i\lambda^2$. Hence equation 1 may also be written as

$$1/P_d = 2\lambda/D_{sm} + \delta/D_mK \qquad (2)$$

which clearly shows the dependence of the permeability coefficient on the two diffusion steps. Further insight into the relation between the separate terms may be gained by considering that resistances to diffusion, which are frictions, are additive, although permeability coefficients are not. Resistance can be defined by $R_i = P_i^{-1}$ so that

$$R_d = R_{sm} + R_m \qquad (3)$$

All three coefficients, the two rates k_{sm} and k_m, and the partition coefficient, K, are related to the particular character of solvent and solute. The physical chemical nature of the matrix that permeating solutes cross may be inferred from the thermodynamic and kinetic parameters that describe solute: matrix interactions. This paper is concerned with the information about membranes that can be provided from such measurements in lipid vesicles and spherical bilayers.

The Partition Coefficient

The partition of small solutes between water and bulk hydrocarbon is a guide in understanding the more complex

process of partition between bathing solution and structural arrays of lipids. Even the apparently simple partition of a small solute from water into bulk hydrocarbon goes by two steps: adsorption into the interface, followed by transfer from the interface into the bulk hydrocarbon. In order to compute the entropy, enthalpy and the free energy changes in these processes it is necessary to combine information from very diverse sources. It is necessary also to know how the free energy changes are dependent on temperature to compute the enthalpy and entropy changes of adsorption and partition.

One of the very few solute-solvent systems for which much of the information is in the literature is the partition of butanol, between water and an alkane solvent. The adsorption of butanol to a water: petroleum ether interface has been measured by HAYDON and TAYLOR [3] and the temperature dependence of butanol partition in a water:decane system has been reported by JOHNSON and BANGHAM [4]. Since petroleum ether is principally a mixture of pentanes and heptanes, the physical properties of the two hydrocarbon phases are nearly identical.

HAYDON and TAYLOR have shown that the free energy of adsorption of a homologous series of monohydric alcohols is linearly dependent on chain length, as illustrated in figure 1 which also contains similar data for the homologous amides whose adsorption in a water:decane system was studied by WANG *et al.* [5]. Since ΔG° is linearly dependent on chain length, the contribution of the methylene groups to ΔG° may be computed from the slope. The results of HAYDON and TAYLOR have been confirmed by WANG *et al.*; both groups report that ΔG° declines by 820 cal mole^{-1} per methylene group. These linear plots may be readily extrapolated to zero chain length to determine the ΔG° of the polar group, which is -800 cal mole^{-1} for the hydroxyl group and -400 cal mole^{-1} for the amide group. From the data of JOHNSON and BANGHAM [4], we have calculated from $\Delta G^\circ = -RT\ln K$ that ΔG° is 1150 cal mole^{-1} at 20°C and, from the temperature dependence of ΔG°, that ΔH° is 6100 cal mole^{-1}.

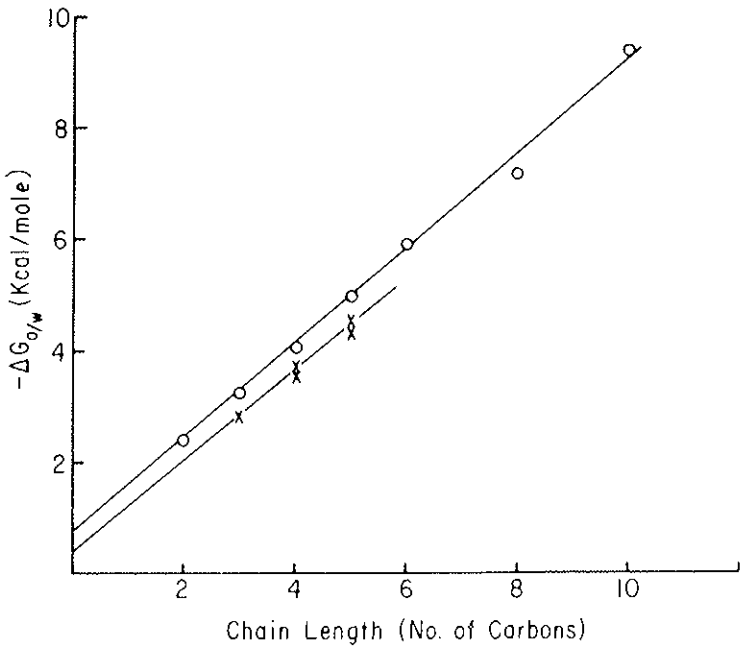


FIG. 1 — Free energy of adsorption of alkane monohydric alcohols and amides as a function of the number of carbons in the chain. This figure is reproduced from WANG *et al.* [5] who observed the convention followed in adsorption studies of plotting the negative free energy change rather than the positive value which we have used in this paper and particularly figure 2. The crosses are points for the alkane monoamides which WANG *et al.* measured and the circles represent the alkane monohydric alcohols as determined by HAYDON and TAYLOR [3].

These data have been measured in different systems and expressed in different units. For comparative purpose, it is necessary to convert them all to the same standard state. As TANFORD [6] has pointed out, mole fraction units should be chosen so that the standard free energy contains no contribution from the entropy of mixing. With this choice, ΔG° represents only the free energy of the solute molecule and that of its interaction with the solvents. Partition coefficients are

usually expressed in units of moles liter^{-1} and the transformation to mole fraction units is straightforward.

However, the transformation is not straightforward for the adsorption data since the standard state for the adsorbed phase was taken by HAYDON and TAYLOR [3] in terms of surface areas. To transform their adsorption equations into mole fraction units, we have treated the surface as a volume one molecule thick, and have computed the mole fraction from the ratio of the projected areas of solute and solvent, taking the limiting close packed area as the projected area. For amide solutes, WANG *et al.* have measured [5] the close packed area to be 24 Å^2; the value for the alcohols is given by HAYDON and TAYLOR as 18 Å^2, which we have also used for the alkane hydrocarbons that comprise the oil phase, since the small hydroxyl group does not project beyond the alkane chain in molecular models. Though the procedure is complex, the correction itself is quite small, amounting to only 150 cal mole^{-1} for butyramide; no correction is required for the alcohols.

The partition coefficients of monohydric alcohols have been measured in a variety of other solvent systems, including water: octanol by LEO *et al.* [7], and water: alkane by AVEYARD *et al.* [8, 9]. The partition coefficient for some of the higher alcohols into the human red cell membrane has been measured by SEEMAN *et al.* [10]. From figure 2, which gives ΔG° for the buffer: red cell membrane system and for water: octanol, it can be seen that ΔG° is linearly dependent on chain length, as for adsorption. Hence, the butanol partition coefficient in the red cell membrane may readily be obtained by extrapolation. Transformation into mole fraction units is straightforward when octanol is the solvent. For the red cell, butanol is considered to be dissolved solely in membrane lipids which represent 43.6% of membrane solids according to GUIDOTTI [11]. We have used a molecular weight of 750 as representative of the lipids.

As a step towards understanding the interactions of small nonelectrolytes with membranes, TING and SOLOMON [12]

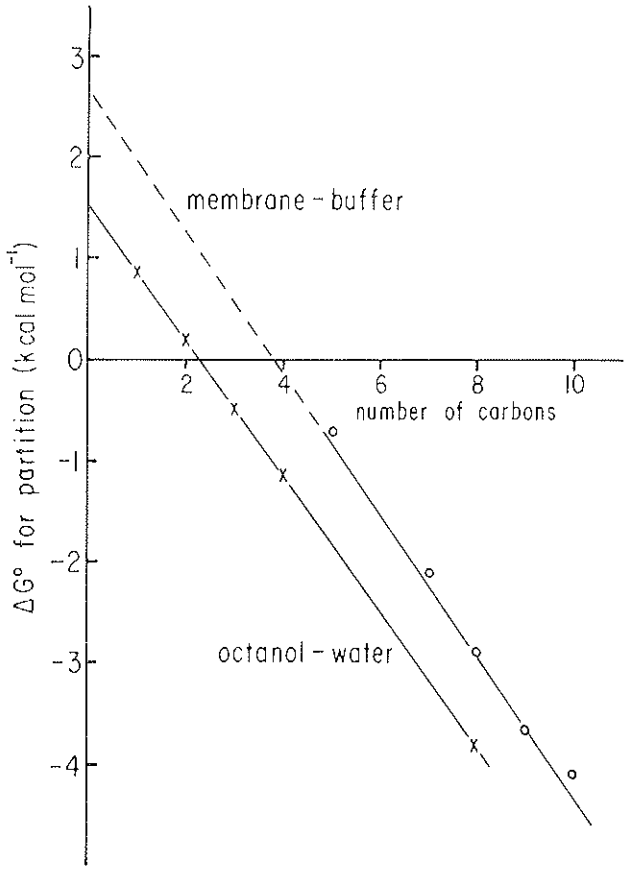


FIG. 2 — The free energy change for partition of the monohydric alcohols as a function of chain length. The values for the water:octanol system are taken from LEO *et al.* [7] and those for the buffer:red cell membrane system from SEEMAN *et al.* [10].

have studied the association of egg phosphatidyl choline vesicles with homologous short chain solutes such as the alkane amides and the alkanols. The vesicles used by TING and SOLOMON were prepared by a modification of the method of HUANG [13] which produces a homogeneous population of single walled

vesicles with an outside diameter of ≃ 210 Å. The association constants were determined from the displacement of fluorescent probes from the vesicle by competition with amides or alcohols in the aqueous medium. Since the probe fluoresces much more intensely in the nonpolar vesicle than in the aqueous medium, its fluorescence can be used to provide an accurate measure of solute-vesicle binding.

The binding reactions in the vesicle system may be described by the following scheme

$$P + S \rightleftharpoons PS$$

$$A + S \rightleftharpoons AS$$

in which P refers to free probe concentration, and PS to the concentration of probe bound to a lipid site. S is the concentration of free sites. A similar nomenclature is used for the competing solute, A, which may be an alcohol or an amide. The association constant, $K_{assoc} = [AS]/[S][A]$. K_{assoc} may be determined after control experiments have been carried out to determine the association constant of probe-site binding. TING and SOLOMON have used two probes to demarcate different regions of the lipid, ANS^-, 1-anilino-8-napthalenesulfonate, and NPN, N-phenyl-1-napthylamine. Despite the difference in nomenclature the two fluorescent probes are very similar in chemical structure, differing only in that ANS contains a charged sulfonate group absent in NPN.

These two probes have been chosen because they report different regions of the lipid vesicle. TRÄUBLE and OVERATH [14] have shown how the fluorescence intensity and wave length maximum of NPN varies with the polarity of the medium in which the probe is placed. They have given good evidence that NPN is to be found within the hydrocarbon of lipid dispersions, effectively shielded from agents external to the lipid. This conclusion is in agreement with the findings of FLANAGAN and HESKETH [15] who also localized NPN

within the membrane and concluded that ANS^- was at the interface. COLLEY and METCALFE [16] arrived at the same conclusion from nuclear magnetic resonance studies. LESSLAUER *et al.* [17] used x-ray diffraction to place ANS^- with the napthalene ring in the hydrocarbon region of the bilayer and the sulfonate group in apposition to the polar head groups of the lipid.

These localizations have enabled us to use the displacement of ANS^- by competitive solutes to determine ΔG° for association of the competitor with the interface, and NPN displacement to give ΔG° for the bulk lipid phase. The temperature dependence of K_{assoc} gives ΔH° so that ΔS° may readily be calculated. Though association constants are normally expressed in concentration units, they may easily he converted to mole fractions.

Figure 3 shows the free energy changes associated with butanol distribution in several systems. Adsorption at a water: hydrocarbon interface is a spontaneous process, but free energy needs to be supplied to remove the alcohol from the interface and place it in the bulk solution beyond. More free energy is required to transfer butanol from the interface to decane than to octanol, reflecting the fact that decane is significantly less polar than octanol. The free energy profile for egg lecithin vesicles is quite similar. Since the lecithin interface is more structured and hence more ordered than the water: decane interface, ΔG° for transfer to the interface is less, but the climb back to the vesicle interior is also smaller.

It is interesting that ΔG° for transfer into vesicle hydrocarbon (- 2950 cal $mole^{-1}$) is similar to that for red cell membrane (- 2100 cal $mole^{-1}$). Both are very similar to octanol (- 2400 cal $mole^{-1}$). This correspondence of the properties of octanol with those of red cell membranes and vesicles accords nicely with the finding of DIAMOND and KATZ [18] that the distribution coefficients water: dipalmitoyl lecithin-vesicles (above the transition temperature) are similar to distribution coefficients water: isobutanol.

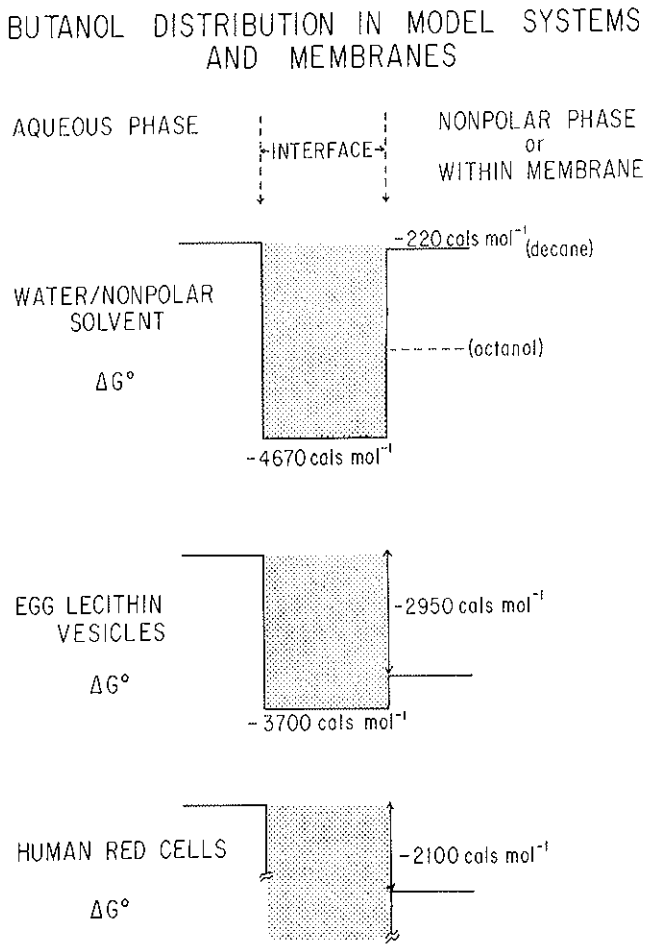


FIG. 3 — Schematic diagram showing the free energy changes for adsorption and partition of butanol in nonpolar solvents, vesicles and red cell membranes.

Figure 4 shows ΔG°, ΔH° and ΔS° for transport of butanol and the hydroxyl group across the interface water:decane. The thermodynamic parameters for water:butane partition given by TANFORD [6] have been subtracted from those for butanol to give the hydroxyl data. It is generally accepted that the cage effect in water is the dominant process which regulates water:hydrocarbon partition. This is clearly illustrated by comparing the entropy changes for butanol with those for the hydroxyl group alone. There is an increase of 21.6 e u in ΔS° for the transfer of butanol into decane, whereas ΔS° decreases by 1.4 e u for the hydroxyl group alone. The difference of 23.0 e u represents the entropy change associated with the transfer of butane into the hydrocarbon phase. Such a large change in ΔS° is to be attributed to the fact that hydrogen bonds in water are readily made in the neighborhoord of a nonpolar hydrocarbon chain and can form a more ordered arrangement than is possible in water alone. There is a minimal entropy change associated with the removal of the hydroxyl group from water to hydrocarbon; instead the change is in ΔH°; its increase of 5300 cal $mole^{-1}$ corresponds roughly to the enthalpy required to break two hydrogen bonds when the hydroxyl group crosses from water into bulk hydrocarbon (1).

With this information, it is possible to interpret the data for partition into egg lecithin vesicles shown in figure 5. We have used butyramide to illustrate the process since we want to use the results in connection with permeation of spherical bilayers of egg phosphatidyl choline. These measurements have been made with the amides and not with alcohols. TING

(1) The partition coefficient of octanol in water:dodecane has been measured by AVEYARD and MITCHELL [8]. Combining their results with those on the temperature dependence of the adsorption of octanol on a water:dodecane interface given by AVEYARD and BRISCOE [19], it is possible to compute ΔH° and ΔS° for all the processes involved in octanol partition into dodecane. The conclusions drawn from these computations are in general agreement with our conclusions concerning butanol, for which ΔH° and ΔS° are not directly available.

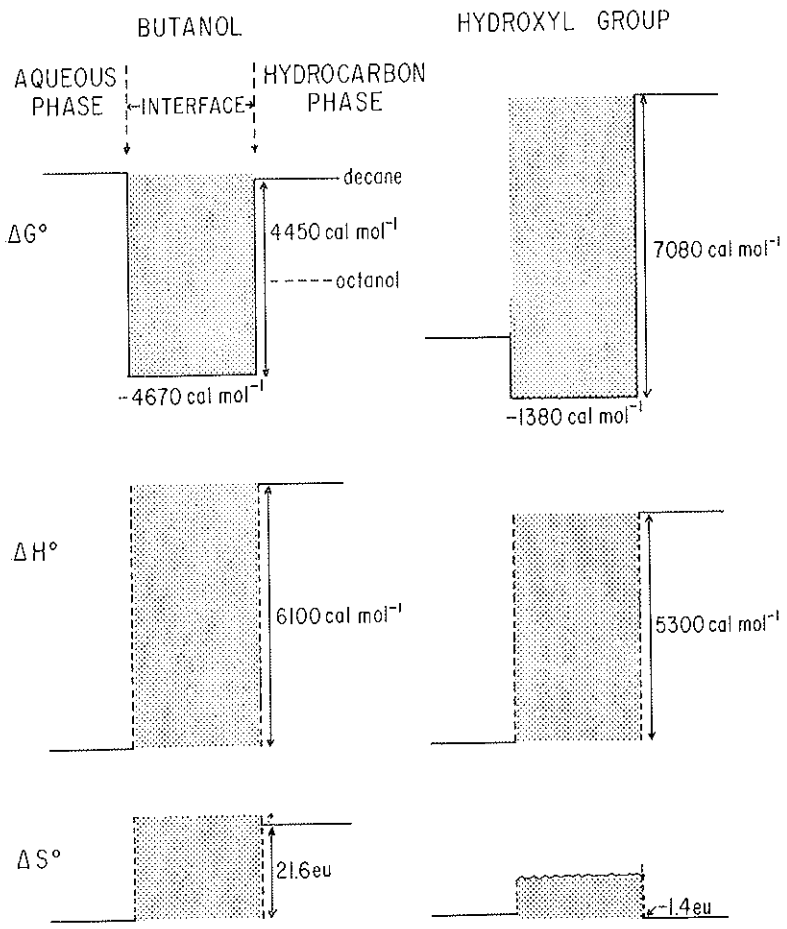


FIG. 4 — Thermodynamic parameters for the partition of butanol and the hydroxyl group from the aqueous phase into decane or octanol.

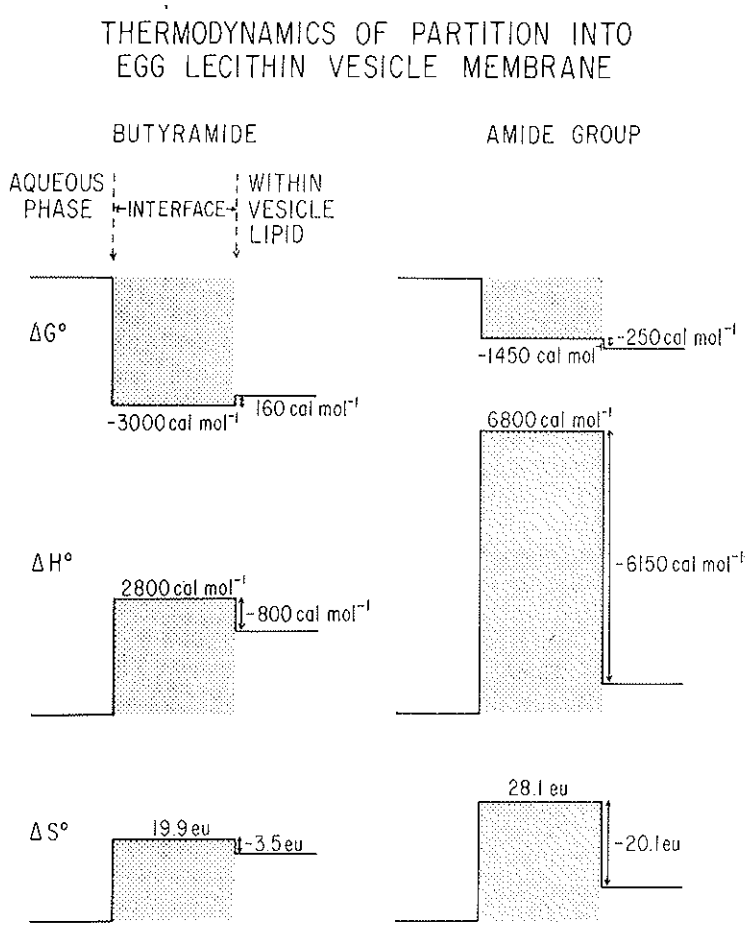


FIG. 5 — Preliminary estimate of thermodynamic parameters for the partition of butyramide and the amide group from water into egg lecithin vesicle membranes.

and SOLOMON [12] measured the temperature dependence of the association of the homologous amides with egg lecithin vesicles. They found ΔG° to be a linear function of chain length, as is the case for the partition coefficients shown in figure 2. Hence the temperature dependence of the intercept at zero chain length can give us a preliminary estimate of ΔH° for the amide group ([2]).

The most interesting clue to the causes underlying butyramide permeation into lecithin vesicles is provided by the enthalpy of the amide group. Transporting the amide group into the interface is up an enthalpy gradient of 6800 cal $mole^{-1}$; transport from the interface to the hydrocarbon is down a gradient that is almost as large, -6150 cal $mole^{-1}$. The upward step can be attributed to disruption of the bonding of adjacent phosphoryl choline head groups at the surface of the vesicle. Conversely, when the amide group is removed from the interface and enters the hydrocarbon, most of the enthalpy is returned to the system. Consideration of the entropy change is consistent with this view, since introduction of the amide group into the interface causes an entropy increase of 28.1 e u, a surprisingly large figure for the introduction of so small a moiety of the solute. Again, much of the entropy gain is lost when the amide group leaves the interface and enters the hydrocarbon. These very marked changes in entropy and enthalpy mean that the interface region is a highly organized and tightly coupled structure, which is entirely consonant with information about lipid bilayers obtained from the behavior of many other probes.

When a hydroxyl group is transferred from water to bulk hydrocarbon, two hydrogen bonds are broken and there is a gain in enthalpy of 5300 cal $mole^{-1}$. This may be contrasted to the total ΔH° for transfer of an amide group from water to the hydrocarbon region of the lipid vesicle. Three hydrogen

([2]) More recent, but not yet final, estimates give 1300 cal $mole^{-1}$ for ΔH° for butyramide and 4780 cal $mole^{-1}$ for the amide group at the interface.

bonds are broken and yet the net enthalpy gain is only 650 cal $mole^{-1}$. From the behavior of the hydroxyl group we would expect an increase of about 8500 cal $mole^{-1}$. This discrepancy means that some other bonds must be formed within the hydrocarbon region. The polar amide group can not interact with the hydrocarbons of the fatty acid chains. It seems much more probable that there are associations between the polar groups themselves. This seems the more likely since the oxygen of the amide is a hydrogen bond acceptor, whereas the nitrogen group of the amide is a hydrogen bond donor. Furthermore the dimerization of amide groups is well known to occur in nonpolar solvents. Thus, LONGSWORTH [20] has shown that dimers and higher polymers of N-methylacetamide are formed in carbon tetrachloride, at solute concentrations in the range of 10^{-1} to 10^{-2} M, whereas dimethylacetamide does not polymerize.

There is an additional feature to be considered in comparing the environment of a polar group in the hydrocarbon region of a lipid vesicle with that in bulk solvent. A solute in decane, for example, may diffuse isotropically, whereas in the hydrocarbon region of the vesicle, the motion is restricted and diffusion along an axis parallel to the hydrocarbon chain would appear to be favored over diffusion perpendicular to this axis. This means that the solute concentration within the vesicle hydrocarbon would not be uniform, some regions being preferred to others, thus promoting hydrogen bond formation in the more densely populated regions.

Further evidence supporting the structured nature of the vesicle hydrocarbon is found by considering the entropy changes. When a hydroxyl group is removed from water and inserted into an alkane, the entropy is reduced by 1.4 units which means that the order introduced into the aqueous region more than counterbalances the disorder arising from the presence of the hydroxyl in the alkane. The situation is reversed in lipid vesicles, where transfer of an amide group from the aqueous region to the hydrocarbon results in an increase of 8 e u. This

difference is attributable to the disorder introduced into the lipid hydrocarbon region and the large value of ΔS° indicates that the lipid region is highly ordered. Comparison of amide partition with hydroxyl partition is justified because ΔG° for butanol water: vesicle partition is -2950 cals mole^{-1} (figure 3) as compared to -2840 cals mole^{-1} for butyramide (figure 5).

In order to complete the picture it is necessary to examine the effect of the hydrocarbon moiety of butyramide on the partition process. Whereas amide partition was dominated by the interface, interactions with water are of greater importance for methylene group partition. ΔH° in the interface is some 4000 cal mole^{-1} less for butyramide than for the amide group alone, which means that ΔH° is negative for methylene transfer to the interface. Preliminary computations of TING and SOLOMON [12] give $\Delta H^\circ = -1000$ cal mole^{-1} per methylene group (water: interface), which is consistent with the butyramide-amide difference. From the data given by TANFORD [6] we can determine that ΔH° for methylene transfer water: alkane is -850 cal mole^{-1}. This enthalpy change can be attributed to the formation of new hydrogen bonds by water molecules which had previously been adjacent to the methylene group. A similar explanation should apply for the methylene transfer, water: interface.

ΔS° for butyramide water: interface is 19.9 e u, 8.2 e u less than ΔS° for amide partition. Thus transfer of the methylene group out of water into the interface results in an increase in order, contrary to the decreased order when methylene groups are transferred water: alkane. The preliminary data of TING and SOLOMON give $\Delta S^\circ = -2$ e u per methylene group for the transfer water: interface. For water: alkane the data of TANFORD give a large positive entropy change which arises from the more orderly array of water in the neighborhood of the methylene group, as previously discussed.

To understand the unexpected decrease in ΔS° for methylene transfer water: interface it is necessary to examine events at the interface rather than in the water. As butyr-

amide molecules enter the interface region, the methylene groups will intercalate into vesicle hydrocarbon, leaving the amide groups at the interface. In view of the ability of amides to dimerize, hydrogen bonds will probably form with butyramides in the aqueous region adjacent to the adsorbed group. Dimerization should be promoted because the environment of the adsorbed amide group is significantly less polar than bulk water. This process should result in an increased concentration of butyramide methylene groups between the polar heads of the phospholipid. The presence of the methylene groups in this region reduces the polarity and the dielectric constant. The force between the electrostatic charges on the head group is inversely proportional to the dielectric constant and thus should increase as $-CH_2-$ groups penetrate the space between the charged phosphoryl choline head groups. If the interactions between the charged groups are attractive, they will be increased in magnitude, and the decrease in entropy will result from this increased order. Conversely, if the forces between the charges are repulsive, the repulsion will increase and the freedom of motion of one positive charged choline with respect to its neighboring choline will be restricted. The system will be more ordered in either configuration, thus accounting for the decreased entropy.

One final question may he approached in considering the partition coefficients. Why are the solubility properties of the vesicle hydrocarbon so different from those of decane? ΔG° for water:decane partition of butanol is -200 cal mole^{-1}, whereas ΔG° for water:vesicle-hydrocarbon partition for butyramide is -1700 cal mole^{-1}. The difference may not be attributed to the difference between the hydroxyl and amide groups, since the preliminary data of TING and SOLOMON indicate that the water:vesicle-hydrocarbon partition coefficient for butanol is very nearly the same as that for butyramide. One intriguing possibilitty should be considered. Both alcohols and amides associate with one another, as LONGSWORTH has shown [20]. AVEYARD and BRISCOE [19] have observed

auto-association of octanol in dodecanol at concentrations as low as 2 mM. We have already suggested that amide-amide hydrogen bonding is an important factor in explaining the ΔH^o of partition into vesicle hydrocarbon, and pointed out that this association should be favored by the steric restraints of the fatty acid chains which effectively increase local concentrations of solute. Thus if equal volumes of bulk alkane and vesicle hydrocarbon are compared at the same overall concentration of solute, some regions within the vesicle will have a significantly higher solute concentration, thus promoting solute-solute interaction which must be concentration dependent.

One alternative explanation for the large partition coefficient of vesicles should be examined, the possibility of solute hydrogen bonding with the double bonds of the lipid fatty acid chains. PIMENTEL and MCCLELLAN [21] report evidence of hydrogen bonding with acetylenic bonds, so that the possibility of solute bonding to ethylenic groups should be considered. LANGE *et al.* [22] studied the effect of the double bond on amide solubility by measuring the partition of several solutes, including valeramide, in a mixed solvent system consisting of hexadecane, 1-octadecene and 1,7-octadiene. They found that the partition coefficient increased as the mole fractional content of double bonds increased. However, at the highest double bond content they studied, when there were 1.5 double bonds per hydrocarbon chain, the partition coefficient water:hydrocarbon was more than two orders of magnitude lower than the partition coefficient water:lecithin-vesicle which they also determined. Thus chemical interactions between amides and double bonds do not appear to be the determining factor in amide partition into vesicle hydrocarbon.

If the high partition coefficient of butyramide into lipid vesicles is to attributed to dimer formation, we would expect that the partition coefficient would reveal some sort of concentration dependence. Exactly how the concentration dependence would be reflected in the partition coefficient is not immediately obvious. The problem is particularly complex because partition

may be mediated by dimerization between a mobile butyramide and one fixed at the interface and because the concentration within the lipid hydrocarbon is not uniform.

Dynamic aspects of permeation

POZNANSKY *et al.* [23] have prepared spherical lipid vesicles according to the method of JUNG [24] and measured their permeation by the homologous monoamides. They have plotted the permeability coefficient, ω ($P_d = \omega RT$), as a function of solute molar volume, $\bar{V}_s$, and their graph is shown as figure 6. There is a sharply defined permeability minimum at acetamide, which means that there must be two rate limiting processes, one declining with molar volume while the other increases. As we have seen, the logarithm of lipid solubility increases linearly with chain length, as illustrated in the bottom graph in figure 7 for the water:ether system, using the data of COLLANDER [25]. POZNANSKY *et al.* divided the permeability coefficient by K_{ether}, and found that ln (P_d/K_{ether}) fell linearly with molar volume as illustrated in the top graph in figure 7. They identified the second rate limiting process as steric hindrance which increases regularly with the solute molar volume.

The reason that these two processes are delineated so clearly by logarithmic functions is that: 1) the free energy of partition, $\Delta G^o = -RT \ln K_{ether}$, is linearly related to chain length as already discussed and 2) the free energy of activation of the permeation process is related to molar volume as will be shown below. ZWOLINSKI *et al.* [2] expressed the permeability coefficient in terms of rate constants as

$$P_d = (k_{sm} k_m \lambda)/(2k_m + mk_{ms}) \qquad (4)$$

in which the symbols have the meanings given with equation 1; m is the number of jumps across the membrane ($\backsimeq \delta/\lambda$). Since

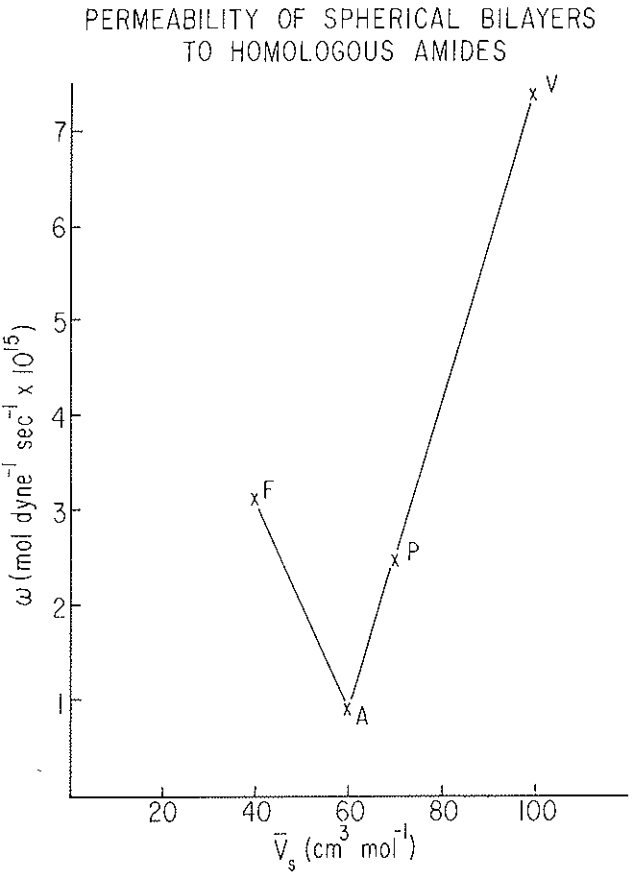


FIG. 6 — Relation of the permeability coefficient, ω, for amide permeation of spherical lipid, bilayers to solute partial molar volume, $\bar{V}_s$. F is formamide; A, acetamide; P, propionamide and V, valeramide.

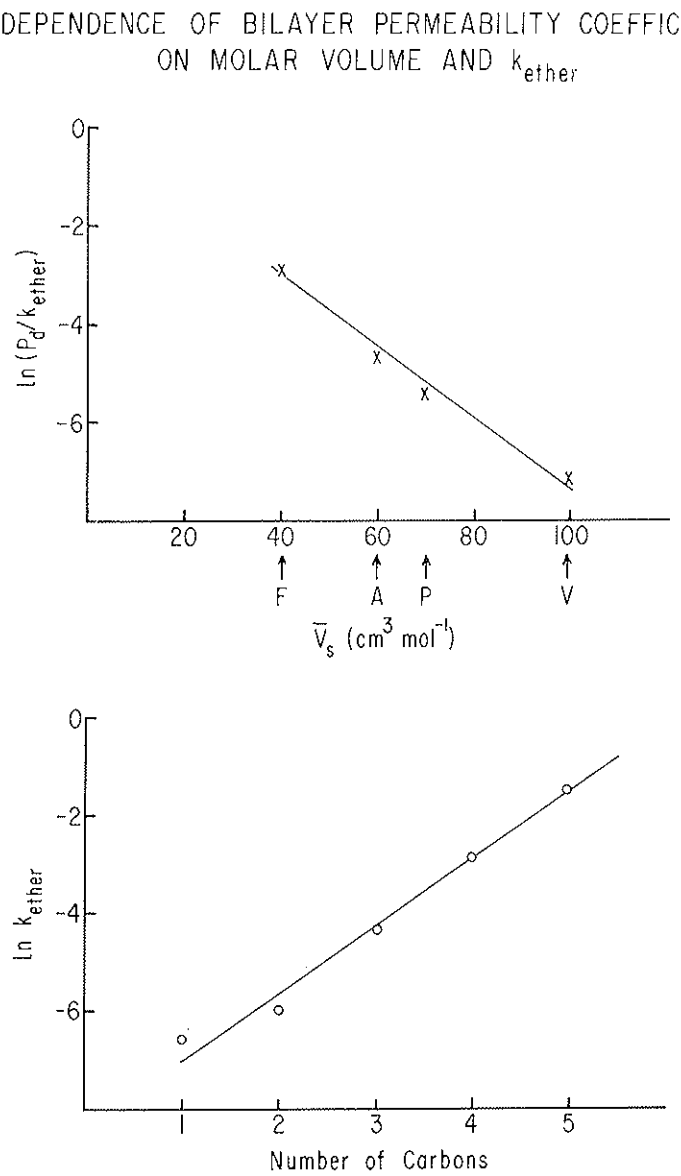


FIG. 7 — Top: Relation of $\ln(P_d/K_{ether})$ to solute molar volume for the solutes specified in figure 6.
Bottom: Relation of $\ln K_{ether}$ to chain length for the alkane monoamides.

the partition coefficient $K = k_{sm}/k_{ms}$, equation 4 may be written as

$$P_d = (k_m K\lambda)/(2K[k_m/k_{sm}] + m) \qquad (5)$$

Each rate constant may be expressed in terms of the free energy for activation of the activated complex, $\Delta G^{\ddagger}$, since $k_i = (kT/h)e^{-\Delta G_i^{\ddagger}/RT}$, in which k and h are Boltzmann's and Planck's constants.

Values of several components of equation 5 are already known for butyramide, the solute for which we will solve the equation. K has been determined, by TING and SOLOMON [11] to be 2.34 in concentration units, which are the appropriate ones for rate equations, λ is 5.2 Å. Taking the membrane thickness as 62 Å as given by CHERRY and CHAPMAN [26], approximately 12 jumps are required to cross the membrane. Reserving one jump for each interface, $m = 10$. It remains to determine k_m and k_{sm}. When P_d^{-1} is plotted against K^{-1}, as done in figure 8, according to equation 1, the intercept is $2/k_{sm}\lambda$. The points in this plot do not lie on a straight line, so the extrapolation is not straightforward. The permeability coefficient of 11.8×10^{-5} cm sec^{-1} for butyramide in figure 8 has been determined by interpolation in figure 7 at the butyramide molar volume of 84.4 cm^3 mole^{-1}. This point is denoted in figure 8 by a dotted circle. Continuation of the smooth curve through the data points gives the most probable y intercept as 2.5×10^3 sec cm^{-1}. A less probable intercept is 0 which is obtained by drawing the line through the two points closest to the origin. The area between these two values has been stippled to indicate the uncertainty; we have chosen the larger value for our calculation.

Using this value for the intercept, denoted as R_{sm} (since $R_i = P_i^{-1}$), the ratio k_m/k_{sm} is given by

$$(k_m/k_{sm}) = mR_{sm}/(2K[R_d - R_{sm}]) \qquad (6)$$

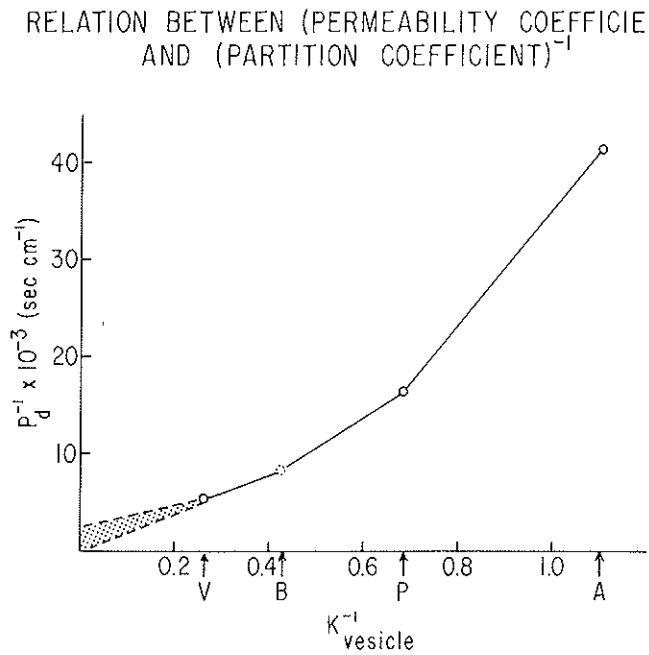


FIG. 8 — Relation between the inverse permeability coefficient for alkane monoamide permeation of spherical bilayers and the inverse partition coefficient (water: vesicle hydrocarbon). B represents butyramide.

In deriving equation 6, we have cancelled λ, thus assuming that the dependence of k_{sm} upon λ is the same as the dependence of k_m upon λ, which seems not unreasonable since both rate coefficients are expected to depend upon solute molar volume. Nonetheless, the major uncertainty in the computation is the assumption that k_{sm} does not depend upon solute molar volume to any higher power of λ. Using equation 6 and figure 8, $k_m/k_{sm} = 0.89$ for butyramide. Equation 5 may now be solved leading to $k_m = 1.37 \times 10^4$ sec^{-1} for butyramide diffusion in spherical bilayer hydrocarbon. The corresponding activation energy is $\Delta G^{\ddagger}_m = 11.55$ kcal mole^{-1}. For the interface $\Delta G^{\ddagger}_{sm} = 11.49$ kcal mole^{-1}, virtually the same figure.

Another approximation may now be made which is useful in understanding the processes which govern the temperature dependence of permeation. The first term in the denominator of equation 5, $2K(k_m/k_{sm}) = 4.16$, which is appreciably smaller than $m = 10$. Neglecting the k_m/k_{sm} term,

$$P_d \backsimeq K k_m \lambda/m \backsimeq K k_m/\delta \tag{7}$$

On the basis of this approximation, we are in a position to compute all the thermodynamic parameters that describe the permeation of butyramide across a spherical lipid bilayer.

One additional piece of experimental information is required. POZNANSKY *et al.* [23] have measured the apparent enthalpy of activation of butyramide permeation across liposomes according to the method of COHEN and BANGHAM [27] and computed $\Delta H'$ ($d(\ln P_d/T)/d(1/T) = -\Delta H'/R$). Because of the multilamellar nature of the liposomes, they are not an ideal model for lipid bilayer permeation. However POZNANSKY *et al.* measured the apparent activation enthalpy of urea permeation across both spherical lipid bilayers and liposomes and found the enthalpies to be in good agreement. Thus we may take their value of $\Delta H' = 12.7$ kcal mole^{-1} as a reasonable estimate of the true figure for butyramide.

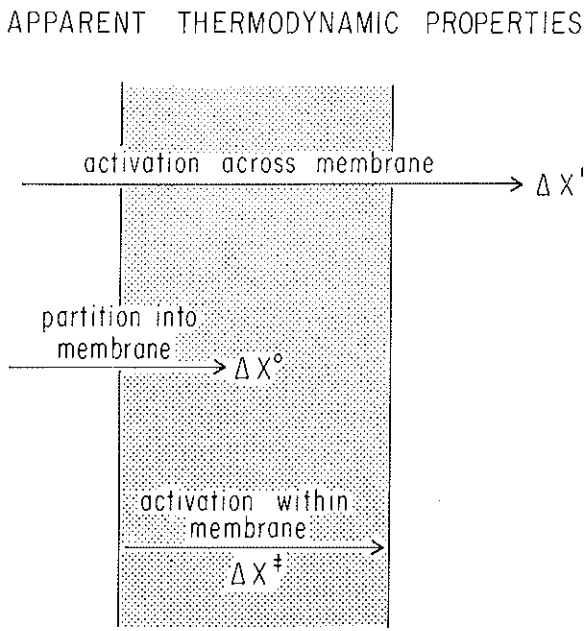
Equation 7 may be written as

$$P_d \backsimeq (kT/h\delta)e^{-(\Delta G^o + \Delta G^{\ddagger})/RT} \tag{8}$$

When $\ln P_d/T$ is differentiated with respect to T^{-1}, we obtain

$$\Delta H' \backsimeq \Delta H^o + \Delta H^{\ddagger} \tag{9}$$

and a similar relation holds for ΔG and ΔS. When the value for $\Delta H'$ is combined with $\Delta G^{\ddagger}_m$, and the preliminary values of ΔH^o, ΔS^o and ΔG^o from TING and SOLOMON, we can compute the remaining thermodynamic parameters which are



	$\Delta X'$	ΔX°	$\Delta X^{\ddagger}$	
H	12700	2050	10650	cal mol^{-1}
S	13.6	16.7	-3.1	eu
G	8700	-2850	11550	cal mol^{-1}

FIG. 9 — Preliminary estimate of apparent thermodynamic properties for butyramide permeation of spherical bilayers.

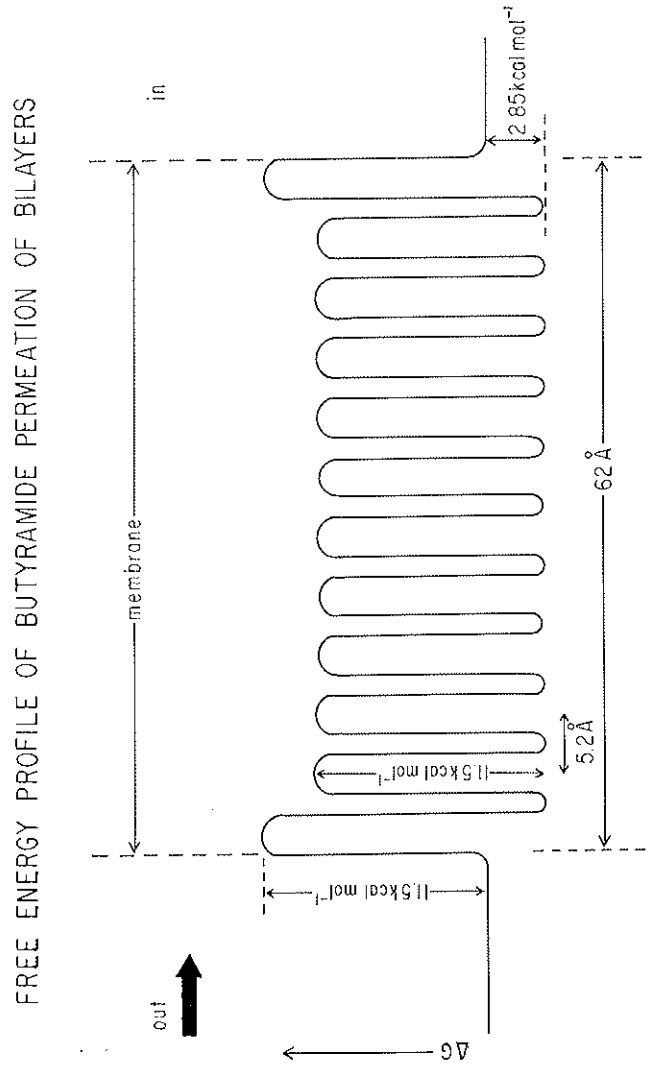


FIG. 10 — Free energy profile for butyramide permeation of spherical bilayers.

shown in figure 9. It is also now possible, with the aid of $\Delta G^{\ddagger}_{sm}$, to generate the free energy profile in figure 10 which shows all the steps involved in the transport of butyramide across a lipid bilayer ([3]).

There are several interesting conclusions that may be drawn from the rate constants and the energy barriers illustrated in figure 10. The energy barrier for crossing the interface is large, even though the step is down a free energy difference of 2.85 kcal mole^{-1}. This means that the molecular organization of the phospholipid molecules at the interface is highly organized and that strong cohesive forces keep the phospholipids together. This high order of organization must persist for much of the distance across the membrane because the average free energy of activation of the steps within the membrane is not perceptibly different from that for the interface step. It has been generally accepted that the viscosity of the membrane is less at its core than near the interface, based primarily on electron spin resonance studies of SEELIG [28]. However, recently SEELIG and SEELIG [29] and SMITH *et al.* [30] have used NMR methods to indicate that order persists much further down the chain than had previously been thought. Our evidence is entirely consonant with these results.

ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the contributions of members of the Biophysical Laboratory to the development of the ideas in this paper, particularly Mr. M. JENNINGS and Drs. E. A. DAWIDOWICZ, Y. LANGE, D. KIRKWOOD and J. HERZFELD. The unpublished results which have been presented come from research supported in part by the National Institutes of Health under grants 5 RO1 HL14820-03, 5 RO1 GM15692-07 and 2 TO1 GM00782-16.

([3]) These compilations assume that data obtained with egg lecithin vesicles may be applied to egg lecithin bilayers and make no allowance for the differences in thickness, shape and solvent content, whose effects on the thermodynamic parameters are, in any case, not known.

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DISCUSSION

Chairman: Prof. A. K. SOLOMON

STAVERMAN

I did not hear to what extent you have controlled that the states in those permeation measurements are stationary. You would expect, particularly in the case where you have a concentration dependence you mention of the chemical composition of the solution, the state will never become stationary. You will have constant changing DG and DH during the process, or did you in any way check that the state was stationary?

SOLOMON

We have to consider the order in which these experiments were done. When we made the vesicle permeability masurements, we did not know that there was dimerization and we thought we had a stationary state because the permeability coefficient appeared to be independent of the time of observation. We measured the permeability coefficients that I have presented at a single concentration, 20 mM. It is now clear that one of the tests of whether dimerization indeed takes place will come from a study of the dependence of the rate constant on concentration. This we have not yet done, and obviously it should be done. Furthermore, one has to look at the dependence of the partition coefficient on concentration. This is not a trivial question because if the dimerization takes place at the

interface, it is not clear what the kinetics of the process will be. I've thought about it a little, but all I know so far is that it isn't simple to predict how the partition coefficient will depend upon solute concentration. Some preliminary measurements were made at solute concentrations of 2 to 8 mM with evidence that they were in reasonable agreement with those at 20 mM).

STAVERMAN

I understand that you have just taken the state as it happened, and you have not checked whether the state was gradually changing. Of course, this gives you useful average coefficients.

SOLOMON

Indeed this is so — we have not — but we waited some minutes for the system to reach an apparent steady state before we made our measurement. The difficulty with this method is that you destroy the bilayer membrane when you take your sample, and you have to try to form a great many membranes to get a good one. Hence, the stationary state problem is not trivial, but I think it's got to be dealt with. We've got to see whether there is a concentration dependence of the fluxes.

SPIEGLER

Regarding your slide about the relation between permeability coefficient and a partition coefficient, it is certainly very satisfying to see that there is a monotonous relationship, that the higher the partition coefficient, the higher also the permeability. However, it seems to me that one had to be careful when comparing permeates which are not of chemically similar nature, because there are cases

known where the partition coefficient does not vary monotonously with the permeability, where you have a very favourable partition coefficient but not much permeability. I am just wondering whether your difficulty in extrapolating this curve may not be related to this problem.

SOLOMON

There are two things. The permeability coefficient does not really vary monotonically with the partition coefficient though its inverse does increase monotonically with the inverse of the partition coefficient. Obviously if you deal with molecules with different polar groups you will obscure the simplicities which we have observed with the amides. I agree that there are several factors that control permeation; either three-rate constants, or two-rate constants and a partition coefficient. You have to find some means of separating these factors in order to understand the physical and chemical basis of the process.

KEDEM

Have you possibly considered the dimerization of small peptides? There is a lot of interest in trying to estimate the pore size in organelle membranes by looking at a series of peptides. The relation may not be as simple as often assumed.

SOLOMON

We are also anxious to study the interactions of polypeptides. We have a set of preliminary data which PETER TING and I have obtained, on a linear series of polydipeptides, gly-pro I think, and our measurements went from $n=1$ up to 4. The plot of ΔG° for partition, against n, appears to be linear with respect to NPN

displacement. We have also studied the same polypeptides when they have been circularized, and it appears that the cyclic ones are somewhat less lipid soluble than the linear ones, probably because of the end group, in the linear polymers. It looks as if there is going to be some regularity in the partition coefficients. We hope to carry out the same kind of studies on the interactions between the polypeptides and the lipids, as with the amides.

MEARES

Can you explain, please, why, when considering the uptake of butanol into a membrane, you accounted for the heat as being due to the loss of two hydrogen bonds? I can see an argument for one, and I can see an argument for one and a half, but I can't find an argument for two.

SOLOMON

My understanding is that with water there are two donors with the two hydrogens, and there is an acceptor with the oxygen. Removal of the hydroxyl would mean removal of an acceptor with the oxygen and a donor with the hydrogen, but this may not be a water-tight argument.

MEARES

May I come back on that? There are two ion pairs on the oxygen. If all possible hydrogen bonds were formed, there would be one through the hydroxyl hydrogen and two, with the hydrogens of water molecules, through the oxygen. Probably the steric arrangement is such that you may not get all three formed. When you have taken the butanol into the oil phase, you have the « loose ends » of the hydrogen bonds in the water which will recouple in some way.

Thus you will get a part of the energy back. That is why I feel that if you broke two and remade one, you would have a net loss of one, or if you lost three and remade one and a half, you would have a net loss of one and a half.

SOLOMON

I would agree with this since I wouldn't argue in quantitative terms about the actual number of calories per mole per hydrogen bond, expecting very high accuracy. We are really describing things in relatively simple terms. The exact detailed description of each step, of course, will be much more complicated.

MEARES

Your figure of 5 kcal/mol appears small for two really strongly formed hydrogen bonds.

SOLOMON

My general feeling is that there is a approximate range of 2 or 3 kilo-calories per hydrogen bond so anywhere in this range seems not unreasonable.

[*10*] IV, 1 - *Solomon* - p. 33

KINETICS AND ENERGETICS OF CALCIUM TRANSPORT IN SQUID GIANT AXONS

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The central importance of calcium in the regulation of many aspects of cell function and behaviour provides a strong reason for studying the mechanisms that regulate and control the concentration of ionized calcium inside cells. In general, these mechanisms involve the movement of calcium ions across cell membranes either those of special intracellular compartments such as the mitochondria and sarcoplasmic reticulum or across the surface membrane. We shall be hearing in detail about calcium pumps in the sarcoplasmic reticulum later in the meeting and it is my task to discuss calcium movements across the surface membranes of nerve cells. Although the intracellular binding systems may be very important in the short term regulation of intracellular ionized calcium, in the long term any calcium that enters the cell must be expelled and this seems to be effected by the surface membrane.

Unfortunately the movements of calcium across the cell membrane are usually small and difficult to follow and our knowledge of calcium transport is still at a rather elementary stage. Nevertheless, recent experiments on the large nerve fibres of squid have enabled both active and passive components of calcium transport to be characterized (see BAKER, 1972).

Many of the properties of the calcium transporting systems present in the squid giant axon find close parallels in mammalian cells which suggest that studies on the squid giant axon may have a general relevance. I should like to review this work under three headings: 1) Evidence that only a small fraction of the intracellular calcium is ionized. 2) Evidence for a specific voltage-sensitive calcium permeability channel controlling the movement of calcium ions into the axon down the existing electrochemical gradient, and 3) Evidence for a sodium-calcium counter transport system maintaining the low intracellular concentration of ionized calcium. The properties of sodium-calcium counter transport share many features in common with other sodium-dependent co- and counter-transport systems and some of these will be discussed with particular reference to the possibility that metabolism plays a specific role in modulating many gradient linked transport systems over and above that of maintaining the necessary electrochemical gradients through the operation of the sodium-potassium exchange pump.

1) *The calcium gradient*

It is a relatively simple matter to obtain from a squid giant axon a pure sample of nerve protoplasm. The axon is cut open at one end and by sqeezing gently towards the cut end axoplasm can be extruded rather like toothpaste from a tube (BEAR, SCHMITT and YOUNG, 1937). Analysis of this axoplasm gives values for intracellular Ca and Mg of about 0.5 and 7.0 m mole/kg axoplasm respectively. As the concentration of these cations in the external solution is about 10 mM for Ca and 55 mM for Mg and the interior of the axon is negative with respect to the external solution, it is clear that for both Ca and Mg the electrochemical gradient is inwardly directed. For calcium, the electrochemical gradient is much steeper than the above figures suggest because much of the intracellular calcium

is bound. Evidence for the binding of calcium in axoplasm is based on the following observations:

a) Ca does not diffuse in axoplasm. A small patch of ^{45}Ca injected into axoplasm is still located close to the site of injection 4-6 hours later. A patch of ^{28}Mg introduced at the same time broadens considerably suggesting that much less of the Mg is bound (BAKER and CRAWFORD, 1972).

b) Ca does not move in axoplasm under the influence of an electric field (Fig. 1). If, after injection of a patch of ^{45}Ca, an axon is subjected to a longitudinal electric field, the patch of ^{45}Ca remains located at the site of injection whereas a

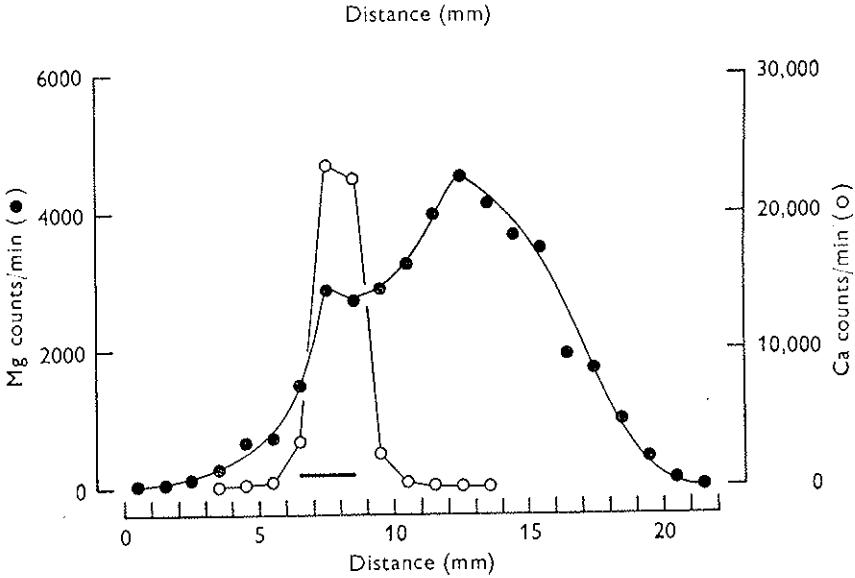

FIG. 1 — Mobility of Ca and Mg in axoplasm. The injector was inserted from the left-hand side. The short horizontal bar marks the site of injection. 1.025 V was applied over 4.3 cm for 240 min. Temp. 19°C. Axon diameter 790 µm. If the loss of ^{28}Mg from the axon is neglected and if Mg has the same mobility in axoplasm as in free solution, the peak should have moved 13.7 mm. (From BAKER and CRAWFORD, 1972).

similar patch of ^{28}Mg both broadens and moves towards the cathode (Fig. 1).

These observations suggest that although about 1/3-1/2 of the intracellular magnesium is probably ionized, the concentration of ionized Ca must be very low and certainly not greater than 5-10 μM. A discussion of the axoplasmic systems binding calcium is outside the scope of this meeting; but it should be noted that they include mitochondrial and non-mitochondrial binding systems that are sensitive to both the metabolic state of the cell and also to the intracellular ionic composition (BAKER and SCHLAEPFER, 1975).

New information on the level of ionized Ca in axoplasm was obtained from experiments using the calcium-sensitive photoprotein aequorin. This protein was first isolated from the jellyfish *Aequorea forskalea* by SHIMOMURA, JOHNSON and SAIGA (1963) who showed that the mechanism of light generation is quite unusual in that the only co-factor required for the light-generating reaction is the Ca ion — or more accurately two calcium ions. The reaction is extremely sensitive to Ca and it is possible to detect Ca concentrations as low as 10^{-8} M. Injection of purified aequorin into squid axons provides a very simple reporter molecule for the level of ionized Ca inside the cell. By injecting various Ca-EGTA buffers into aequorin-loaded axons it is possible to find a buffer that produces no change in light emission and BAKER, HODGKIN and RIDGWAY (1971) argued that this buffer must be stabilizing within the axon on ionized Ca concentration very close to that which normally exists inside the cell. Experiments of this type gave values for axoplasmic ionized Ca in the region 0.1-0.5 μM. It follows that less than 0.1% of the total intracellular Ca is ionized and the ratio calcium activity outside/calcium activity inside is about 10,000:1. Coupled with the data already described for Mg, it seems that inside the axon, the ratio ionized Mg/ionized Ca is probably also about 10,000:1.

The very steep inwardly directed Ca gradient, coupled with

the extremely low absolute concentration of intracellular Ca, has been exploited by cells to provide a system for controlling many aspects of cell function and behaviour. If the membrane permeability to Ca is increased in some way, for instance by chemical or electrical means, Ca will move into the cell raising the intracellular concentration of ionized calcium. A rather small entry of Ca can produce a large percentage change in intracellular ionized calcium. It is of interest to note that apart from the well known action of Ca in activating contraction, many of the actions of intracellular Ca are on membrane systems. Thus a rise in intracellular Ca in nerve terminals seems to promote the release of neurotransmitter substances by a process of membrane fusion known as exocytosis and in other cells a rise in intracellular Ca can lead to alteration in the permeability properties of membranes including increasing the potassium permeability of the cell membrane in some cells (GARDOS, 1958) and reducing the effectiveness of low resistance junctions and hence of electrical coupling between cardiac and many epithelial cells (see WEIDMANN, this symposium).

2) *Voltage-sensitive changes in the permeability to calcium*

The permeability of nerve cell membranes to calcium is not constant but can alter in response to changes in membrane potential and the squid giant axon provides an ideal preparation for studying the properties of these voltage-sensitive changes in permeability. An increased uptake of calcium during nervous activity was first reported by HODGKIN and KEYNES (1957). They measured the influx of ^{45}Ca into pairs of squid axons and it was necessary to cause one axon of the pair to carry a large number of impulses in order to obtain a measurably greater Ca uptake in stimulated axon as compared to their unstimulated controls. Nevertheless, the results show quite clearly that nervous activity increases calcium uptake and this uptake increases in a roughly linear manner with increasing

external Ca concentrations. Stimulation had no effect on the efflux of ^{45}Ca. In an axon immersed in sea water at 20°C, the extra entry of calcium averaged 0.01 pmole/cm^2 impulse or about 1/400 of the net entry of Na under the same conditions.

Intracellular aequorin provides a much more sensitive method for detecting calcium entry and under suitable conditions it is possible to detect a rise in internal Ca during a single nerve impulse (BAKER, HODGKIN and RIDGWAY, 1971; HALLETT and CARBONE, 1972).

A feature of the records that are obtained with aequorin is that the light output from the axon increases during stimulation: but falls back close to its initial value when stimulation ceases. The increased light output seems to result from entry of calcium into the cell because it is not seen when axons conduct impulses in solutions lacking Ca ions. The return after stimulation to the initial rate of light emission probably reflects uptake of Ca by intracellular binding systems.

Depolarization is known to open channels through which sodium and potassium ions can cross the nerve cell membrane, the well-known Na and K channels that underlie the action potential, and it is pertinent to enquire whether the observed entry of Ca occurs through either or both of these channels or whether Ca enters by a separate route. The aequorin reaction is too slow to enable Ca entry to be followed directly; but by measuring the light output from intracellular aequorin in response to voltage clamp pulses of different duration, BAKER, HODGKIN and RIDGWAY (1971) were able to demonstrate two phases of Ca entry (Fig. 2). An early phase that occurred at roughly the same time as the rise in sodium conductance and a later phase that roughly parallelled the rise in potassium conductance. Although the time course is consistent with Ca entry occurring through both the Na and K channels, other evidence suggests that this explanation is only partly correct (BAKER, MEVES and RIDGWAY, 1973a-b). This evidence rests heavily on pharmacological methods of dissecting the calcium

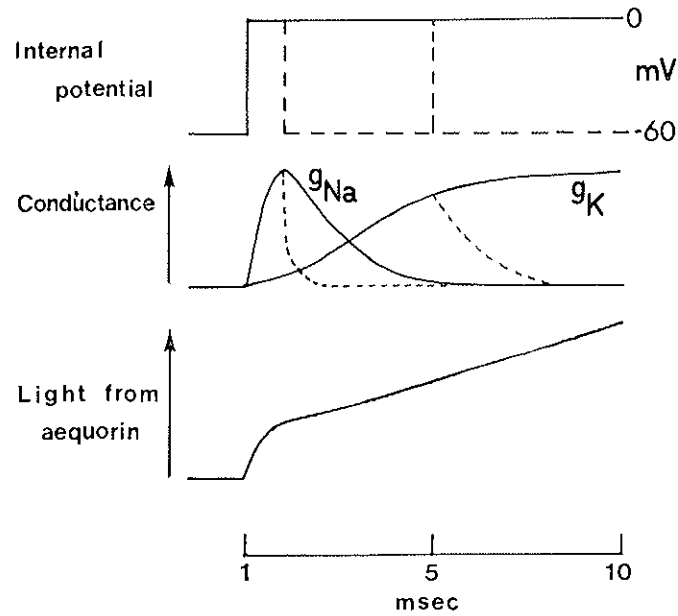

FIG. 2 — Diagramatic representation of the method used by BAKER, HODGKIN and RIDGWAY (1971) to elucidate the time course of Ca entry in response to depolarization. Changes in the light output from intracellular aequorin are shown in response to repetitive depolarization from −60 mV to zero potential for different lengths of time. The time courses of the sodium and potassium conductances in response to a maintained depolarization of the same magnitude are shown for comparison.

entry. The sodium conductance is blocked very specifically by low concentrations of tetrodotoxin (TTX) and application of TTX in concentrations adequate to block the Na conductance also blocks the early phase of Ca entry suggesting that this component of uptake probably reflects Ca entry through the sodium channels. The late phase of Ca entry cannot be explained so easily. Agents such as tetraethylammonium ions (TEA) that block the K channel have no effect on the late phase of Ca entry whereas various divalent cations including Mg^{2+}, Mn^{2+}, Ni^{2+} and Co^{2+} and the organic calcium antagonists

iproveratril and D600 block the late phase of calcium entry without affecting the K channels appreciably (see Fig. 3). These experiments are most consistent with the existence of a separate Ca channel which has been called tre " late Ca channel " to distinguish it from the early TTX-sensitive entry of Ca through the Na channel. Another difference between the late Ca channels and the potassium channels is their response to maintained depolarization. Both channels ultimately cease to conduct ions, a process knows as inactivation, but the time coures of onset and especially recovery from inactivation are quite different.

The main criticism of these conclusions is that they rely entirely on the use of aequorin and it would be more satisfactory if they could be corroborated by some other technique.

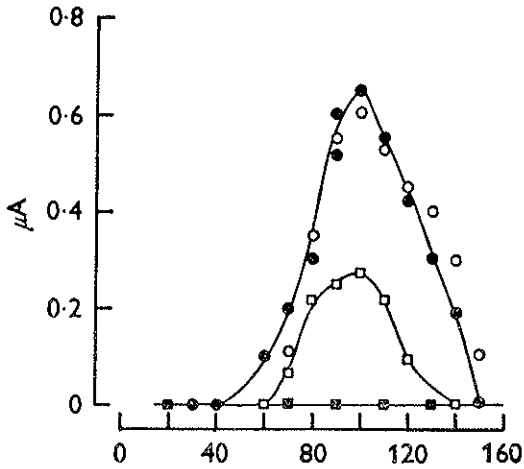

FIG. 3 — Inhibition of Ca entry through the "late Ca channel" by cobaltous ions. The light output (μA) from intracellular aequorin is plotted as a function of the depolarizing pulse amplitude (mV) expressed relative to the resting potential. O, Artificial sea water (ASW) containing 112 mM Ca; □, ASW with 112 mC Ca and 5 mM Co^{2+}; ■, ASW with 112 mM Ca and 25 mM Co^{2+}; ●, ASW containing 112 mM Ca after removal of cobalt. All solutions contained 1.6 μM tetrodotoxin to block the Na channels. Axon diameter 675 μm. Temp. 20.5°C (from BAKER, MEVES and RIDGWAY, 1973).

It might, for instance, be possible to detect a Ca current associated with Ca entry. This has not so far proved possible in intact squid giant axons, although under certain conditions calcium action potentials can be detected at the squid giant synapse and in a variety of other nerve cell preparations. These action potentials are blocked or reduced by Co, Mn and D600 and calcium entry seems to take place through a channel that exhibits many of the properties of the " late Ca channel " (REUTER, 1973). The only Ca currents that have been characterized in the squid giant axon have been studied in perfused axons that have been specially treated to minimize the currents of Na and K. Under these conditions MEVES and VOGEL (1973) were able to detect a small inward current carried by Ca ions. This inward current showed many features in common with the Na channel including being blocked by TTX and it seems most likely that this current reflects the early phase of Ca entry. Failure to detect any currents associated with the late phase may simply reflect the fact that Ca entry during the second phase occurs over a much longer period of time than the early phase. This would make detection of a " late Ca current " very difficult. It is of course possible that the late entry of Ca may occur in an electroneutral fashion; but this seems unlikely in view of the clear evidence in other tissues for a Ca current with remarkably similar properties to the late phase of Ca entry.

Some independent evidence for the late Ca channel was obtained using a combined voltage clamp electrode and internal glass scintillator (BAKER, COOK and GLITSCH, 1974). The principle of this method is to thread down the long axis of the axon a 100-150 μ diameter rod of glass scintillator. Immersion of the axon in sea water containing ^{45}Ca produces very few scintillations until ^{45}Ca begins to enter the axon when the counts from the scintillator begin to rise in a linear fashion. By winding around the scintillator wires for passing current and measuring potential, it is possible to monitor ^{45}Ca uptake under voltage clamp conditions. Although the technique is

very insensitive, it does show increased ^{45}Ca uptake in response to trains of depolarizing pulses and the increased uptake persists in the presence of enough TTX to block the Na conductance. Hyperpolarizing pulses gave no extra uptake of ^{45}Ca.

Taken together these results suggest that in response to depolarization some Ca enters through the TTX-sensitive Na channel: but the rest enters through a channel that is insensitive to both TTX and TEA and seems to be separate from the well known Na and K channels of the action potential. The relative amounts of Ca entering through the Na channel and " late Ca channel " varies widely. In some cells it is about equal while in others Ca entry through the late Ca channel predominates.

The " late Ca channel " is particularly interesting because its properties parallel very closely those of the mechanism that evokes release of transmitter substances and neurosecretory products from nerve terminals. Thus transmitter release appears to depend on Ca entry and can be activated by iontophoresis of Ca into the nerve terminal (MILEDI, 1973). Transmitter release in response to depolarization is unaffected by both TTX and TEA but is blocked by Mg, Mn, Co and D600. The insensitivity of transmitter release to TTX does not imply that Ca entry through the Na channel is of no significance in release; but merely that Ca entry can occur by a route that persists in the presence of TTX. This route has all the properties of the late Ca channel.

The only area where there is some dispute about whether the properties of the " late Ca channel " and release do alter in parallel is in response to maintained depolarization. Under these conditions the " late Ca channel " inactivates but there is rather conflicting evidence on what happens to transmitter release mechanisms. We have recently obtained evidence that the most likely explanation for the transient release of catecholamines from the depolarized adrenal medulla is that depolarization promotes first activation followed by inactivation of Ca entry in this tissue (BAKER and RINK, 1974). NORDMANN (1975) has interpreted his results on the rat neurohypophysis

in a similar way. The available evidence suggests that inactivation of Ca entry probably does occur in most secretory systems but at widely different rates.

3) *Calcium pumps in the nerve cell membrane. Evidence for Na-Ca counter-transport*

In order to maintain the low intracellular concentration of ionized Ca, calcium ions must be expelled from the cell against a steep chemical and electrical gradient. This process requires energy, the most obvious source of which is cytoplasmic ATP. Poisoning a squid axon with either cyanide or dinitrophenol results in a marked fall in the cytoplasmic ATP content which might be expected to inhibit Ca efflux if it depends directly on ATP. On some occasions a small initial fall in efflux is seen but on all occasions the efflux ultimately rises to a value 5-10 times higher than its resting level (BLAUSTEIN and HODGKIN, 1969). Experiments using aequorin show that the rise in efflux occurs at a time when the intracellular Ca also rises, apparently because Ca is released from mitochondrial binding sites. The rise in intracellular ionized Ca is usually about 30 fold whereas the Ca efflux usually increases about 10 fold. This suggests either that the Ca efflux mechanism has begun to saturate with internal Ca or that the efflux process is less efficient in poisoned cells. Which ever of these explanations is true, and I shall return to this point later, it is pertinent to enquire what, in the apparent absence of ATP, is providing the energy for Ca extrusion.

The first possibility is that the Ca efflux is not really a net efflux at all but is entirely a one for one exchange of internal Ca for external Ca. This is easily tested by examining the effect on Ca efflux of removing external Ca. In the absence of Ca, the Ca efflux from cyanide-poisoned axons is reduced; but about half of the efflux persists in the complete absence of external Ca. This component of the efflux is reduced to less than 10% on replacing external Na by choline or lithium suggesting

that Ca efflux may be coupled to Na influx (BAKER, BLAUSTEIN, HODGKIN and STEINHARDT, 1967): the energy for the uphill movement of Ca being provided by the downhill movement of Na into the axon. Although highly attractive, in order to gain acceptance this model must meet a number of quite stringent requirements.

1) A dependence of Ca efflux on the last traces of ATP must be excluded.

2) The coupling of Na influx to Ca efflux must be such as to provide enough energy to maintain the observed calcium gradient and it must be shown that Na entry is stoichiometrically associated with Ca efflux.

3) The same mechanism must be shown to occur in unpoisoned axons where the ionized Ca is at least one to two orders of magnitude lower than in poisoned axons.

The first point is difficult to exclude in intact axons where the residual ATP may be as high as 100 μM. Some information against a direct involvement of ATP is that injection of the ATP-destroying enzyme apyrase into a cyanide-poisoned axon does not inhibit the large Ca efflux. Another approach is to use axons that have been internally dialysed to lower their ATP content to less than 5 μM. The Ca efflux from these axons has components that depend on external Ca and external Na respectively, suggesting that a Na-dependent Ca efflux can persist at very low ATP levels; but it is still possible that the small residual ATP concentration is involved.

The second point is quantitative. If 2 Na_o exchanged with 1 Ca_i, the ratio $[Ca]_i/[Ca]_o$ should approach $[Na]_i^2/[Na]_o^2$. As $[Na]_i/[Na]_o$ is roughly 1/10, this would give $[Ca]_i/[Ca]_o$ of 1/100 and for an axon immersed in sea water containing 10 mM Ca, $[Ca]_i$ should approach 100 μM. Experiments with aequorin indicate that in cyanide-poisoned axons Ca_i may be between 10 and 100 μM which suggests that a simple exchange of 2 Na_o for 1 Ca_i may suffice to maintain

the observed $[Ca]_i$ in poisoned axons; but it would not be adequate to maintain the much lower $[Ca]_i$ found in unpoisoned axons. More complex exchange schemes can give values for $[Ca]_i$ close to those observed. For example if 3 Na_o exchange for 1 Ca_i, the exchange will no longer be electroneutral and one Na ion will enter the axon down both a chemical and electrical gradient. In this case

$$\frac{[Ca]_i}{[Ca]_o} = \frac{[Na]_i^3}{[Na]_o^3} e^{VF/RT}.$$

Alternatively, electroneutrality may be maintained by $1K_i$ ion moving out of the axon with each Ca_i ion in which case

$$\frac{[Ca]_i}{[Ca]_o} = \frac{[Na]_i^3}{[Na]_o^3} \frac{[K]_o}{[K]_i}$$

Both these last two models would give values of $[Ca]_i$ that approximate those found in unpoisoned axons.

There is excellent experimental evidence for some form of Na-Ca exchange in unpoisoned axons. The Ca efflux consists of Ca-dependent and Na-dependent components and if axons are loaded with Na and immersed in Na-free media, it is possible to detect a Ca-dependent Na efflux that is associated with and seems to be coupled to a net inflow of Ca into the axon. This observation is consistent with transmembrane Ca fluxes being determined by the magnitude and direction of the electrochemical gradient for Na. Under conditions where the Na gradient is reversed, more than 1 Na ion moves out of the axon in exchange for each Ca ion that enters. The available data is more consistent with an exchange of 2 Na_i for 1 Ca_o than an exchange of 3 Na_i for 1 Ca_o; but the data is not good enough to exclude an exchange of 3 Na_i for 1 Ca_o.

To return to the more physiological condition of Ca moving out of the axon in a Na-dependent fashion, if more than 1 Na ion is required for the extrusion of each Ca ion, some evidence for this might be found in the shape of the curve relating Ca efflux to the external Na concentration. Unfortunately, this is rather difficult to examine experimentally as lowering $[Na]_o$ leads to uptake of Ca and alteration, in the specific activity of Ca inside the axon. In order to avoid this complication, it is usually necessary to work in the absence of external Ca which, of course, means that the extrusion of Ca is no longer against such a steep chemical gradient. In the absence of external Ca, the curve relating Ca efflux to external Na closely approximates a section of a rectangular hyperbola over the range of Na_o concentrations from 25 mM to 460 mM. Below 25 mM, the fluxes are too small to measure accurately. The shape of the Na-activation curve in unpoisoned axons contrasts sharply with fully poisoned axons where the same curve is markedly sigmoid (BAKER and GLITSCH, 1973).

These observations reveal an interesting difference in the properties of the Ca efflux mechanism in unpoisoned and poisoned axons. In poisoned axons where the intracellular Ca is much higher than normal, an exchange of 2 Na_o for 1 Ca_i may suffice to maintain the observed gradient. In both poisoned axons and axons dialysed with solutions lacking ATP, the curve relating Ca efflux to external Na is markedly sigmoid and consistent with more than one (2 or 3) Na ions being required to activate the Ca efflux and recently BLAUSTEIN and RUSSEL (1975) have obtained evidence that in the absence of ATP part of the Na influx in dialysed axons is associated with Ca efflux. Roughly 3 Na ions enter for each Ca ion that leaves the axon. This data is, however, open to objection. In order to obtain measurable fluxes, it was necessary to use very high internal Ca concentrations. In addition, the evidence for a Ca_i-dependent Na influx is based on including EGTA in the dialysis fluid. EGTA is known to have other, as yet unexplained, effects on Ca transport in squid axons (BAKER,

1970) and as BLAUSTEIN and RUSSELL point out their evidence in favour of a stoichiometry of 3 Na: 1 Ca should be treated with some caution.

The situation in unpoisoned axons is much less clear. The shape of the curve relating Ca efflux to external Na gives no indication of more than 1 Na_o ion being required to activate the efflux and so far no one has detected a component of Na entry that depends on Ca_i. Failure to observe a component of Na entry coupled to Ca efflux is not surprising as the Na flux would be extremely small and difficult to detect in the presence of other much larger components of the Na influx. The shape of the Na-activation curve is also not conclusive, a curve that approximates closely to a section of a rectangular hyperbola would be seen if more than 1 Na_o ion were required but binding of all but one occurs with very high affinity. It will be recalled that activation of the Na-K exchange pump by external K is clearly sigmoidal in the presence of external Na but approximates to a rectangular hyperbola in Na-free media (BAKER *et al.*, 1969), yet there is strong evidence that the pump cycle takes in 2 K ions under both sets of conditions.

In view of the absence of clear cut evidence for a quantitatively satisfactory explanation of Ca efflux from unpoisoned axons in terms of Na-Ca counter-transport, we re-examined the possibility that metabolic energy may participate directly in calcium extrusion. The large rise in Ca efflux seen in cyanide-poisoned axons is often preceded by a small fall suggesting that if Ca were maintained constant, Ca efflux may be sensitive to the metabolic state of the cell. In order to maintain a constant $[Ca]_i$, we injected into the axon a Ca-EGTA buffer to give a final buffer concentration of 5 mM and an ionized Ca close to physiological. In these axons cyanide always produced a maintained fall in Ca efflux that recovered to its original level on removing the poison. The efflux usually fell to about 20-30% of its initial value and the efflux could be restored to its original or even higher levels by injecting ATP (Fig. 4), but not AMP or cyclic AMP, into the poisoned axon.

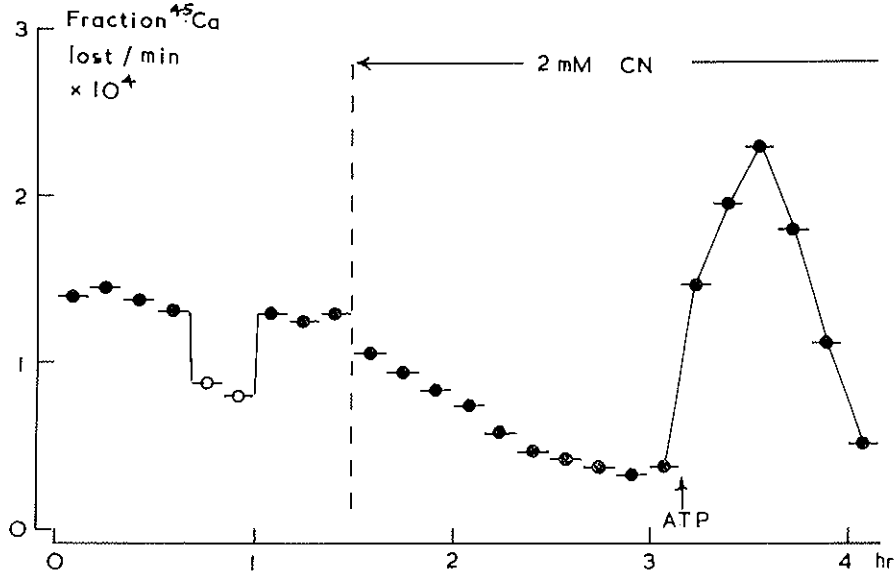

FIG. 4 — Dependence of Ca efflux from squid axons on ATP. Ordinate: fraction of ^{45}Ca lost/min. Abscissa: time (hr). The axon had been preinjected with a mixture containing 1 Ca: 2 EGTA to give a final EGTA concentration of 5mM. If injected into an axon containing aequorin, this mixture gives little change in light output suggesting that the buffered ionized Ca is close to physiological. ATP (final concentration 3 mM) was injected at the time indicated. -●- Na-ASW; -o-, Ca-free Li ASW. Temp. 21°C. Axon diameter 760 μm. (Unpublished data of BAKER and MCNAUGHTON).

Injection of ATP into a poisoned axon gave an increased efflux even in media that lacked both Ca and Na ions which suggests that part of the ATP-dependent Ca efflux is not dependent on these ions and may be similar in properties to ATP-dependent transport systems in other cells and intracellular structures (SCHATZMANN, 1975).

ATP also increases the size of the Na-dependent component of the Ca efflux and alters the form of the dependence of Ca efflux on external Na. The Na activation curve, initially very sigmoid in the absence of ATP, becomes much less sigmoid

and even approximates to a rectangular hyperbola after injection of ATP. This suggests that ATP or some derivative of it interacts with the Na-dependent Ca efflux system altering the affinity for external Na ions. DIPOLO (1974) has recently confirmed this finding in dialysed axons where addition of ATP to the dialysis fluid both increases the Ca efflux and also increases the affinity of the Ca efflux system for external Na ions. Rather high concentrations of ATP (0.6 mM) are required for half maximal activation of this effect. The data provides no clear evidence whether ATP is consumed stoichiometrically during Ca extrusion or whether ATP merely favours the existence of a Ca efflux mechanism with a high affinity for external Na ions. There is also evidence that the affinity of the Ca_o-dependent component of Ca efflux also depends on the metabolic state of the cell (BAKER and BLAUSTEIN, 1974); but I cannot go into this today.

In view of the evidence in poisoned axons and axons dialysed in the absence of ATP that the Na_o-dependent Ca efflux does involve exchange of internal Ca for more than one external Na ion, the most economical explanation of these results on unpoisoned axons is that the presence of ATP increases the affinity of the Ca transporting system for Na_o; but it has yet to be proved that during Na_o-dependent Ca extrusion ATP is not consumed in a stoichiometric fashion. The view that Na-Ca counter-transport operates in unpoisoned axons is supported by the observation that in Na-loaded axons in Na-free media, internal Na exchanges with external Ca and it is of interest to note that this exchange is not seen in poisoned axons (BAKER *et al.*, 1969b).

4) *Metabolic effects on other gradient-linked transport systems*

Other experiments on squid axons provide further support for the view that Ca transport is not the only gradient-linked

transport system that may be sensitive to the metabolic state of the cell.

The efflux of Mg is dependent on Na_o; but is also reduced to less than 10% in fully-poisoned axons (BAKER and CRAWFORD, 1972). This observation may indicate that Mg is transported by a Na_o-dependent Mg-ATPase, but there is some data that is more consistent with a coupling of Mg efflux to Na influx.

A much clearer example is the uptake of glutamate by squid axons. Amino acid uptake by many tissues is dependent on external Na and seems to result from co-transport of Na and the amino acid into the cell (see review by SCHULTZ and CURRAN, 1970). In the case of glutamate uptake by the squid giant axon, the axon contains a much higher concentration of the negatively charged glutamate anion than is present in the squid's blood. It follows that to maintain this difference in distribution, mechanisms must exist either for synthesizing glutamate inside the cell or for transporting glutamate into the cell against a steep electrochemical gradient. Co-transport of glutamate with one Na ion is unable to maintain the observed glutamate distribution and if the gradient hypothesis is to hold, at least two Na_o must move into the axon with each glutamate molecule.

When this was examined in both squid axons and crab nerve, the kinetics of uptake were consistent with the involvement of 2 Na_o in the transport of each glutamate molecule into the cell (BAKER and POTASHNER, 1971). Despite this evidence that a simple co-transport system could maintain the observed gradient, we also obtained very clear evidence that poisoning the axons inhibited Na-dependent glutamate uptake (BAKER and POTASHNER, 1973) (1). The inhibition of glutamate influx was not secondary to changes in the ion gradients

(1) Poisoning has no effect on glutamate efflux contrary to an earlier report by BAKER and POTASHNER (1973) who failed to achieve complete separation of ^{14}C-glutamate from labelled carbon divixide.

and kinetic analysis of the glutamate uptake in partially and fully-poisoned cells showed that poisoning reduces the apparent affinity for glutamate without affecting either the apparent affinity for Na_o or the maximum rate of transport. As with Na-Ca counter transport, this data shows quite clearly that the metabolic state of the cell affects Na_o-dependent glutamate uptake. Although BAKER and POTASHNER (1973) suggested that the glutamate transport system may exist in two forms, one with a high affinity for glutamate and one with a low affinity, the available data — especially on the variation in apparent affinity for external glutamate with internal ATP — is more consistent with a model of the following kind where ATP or some derivative of it must first bind to or react with the membrane (M) before external glutamate can be bound and transported into the cell:

$$M + ATP \underset{}{\overset{K_m^{ATP}}{\rightleftharpoons}} M.ATP \underset{Glutamate_o}{\overset{K_m^{Glut_o}}{\rightleftharpoons}} M.ATP.\ Glut \rightarrow \text{transport}$$

According to this model

$$\frac{v}{V_{max}} = \frac{1}{1 + \dfrac{K_m^{Glut_o}}{[Glut_o]}} \left(1 + \frac{K_m^{ATP}}{[ATP]}\right)$$

and the apparent K_m for Glutamate is

$$K^{Glut}_{apparent} = K_m^{Glut} \left(1 + \frac{K_m^{ATP}}{[ATP]}\right)$$

From which it follows that as ATP falls the apparent K_m glutamate will increase in the observed manner.

At present we do not know how ATP modifies these trans-

port systems but an obvious candidate is phosphorylation of the membrane perhaps by a reaction involving a cyclic AMP-dependent phosphokinase.

5) *Metabolism and other permeability systems*

In view of the evidence that gradient-linked transport systems may be subject to regulation by the metabolic state of the cell, it seems pertinent to enquire whether there is similar evidence for an involvement of metabolism in controlling any of the passive permeability systems in membranes. It is usually assumed that these systems are rather insensitive to changes in the metabolic state of the cell. Thus the action potential persists in fully-poisoned squid axons and these axons continue to conduct impulses when perfused with media lacking ATP or substrate. Although the Na and K permeability systems may be quite stable, there is growing evidence that the " late Ca channel " may not be. The evidence is still tenuous but it seems a strong possibility that the " late Ca channel " may be subject to metabolic control. Perhaps the clearest example is in the heart where Ca entry during the action potential can be increased by adrenaline (REUTER, 1974). The cardiac calcium channel shares many properties with the " late Ca channel " of nerve and if modification can occur in one tissue it is a very real possibility that it may occur in others. One possible example in the nervous system is the well-known rapid failure of many synapses following exposure to anoxia. In the squid giant synapse, synaptic transmission fails without loss of the presynaptic action potential and it also occurs with little change in the frequency of miniature potentials (CRAWFORD, unpublished data). This suggests that transmission has failed with minimal alteration in resting potential and ion concentrations. Prolonged exposure to anoxia results in a rise in the frequency of miniature potentials presumably as a result of the release of Ca from internal stores. The rapid

failure of evoked release is difficult to explain but could be rationalized if Ca entry into the presynaptic terminal is extremely sensitive to the metabolic state of the cell. As transmitter release depends on a high power of $[Ca]_i$, a relatively small reduction in Ca entry would result in a much larger reduction in transmitter release.

Regulation of calcium permeability systems would seen to offer considerable scope for effecting fine control of a number of aspects of cell behaviour and function and further investigation of this possibility seems desirable.

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DISCUSSION

Chairman: Prof. A. K. Solomon

Post

What affects have you seen from injected nucleotides?

Baker

In axons that have been injected first with EGTA to control the ionised calcium and then poisoned with cyanide, the Ca efflux can be restored by injection of ATP. If you inject AMP or cyclic AMP you get no effect, and if you inject certain analogues of ATP, for example the methylene analogue that is not split, you obtain results that are difficult to interpret. Some activation of Ca efflux is seen; but analysis of these analogues usually reveals some ATP in them and we are inclined to think it is this small contamination with ATP that is doing the job rather than the analogues.

Ritchie

The first slide with aequorin showed that as the light signal increased as stimulation progressed, it became progressively more noisy. More interesting, by when the stimulation was stopped, the light response returned to the base line, but the noise persisted.

Was that just chance, and if not, have you made any analysis of the noise?

BAKER

I think it was just chance and nothing else.

KEYNES

Is there any evidence for a similar coupling with the ATP supply and the sodium in sugar transport?

BAKER

We haven't looked into this, and I don't think anybody has looked at glucose transport in squid axons although glucose transport into nerve tissue is said not to be dependent on Na ions. In those preparations where you do have a sodium dependent transport of sugar (e.g. intestine and kidney) there is evidence that the transport of sugar is sensitive to the metabolic state of the cell, but you can't differentiate very easily in these tissues between effects that are secondary to changes in potential or ion concentrations and effects that are produced directly by metabolism. This problem arises because you are dealing with much smaller cells with an enormously higher surface area/volume than exists in the squid axon.

SLAYMAN

In a system where you have what looks like counter-transport with the possibility of different numbers of charges coupled together, moving in opposite directions, it might be fun for you to see whether you can drive glutamate with aspartate. In heart muscle mitochondria

a system has been described by which glutamate and aspartate exchange is driven by the hydrogen ion gradient. It would be interesting to determine whether your glutamate system could be put into an analogous state.

BAKER

If you look at the efflux of glutamate, there is very little evidence for glutamate-glutamate exchange, so I doubt whether there would be any glutamate-aspartate exchange either, but for other aminoacids CALDWELL and LEA have shown very nicely that glycine efflux can be coupled to movement of glycine, alanine and other aminoacids in the opposite direction. It would seem that the gradient for one provides the energy for the movement of the other in the opposite direction.

DE MEIS

Did you use iodoacetate or other inhibitor of glycolysis? I wonder when you squeeze the axon or dialyse it, would you really be able to get rid of all cytoplasm, and therefore be sure that no ATP would be generated?

BAKER

In poisoning intact axons one tends to use cyanide in rather high concentrations (2 mM) and ATP doesn't totally disappear. But in a dialysed axon you're maintaining a steady flow of solution without any ATP in it and there's no substrate or cofactors from which ATP can be synthesized, so its very difficult to see how there could have been an appreciable amount of ATP generated there. Experiments with iodoacetate are difficult as this substance effects Ca transport in squid arons.

When you dialyse an axon you only thread your dialysis tube through the middle of the axon and by flowing solutions through the tube hope to dialyse out the small molecular weight materials from the axoplasm which remains in situ. Alternatively you can squeeze out the protoplasm from an axon and when you do this the axoplasm tends to break about 10 microns inside the axolemma giving you a rather pure sample of protoplasm, but the membrane is left with 10 microns of protoplasm inside it. Now if you flow solutions through these axons, that is perfuse them and I wasn't discussing this type of experiment, it is possible to reduce the amount of residual protoplasm by various enzyme treatments that seem to leave you with virtually bare membrane at the inner face of the cell.

DE MEIS

Thus, you feel that no ATP will be left available to the membrane. What I wonder is that most of the pumps have a very high affinity for ATP with a K_m in the order of 10^{-6} to 10^{-7}. So you really need very little concentrations of ATP to put the Na^+ K^+ or Ca^{2+} pump to work.

BAKER

Yes, that is, of course, true. If you had a very small amount of ATP you could conceivably operate the system. Now that's always a very difficult problem to get around. The fact that, in an intact axon, even though there is probably 50 to 100 micromoles of ATP left in it, poisons still reduce the calcium efflux to a very low level, indicates that under those conditions the affinity of the Ca efflux system for ATP is quite low, but it is possible that the various breakdown products of ATP (ADP, Pi etc.) may interfere with the utilization of ATP. The fact that in a dialysed axon in order to activate the system Dipolo has to add not 1 to 10 micromolar ATP

but nearly 1 mM ATP is again suggestive that activation requires a lot more ATP than 1 μM, but you could say that you get a first stage of activation with micromolar ATP and further I don't think there is any proof for or against that.

DE MEIS

The question is related to the properties of the pump which have been shown to vary with the concentration of substrate. For instance the Na^+ plus K^+ pump. If one uses a substrate of low affinity such as UTP or ATP at concentration in the range of 10^{-6} to 10^{-5} M, it does not require potassium, only sodium to work. If the ATP concentration is raised to 1 mM then it requires both sodium and potassium. For the Ca^{2+} pump of sarcoplasmic reticulum, using ATP concentrations in the range of 10^{-6} to 10^{-5} M, the Ca^{2+} transport is inhibited by sodium or potassium ions. If one goes up with the ATP concentration (1 mM) the properties of the pump change and sodium or potassium no longer act as inhibitors, but they now activate the Ca^{2+} transport.

BAKER

How do you suggest getting rid of the last trace of ATP?

DE MEIS

In addition to the inhibitors of oxidative phosphorylation, we use also inhibitors of glycolisis.

KEYNES

In one of your earlier experiments you showed an entry of calcium going up to a peak with pulses of a certain size and going down

again beyond it. Does that give you a calcium reversal potential which fits with the other data you presented?

BAKER

If you look at calcium entry as a function of potential, you find a bell-shaped curve that rises to a peak and with increasing depolarizations, then falls towards zero but never actually below zero.

To obtain a true reversal potential one would like to see a reversal in the direction of Ca movement, this is never seen. A possible explanation is that depolarization is changing more than one thing, perhaps as well as opening Ca channels, it also alters the rate of Na-Ca counter transport, causing Ca efflux and promoting Ca influx. All that aequorin measures is ionized calcium and it gives no information on where the calcium come from. If one ignores the light at large depolarizations and draws a line through the falling part of the curve one can obtain a value for the reversal potential, but the value obtained is not as positive as you might expect.

There are plenty of possible explanations for this discrepancy but an interesting one is that the channel may also allow Mg ions to pass. The ionized magnesium inside the cell is quite high and magnesium enters axons during nervous activity. The property of this Mg entry suggests that it passes through the late calcium channel. If there is interaction between Ca and Mg in passing through the calcium channel, the apparent equilibrium potential measured with aequorin could be less positive than the equilibrium potential for calcium alone; but this involves a lot of assumptions.

KEDEM

The simple relation between the calcium reversal potential and the other parameters would require that the sodium and calcium be totally coupled-otherwise you cannot predict the potential at which the Ca-flow vanishes.

BAKER

We are talking about reversal potentials in connection with the passive voltage-sensitive movement of calcium and this is unlikely to be much affected by Na-Ca exchange; but certainly, as I mentioned earlier, if the calcium and the sodium are not coupled in a neutral function we will get some changes in calcium movement when the potential is altered.

POST

I would like to suggest a supplementary poison for getting rid of ATP. This is arsenate in concentrations high enough to compete effectively with the inorganic phosphate.

ELECTRICAL BEHAVIOUR OF "EXCITABLE" ARTIFICIAL MEMBRANES

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I - Diversity of membranes made from lipidic derivatives.

Excitability and ionic selectivity are two of the most important properties of cells membranes. Many artificial membranes display such features. Mueller and Rudin (1962) proposed phospholipidic bilayers as thin as cell membranes. But these bilayers show ionic permeability only if they are "doped" with cyclic polypeptides, that is annular molecules having peripherical lipophilic groups and internal hydrophilic groups. These molecules can thus carry into a phospholipid membrane an hydrated cation inserted in the ring. Bilayers display excitability only if they are in contact with specific agents. The most used has been a protein of a culture of an intestinal bacterium. Phospholipids are not necessary constituants of bilayers. These can be made with oxidized cholesterol (Yafuso and coll., 1974). Furthermore "model" membranes do not require the extreme thinness (60-80 Å) of the bilayer structure. Our group (Monnier, Monnier, Goudeau, Reynier-Rebuffel, Sanchez, Perrin, 1964, 1965, 1967, 1968, 1972, 1973, 1974) has shown that many lipidic derivatives form easily, without any specific additives, excitable membranes

a few microns thick (i.e. 100-500 times thicker than bilayers). These lipidic derivatives are:

a) *unsaturated fats* (*that is drying oils, such as linseed or Tung oils*) previously subjected to oxidizing polymerization through spreading on an oxidizing solution (MnO_4K, 1g p. 1000). This treatment produces membranes 1-2 microns thick (which show interference coloured rings) sufficiently strong to separate two aqueous compartments. Furthermore oxidation creates in the membrane fixed carboxylic acidic groups, which appear a necessary condition for excitability and ionic selectivity;

b) "*alkyds*" that is polymeric condensates of glycerol, fatty acid and an anhydride (for instance succinic);

c) *aqueous gels of monoglycerides,* for instance glycerol monooleate. These substances form aqueous transparent gels, containing 10-30 p. 100 water. This through hydrogen bonds linking water molecules to the OH groups of the glycerol monooleate. Electrical excitability requires that some fixed acidic charges are inserted in the gels. This is obtained by adding previously 5-10 p. 100 oleic acid to the monooleate;

d) KOBATAKE and coll. (1973) have recently obtained excitable artificial membranes by incorporating cellophane "millipore" filters with dioleylphosphate. This phosphoric ester of oleylalcohol, in contact with aqueous phases, undergoes a slight hydrolysis which provides the necessary fixed acidic groups;

e) Dr. LAKATOS from Pecs (Hungary), in my laboratory has just found that bitumen gives excellent results. A benzene solution of 1-2 p. 100 bitumen plus 1 p. 100 oleic acid, spreads easily on water and leads to fairly strong membranes about 1 micron thick. The membranes prepared with this odd material are electrically excitable practically over all their surface (Fig. 2). The above types of membranes (a, b, c, d) require usually that the exploring pipette electrode should be moved across the membrane surface until a sensitive spot is found.

II - Essential features of electrical excitability of artificial membranes.

For the nerve axon membrane, the most general character of excitability is a large and sudden temporary increase in conductance, as the essential response to excitation (Cole and Curtis, 1938). The above membranes can show a 10-20 fold transient increase in conductance when the field across the membrane exceeds 50-500 volts cm^{-1}. These responses usually repeat with a frequency which rises with the field. Objection has been raised that these responses may be only statistical "noise". Such "noise" can be sometimes visible on the oscillograms. But the responses can exceed this "noise" by one or two orders of magnitude and occur with a steady frequency, thus they do not meet the essential criteria of a noise phenomenon (Fig. 1). Under weak fields small stepwise responses occur, indicating the opening and closing of a pathway (Fig. 3). This indicates that the conductance of such a pathway oscillates, in an all or none fashion, between a "closed" and "open" value (Fig. 3), (Reynier, 1971). The same phenomenon exists in bilayer membranes (Ehrenstein). When large responses occur, they appear to involve many such pathways that open and close synchronously (Fig. 4). Thus large responses appear as a cooperative process. Sometimes this process developes slowly (in 10-20 seconds), while the magnitude of the rythmical responses increases. This is an image of the neurophysiological "recruitment".

III - The essential difference between axon membrane and artificial membrane excitability.

Excitability of the axon membrane depends upon a bionic process (Hodgkin and Huxley, 1952). Na^+ predominates outside of the membrane and K^+ inside the axon. Response to excitation corresponds to a large and transient in-

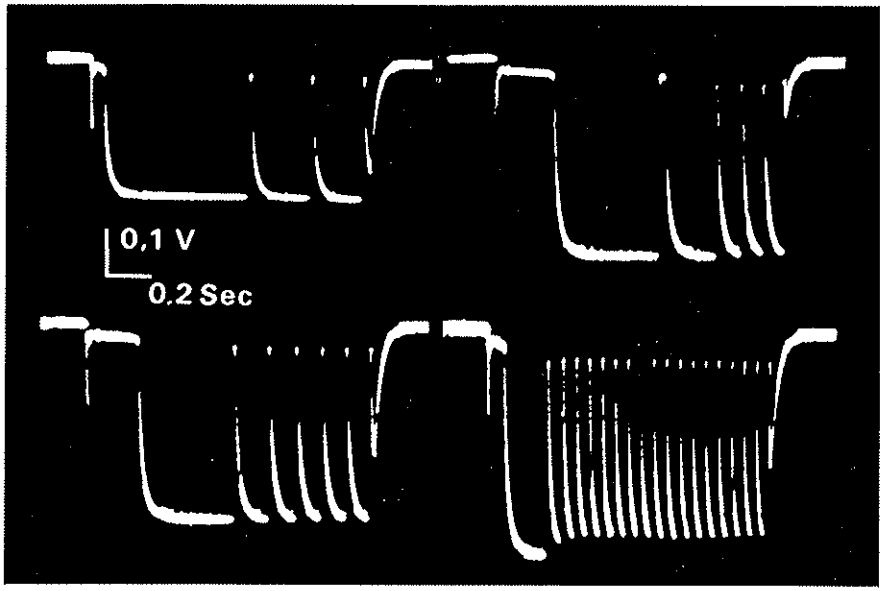

FIG. 1 — Membrane obtained after spreading a drop of a mixture of linseed oil and tung oil, on a Mn O_4K (1g p. 1000) solution. The membrane is placed between KCl, 0,1M. solutions, connected to Ag-AgCl electrodes. Constant currents of increasing intensity apply, voltages (indicated down wards) to the membrane. When responses occur, the voltage drops almost completely, indicating a marked increase in membrane conductance. Latencies decrease and frequency of responses increases as the initial voltage augments (After REYNIER-REBUFFEL, 1971).

crease in conductance due to a great enhancement of the permeability of the membrane towards Na^+. Thus a dose of outside Na^+ ions penetrates into the fibre. This entrance is followed by a temporary increase of the permeability to K^+, accompanied by the exit of an equivalent dose of K^+. Therefore response of the nerves axon to excitation is a "passive" process that requires a ionic dissymetry on both sides of the membrane. Maintenance of this dissymetry requires an "active" process that is a pumpwise system, animated hy metabolic energy, which accumulates K^+ in the fibre and extrudes Na^+ from the

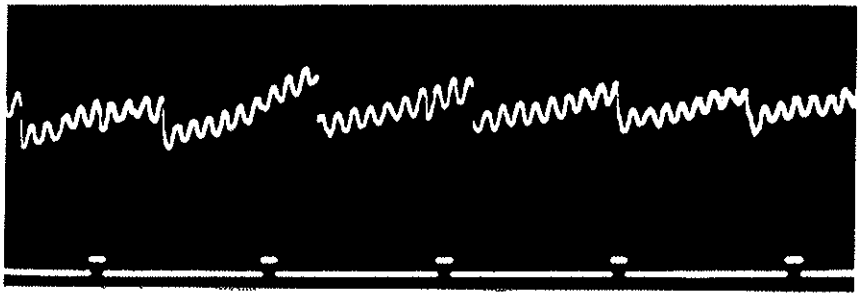

FIG. 2 — Membrane obtained by spreading a drop of a benzene solution containing 5 g. p. 100 bitumen and 4 g. p. 100 oleic acid, on a KCl 0,1 M solution. The membrane is about 1 micron thick. Its resistance (1000 ohms. cm^{-2}) is much lower than that of a drying oil membrane (10^4-10^5 ohms. cm^{-2}). Under an applied voltage of 2 volts, it falls more than 10 p. 100 of its initial value. On the main responses smaller sine-waves occur, showing that two sites, with different characteristics are involved. 100 ms. time markings (After LAKATOS, 1975).

latter. On the contrary the response of the above artificial membranes depends upon a mono-ionic process. It is observed when the saline solutions on both sides of the membrane are identical. But it is probable that, in natural and artificial membranes, the "gating" process which opens and closes the ionic pathways has common features.

IV - POSSIBLE GATING MECHANISM.

The above membranes have a common feature: they form a "mixed body" having an hydrophilic and lipophilic component. For type *a* membranes, the dielectric constant is 10-20, that is intermediary between that of water and that of a pure lipid (SANCHEZ and REYNIER, 1967). This low dielectric constants agree with the 10 p. 100 water content of the membrane (GOUDEAU, 1969). Besides water in thin layers inserted between solid surfaces shows a very small dielectric constant (PALMER and coll., 1952). Therefore in the narrow aqueous

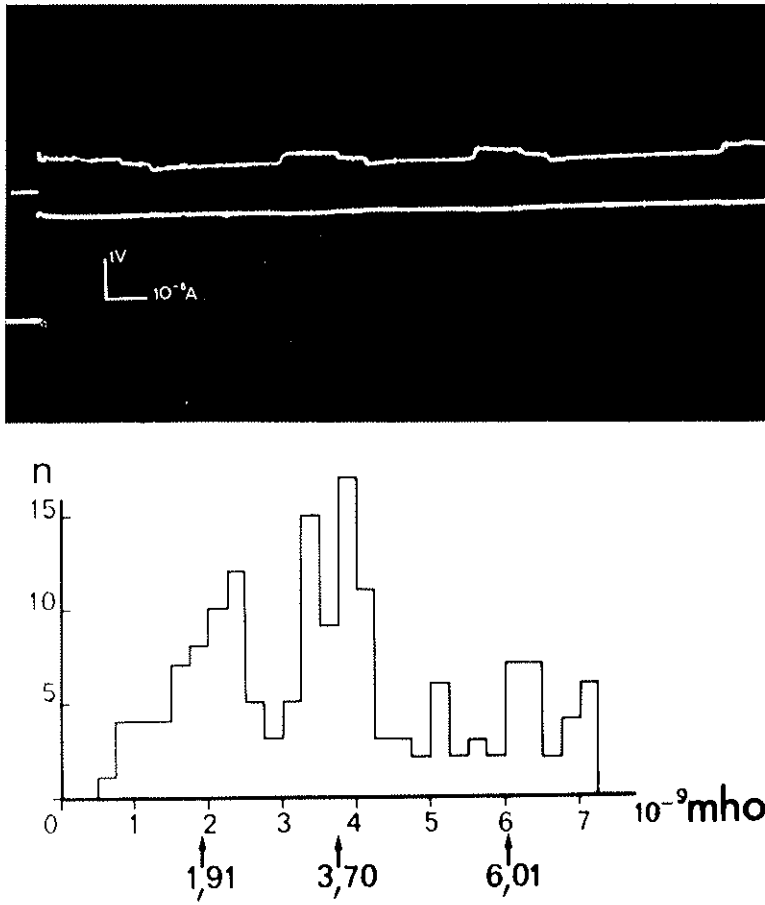

FIG. 3 — Similar membrane as in fig. 1. *Above,* top record shows discrete jumps in voltage, under current-clamp conditions. The constancy of the current is indicated by the bottom record. *Below,* histogram of calculated conductance steps (per cm² of membrane). The smaller steps form two distinct groups, of which the averages are in the ratio 2:1. This indicates a tendency to the mutual triggering of double groups of sites, as pathways, which open and close simultaneously (REYNIER-REBUFFEL, 1971).

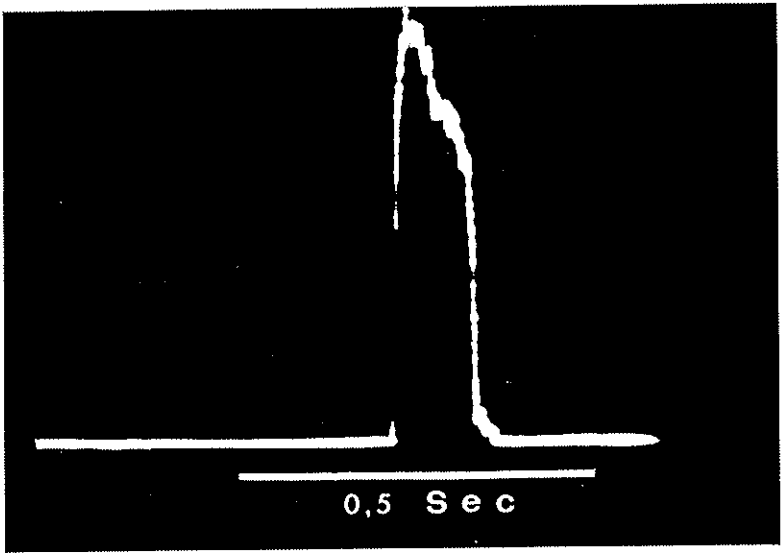

FIG. 4 — Typical large response usually occuring in rhythmic succession, as in Fig. 1. Peak conductance over 10 times that of resting conductance. A step-wise time-course is perceptible, especially in the inactivation phase. Therefore the closing of pathways is less synchronous than their opening.

channels of a lipidic reticulum, alkaline cations are subjected to a small dielectric constant. Their electrostatic attraction of dipolar water molecules is stronger. Thus the cations in the aqueous channels should carry more water molecules than in free water. These highly hydrated cations are attached to the channel wall by H-bonds linking water molecules of their hydration shell, to the H accepting groups (OH, COOH) fixed upon the channel wall. The proposed "gating" process would comprise the following phases: 1) When the field exerts on the cations a force sufficient to break those H-bonds, these cations become mobile. Thus membrane conductance occurs and a current is established. But cations while they are in the channel are more hydrated than the incoming cations from the outside solutions. 2) Therefore motion of the cations in the aqueous channel dehydrates the latter. 3) As dehydration

proceeds the dielectric constant becomes still smaller, approaching the low value which characterizes the lipidic component. 4) As the current gradually dehydrates the channel, electrostatic attractions become stronger and consequently H-bonds are reconstituted more tightly. The constant force exerted by the field upon the cation is then not strong enough to break them. 5) Thus the current stops. 6) As water from the outside solution diffuses slowly into the membrane, it increases the dielectric constant and thus weakens the H-bonds to the point where the force exerted on the cation by the field can break anew these bonds. This is phase 1 again, which would initiate as above all the following phases. Such response would repeat indefinetely with a frequency increasing with the field. This tentative explanation of the "gating" process can be amenable to calculation. It receives some support from the data concerning ionic selectivity of the above membranes, as shown in the next paragraph.

V - Cationic selectivity. (Goudeau)

The data concern mostly the drying oil membranes. When they separate two solutions containing the same cation, but of different concentration, a potential occurs, across the membrane, which follows accurately, in sign and magnitude, Nernst law. This indicates that the membranes are exclusively permeable to cations.

VI - Inorganic cations. Intercationic selectivity.

The membrane permeability is not the same for all cations. This is shown by Sollner's bi-ionic potentials, observed when the membrane separates two solutions of the same concentration but each containing a different cation. The sign and magnitude of this bi-ionic potential indicates the ratio between the membrane permeabilities towards each cation. The perme-

abilities of alkaline cations follow the sequence: $Li^+ < Na^+ < K^+ < Rb^+ < Cs$. The heavier cations, that is those that are known to be more hydrated in free water, penetrate the membrane more readily. For instance the membrane is about 10 times more permeable to K^+ than to Na^+. Excitability is more frequently observed when the aqueous solutions in contact with the membrane contain K^+, than when they include the more hydrated Na^+. Furthermore when the adjacent solutions contain only lipophilic organic cations, excitability is seldom observed. This shows that a hydration shell of a certain size is an important factor for the occurence of excitability. This property thus seems located in the aqueous channels of the membrane.

VII - Intercationic selectivity - Organic cations.

With organic cations high bi-ionic potentials may be observed. These potentials increase with the number and length of the carbon chains linked to the nitrogen atoms, that is increase with the "liposolubility" of the cation. For instance, K^+ being the reference cation, tetraethylammonium and tetrabutylammonium give potentials respectively of 57 mv and 125 mv, from which can be calculated according to the bi-ionic potential formula, the ratio of membranes permeabilities.

$$\frac{P_4\ \text{ethyl}^+}{P_{K^+}} = 9{,}4 \text{ and } \frac{P_4\ \text{butyl}^+}{P_{K^+}} = 138.$$

Many cationic drugs give high permeability ratio versus K^+. For ephedrine:

$$\frac{P\ \text{eph}^+}{P_{K^+}} = 26.$$

Therefore membranes are very permeable towards "lipophilic" cations. These high permeabilities appear to be also due to an ion-exchange process involving the carboxylic acid groups

attached to the lipidic reticulum of the membrane. This hypothesis can be given an indirect test: the ion-exchange between a fatty acid and a cation included in an aqueous solution.

VIII - Cation exchange between oleic acid and an aqueous solution - Correlation with membrane permeabilities.

When a fluid fatty acid (oleic acid) and an aqueous chloride solution of a cation are stirred together, the pH of the aqueous phase drops (Fig. 5, 6, 7). This is due to an exchange between an H^+ from the acid and a cation from the aqueous solution. From the pH change the magnitude of the exchange can be calculated, by a formula indicated by Marcus and Kertes (1969). Some precautions are necessary to insure an accurate measurement of the exchange coefficient pK_e: the oleic acid must be free of water soluble short fatty acids. The pH measuring electrodes must be coated with a thin layer of agar gel. This highly hydrophilic film prevents the electrodes from being soiled by the fatty acid. Usually the experiment is made with equal volume of solution and oleic acid. When the exchange is completed (a few minutes of stirring is sufficient) the pH is noted. A linear relation appears between this pH and the cationic initial concentration in the solution (expressed in the logarithmic notation $p[M_o]$).

That is $p[M] = -\log[M_o]$. (Fig. 5) shows the linear relation for tetrabutyl-ammonium. It can be easily derived from the exchange reaction proposed by Marcus and Kertes, and valid only for cations that are strong bases

$$\overline{AH} + [M^+] \rightleftarrows \overline{AM} + [H^+]$$

Square brackets denote concentrations in the aqueous phase. The corresponding equilibrium expression is

$$A) \qquad \frac{\overline{AM} \cdot [H^+]}{\overline{AH} \cdot [M^+]} = K_e$$

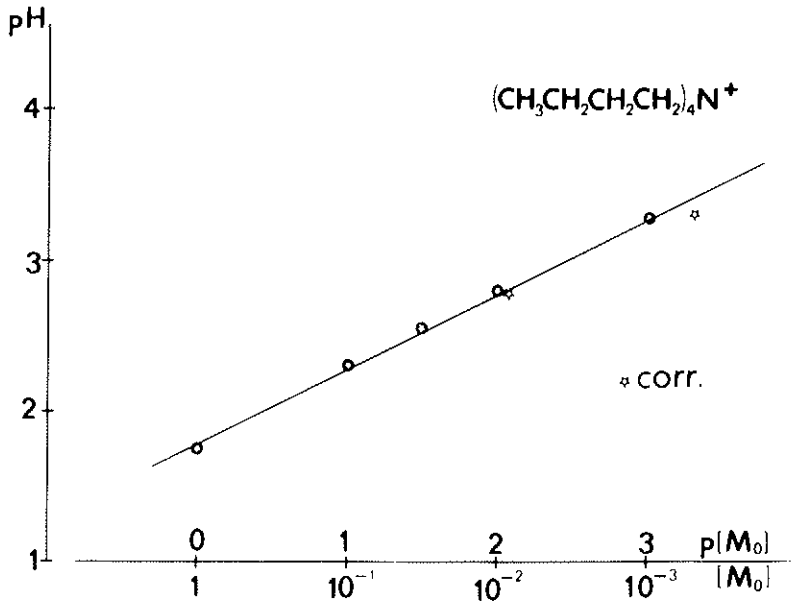

FIG. 5 — Exchange of tetrabutylammonium with H ion of oleic acid. Abscissae, initial concentration of the aqueous chloride solution of the organic cation. Exchange being important, final value of this concentration has to be taken into account for dilute solution (starlike dots). Ordinates, pH value of the aqueous phase when exchange is terminated. The straight line has the theoretical slope of 0.5, according to equation (B). Equal volumes of oleic acid and aqueous chloride solution.

As the oleic acid and aqueous solution have the same volume,

$$AM = H^+$$

If we take the concentration of oleic acid in its own phase as unity, the above equation becomes, in logarithmic notation:

B) $$pH = \frac{p[M_o]}{2} + \frac{pK_e}{2}$$

Experiment shows that correction for activity is unnecessary, at least over a wide range of concentration.

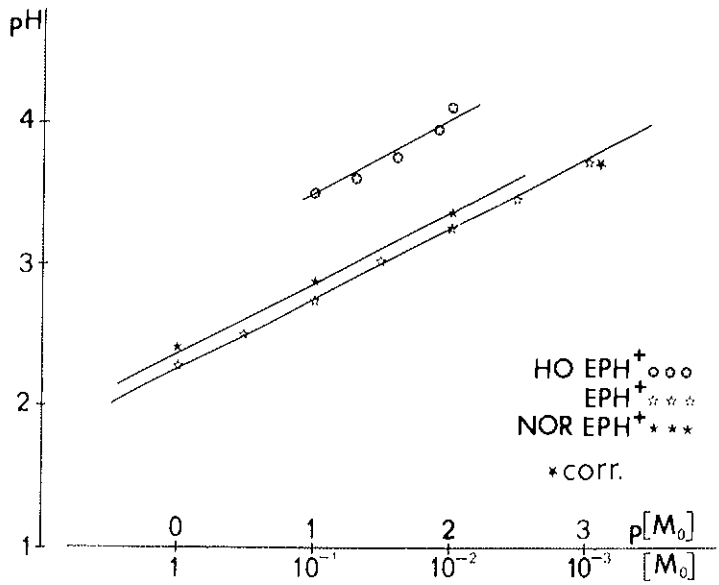

FIG. 6 — Exchange of ephedrine and its derivatives when in contact with oleic acid. Same conditions and notations as in Fig. 5. Parahydroxy and nor ephedrine, more hydrophilic than ephedrine, are less exchanged.

The pK_e thus defined is not an absolute constant but it leads to significant comparisons. The smaller the pK_e the greater the exchange, that is the ability of the cation to penetrate into an acidic lipid phase. From the difference between the pK_e of two cations, the ratio of their exchange with the oleic acid can be calculated (Table 1). The exchange for tetrabutylammonium is 560 times that for K^+. The ratio of their permeances into a membrane was smaller (138) but of the same order. The difference results from the bi-ionic potentials method used to determine permeabilities. It considers the cation transport number in the membrane, that is the product concentration mobility.

Table 1 shows the exchange of two cationic drugs, ephedrine and amphetamine. Addition of a hydrophilic group

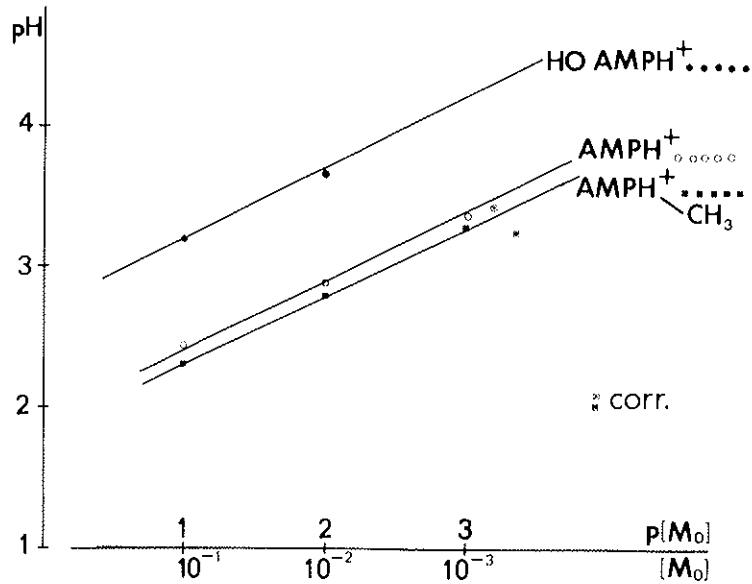

FIG. 7 — Exchange of amphetamine and its derivatives when in contact with oleic acid. Same conditions and notations as in Fig. 5 and 6. Hydroxy amphetamine, more hydrophilic, is less exchanged. Methylamphetamine, more lipophilic than amphetamine, is more exchanged.

(- OH) to their molecule reduces their exchange with the lipidic acid phase. Suppression of lipophilic group (- CH_3) as in nor-ephedrin, does the same. On the contrary addition of a methyl group as in methylamphetamine increases the exchange. The rates of penetration of these drugs into the central nervous system are affected in the same way by the above modifications of their molecules (LINDENBAUM, 1972). The structure of morphine, heroin, methadone, explains in the same way their different permeabilities into the nervous system as observed by OLDENDORF (1972). The first condition of the pharmacological action of a cationic drug appears to be its liposolubility, which implies the existence of a lipidic acid component in cell membranes. This condition of liposolubility

was known to apply only to neutral molecules (OVERTON-MEYER's oilwater partition coefficient).

Exchange of divalent cations with oleic acid can also be determined, the formula being slightly different. The pK_e of the cations Cu^{++}, Hg^{++}, emetine^{++} are 8,6 - 8,0 - 7,7, much smaller than the pK_e of other cations such as Ca^{++} or Mg^{++} (11,3 - 12,7). Perhaps this important difference explains, in part, the respective antifungal, antibacterial, antiamoebial actions of the above cations.

TABLE 1 — (HOUDU-BINTEIN, 1974).

Cation	pK_e	Magnitude of the exchange to that of K^+	
K^+	6,81	1,0	
Tetrabutyl-ammonium	4,06	560	
Parahydroxy-ephedrine	6,40	2,6	
Norephedrine	5,24	37	
Ephedrine	5,02	62	
Parahydroxy-amphetamine	5,90	8,1	
Amphetamine	4,30	220	
Methyl-amphetamine	4,10	510	
Malachite Green	2,85	9100	figures obtained by colorimetry
Methyl Violet	2,37	27000	figures obtained by colorimetry

IX - Photo-excitability of artificial membranes.

Recently many authors have shown that bilayers in contact with dye containing solution show conductance increases under illumination. The same has been observed on the above membrane (a and e types) (Wallon 1973). In these cases the effect is quite large (Fig. 8). A necessary condition is that the dye must be a very lipophilic and strong cationic base, such as Malachite Green or Methyl Violet. In other words it must undergo a large ion-exchange with the fixed acidic groups of the membrane, exchange which gives a strong colouring to the latter, even if the dye is very diluted in the adjacent solution. The wavelength corresponding to the main absorption band of the dye are the most efficient. The effect is genuine and is not due to warming of the membrane under illumination, adequate filters excluding infra-red radiations. As a precaution against photo-electric artefacts at the metal of the electrodes (Ag-AgCl-KCl) these are carefully shielded: these artefacts are always very brief and thus never blurr the genuine photoconductance effect. Photoconductance is independent of temperature. But as conductance in the dark is very much increased by temperature (an activation energy of 15-25 Kilocalories/mole), the relative change of conductance to illumination is much larger at low temperature.

When the dye is a weaker base such as methylene Blue, that is when the aqueous solution contains the dye in its neutral and its cationic form, the changes of conductance under illumination are more complicated (Fig. 9). First there is a brief increase in conductance followed by a large decrease, which is slowly reversible. Thus several reactions of different velocities are involved.

A tentative explanation can be proposed. As the dielectric constant of the lipidic component of the membrane is low, the electrostatic inter-ionic attractions are strong. In this component the cationic dye forms certainly a weakly dissociable ion-

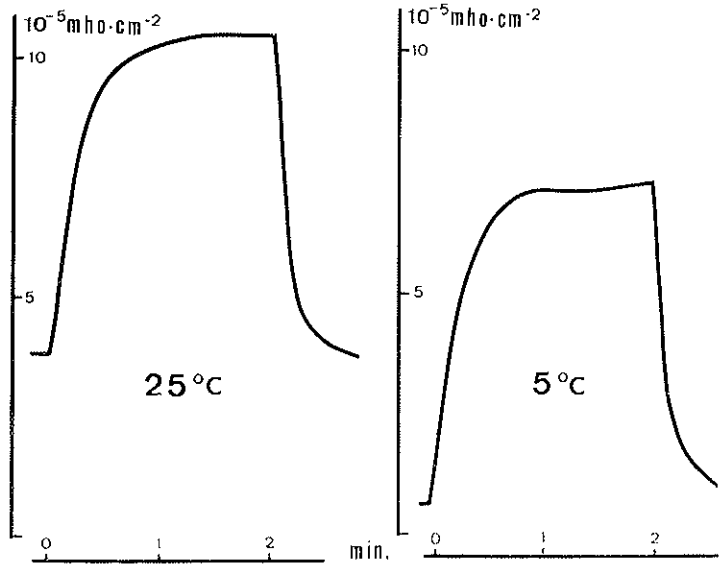

FIG. 8 — Photo conductance of drying oil membranes (see Fig. 1) in contact with 0.1 M KCl solutions containing Malachite Green (1 g. p. 1000). Illumination (white light) 2.10^4 lux. Thermal effects avoided by adequate filters The photo conducting effect is independent of temperature (WALLON, 1973). But the dark conductance decreases markedly under cooling.

pair, as shown by BJERRUM (1926) and KRAUSS and FUOSS (1933). Under illumination the dissociation constant K_1 of the $[A^- \; R-NH_3^+]$ pair would increase, thus more $R-NH_3^+$ cations would be free, then increase of conductance would occur. A^- and $R-NH_3^+$ denoting the fixed acidic groups and the cationic dye, the equilibrium equation would be:

$$(1) \qquad \frac{[A^-] \cdot [R-NH_3^+]}{[A^- \; R-NH_3^+]} = K_1$$

When the dye is a weak base the ion-pair would be also under illumination dissociated in non-ionic components

$$[A^- \; R-NH_3^+] \rightleftharpoons [AH] + [R-NH_2]$$

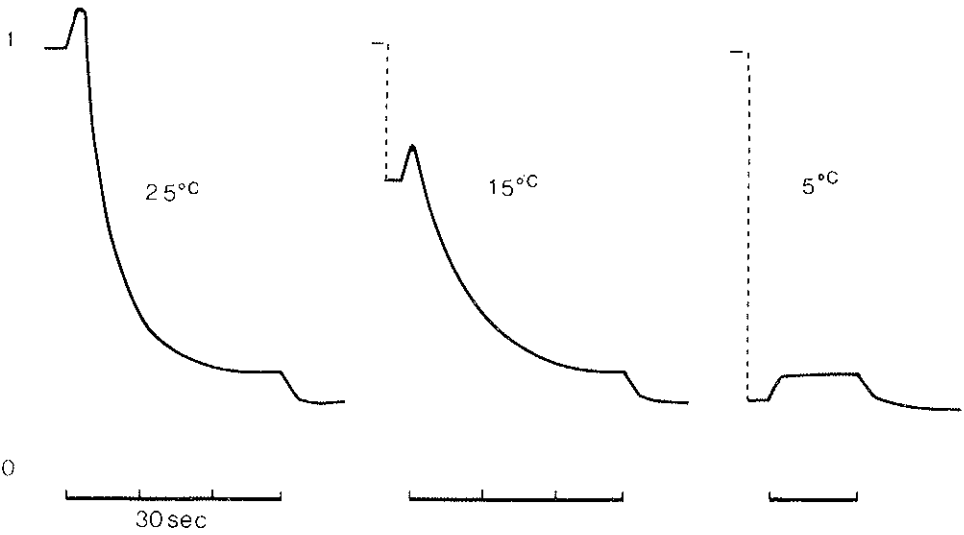

FIG. 9 — Dual photoconductance effects on drying oil membranes (see Fig. 1) in contact with KCl 0.1 M solutions containing Methylene Blue (1 g. p. 1000) 30 sec. illumination. At room temperature (25°C) a transient increase of conductance appears immediately at the onset of illumination, to be followed by a large decrease of conductance, which is reversible only after 5-10 minutes in the dark. This negative photo-conductance effect disappears at low temperature. Then only the photoconductance increase remains (WALLON, 1973). For a tentative explanation of this dual effect (see text).

the corresponding equilibrium being:

$$(2) \qquad \frac{[AH] \cdot [R-NH_2]}{[A^- R-NH_3^+]} = K_2$$

combining equations (1) and (2):

$$\frac{[A^-] \cdot [R-NH_3^+]}{[AH] \cdot [R-NH_3^+]} = \frac{K_1}{K_2}$$

If under illumination K_1 is more increased than K_2, an augmentation of the ionic species $R-NH_3^+$ would result, that is a greater conductance. On the contrary if K_2 is more increased

than K_1 by the illumination, a decrease of conductance would occur.

If the velocities of these photo reactions are different, the opposite conductance changes will proceed somewhat one after the other. Of course such theoretical essay is only an approach. Equation (2) is only a global reaction. Intermediary processes are certainly involved. But the following experiments give some support to this approach. An aqueous solution of Malachite Green is stirred with oleic acid. The exchange is important (see Table 1) as shown by the intense colour of the acid. If the latter is centrifuged so as to be free of water droplets, and placed into a conductivity cell its conductance, though quite small, increases markedly under illumination. In that case oleic acid contains some water (1-2 p. 100). This raises its dielectric constant to about 4. Dehydratation under vacuum lowers the dielectric constant to about 2,5. But then no perceptible photoconductance occurs. The energy of ion-pair attraction is too strong to be overcomed by the radiant energy. Rehydratation of the acid restores the photo-conductance. But further increase of dielectric constant by addition of alcohol slowly reduces the photo-conductance. Then the ion-pairs are already markedly dissociated and illumination adds but little to the dissociation (WALLON, 1974-75).

X - CONCLUSION.

Many lipidic derivatives can form membranes which, though much thicker than bilayers, show readily, without requiring as the latter any additive, the essential features of ionic-selectivity and electro-and photoexcitability. Thus they are attractive "models" of some functions of cell membranes, that is those functions that do not require metabolic energy and which are therefore labeled "passive" processes. These models may be useful in indicating the essential role of ionic hydration and dielectric constant in membrane functions.

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DISCUSSION

Chairman: Prof. A. K. SOLOMON

MUELLER

Did you ever voltage clamp these membranes?

MONNIER

The pattern of responses is much the same under voltage clamp, but the voltage clamp has some disadvantages in that the surge of current during the response becomes so great that the reversibility of the membrane is slowly impaired. When you hit a good spot on the membrane responses can repeat for an hour or so. Under voltage clamp the repetition would not last so long and after a few minutes would die out, but basically both current and voltage clamps work.

MEARES

Have you any information or experience on whether the behaviour is specific to having fixed ionic groups in the membrane or whether mobile ions, confined to the membrane phase like liquid ion exchangers but which nevertheless constitute mobile sites in the membrane, are effective?

MONNIER

In the classical naphtalene sulphonic acid which are used as com-

mercial liquid exchangers, anions are mobile. They are mobile too in oleic acid, but in the membranes they seem to be fixed, for instance in drying oil membranes the membrane is naturally becoming acidic through oxidation and the acidic groups according to the experts are fixed or have a very low mobility.

TEORELL

In the introduction to your beautiful lecture, Prof. MONNIER, you mentioned that many more oscillatory systems coud be invented. You have focussed on « mixed » membranes. You can however have perfectly homogenous membranes without lipophilic groups and yet get oscillations. I have used a very simple membrane being sintered glass across which a constant current was led. Above a « threshold » current density we got oscillations.

MONNIER

What kind of membrane?

TEORELL

It was sintered glass (as « Pyrex » filter discs). This contains silicic acid residues wbich introduce a negative charge to the pore walls. When one shoots current across it electro-osmosis takes place. This model had the advantage that it is sensitive to perturbations of pressure (hydraulic pressure) as well as of the electrical current. This « membrane oscillator » may be of interest from a biological point of view. In biology we know of many pressure sensitive « transducers », for instance, a device sitting in the neck which controls the blood pressure (Sinus caroticus), and in the muscles there are also stretch pressure sensitive receptors. My question to Prof. MONNIER is are your memhranes pressure sensitive (to distension)?

MONNIER

We have not yet looked for pressure sensitivity in our membranes. This is in our programme. If I may offer a suggestion — I think the mutual triggering of responses is due to a change of lateral pressure of the channels involved.

TEORELL

I should like to expand a little on Prof. MONNIER's idea on the « membrane hydrolysis » (a modern term is « protolysis »). By the application of this process to a « lipophilic » membrane you can create a simple « proton pump », i.e. accumulation of hydrogen ions, H^+. First let us recall the hydrolysis formula (for a salt (NaA) of a weak acid (*HA*):

$$Na^+ + A^- + H_2O \rightleftharpoons Na^+ + OH^- + HA \text{ (undissoc).}$$

Assume that of the constituents present in an aqueous phase only the undissociated acid HA is soluble in an « oil membrane », then we can depict the following system:

H_2O (i)	Oil	H_2O (o)
Na^+_i		—
A^-_i		A^-_o
HA	HA	HA
OH^-_i		OH^-_o
H^+_i		H^+_o

$$H^+_i \times A^-_i = H^+_o \times A^-_o = (H^+_o)^2$$

A^-_i approx. = NaA

$$H_o = \sqrt{H^+_i \times A^-_i} = \sqrt{10^{-6} \times 10^{-1}} = 10^{-3.5}$$

The oil membrane dissolves the HA (which operates as a « carrier » for the H^+ and A^- ions). Now, we observe that we have a real Donnan equilibrium system present, because there is one ion which is impermeable, that is the Na^+. One can now apply the wellknown « cross product » relation of Donnan and calculate the steady state H^+ ion distribution between the « inside » (i) and the « outside » (o) as indicated in the diagram. The example, cited from an early work of mine (dealing with the production of the gastric acid juice (pH 1-2)), shows that one can « produce » pH 3.5 from a « source » of pH 6 of a N/10 normal salt (NaA), a rather good « proton pump » and entirely passive. Apparently this model fell short of the living stomach. Maybe that this type of process nevertheless can occur at very local levels in the living system.

In this context I recall that DANIELLI and DAVSON long ago pointed out that surface or interfaces could have a pH several units different from that of the adjacent aqueous solution.

V

IONIC PERMEABILITY AND TRANSPORT IN BIOLOGICAL AND ARTIFICIAL MEMBRANES. II

CALCIUM TRANSPORT BY MUSCLE MICROSOMES

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INTRODUCTION

The ATP driven calcium pump of the sarcoplasmic reticulum was discovered 1961 (HASSELBACH and MAKINOSE, 1961) shortly after it has been established by CALDWELL et al. (1960), DUNHAM and GLYNN (1961) that the sodium potassium pump of the membranes of the cell surfaces was fueled by energy rich phosphate compounds. The two ion pumps share a number of essential features as far as the basic mechanism of ion transport is concerned. In various structural and functional respects, however, the ion transport systems differ considerably according to their respective functions in the organism. The essential function of the sodium potassium pump is the maintenance of the osmotic and ionic balance between the intracellular and the extracellular compartments by extruding sodium and absorbing potassium ions. Structure and function of the sarcoplasmic membranes are characterized by a much higher degree of specialization. Their function is restricted to the regulation of muscular activity by calcium release and calcium removal from the myoplasma (cf. HASSELBACH, 1964;

EBASHI and ENDO, 1968). The contractile proteins in the muscle are activated when 0.05-0.2 μmol calcium • ml^{-1} of muscle fiber are liberated during excitation from small specialized membrane areas which are in intimate contact with the tubular invagination of the surface membrane (WINEGRAD, 1970). The inactivation of the contractile protein requires the complete removal of the liberated calcium during the relaxation period lasting 10-100 msec. This removal is mainly performed by the longitudinal elements of the sarcoplasmic reticulum. Accordingly, the reticulum is abundantly developped in fast contracting muscles and less developped in slowly contracting muscles. In fast contracting skeletal muscles the surface of the sarcoplasmic reticulum is approximately 30-100 times larger than the surface of the plasma membrane (PEACHEY, 1965). The working conditions of the sarcoplasmic membranes in vivo are determined by the concentration ratio of calcium between myoplasma and intravesicular space and by the permeability of the membranes for calcium. Since in the resting muscle the concentration of ionized calcium in the myoplasma does not exceed 0.1 μM (PORTZEHL, CALDWELL and RÜEGG, 1964) and the calcium concentration in the intravesicular space can be estimated to be approximately 1 mM, the concentration ratio maintained by the calcium pump approaches 10 000. This concentration ratio is more than 100 times higher than that maintained by the sodium potassium pump. In the resting muscle under steady state conditions the calcium pump works at least by a factor of 100 slower than at maximum activity. This estimate is based on the assumption that the total resting metabolism of the muscle accounts for the activity of the calcium pump. The low activity in the resting muscle means that relatively little ATP is consumed to maintain the high calcium gradient indicating that the passive permeability of the sarcoplasmic membranes for calcium is comparatively low.

This important property of the sarcoplasmic membrane is

not markedly affected by the procedure applied for their isolation which necessarily includes intense fragmentation. The membrane fragments which are mainly derivates of the longitudinal tubules of the sarcoplasmic membranes are characterized by a high healing tendency and spontaneously tightly sealed vesicles are formed (Fig. 1) (MAKINOSE and HASSELBACH, 1965; HASSELBACH, 1966; DUGGAN and MARTONOSI, 1970). The mean diameter of the vesicles is approximately 1000 Å and the sideness of the membranes is maintained during vesiculation (HASSELBACH and ELFVIN, 1967). These properties are in contrast to those of the fragments of the plasma membranes. Only a small fraction of these membrane fragments forms vesicles and the membranes of the vesicles are very often inverted and usually leaky (WALTER and POST, 1975). The unique stability of the sarcoplasmic membranes is the essential prerequisite which allowed us to analyze the calcium pump in vitro.

ENERGY DEPENDENT CALCIUM UPTAKE

Calcium uptake and phosphate liberation.

The calcium pump in the membranes of the sarcoplasmic vesicles transports calcium ions from the solution into the vesicles when they are supplied with magnesium and ATP or various other energy rich phosphate compounds (MAKINOSE and THE, 1965; DE MEIS, 1969; INESI, 1971). Under optimal conditions 1-2 µmol calcium are transported per mg of protein and minute at room temperature. The calcium transport is accompanied by the liberation of inorganic phosphate. The calcium dependent ATP hydrolysis has been called "extra-ATPase" (HASSELBACH and MAKINOSE, 1961; 1963). Under the described conditions the calcium storing capacity of the vesicles is limited to only 100-200 nmol per mg of vesicular

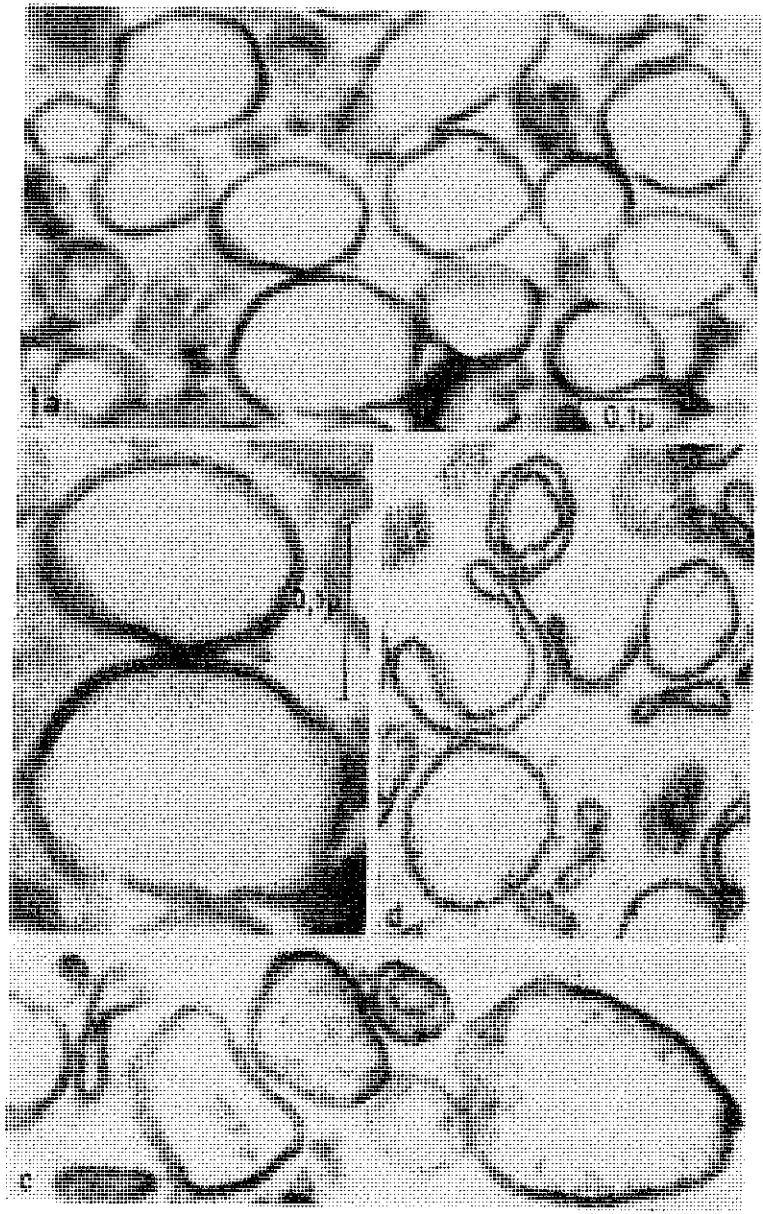

FIG. 1a-d — Sectioned pellet material of FSR membranes after freeze-drying and subsequent fixation with OsO_4 vapour (a and b), after partial dehydration (d). a, c, d 180.000 : 1, b 300.000 : 1.

protein. Therefore calcium uptake terminates after a few seconds. This limitation is due to a steep rise of the internal calcium concentration which inhibits calcium translocation and ATP splitting severely (HASSELBACH and MAKINOSE, 1965; WEBER, 1971; IKEMOTO, 1974). The inhibition is largely prevented in the presence of permeable calcium precipitating anions like oxalate, phosphate or pyrophosphate (MAKINOSE and HASSELBACH, 1965; HASSELBACH and WEBER, 1974). Under these conditions calcium and the anions are taken up in stoichiometrical amounts and the calcium storing capacity increases from 0.1 μ mol.mg to 10 μ mol.mg^{-1} of protein thus prolonging the uptake of calcium considerably. Therefore, the rate of calcium uptake and the accompanying phosphate liberation could be measured precisely. As one of the most important consequences of the presence of calcium precipitating anions the internal calcium activity remains low. It is determined by the solubility product of the respective calcium salt and the concentration of the precipitating anion. Due to the low internal calcium concentration the efflux of calcium during the initial phase of calcium uptake can be neglected. The initial rate of calcium uptake is therefore identical with the influx of calcium and can be related directly to the enhanced rate of Pi-liberation during the uptake phase, the extra-ATPase activity. Figure 2 demonstrates that the calcium pump consumes various phosphate donors with different rates. Yet, the ratio between the rate of calcium uptake and the calcium dependent phosphate liberation has a constant value of two for all phosphate donors. The low substrate specificity of the calcium pump is in marked constrast to the high specificity of the sodium potassium pump. The latter accepts only ATP as energy donors for ion translocation. The hydrolysis of phosphate donors like paranitrophenyl phospbate or acetylpbosphate by the sodium potassium transport system are thought to occur at a late step in the reaction sequence (SKOU, 1974).

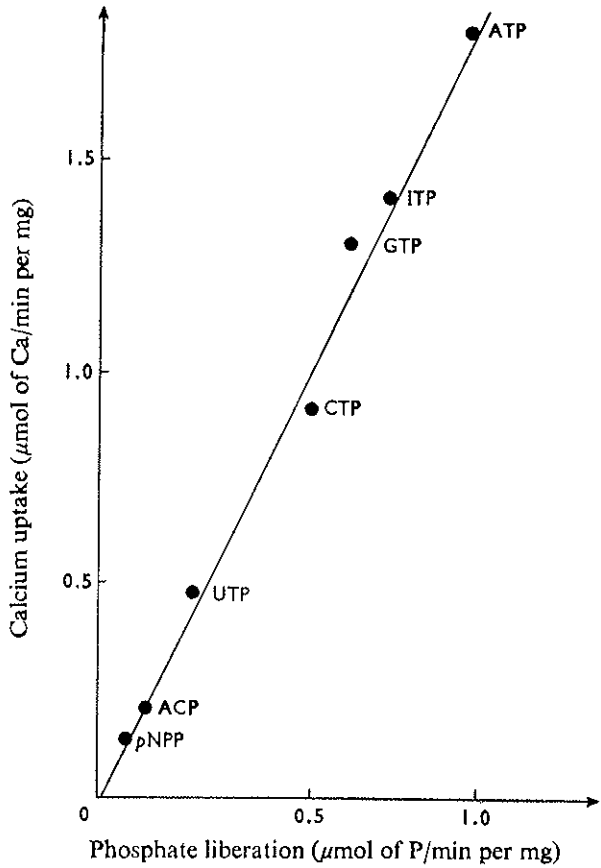

FIG. 2 — Correlation between the rate of calcium uptake and the rate of calcium-activated phosphate liberation. The data for ATP, ITP, GTP and UTP are taken from MAKINOSE and THE (1965). The transport ratio for acetyl phosphate (ACP) was determined at 30° C by measuring proton liberation and calcium uptake simultaneously. [The determination of acetyl phosphate splitting by proton liberation is more accurate than the determination of the unused acetyl phosphate]. The transport ratio for p-nitrophenyl phosphate (pNPP) is taken from INESI (1971).

Phosphoryl transfer and calcium uptake

Sufficient evidence has been provided in different laboratories that phosphate liberation and calcium accumulation are the terminal steps of a sequence of reactions. The sequence starts with the binding of ATP and calcium to the external surface of the sarcoplasmic membranes (FIEHN and MIGALA, 1970; CHEVALIER and BUTOW, 1970; MEISSNER, 1973). The affinity for calcium and ATP found in direct binding studies is in good agreement with those derived from kinetic measurements. Magnesium ions are not required for ATP binding (THE, 1975). Since calcium binding does not influence ATP binding and vice versa, the binding of the two reactants must be considered to occur independently. Low concentration of chaotropic anions can displace ATP reversibly from its binding site. This finding supports the assumption that ATP is bound in a hydrophobic pocket of the protein component of the calcium pump THE and HASSELBACH, 1975). When magnesium ions are present the binding of ATP and calcium is followed by a fast phosphoryl transfer reaction (MAKINOSE, 1966; YAMAMOTO and TONOMURA, 1967). Tbe rate constant of the transfer reaction is approximately 100 times higher than the rate found for phosphate liberation (Fig. 3) (HASSELBACH and SUKO, 1974; TAYLOR, 1973). Tbis explains the close correlation between the level of phosphoprotein and the activity of the calcium dependent ATPase found under a variety of conditions (MAKINOSE, 1969). The phosphoryl group in the membrane protein cannot only hydrolytically be split. In a competing reaction it can be transferred to nucleosiddiphosphates (HASSELBACH and MAKINOSE, 1962). This calcium dependent nucleosiddiphosphokinase activity is approxi mately 5 times higher than the transport related phosphatase activity. ADP is the phosphate acceptor with the highest affinity. It produces a severe inhibition of calcium uptake and ATP hydrolysis. The other nucleosiddiphosphates having

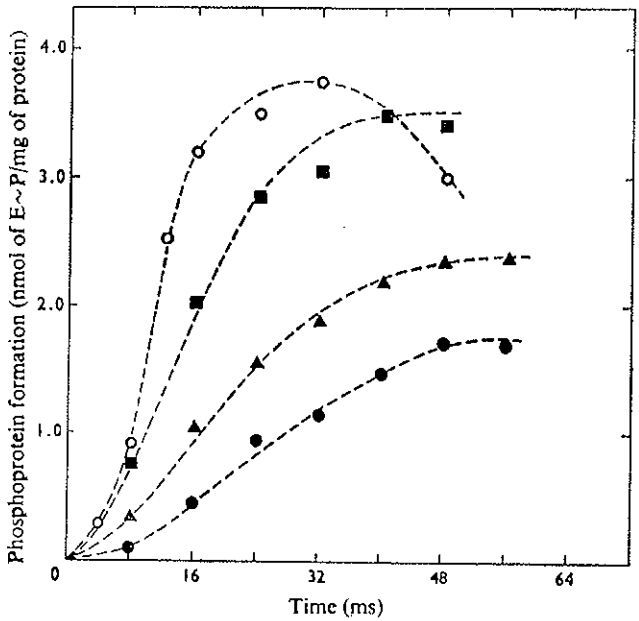

FIG. 3 — Rate of phosphoprotein formation at different ATP concentrations. ATP concentrations: ●, 5 μM; ▲, 10 μM; ■, 20 μM; ○, 40 uM. The assay media contained 1 mg of vesicle protein/ml, $MgCl_2$ (5mM), histidine (20 mM) and KCl (40 mM) at pH 7.0. The labelled ATP was mixed with the vesicular suspension in a fastmixing device at 4° C and quenched in cold 5% $HClO_4$. The precipitated protein was washed repeatedly. The activity incorporated into the protein was measured by liquid-scintillation counting.

a much lower affinity interfere only very little with phosphate liberation and calcium uptake (MAKINOSE, 1966).

All calcium dependent phoyphoryl transfer reactions in the sarcoplasmic reticulum require unimpaired thiol groups. They are located at the external surface of the vesicular membrane (HASSELBACH and SERAYDARIAN, 1966; HASSELBACH and ELFVIN, 1967). Their selective blockage inhibits phosphoprotein formation together with ATP-ADP exchange, calcium transport and phosphate liberation. This is in complete contrast

to the behaviour of the sodium potassium pump which exhibits and ATP-ADP exchange reaction only after thiol blockage (FAHN et al., 1968). The initial step of the reaction chain of calcium transport, the binding of ATP, is not affected by the blockage of the external thiol groups.

ENERGY TRANSFORMING CALCIUM RELEASE

ATP formation and calcium release

From vesicles filled with calcium oxalate or calcium phosphate calcium is released only very slowly, even when the external calcium concentration is kept low by the presence of high concentrations of EGTA (MAKINOSE and HASSELBACH, 1965). This calcium efflux has a comparatively low temperature coefficient and it is only little affected by high concentrations of univalent alkali chloride salts (BARLOGIE, 1971). Obviously, electrostatic interactions between the various membrane constituents do not essentially contribute to the tightness of the vesicles.

The contribution of the lipid phase of the sarcoplasmic membranes to their high resistence for calcium permeation is revealed by the complete leakiness which occurs after lipid perturbation by general anesthetics, bile salts or by enzymatic lipid degradation (FIEHN and HASSELBACH, 1969; AUGUSTIN and HASSELBACH, 1973). Since approximately 70 % of the membrane surface are covered by the transport protein, the permeability barrier for calcium does obviously not only reside in the membrane lipids. The contribution of the calcium transport protein to the high resistance of the membranes for passive calcium movements was revealed by the finding that a fast calcium efflux through the calcium transport protein could be induced by the simultaneous application of ADP and inorganic phosphate or of arsenate to calcium filled vesicles (Fig. 4) (BARLOGIE et al., 1971; HASSELBACH et al., 1972).

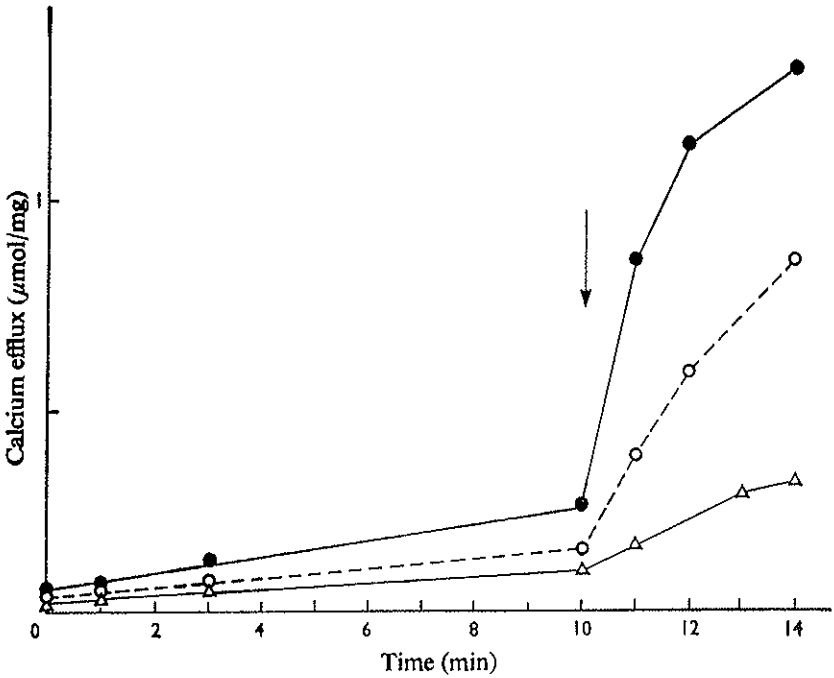

FIG. 4 — Effect of temperature on calcium release induced by ADP. ●, 28° C; ○, 20° C; △, 10° C. The vesicles were loaded with calcium phosphate with acetyl phosphate as energy donor in a medium containing acetyl phosphate (2 mM), $MgCl_2$ (5 mM), histidine (20 mM) and P_i (10 mM) at pH 7.0. At zero time 2 mM-EGTA was added and 10 min later calcium release was initiated by the addition of 2 mM-ADP (shown by the arrow).

10 μM ADP and 3 mM phosphate or 3 mM arsenate are required to activate calcium efflux half maximally. An additional requirement for the calcium efflux stimulation is the presence of magnesium ions. The stimulation of the calcium release is connected with an increase of the activation energy from 10,000-27,000 cal per Mol (HASSELBACH and SUKO, 1974). The Arrhenius graphs of the unstimulated and the stimulated calcium release intersect at approximately 0°C. Consequently,

the ADP + Pi induced calcium efflux as well as the arsenate induced calcium efflux disappear at 0°C (Fig. 5).

The conditions under which calcium efflux stimulation occurs suggest that the stimulated calcium efflux is the reversal of the ATP dependent calcium influx. This suspicion has at once been confirmed by the finding that the ADP + Pi dependent calcium efflux is coupled stoichiometrically to a net synthesis of ATP (MAKINOSE and HASSELBACH, 1971). The exit of two calcium ions leads to the formation of one molecule of ATP. Calcium release and ATP synthesis continue until the calcium stores are empty when the accumulation of ATP in the external medium is prevented by the transfer of its terminal phosphate to glucose (Fig. 6) (cf. HASSELBACH, 1974).

The first step in the reverse reaction is the incorporation of inorganic phosphate into the membrane protein. It is a relatively slow process which requires the absence of calcium ions in the external medium and a high internal calcium concentration (Fig. 7). The latter requirement emerges from the fact that in the presence of oxalate only a small amount of phosphoprotein is formed and decays spontaneously in spite of the existence of a considerable calcium gradient (HASSELBACH and SUKO, 1974). If both conditions, a high concentration of internal calcium and a low concentration of external calcium are fulfilled, phosphoprotein formation continues during calcium efflux and the phosphoryl group can be transferred continuously to ADP (MAKINOSE, 1972). When the calcium release is initiated by arsenate, no transport intermediates could be detected.

CALCIUM EXCHANGE

Calcium exchange at equilibrium

The stoichiometric relationship between calcium uptake and ATP splitting on the one hand, calcium release and ATP

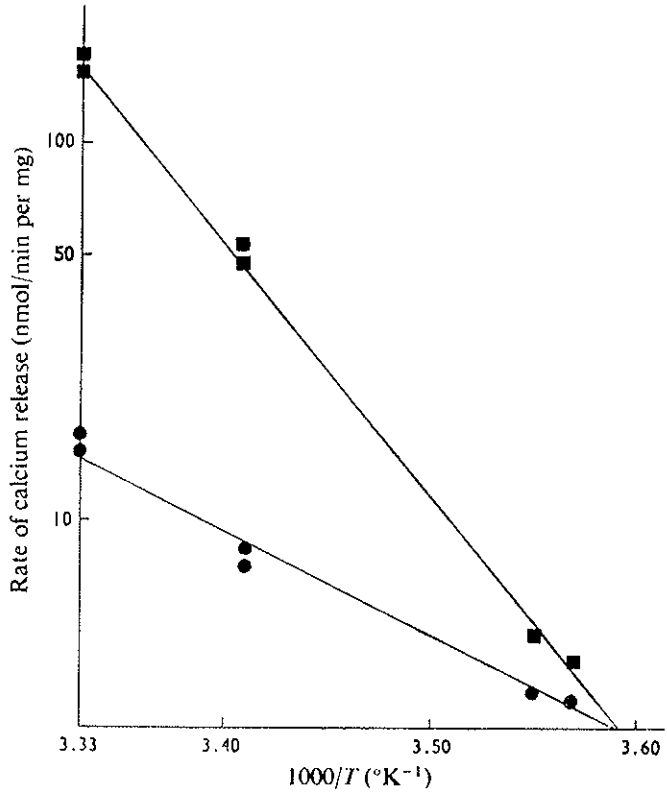

FIG. 5 — Temperature dependence of calcium release. The vesicles were loaded with calcium phosphate in media containing acetyl phosphate (3 mM), $MgCl_2$ (5 mM) and P_i (10 mM) at pH 7. ●, Calcium release was initiated by the addition of 2 mM-EGTA. ■, Calcium release was initiated by the addition of 2 mM-EGTA + 5 mM-arsenate.

synthesis on the other hand are observed under conditions which guarantee unidirectionality of both reactions. If the forward and the backward reactions are allowed to continue, net calcium movements and net phosphoryl transfer reactions finally cease. At this moment the transport system is not far from its

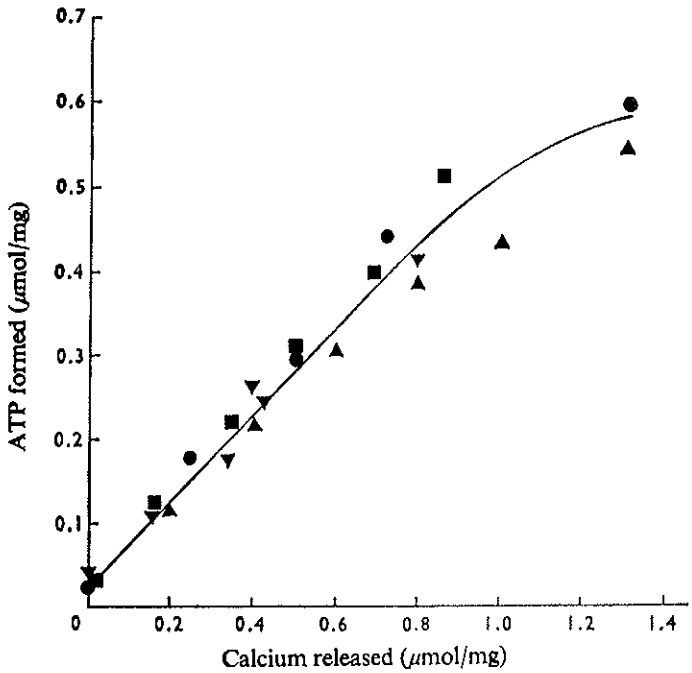

FIG. 6 — Correlation between calcium release and ATP formation. The vesicles were loaded with calcium phosphate, with acetyl phosphate as energy donor as described in Fig. 4. Calcium release and ATP formation was initiated by the addition of 2 mM-ADP. The ATP concentration was kept low by using hexokinase to transfer the γ phosphate of ATP to 0.1 M-glucose. ●, ■, ▲, ▼, Four different preparations.

true equilibrium. This statement is supported by the following consideration. The phosphate ions present in the system are not only reactants but also calcium precipitating agents. Consequently, when calcium phosphate precipitates exist inside the vesicles the internal activity product of calcium and phosphate is identical with the solubility product of calcium phosphate. This value together with the analytically determined activity of the ions in the external solution allow to make an energy

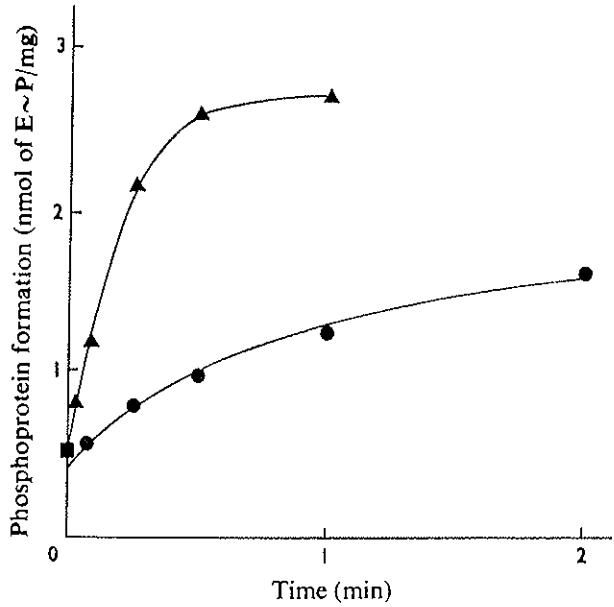

FIG. 7 — Phosphoprotein formation during calcium efflux. ▲, 5.5° C; ●, 0° C. Vesicles (25 mg) were incubated in $CaCl_2$ (20 mM, 2 ml). Calcium efflux and phosphorylation were initiated by adding 5 ml of a solution containing $MgCl_2$ (5 mM), EGTA (50 mM), [^{32}P] phosphate (5 mM) and histidine (0.1 mM) at pH 7.0. The reaction was terminated by the addition of $HClO_4$. After five washings the radioactivity in the protein was measured.

balance. Under steady state conditions the concentration ratios for calcium vary between 3000 and 30.000 corresponding to values existing in the living muscle. These values are in fair agreement with the figures calculated from the standard free energy of ATP hydrolysis and the concentrations of ATP, ADP and phosphate in the system assuming equilibrium conditions. The state of equilibium is characterized by a slow exchange of external for internal calcium which is paralleled by the incorporation of inorganic phosphate into the ATP fraction (MAKINOSE, 1972; DE MEIS and CARVALHO, 1974). If the calcium

gradient is abolished and the calcium concentration in the system is kept low, the Pi-ATP exchange reaction disappears completely. The correlationship between calcium exchange and Pi-ATP exchange is stressed by the finding that agents like prenylamine or propranolol which slow down the calcium movement, inhibit the Pi-ADP exchange equally strong (MAKINOSE, 1972).

Calcium exchange stimulation by ADP.

When the equilibrium is disturbed by the addition of calcium ions, the calcium dependent ATP splitting and calcium uptake are immediately activated while the Pi-ATP exchange reaction is completely suppressed (MAKINOSE, 1973). Since the concentrations of the reactants which determine the rate of the back reaction are not changed by the addition of calcium, the inhibition must be due to a direct inhibiting effect of the calcium ions in the external medium. Another important feature of the calcium transport system has been revealed by this experiment. If the system at equilibrium contains only low concentrations of ADP, the disturbance of the equilibrium by the addition of calcium results in an unidirectional calcium influx. If, however, higher concentrations of ADP (0.2 - 2 mM) are present, the addition of external calcium stimulates calcium efflux. This calcium induced ADP dependent calcium exchange is paralleled by a stimulation of the ATP-ADP exchange reaction. This observation indicates that the ATP-ADP reaction is closely correlated to the movement of the calcium ions in both directions. Furthermore, the fact that the ATP-ADP exchange only occurs after the Pi-ATP exchange is completely suppressed, strongly indicates that calcium is released from the transporting unit at the internal surface of the sarcoplasmic vesicles before inorganic phosphate is liberated. Fig. 8 summarizes the essential steps in the reaction sequence.

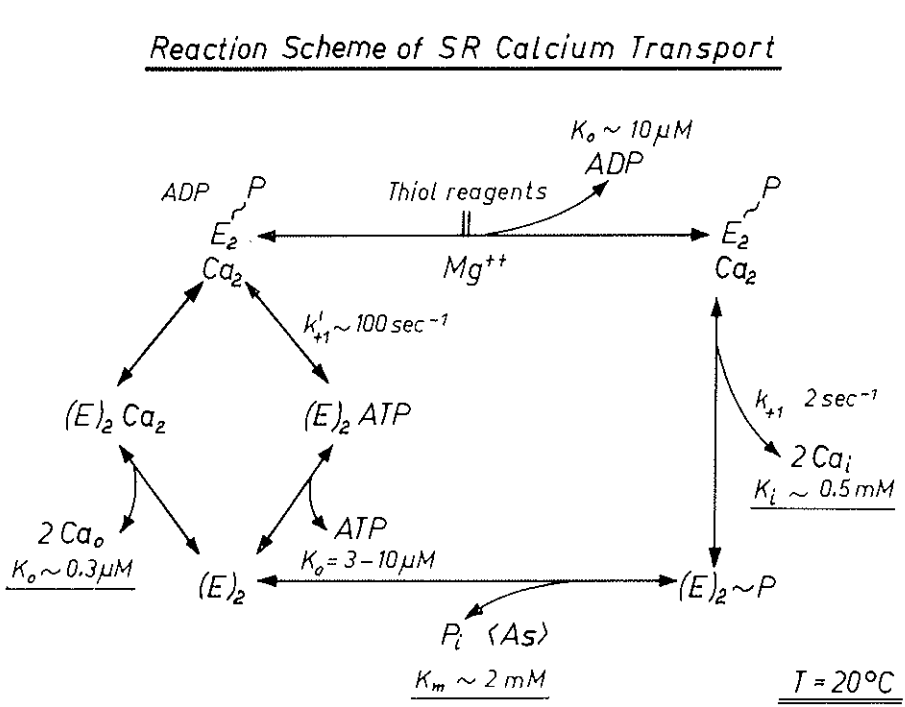

FIG. 8 — Reaction scheme of the sarcoplasmic calcium transport.

THE STRUCTURAL COMPONENTS OF THE CALCIUM PUMP

Size and shape of the pump complex

This survey would remain incomplete if the molecular properties of the calcium translocating unit in the sarcoplasmic membrane would not be considered. In contrast to other transport systems the protein of the calcium pump is the main constituent of the membrane. The transport protein comprises approximately 70-80% of the total membrane protein (cf. HASSELBACH, 1972; 1974). The transport unit is embedded in a lipid matrix which consists mainly of phospholipids containing

highly unsaturated fatty acids but only a small fraction of cholesterol and cholesterol esters (FIEHN and HASSELBACH, 1969; 1970). Mild detergents like cholate or desoxycholate solubilize the sarcoplasmic membrane (MACLENNAN, 1970; MACLENNAN et al., 1971; MEISSNER et al., 1973). The minor membrane protein components like the calcium binding protein can be removed from the calcium pump complex by low concentrations of detergents (MACLENNAN and WONG, 1971). The composition of the lipoprotein complex remains virtually unchanged by this procedure. In contrast, solubilization by Triton X-100 leads to the replacement of the natural lipids by the detergent (WALTER and HASSELBACH, 1973). The calcium transporting potein whose molecular weight has been found to be approximately 100.000 is asymmetrically arranged in the membrane. A considerable part of the molecule protrudes from the cytoplasmic surface of the membrane (DEAMER and BASKIN, 1969; DUPONT et al., 1973). The thiol groups which are essential for calcium translocation are located at the top of these projections. This can be shown by selective labelling with mercuri-phenyl azoferritin (HASSELBACH and ELFVIN, 1967; LENGSFELD and HASSELBACH, 1974). The ferritin label seems to be connected with the thin stalk to the membrane when conventionally dehydrated and embedded (Fig. 9). This characteristic arrangement disappears and is replaced by a more irregular pattern when the lipid phase of the membrane is removed by enzymatic delipidation (AGOSTINI and HASSELBACH, 1971). In contrast, the stability of the membrane structure is not affected when the transport ATPase is fragmented by tryptic cleavage (MIGALA et al., 1973; THORLEY-LAWSON and GREEN, 1973; STEWART and MACLENNAN, 1974; INESI and SCALES, 1974). The tryptic fragments remain in their original position and it can be shown that they are accessible from outside (Fig. 10). Obviously, intramolecular hydrophobic forces are strong enough to keep the fragments together. Most astonishingly, the transport system can still translocate calcium and hydrolyze ATP.

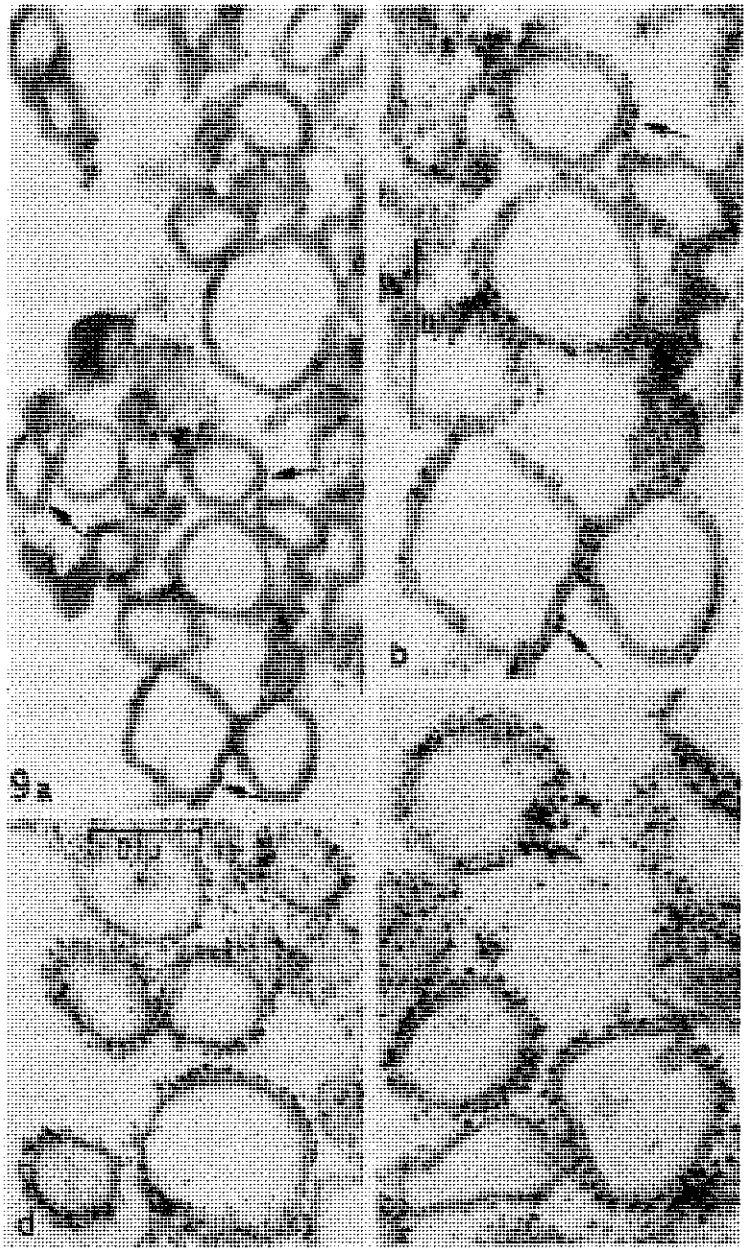

FIG. 9 — Hg-phenylazoferritin incubated membrane preparations, after freeze-drying and subsequent fixation with OsO_4 vapour (a and b), after partial dehydration (c) and after routine dehydration and embedding (d). a, c, d 180.000 : 1, b 300.000 : 1.

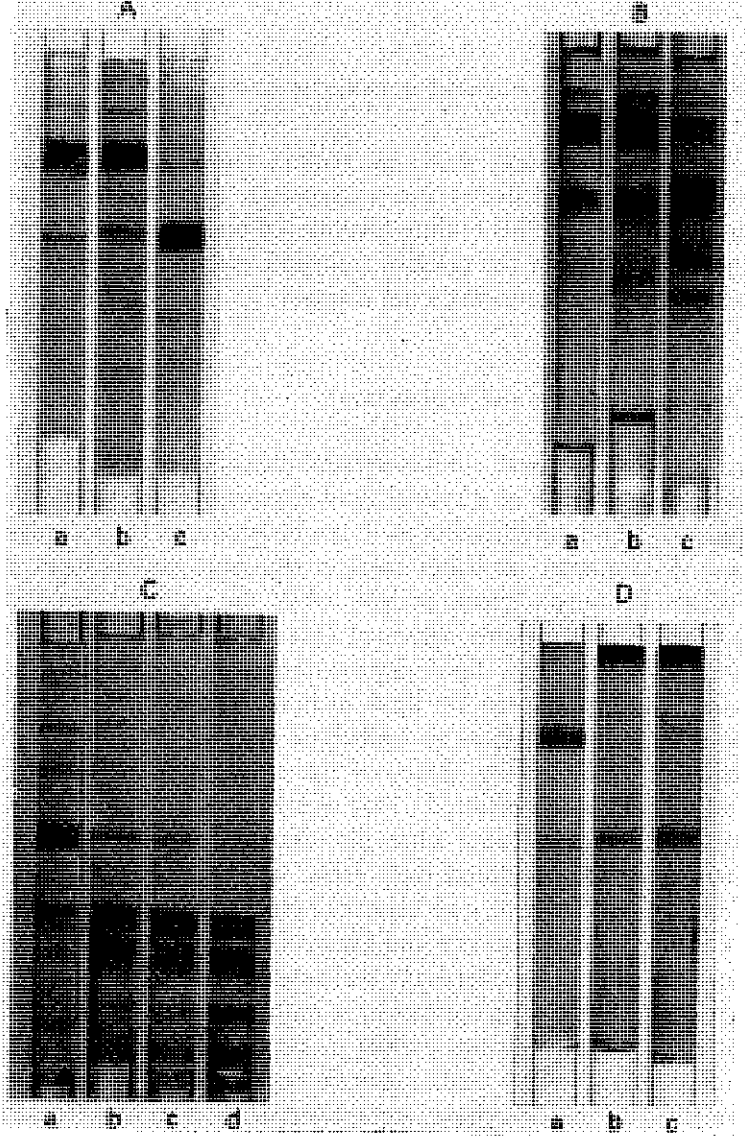

FIG. 10 — Separation of different SR protein preparations by gel electrophoresis. - A. Separation in acetic acid-phenol-water: a. Normal SR protein band (1) ∞ 100.000 dalton, band (2) ∞ 60.000 dalton; b. SR protein labelled with ^{14}C-NEM, 4 moles per 100.000 dalton; c. ^{14}C-NEM labelled protein was digested with trypsin for 5 min (trypsin-membrane protein ratio 1/20). - B. Separation in acetic acid-phenol-water: a. Normal SR protein; b. phosphorylated SR protein; c. the protein was digested with trypsin (trypsin-membrane protein ratio 1/200) and subsequently phosphorylated with ^{32}P-ITP. - C. Separation in tris-bicine buffer: a. Normal protein; b. c. d. protein digestion for 2 min (b), for 5 min (c) and 30 min (d) with trypsin (trypsin-membrane protein ratio 1/200). Note that the less intense band (2) which corresponds to a molecular weight of ∞ 60.000 dalton remains unchanged by digestion. - D. Separation in acetic acid-phenol-water: a. Normal SR protein; b. SR protein shortly digested with typsin and subsequently treated with the surface reagent dextranaminophenyldiazotate; c. SR protein crosslinked with dextranaminophenyl diazotate.

Q'9Bu00 In; shrdl

Obiously, the system does not require the covalent linkages which are cleaved by trypsin to maintain structural and functional integrity. This finding favours the mechanism of calcium transport in which the calcium transport system functions with relative modest conformational changes.

Lipid modification and replacement

As small as these conformational changes may be, they require the presence of a special lipid environment of the protein moiety. Lipid modification as it can be produced by phospholipase A treatment abolishes calcium transport and reduces the nucleosiddiphosphokinase activity while the calcium dependent ATPase is considerably activated (HASSELBACH et al., 1975). On the other hand, lipid deprivation as it can be achieved under mild conditions by lipid hydrolysis connected with the removal of the products of hydrolysis produce complete inactivation. However, the ability of the membranes to bind ATP and calcium is not affected. Part of the natural function of the system can be restituted when the membrane protein is supplemented with unsaturated fatty acids, with lysolecithin or with Triton X-100 (FIEHN and HASSELBACH, 1970; the and HASSELBACH, 1973; WALTER and HASSELBACH, 1973). The function which is most effectively restituted is the ATPase activity while phosphoprotein formation is only partially repaired. The most vulnerable function proves to be the nucleosiddiphosphokinase activity which could never be restituted. The lipid requirement for the described reconstitution is not very specific. It indicates an unspecific kind of hydrophobic interaction which provides a certain mobility of the transport ATPase molecule or its parts in the membrane (SEELIN and HASSELBACH, 1971). This explanation is supported by the experiments of WARREN et al. (1974) who exchanged the natural lipids of the sarcoplasmic membranes for synthetic phospholipids.

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DISCUSSION

Chairman: Prof. R. D. KEYNES

BAKER

Is the movement of calcium coupled to the movement of other ions? If it is not, a potential should be generated and if a potential is generated this should be measurable by using a fluorescent potential-sensitive probe, and I wonder whether you had looked at this under conditions of uptake and reversal to see whether there are potential changes and if so, how big they are.

HASSELBACH

The calcium transport is usually connected with the uptake of oxalate or phosphate. If there are no anions present, the internal calcium concentration rises and you can see an increase of the fluorescence of ANS during calcium uptake. This is an effect of the ionic strenght because at the moment you add 0.1 M Na acetate or KCl, the fluorescence change disappears.

BAKER

But how do you think the oxalate is being taken into the vesicles? Is it being drawn in by the potential?

HASSELBACH

Either an electrical potential is generated during the calcium transport or calcium is exchanged for a cation as potassium. The membranes seem to be very permeable for monovalent cations and chloride.

BAKER

But under normal conditions when you don't have these unphysiological things like oxalate around, you think there's quite a high concentration of free calcium inside and presumably there you might be able to detect a potential. Have you actually looked at that?

HASSELBACH

This is a question asked by all physiologists. I am really worried about the very high calcium concentration inside the sarcoplasmic vesicles. Under such conditions the system wouldn't work because high internal calcium concentration severely inhibits calcium transport. There must be some device that keeps the calcium concentration lower than we think, or in the living muscle the membranes are less sensitive to internal calcium.

BAKER

Is there no evidence for a calcium binding system inside the sarcoplasmic reticulum?

HASSELBACH

I do not believe that calsequestrin functions as a calcium binding substance. It is located in the membrane and not inside the vesicles.

POST

I would like to ask about the calcium binding protein, which was 10 to 15% of the total protein. Please state the type of protein. Do you know about its sideness, and is it required for function?

HASSELBACH

I think it is not required for function and it is exposed to the external surface of the membranes.

POST

Is it a glyco-protein?

HASSELBACH

We isolated the calcium binding protein by a very simple procedure and looked for its calcium binding properties. I do not know if it is a glyco-protein, and as far as I know sugar residues have not been found in this protein. Furthermore, its content in the isolated vesicles depends on a number of facts and varies considerably. These variations do not affect the activity of the calcium transport system. It is true that this protein has a great number of calcium binding sites, but its calcium affinity in the presence of KCl is low. The dissociation constant is in the order of 10^{-3}M. Therefore, the amount of bound calcium in relatively low and the free calcium concentration relatively high.

CONTROL OF ATP HYDROLYSIS, ATP⇌P_i EXCHANGE AND MEMBRANE PHOSPHORYLATION BY THE Ca^{2+} CONCENTRATION GRADIENT IN SARCOPLASMIC RETICULUM VESICLES

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The membranes of the sarcoplasmic reticulum can be separated in vesicular form from skeletal muscle homogenates. These vesicles retain the ability to accumulate Ca^{2+} at the expense of ATP hydrolysis. The relative simplicity of the membranes makes them useful for studying the energetic transformations involved in ion transport. A large proportion by weight of the proteins in these membranes consists of a highly efficient $Ca^{2+} + Mg^{2+}$ – dependent ATPase which has an affinity for Ca^{2+} in the micromolar range [1, 2].

The hydrolysis of ATP by the $Ca^{2+} + Mg^{2+}$ – dependent ATPase of isolated sarcoplasmic reticulum vesicles (SRV) is stoichiometrically coupled to the transport of calcium ions into the vesicles [1]. In the simplest reaction scheme [3], Ca^{2+} and ATP bind to the external surface of the membrane. An intermediate acyl phosphoprotein (E∼P) is formed, and ADP

is released (I). The phosphoprotein is subsequently hydrolyzed, liberating P_i (II):

$$ATP + E \underset{Mg^{2+}}{\overset{Ca^{2+}}{\rightleftharpoons}} E\sim P + ADP \qquad (I)$$

$$E\sim P \longrightarrow E + P_i \qquad (II)$$

In the process, two calcium ions are carried across the membrane and released to the interior of the vesicle. Under suitable conditions, a Ca^{2+} concentration gradient greater than 1000:1 can be maintained by the vesicles [1].

MAKINOSE [4-6] and MAKINOSE and HASSELBACH [7] were the first to demonstrate that the whole reaction can be completely reversed, culminating in a rapid efflux of Ca^{2+} and a net synthesis of ATP from P_i and ADP [8]. These observations have been confirmed by others [9, 10]. The reversibility of the individual reactions has also been demonstrated [5, 11]. The correlation of reversal with the existence of a Ca^{2+} concentration gradient led MAKINOSE [4] and MAKINOSE and HASSELBACH [7] to postulate that the gradient provides osmotic energy for the synthesis of ATP.

However, there exist a number of observations in leaky or solubilized vesicles with no Ca^{2+} gradient, showing both the formation of $E\sim P$ from P_i and an $ATP\rightleftharpoons P_i$ exchange that results in the formation of ATP from P_i and ADP while hydrolysis is still going on [12-17]. This report summarizes our attempts to understand how the Ca^{2+} concentration gradient might act to stimulate reversal of the reactions shown above. For this purpose, we have used the $ATP\rightleftharpoons P_i$ exchange reaction which has been shown by MAKINOSE [4] to be triggered in intact vesicles by the formation of a transmembrane Ca^{2+} concentration gradient.

METHODS

Sarcoplasmic reticulum vesicles were prepared from rabbit skeletal muscle as previously described [18]. $\gamma^{32}P_i$ obtained from the Brasilian Institute of Atomic Energy and purified by means of a column of Dowex AG1 X10 [15, 19].

Leaky SRV were prepared by treating intact vesicles with alkaline EGTA for 20 min at room temperature. As described earlier [15, 20, 21], the leaky vesicles no longer accumulate Ca^{2+}, although the ATPase activity is increased.

Solubilized SRV were prepared by treatment with Triton X-100 in 20% (v/v) glycerol and 4 mM $CaCl_2$ at pH 8 (Triton: protein 2:1, w/w [22].

Ca^{2+} uptake was measured by the use of ^{45}Ca and Millipore filters [23].

$ATP \rightleftharpoons P_i$ exchange and *ATPase activity* were assayed using $^{32}P_i$ or (γ - ^{32}P)ATP and a modification of the phosphomolydate — isobutanol — benzene extraction described by AVRON [15, 24]. As a control in the exchange experiments, the (γ - ^{32}P)ATP formed in the different experimental conditions described in the results was identified enzymatically [25] using hexokinase (EC 2.7.1.1) and glucose-6-phosphate dehydrogenase (EC 1.1.1.4.9).

In this report we will distinguish two different ATPase activities [1]. The *Mg^{2+} - dependent ATPase* requires only Mg^{2+} for its activation and is tested in the presence of EGTA to remove contaminating Ca^{2+}. The *$Ca^{2+} + Mg^{2+}$ - dependent ATPase* requires Ca^{2+} and Mg^{2+} for full activity and is calculated by substracting the Mg^{2+} - dependent activity from the total activity measured in the presence of Ca^{2+} and Mg^{2+}.

Membrane phosphorylation from either ($\gamma^{32}P$)ATP or $^{32}P_i$ was assayed and corrected for non-specific binding as previously described [14, 19].

RESULTS AND DISCUSSION

In the first series of experiments, $ATP \rightleftharpoons P_i$ exchange was used as an index of the rate at which the overall reaction described in the introduction can be reversed. As shown by MAKINOSE [4], intact vesicles incubated with ATP, ADP, Mg^{2+} and 0.1 mM $CaCl_2$ incorporate $^{32}P_i$ into $(\gamma^{32}P)ATP$ at a very low rate until all but a few micromolar Ca^{2+} has been removed from the medium and a Ca^{2+} gradient has been established (Fig. 1). Then the rate of $ATP \rightleftharpoons P_i$ exchange rather abruptly increases to a new, steady level which depends on the concentration of P_i in the medium. The exchange in this medium becomes negligible if the Ca^{2+} gradient is abolished by adding agents which make the vesicles leaky [4, 15].

The Ca^{2+} dependence of $ATP \rightleftharpoons P_i$ exchange.

One of the conditions which builds up along with the gradient in the experiment of Fig. 1 is a high Ca^{+2} concentration

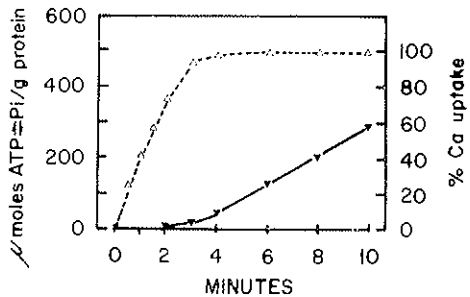

FIG. 1 — *Rate of $ATP \rightleftharpoons P_i$ exchange and of Ca^{2+} uptake.* The assay medium composition was 20 mM Tris-maleate buffer pH 6.8, 4 mM ATP, 15 mM $MgCl_2$, 0.1 $CaCl_2$, 0.2 mg/ml of intact SRV protein and 4 mM potassium phosphate. The reaction was started by the addition of SRV and stopped after different incubation intervals by filtration. For Ca^{2+} uptake (△ - - - △), non-radioactive Pi and ^{45}Ca were used. For $ATP \rightleftharpoons$ Pi exchange (▼——▼), non-radioactive Ca^{2+} and ^{32}Pi were used. The reaction was performed at 30°.

(in the millimolar range) inside the vesicles [1, 15, 26]. Figure 2 shows that this factor alone can account for part of the activation of exchange when a gradient is formed. In vesicles solubilized with Triton X-100 or made leaky by alkaline-EGTA treatment, $ATP \rightleftharpoons P_i$ exchange is negligible in 0.1 mM $CaCl_2$, but increases to a maximum as the Ca^{2+} concentration is raised to 4-5 mM. Hydrolysis is concomitantly depressed, and the Ca^{2+} concentration required to achieve 50% of both effects is about 2 mM. As in intact vesicles, the rate of exchange in leaky vesicles at an optimum Ca^{2+} concentration depends on the concentration of P_i, up to the limit imposed by the precipitation of calcium phosphate in the assay medium [15]. The requirement for low concentrations of ADP is also retained: if phosphoenol-pyruvate and pyruvate kinase (EC 2.7.1.40) are included in the assay medium, exchange is virtually abolished [15].

Activation of $ATP \rightleftharpoons P_i$ exchange by Ag^+.

Although these experiments demonstrate that significant exchange can occur in the absence of a Ca^{2+} concentration gradient if a high Ca^{2+} concentration is provided, the rates are disappointingly low compared with those found in intact vesicles (Table I). As shown in Fig. 2 and Table II the total ATPase activity of leaky vesicles in 4 to 10 mM $CaCl_2$ is still substantial. In contrast, as Ca^{2+} is accumulated by intact vesicles in the experiment of Fig. 1, the concentration in the medium falls below the level required to activate maximally the $Ca^{2+} + Mg^{2+}$ - dependent ATPase activity [1]. Consequently, the rate of hydrolysis falls to little more than that of the Mg^{2+} - dependent ATPase [1]. We were fortunate to discover an agent which allows us to duplicate, in leaky vesicles, this second condition which accompanies formation of a gradient and activation of exchange.

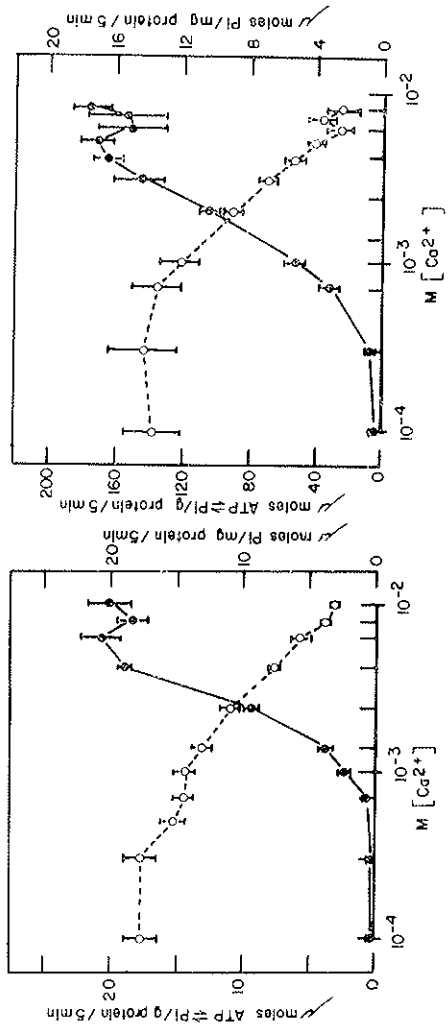

FIG. 2 — *Ca^{2+} dependence of ATP⇌Pi exchange.* The assay medium contained 30 mM Tris-maleate buffer pH 6.8, 10 mM ATP, 6 mM Pi, 20 mM $MgCl_2$ and 0.3 mg/ml of solubilized (*left*) or leaky (*right*) SRV protein. The reaction was performed at 37°. For the ATPase activity (O----O), (γ—^{32}P) ATP was used. For ATP⇌Pi exchange (●——●), ^{32}Pi was used. The values shown in the figure represent the average ± S.E. of 5 experiments. Reprinted with permission from [15].

TABLE I — *Rate of ATP⇌Pi exchange with or without a Ca^{2+} concentration gradient.*

SRV Treatment	μmoles ATP ⇌ Pi/mg protein. 5 min
(A) Intact, with gradient	0.200 ± 0.034 (4)
(B) Leaky	0.064 ± 0.013 (5)
(C) Leaky + $AgNO_3$	0.202 ± 0.033 (5)

The assay medium contained 30 mM Tris-maleate buffer pH 7.0, 5 mM ATP, 20 mM $MgCl_2$, 6 mM ^{32}Pi, 4 mM $CaCl_2$ (B and C) or 0.1 mM $CaCl_2$ (A), and 0.2 mg SRV protein/ml, at 30°. For experiment (A), ADP was omitted and the rate of ATP ⇌ Pi exchange was measured after 99% of the Ca^{2+} of the assay medium had been removed by the SRV (see Fig. 1). For (C), each preparation was tested in a range of $AgNO_3$ concentrations as in Fig. 4, and the maximal rate of exchange in each case was used to calculate the values shown. (B) and (C) contained 0.5 mM ADP. The values represent the average ± the standard error of the number of experiments indicated in parentheses.

TABLE II — *The effect of Ag^+ on SRV ATPase activity and ATP⇌Pi exchange.*

Additions to assay medium or SRV treatment	ATPase activity μmoles Pi/mg protein 5 min		ATP ⇌ Pi exchange: μmoles/mg protein 5 min
	Mg^{2+}-dependent EGTA	Ca^{2+} + Mg^{2+}-dependent 4 mM Ca^{2+}	4 mM Ca^{2+}
None	0.98±0.05	1.47±0.18	0.10±0.01
$AgNO_3$ added to assay medium (5×10^{-5}M)	0.84±0.02	0.08±0.08	0.42±0.04

The assay medium composition and experimental conditions were as described in Fig. 5. For each experiment, the Ca^{2+} + Mg^{2+}-dependent ATPase was calculated as described in Methods. The values represent the average ± S.E. of four experiments.

We find that micromolar concentrations of Ag^+ block Ca^{2+} uptake by intact vesicles and completely inhibit $Ca^{2+} + Mg^{2+}$ - dependent ATP hydrolysis in leaky vesicles (Fig. 3), simultaneously activating $ATP \rightleftharpoons P_i$ exchange (Fig. 4). Although the effect of Ag^+ on hydrolysis under the conditions of Fig. 4 is sometimes hiphasic [17], there is always a narrow range of

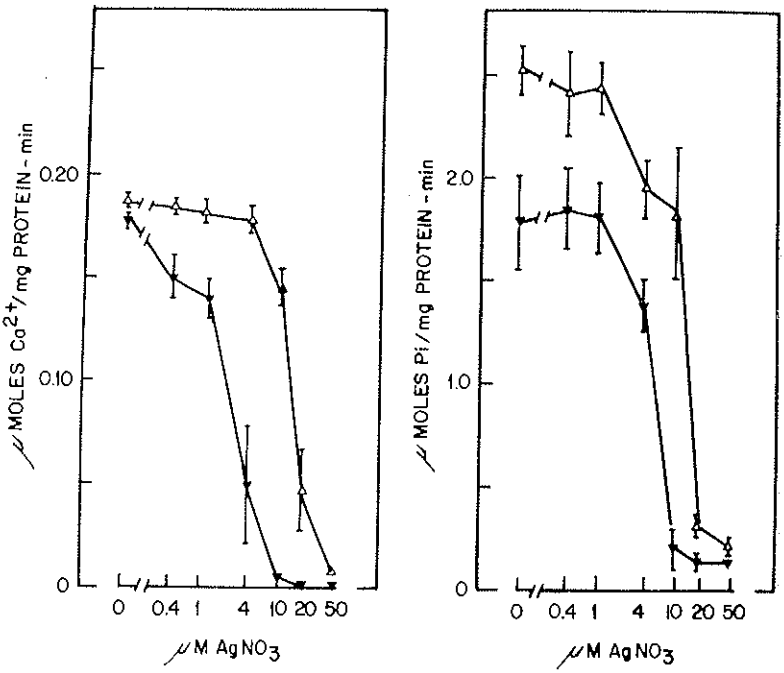

FIG. 3 — *Inhibition of Ca^{2+} uptake and ATPase activity of SRV by Ag^+.* For the Ca^{2+} uptake (*left*), the assay medium contained intact vesicles and 30 mM Tris-maleate buffer pH 7.0, 20 mM $MgCl_2$, 5 mM ATP, 8 mM Pi and 0.1 mM $^{45}CaCl_2$. For the total ATPase activity (*right*), leaky SRV, non-radioactive Ca and ($\gamma - ^{32}P$) ATP were used. The reaction was started by the addition of 0.05 (▼) or 0.20 (△) mg SRV protein/ml. After 4 min incubation at 30° the reaction was stopped either by the removal of SRV with Millipore filters or by the addition of trichloroacetic acid. The values shown in the figure represent the average ± the standard error of 7 (▼) or 4 (△) experiments. Reprinted with permission from [17].

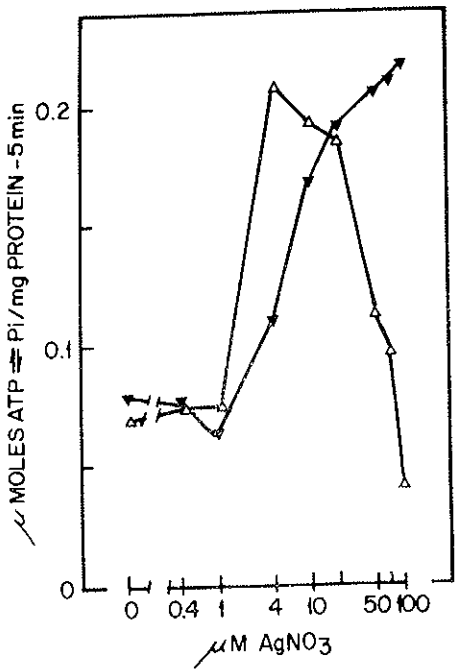

FIG. 4 — *Effect of Ag^+ on ATP $\rightleftharpoons$ Pi exchange.* The assay medium contained 30 mM Tris-maleate buffer pH 7.0, 5 mM ATP, 0.5 mM ADP, 8 mM (^{32}P) Pi, 20 mM Mg $MgCl_2$, and 4 mM $CaCl_2$, at 30°. The reactions were started by the addition of leaky SRV and aliquots were mixed with trichloroacetic acid after 5 min incubation. Formation of ($\gamma - ^{32}$P) ATP was analyzed as described in the Methods. The figure shows a typical experiment. With 0.1 mg leaky SRV protein/ml, the concentration of $AgNO_3$ required for maximum activation of the exchange reaction varied from 4 to 20×10^{-6} M in 5 different SRV preparations tested. (△) leaky SRV, 0.1 mg protein/ml; (▼) 0.3 mg protein/ml. Reprinted with permission from [17].

Ag^+ concentrations in which the $Ca^{2+} + Mg^{2+}$ – dependent ATP hydrolysis is fully inhibited and the ATP$\rightleftharpoons$P$_i$ exchange is stronlgy activated. The Mg^{2+} – dependent ATPase activity does not seem to be significantly inhibited (Table II and fig. 5). The inhibition of ATP hydrolysis by Ag^+ had been seen earlier by WALTER and HASSELBACH [27]. Fig. 4 also shows that the concentra-

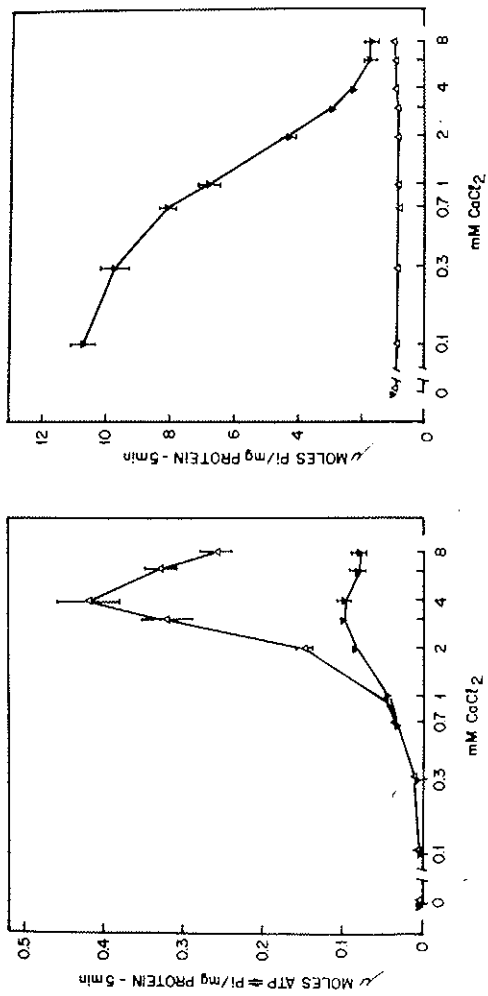

FIG. 5 — *Ca^{2+} dependence of ATP $\rightleftharpoons$ Pi exchange and ATPase activity in the presence of $AgNO_3$.* For ATP $\rightleftharpoons$ Pi exchange (*left*), the assay medium composition was 30 mM Tris-maleate buffer pH 6.8, 5 mM ATP, 0.5 mM ADP, 6 mM ^{32}Pi, 20 mM $MgCl_2$, and different $CaCl_2$ concentrations as shown in the figure. For zero Ca^{2+}, $CaCl_2$ was omitted and 5 mM EGTA was added. For the total ATPase activity (*right*), non-radioactive Pi and ($\gamma - ^{32}P$) ATP were used. The reaction was started by the addition of leaky SRV (0.2 or 0.3 mg protein/ml) and stopped after 5 min incubation at 30° by the addition of trichloroacetic acid. The values shown represent the average ± the standard error of 4 experiments. (▼) Control, without $AgNO_3$; (△) with 5×10^{-5} M $AgNO_3$. Reprinted with permission from [17].

tion of Ag^+ required to activate exchange depends on the protein concentration (*cf.* also Fig. 3), and in general Ag^+ behaves as if it were binding to the protein [17].

Choosing a concentration ratio of Ag^+ to SRV protein that is optimal for activating exchange, we can show that exchange is activated 2- to 4-fold at Ca^+ concentrations above 1 mM, although the Ca^{2+} dependence of the ATP⇌P_i exchange reaction in leaky vesicles is unaltered by the presence of Ag^+ (Fig. 5, left). Hydrolysis, however, is inhibited to the level of the Mg^{2+} - dependent ATPase activity at all Ca^{2+} concentrations (Fig. 5, right). Table I shows that Ag^+ increases the rate of exchange in leaky vesicles at high Ca^{2+} concentrations to the level found in intact vesicles having a Ca^{2+} concentration gradient. It seems reasonable to associate this activation by Ag^+ with its inhibitory effect on the forward reaction. In intact vesicles, the inhibition of hydrolysis is achieved by the reduction of external Ca^{2+} to subsaturating levels.

The Ca^{2+} dependence of E∼P formation.

Measurement of the steady-state levels of phosphoprotein formed from P_i has provided us with more clues about the Ca^{2+} requirement for the ATP⇌P_i exchange reaction [13, 14, 16]. When either leaky or intact vesicles are incubated with $^{32}P_i$ and Mg^{2+} in the absence of ATP and Ca^{2+} (1 mM EGTA), steady-state levels of E - ^{32}P are formed which depend on the concentration of P_i (Fig. 6). Once E∼P is formed, the addition of Ca^{2+} causes an immediate decrease (Fig. 7). This inhibition can be as rapidly reversed by adding EGTA (Fig. 7). The concentration of Ca^{2+} required for half-maximal inhibition of the reaction in empty, intact vesicles is about 10 μM (Fig. 8), not too different from the 2-5 μM required for activating the formation of E∼P from NTP [1, 3, 28]. The E∼P formed from P_i thus seems to be constantly exchanging with the P_i of the medium [12, 13]. However, if ADP is added to the medium,

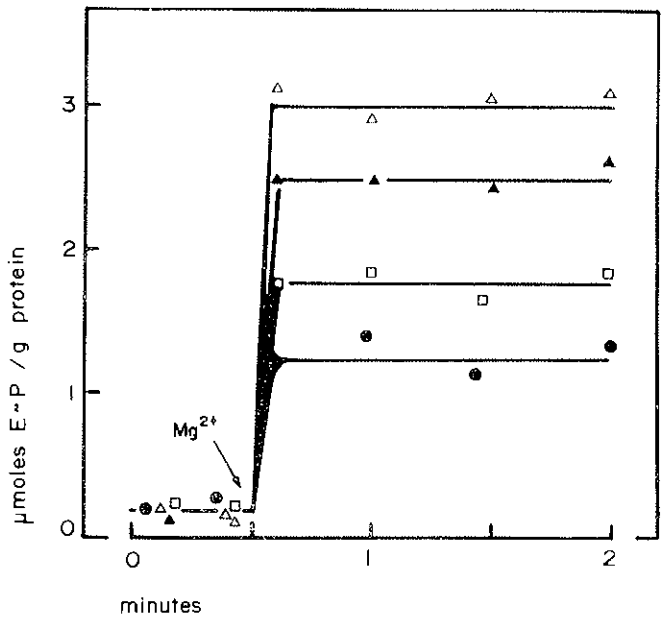

FIG. 6 — *Time course and Pi dependence of E-P formation in the absence of* Ca^{2+}. The assay medium composition was 10 mM Tris-maleate buffer pH 6.0, 1 mM EGTA, 0.6 mg SRV protein/ml and (●) 0.5 mM, (□) 1 mM, (▼) 2 mM, (△) 4 mM ^{32}Pi. The reaction was performed at 37°. At the arrow, $MgCl_2$ was added to a final concentration of 10 mM. The reaction was stopped after different incubation intervals at 37° by injecting it into cold perchloric acid. The amount of E-^{32}P formed was measured as previously described [13]. Reprinted with permission from [13].

the membrane-bound phosphate is not incorporated into ATP. In a low-Ca^{2+} medium, this transfer is only observed in intact vesicles loaded with Ca^{2+} [5, 15].

The role of Ca^{2+} *in regulating* $ATP \rightleftharpoons P_i$ *exchange in intact vesicles.*

With the foregoing information we may propose that the rates of the forward and reverse reactions in intact vesicles are

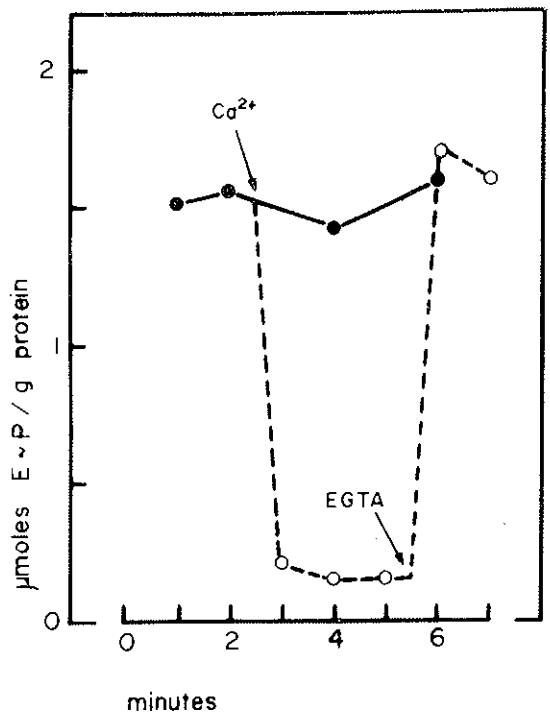

FIG. 7 — *Reversible inhibition by Ca^{2+} of E-P formation from Pi.* The assay medium composition was 10 mM Tris-maleate buffer (pH 6.0), 0.1 mM EGTA, 10 mM $MgCl_2$, and 4 mM ^{32}Pi. To a separate aliquot of this medium, $CaCl_2$ to a final concentration of 0.3 mM and EGTA to a final concentration of 2 mM were added as shown by arrows. Other additions and experimental conditions were as described in Fig. 6. Essentially the same results were obtained in four different SRV preparations tested. Reprinted with permission from [13].

controlled by the internal and external Ca^{2+} concentrations in the following way:

a) The membrane is phosphorylated by the binding of either P_i or ATP to a common site on the external surface of the membrane.

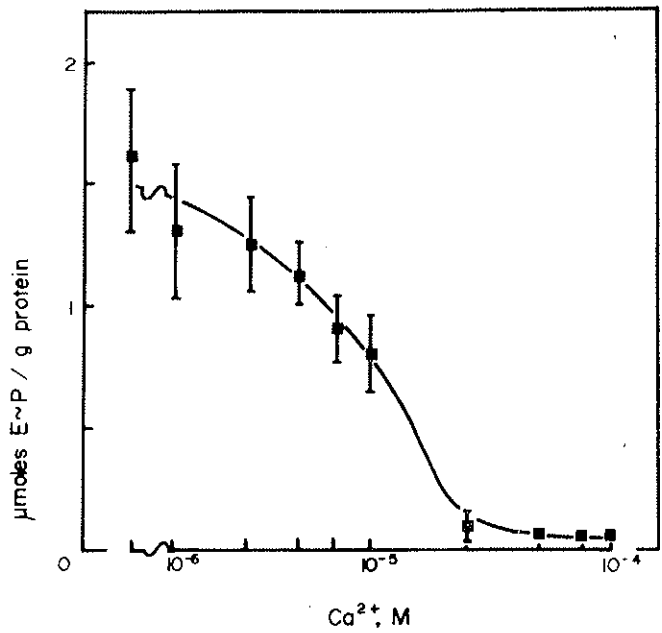

FIG. 8 — *Inhibition of E-P formation by* Ca^{2+}. The ^{32}Pi concentration was 4 mM. For zero Ca^{2+}, no $CaCl_2$ was added and the EGTA concentration was 1.0 mM. For calculated free Ca^{2+} concentrations of 1.5, 3.0, 5.0, 7.0, and 10.6 μM, 0.2 mM $CaCl_2$ and 0.70, 0.46, 0.35, 0.30, and 0.26 mM EGTA, respectively, were added to the assay medium. For the Ca^{2+} concentrations of 25, 50, and 100 μM, no EGTA was used. The free Ca^{2+} concentrations was calculated using the value of 4×10^{-6} for the dissociation constant of the complex Ca-EGTA [18]. Other additions and experimental conditions were as described in Fig. 6. The values represent the average ± the standard error (SE) of four experiments. Reprinted with permission from [13].

The choice of substrate is regulated by the binding of Ca^{2+}, also externally: low Ca^{2+} favors the binding of P_i, and high Ca^{2+}, the binding of ATP [13]. This effect of Ca^{2+} is saturated in the range of 10^{-5} M Ca^{2+}.

b) Ca^{2+} must be bound at the inner surface of the vesicle to permit the transfer of P_i from the phosphoprotein to ADP, to

form ATP and complete the exchange reaction. The affinity for Ca^{2+} at this surface is in the millimolar range [1, 15]. In the exchange reaction, therefore, the formation of E∼P must be followed by the formation of a Ca:E∼P complex. This sequence was also inferred by MAKINOSE [6] from Ca^{2+} exchange data.

This formulation predicts the results shown in Fig. 9: Empty, intact vesicles placed in a medium containing 0.1 mM

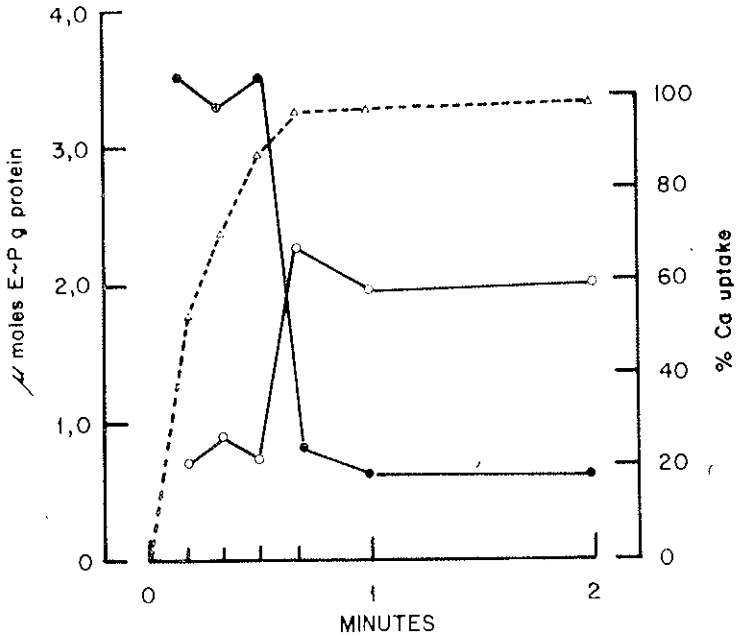

FIG. 9 — *Time course of membrane phosphorylation from Pi and from ATP as a Ca^{2+} concentration gradient is established.* The assay medium composition was 30 mM Tris-maleate buffer pH 7.0, 20 mM $MgCl_2$, 6 mM Pi, 0.1 mM $CaCl_2$, 20 mM KCl, 4 mM ATP, 5 mM phosphoenolpyruvate and 0.1 mg/ml pyruvic phosphotransferase (EC 3.7.1.40). The reaction was started by the addition of intact SRV at 30° and stopped after different incubation intervals by filtration or by perchloric acid. For Ca^{2+} uptake (△---△), ^{45}Ca was used. For E-^{32}P formation, (○) ^{32}Pi or (●) (γ-^{32}P)ATP was used.

$CaCl_2$ initially undergo step (*a*), membrane phosphorylation from ATP. As the external concentration of Ca^2 falls, the preference for ATP is reduced and the phosphorylation site is dominated by P_i. As shown in Fig. 1, once a high level of internal Ca^{2+} is reached and when ADP is present, this phosphate can be incorporated into ATP. In fact, the regenerating system present in the experiment of Fig. 9 is necessary to permit the demonstration of E~P formation from P_i. If ADP is present, the transfer of the membrane-bound phosphate to ADP appears to proceed at a very fast rate [5]. Therefore little of the E~P formed from P_i can be detected.

Using leaky vesicles, we can imitate the phosphorylation data from intact vesicles (Fig. 9) by adding increasing concentrations of Ag^+ to the assay medium (Fig. 10). In the control without Ag^+, the membrane is preferentially phosphorylated from ATP. With concentrations of Ag^+ that inhibit hydrolysis and activate exchange in this system (*cf.* Fig. 4), membrane phosphorylation from ATP is correspondingly depressed and phosphorylation from P_i is enhanced. Evidently the Ag^+ can override the inhibitory effect of Ca^{2+} on membrane phosphorylation from P_i, (*cf.* Fig. 7). Overoptimal concentrations of Ag^+ inhibit phosphorylation at both sites. The appearance of similar levels of E~P in 0.1 mM and 4 mM $CaCl_2$ supports step (*b*) of the scheme presented above. The high Ca^{2+} concentration required for ATP⇌P_i exchange in leaky vesicles is not necessary for formation of the E~P complex; it must be required for the transfer of phosphate to ADP. In intact vesicles this requirement is met by the accumulation of Ca_2^+ inside the vesicles.

A possible role for the Mg^{2+} - dependent ATPase.

If the Ca_2^+ concentration gradient is not a necessary source of energy for the ATP⇌P_i exchange reaction, what provides the energy for the formation of the terminal phosphate bond of

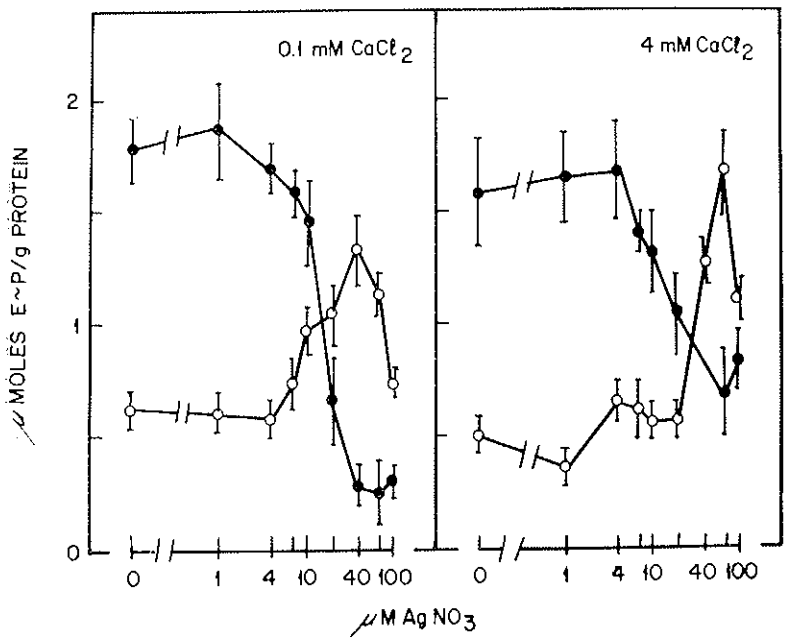

FIG. 10 — *Membrane phosphorylation by ^{32}Pi and $(\gamma-^{32}P)$ ATP in the presence of Ag^+.* The assay medium composition was 30 mM Tris-maleate buffer pH 7.0, 5 mM ATP, 0.5 mM ADP, 6 mM Pi, 20 mM $MgCl_2$ and 0.1 (*left*) or 4.0 mM (*right*) $CaCl_2$. The reaction was started by the addition of leaky SRV (0.33 mg protein/ml) and stopped after 20 or 40 sec incubation at 30° by the addition of trichloroacetic acid to a final concentration of 20% (w/v). The degree of membrane phosphorylation from either compound was essentially the same for either of these times, indicating that the steady state was reached within the first 20 sec. (●) $(\gamma-^{32}P)$ ATP and non-radioactive Pi, (○) ^{32}Pi and non-radioactive ATP. The values shown in the figure represent the average ± the standard error of 4 or 7 experiments, respectively. Reprinted with permission from [17].

ATP? Even in the presence of Ag^+, we have never been able to measure a net synthesis of ATP in leaky vesicles. Table II shows that despite the reduction in $Ca^{2+}+Mg^{2+}$ - dependent ATPase activity, the balance remains tilted in favor of hydrolysis by the persistence of a Mg^+ - dependent ATPase activity

that is double the rate of exchange. If they are two independent entities, then we may consider that we have demonstrated net syntbesis of ATP *at the* Ca^{2+} *- dependent site.* WEBER [29] has argued that there are two discrete enzymes, since they have different affinities for MgATP.

On the other hand, if the two systems are coupled — through a conformational change, for example — the energy of hydrolysis by the Mg^{2+} - dependent configuration could be transferred to the Ca^{2+} - dependent configuration for use in the synthesis of ATP. Several recent reports on the properties of the ATPase obtained from SRV after lipid extraction [27, 30] or trypsin digestion [31] could be interpreted to mean that the Mg^{2+} - dependent and $Ca^{2+} + Mg^{2+}$ - dependent ATPase activities reside in the same protein.

ACKNOWLEDGMENTS

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DISCUSSION

Chairman: Prof. R. D. KEYNES

HASSELBACH

Can you tell us if the effect of silver ions is reversible? How active is the enzyme?

DE MEIS

No, I was not able to remove the effect of silver ions. Either silver ions remain tightly bound or they do something to the enzyme which I failed to remove. After treating the vesicle with A_gNO_3 we tried to restore the Ca^{2+}—dependent ATPase activity by washing the vesicles with either mercaptoethanol, ATP, or EGTA, but we failed.

BOYER

What is the specificity of the silver effect. Ag^+ can bind not only to —SH groups but to other groups as you are aware. Do you get any similar effect by mercurials?

DE MEIS

C_uSO_4 will imitate the effect of silver, but in higher concentrations. I did not test mercurials.

POST

Would you state again what you think the function of the magnesium-dependent, calcium-independent ATPase is?

DE MEIS

Looking at the data of Table II, it seems possible that the energy for the ATP formation could be derived from the ATP hydrolysis by the Mg-dependent ATPase. We were not able to measure ATP synthesis just incubating leaky vesicles in a medium containing ADP, ^{32}Pi, Mg^{2+}, Ca^{2+}(3mM) and Ag^{+}. In order to initiate formation of radioactive ATP one had to include in the medium some cold ATP. It looks like the hydrolysis of ATP at one site of the membrane would promote the synthesis of radioactive ATP at another site of the membrane.

BOYER

One other question: these effects of silver that you uncover they are really very intriguing. If your explanation is correct you would be able to make just one ATP per each enzyme molecule by the use of a silver addition.

DE MEIS

Probably yes. If one exposes the membrane to EGTA, Mg^{2+} and Pi and then to a medium containing a high Ca^{2+} concentration and ADP, one should obtain one mol of ATP per mol of enzymes. We did not feel safe with this experimental approach because we feel that the chances of error are very high, due to the very small amount of ATP generated. It is difficult to have a preparation com-

pletely free of, say, miokinase or other source of contamination of ATP. Therefore it will be hard to distinguish whether one is dealing with ATP-Pi exchange or ATP synthesis.

BOYER

Yes, I realize that such experiments are not easy but sometimes they can give a fair amount of information.

DE MEIS

Yes, I agree. I would feel happy if we could repeat several times the cycle EGTA plus ^{32}Pi and then high Ca^{2+} and ADP, in order to accumulate increasing concentrations of ATP in the medium containing ADP. This would allow a more careful determination of whether or not one would get synthesis of ATP instead of ATP-Pi exchange. Controls without Pi would then be possible since one could measure the amount of ATP formed by other techniques, such as with the use of enzymes. We are at present attempting this approach but up to now we have not obtained clear results.

BOYER

The experiments you mention refer, I believe, to the possible Ca^{++}—driven synthesis of ATP. But with silver you should just get one cycle because you can't get the silver back off.

DE MEIS

It could be so, but with leaky vesicles we can still obtain ATP-Pi exchange without a gradient. These experiments can be done without silver, as described recently by E. RACKER.

SYNTHESIS OF ADENOSINE TRIPHOSPHATE BY WAY OF POTASSIUM-SENSITIVE PHOSPHOENZYME OF SODIUM, POTASSIUM ADENOSINE TRIPHOSPHATASE

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The sodium and potassium ion pump is an intrinsic enzyme of plasma membranes. In these experiments it was driven backward in a transient two-step operation involving first, phosphorylation of the enzyme from P_i, and second, transphosphorylation from the enzyme to ADP upon addition of a high concentration of Na^+. Since there was no evidence of a concentration gradient, Na^+ was presumably added to the solutions on both faces of the membrane simultaneously and produced its effect simply as a consequence of ligand binding. An interaction free energy between the free energy of the binding of Na^+ and the free energy of hydrolysis of the phosphate group on tbe enzyme was estimated. The experiments also suggested a feature of tbe enzymatic mechanism.

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This is control by phosphorylation of the access pathways from the solutions in contact with the faces of the membrane to an active center for cation binding. In the phosphoenzyme access would be to the extracellular solution and in the dephosphoenzyme access would be to the intracellular solution.

Introduction

ATP has been synthesized by driving the Na, K-pump backward in a steady state in intact cells with energy from concentration gradients of Na^+ and K^+ across the membrane [1-3]. In the experiments reported here ATP was synthesized transiently by the pump in presumably leaky membranes without a gradient. In a two-step one-shot operation a phosphoenzyme was first prepared from P_i and subsequently was treated with ADP and a high concentration of Na^+ to release ATP. If Na^+ acted in the absence of a gradient, then binding of Na^+ was sufficient. Translocation involves binding of substrate from a solution in contact with one face of a membrane and release of substrate into a solution in contact with the other face of the membrane. If the transport system was energized by Na^+ when the electrochemical potential of Na^+ was the same on both sides of the membrane, then binding of Na^+ was sufficient. In this way the translocation step was partially fractionated. These experiments also help to clarify the reaction sequence of phosphorylation and dephosphorylation.

Per cycle the sodium and potassium ion pump of plasma membranes transports approximately 3 Na^+ outward and 2 K^+ inward and hydrolyses the terminal phosphate bond of one molecule of intracellular MgATP [3] (Fig. 1). Na^+ is unique as substrate for net outward transport. To drive the pump backwards and Garrahan and Glynn [1] prepared erythrocytes containing only K^+ intracellularly in a medium containing only Na^+. Na^+ was transported inward, K^+ outward, and

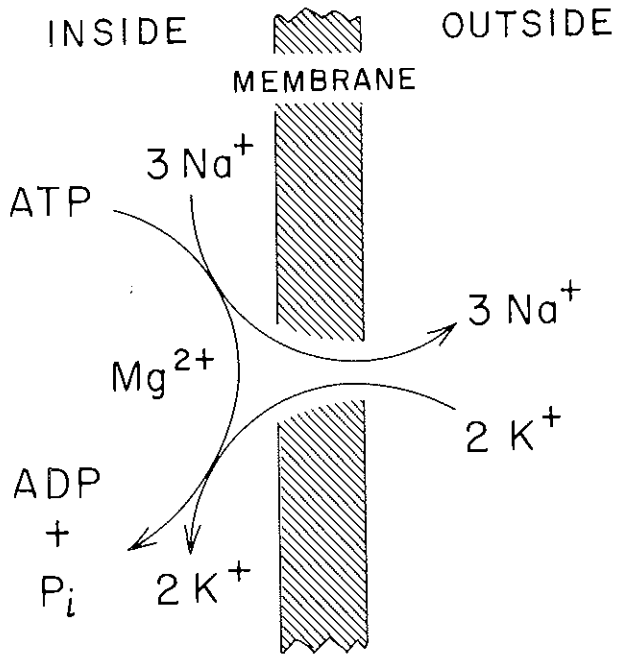

FIG. 1 — Stoichiometry and sidedness of the sodium and potassium ion pump in the plasma membranes of animal cells.

ATP was synthesized at a rate about 1.4% the capacity of the pump to run forward under optimal conditions.

In preparations of broken or leaky membranes, the Na^+, K^+ - dependent ATPase activity of the pump persists and is convenient for study even though intracellular and extracellular phases can no longer be distinguished experimentally. This enzyme is abbreviated Na, K-ATPase. It is an intrinsic enzyme of plasma membranes [4].

Another reason for running the pump backward concerns the reaction sequence of the phosphoenzyme. Na^+, K^+-ATPase accepts a phosphate group from ATP in the presence of Na^+ (uniquely) and Mg^+. The resulting phosphoenzyme exchanges

its phosphate group with inorganic phosphate, P_i (the rate constant is about 0.1 s^{-1} at 0°) and is insensitive to ADP. We have called such a reactive state of the phosphoenzyme, E_2-P [5]. In contrast, after partial poisoning with N-ethylmaleimide or oligomycin the enzyme shows Na^+-dependent ATP $\rightleftharpoons$ ADP exchange and the phosphoenzyme is easily split by ADP. We have called such a reactive state of the phosphoenzyme, E_1-P. We have supposed in the native enzyme that a similar reactive state is a precursor to E_2-P in accordance with the initial suggestion of Albers and coworkers. However, the evidence is not definitive and a precursor-product relationship between E_1-P and E_2-P is controversial [6]. In order to seek evidence for interconversion of these reactive forms in the native enzyme, we attempted reversal of the reaction from E_2-P (prepared directly from P_i) to E_1-P as shown by synthesis of ATP.

From various partial reactions of the Na, K-ATPase, a reaction sequence for phosphorylation and dephosphorylation of the enzyme can be assembled. It reflects in part the work and conclusions of several laboratories but is still controversial. It illustrates our working hypothesis (Fig. 2).

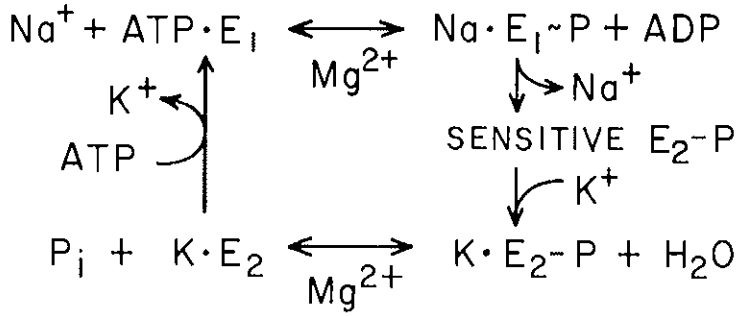

FIG. 2 — Hypothetical reaction sequence for phosphorylation and dephosphorylation of Na, K-ATPase. « E » represents the enzyme and the subscripts « 1 » and « 2 » indicate major reactive forms. « Sensitive » refers specifically to sensitivity to K^+ or its congeners. Reproduced from [9].

In this scheme « E » represents the enzyme. The most important feature of the scheme is the distinction between two reactive forms of the enzyme, E_1 and E_2. E_1 is phosphorylated reversibly from ATP in the presence of Na^+ to give E_1-P. In contrast E_2 is phosphorylated reversibly from inorganic phosphate, P_i, in the absence of Na^+ to give a different reactive form of the phosphoenzyme, E_2-P. Actually, E_2-P is subdivided further into reactive states of which two are shown here. Sensitive E_2-P is the normal product of phosphorylation from ATP at low concentration of Na^+ (about 10 to 100 mM). It is called « sensitive » because it is extremely sensitive to K^+ and rapidly combines with it to become K^+-complexed E_2-P. Sensitive E_2-P exchanges its phosphate group with P_i with a rate constant about $k=0.06$ $(sec)^{-1}$, but $K\cdot E_2$-P exchanges much more rapidly with $k>1$ $(sec)^{-1}$ [5].

The strategy for synthesis of ATP was first tbe preparation of sensitive E_2-P from $^{32}P_i$. This was done by Dr. TODA in this laboratory. The second step was the addition of ADP and a high concentration of Na^+ to sensitive E_2-P with recovery of [^{32}P]ATP in the supernatant after the reaction was stopped with acid. This was done by Dr. TANIGUCHI [7]. It was important to avoid K^+ or its congeners, which prevented synthesis, presumably by forming $K\cdot E_2$-P rapidly at low concentrations.

METHODS

The Na, K-ATPase was prepared in a crude suspension of membranes obtained from a homogenate of guinea pig kidney by differential centrifugation. As part of the purification procedure the membranes were kept overnight at 4° in 1.3 M urea. As a second part of the procedure the membranes were treated with 2 M NaI at 0° for 45 minutes. These treatments greatly reduced ATPase activity insensitive to Na^+ and K^+ and also adenylate kinase activity, which are contaminants in this pre-

paration. These treatments are sufficiently vigorous that one would expect to obtain leaky vesicles.

For phosphorylation 1 to 3 mg of membrane protein were incubated at 0° and pH 7.5 with 10 or 20 μmoles of imidazole glyclyglycine in a final volume of 1 ml in the presence of Na^+, K^+, or neither. Addition of [γ-^{32}P]ATP or $^{32}P_i$ was usually followed by addition of Mg^{2+} to start the reaction. Additions were in volumes of 0.1 ml to produce the final concentrations indicated. After an interval of 3 seconds to a minute (as indicated in the figures) the reaction was stopped with acid. After centrifugation and separation of the supernatant the denatured membranes were usually digested with pepsin. This procedure released radioactive phosphopeptides overlapping the active site into the supernatant for subsequent isolation by paper electrophoresis. The amount of phosphoenzyme estimated in this way is usually expressed as a percentage of near-maximal phosphorylation obtained from [^{32}P]ATP under standard conditions. This procedure avoids uncertainties due to erratic and non-specific binding of $^{32}P_i$ to membranes. Specific details and alternative procedures are described elsewhere [5, 7]. In experiments where ATP was synthesized from $^{32}P_i$ and ADP, the first supernatant was subjected to column chromatography to isolate [^{32}P]ATP [7, 8]. In experiments where [^{32}P]dATP was synthesized, it was isolated from the supernatant by column chromatography on polyethylene imine-Avicel in the presence of borate followed by thin layer chromatography on polyethylene imine-cellulose. Final contamination of dATP with ATP was less than 0.02%.

Results

K^+-sensitive Phosphoenzyme

After Na^+, K^+-ATPase was first phosphorylated from ATP, the rate of dephosphorylation could then be estimated by in-

terrupting further phosphorylation and observing the subsequent rate of disappearance of the radioactive phosphoenzyme. Phosphorylation from [^{32}P]ATP was interrupted either by a chase of cold ATP or by chelation of Mg^{2+} with an excess of a chelator such as EDTA or CDTA (cyclohexylenediaminetetraacetic acid). In order to observe the sensitivity to K^+, KCl was added to phosphoenzyme in the presence of excess EDTA. Dephosphorylation was immediate and complete in less than 4 seconds. In the absence of K^+ the apparent monomolecular rate constant at 0° was $k = 0.06\ s^{-1}$. The high specificity of the reaction is shown by the ratio of $[K^+]$ to $[Na^+]$ which was 1 to 160 (Fig. 3).

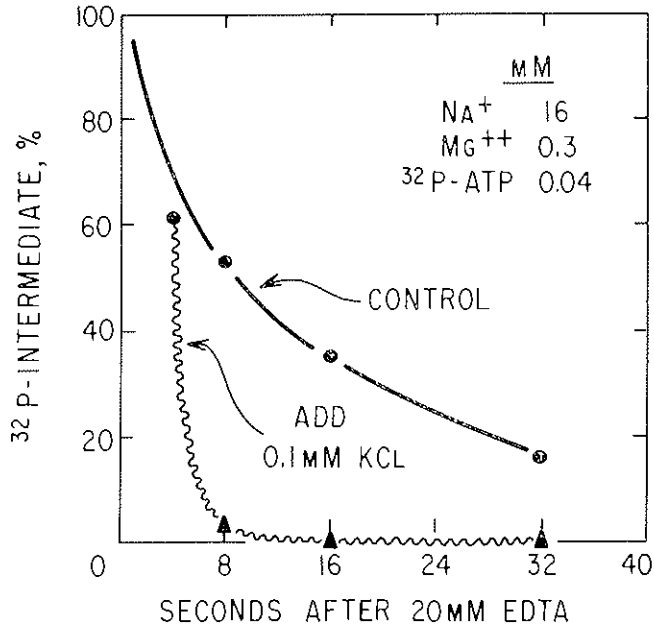

FIG. 3 — Sensitivity to K^+ of phosphoenzyme prepared from ATP. "^{32}P-intermediate" refers to the phosphoenzyme. The initial conditions at 0° and pH 7.6 are shown in the inset. Reproduced from [10].

Sensitivity of this reactive state of the enzyme to ADP was tested by addition of deoxy-ADP during a chase with unlabeled ATP. There was no effect (Fig. 4). Deoxy-ADP was substituted for ADP since it is a poor substrate for adenylate kinase. This experiment serves as a control for the next two experiments.

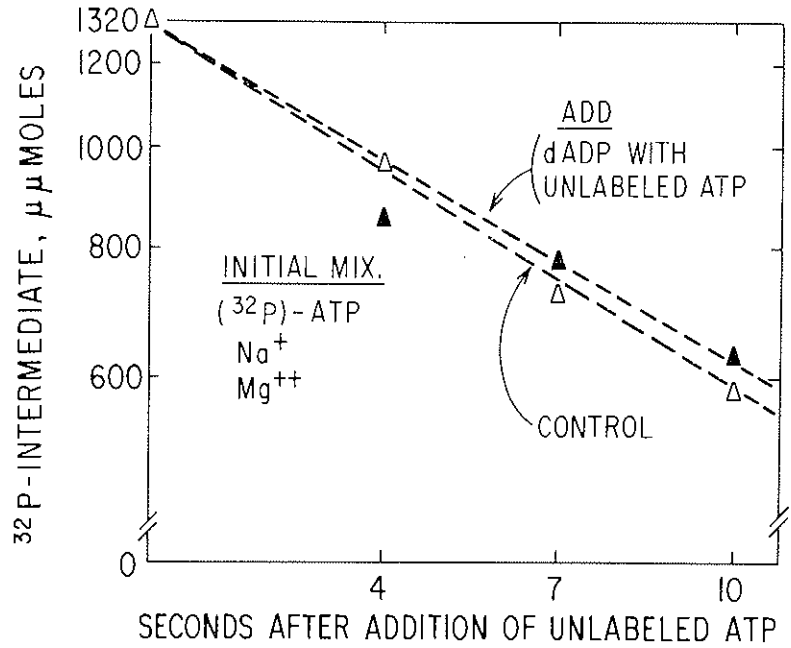

FIG. 4 — Insensitivity to dADP of phosphoenzyme prepared from ATP. In final volume of 2 ml the reaction mixture contained 20 μmoles of imidazole glyclyglycine, 4 μmoles of $(Tris)_3CDTA$, 6 μmoles of $MgCl_2$ and 16 μmoles of NaCl. The reaction was started with 0.04 μmoles of [^{32}P]ATP. Five seconds later a chase of 2 μmoles of unlabeled Mg·ATP was added without, △, or with, ▲, 4 μmoles of Mg·dADP. « ^{32}P-intermediate » refers to the phosphoenzyme.

Is E_1-P a Precursor to E_2-P?

This question is still controversial [6]. In order to make an experimental test the enzyme was incubated with [^{32}P]ATP and Na^+. This allowed ATP to bind to its active site. The reaction was started with Mg^{2+} and a chase of unlabeled ATP. A pulse of radioactive phosphoenzyme was obtained from the bound ATP (Fig. 5). The chase of unlabeled ATP prevented further phosphorylation from [^{32}P]ATP after the initial burst. In order to test for the presence of a transient state of the

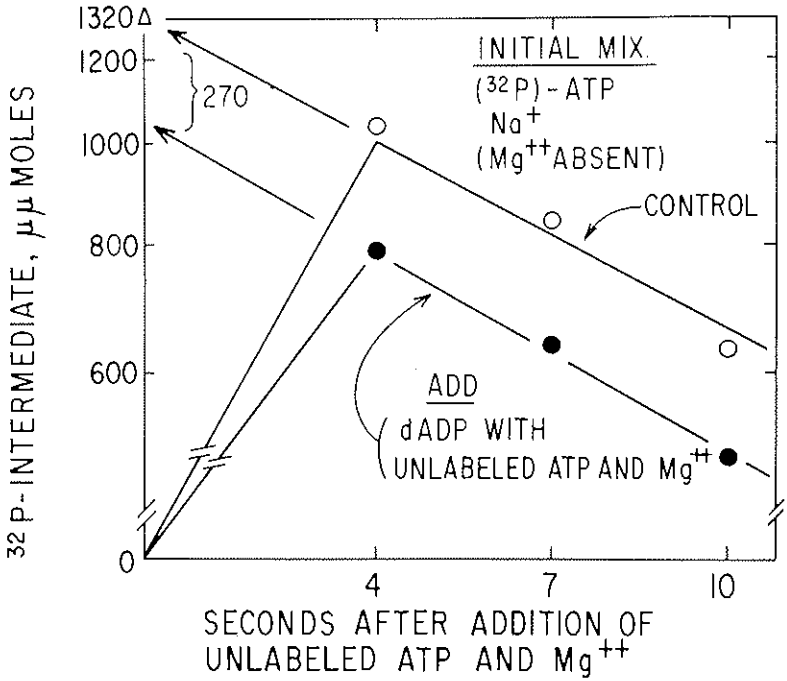

FIG 5 — Partial inhibition by dADP of a transient burst of phosphorylation from [^{32}P]ATP bound beforehand at its active site. The reaction was conducted as in Fig. 4 except that 6 μmoles of $MgCl_2$ were omitted from the initial mixture and were added with the chase of unlabeled Mg·ATP without, ○, or with ●, Mg·dADP.

phosphoenzyme which might be sensitive to ADP, dADP was added with the chase of unlabeled ATP. This addition reduced the initial amount of [^{32}P]phosphoenzyme by 270 pmoles out of a total of 1320 pmoles and did not affect the rate of dephosphorylation (Fig. 5). At least 20% of the [^{32}P]phosphoenzyme produced in this experiment passed transiently through a state in which it was sensitive to deoxy-ADP. Deoxy-ADP cannot be more effective than unlabeled ATP in preventing phosphorylation from [^{32}P]ATP.

In order to find out if the deficiency in phosphoenzyme really represented reversal of phosphorylation, the supernatant fluids were analyzed for [^{32}P]dATP. There were about 270 pmoles more dATP (Fig. 6) when the reaction was started with Mg^{2+} and a chase of unlabeled ATP (Fig. 5) than when the same chase was added to the preformed phosphoenzyme (Fig. 4). These experiments illustrate conditions under which at least 20% of E_2-P formed from ATP passed through a transient reactive state corresponding to E_1-P as a precursor.

But why only 20%? According to a long-standing hypothesis [4] conversion of E_1-P to E_2-P requires Mg^{2+}. We have not found further evidence for this hypothesis. To the contrary, KLODOS and SKOU (Biochimica et Biophisica Acta, 391, 474, 1975) have reinvestigated our earlier experiments [11] and have concluded " that the reported different sensitivity toward potassium and ADP of phospho-enzyme formed in the presence of a low and high concentration of free magnesium, respectively, il due to the EDTA used to decrease the free magnesium concentration and not to the decrease in free magnesium as such ". Rather than Mg^{2+}, Na^+ appears to be the significant ligand. Addition of dADP to the phosphoenzyme (along with a chase of unlabeled ATP) produced 3-fold more dATP in the presence of 200 mM Na^+ than in the presence of 8 mM Na^+ (Fig. 7). These experiments were done by Dr. KUME. To explain the incompleteness of the action of dADP, one might suppose that Na^+ was released about as rapidly as ADP from $Na \cdot E_1\text{-}P \cdot ADP$ and that this release prevented

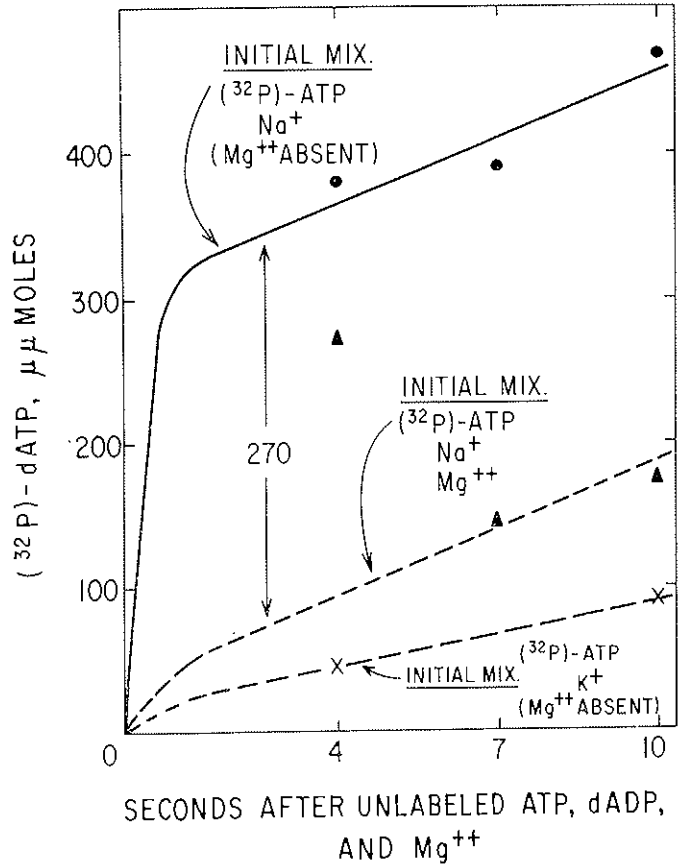

FIG. 6 — Transient synthesis of dATP from [^{32}P] phosphoenzyme preliminary to appearance of K^+-sensitive phosphoenzyme. The closed circles ●, show data from the experiment of Fig. 5. The closed triangles, ▲, show data from the companion experiment of Fig. 4. The crosses, X, show data from control samples in which Na^+ was replaced with equivalent K^+.

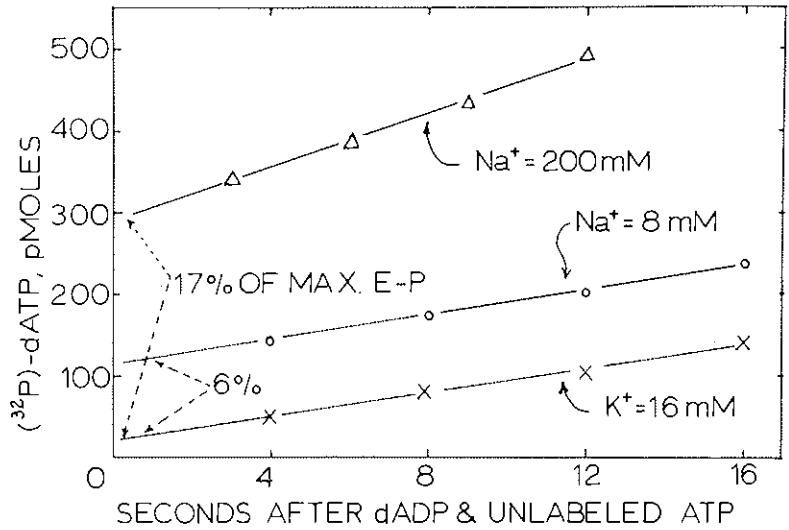

FIG. 7 — Effect of Na^+ concentration on synthesis of dATP from the same amount of phosphoenzyme prepared from [^{32}P]ATP in a steady state. The slopes of the lines show the rate of dADP $\rightleftarrows$ ATP exchange after the specific activity of the [^{32}P]ATP was reduced 50-fold by the chase. The reaction system was similar to that in Fig. 4.

reversal of the reaction. One might suppose further that the affinity of the phosphoenzyme for Na^+ was much less than that of the dephosphoenzyme.

Rather than pursue the forward reaction we began to investigate the reverse reaction after we found that the native enzyme spontaneously accepts a phosphate group from P_i [5, 12].

Formation of K^+-sentive phosphoenzyme from P_i

In order to investigate the effects of Na^+ and K^+ on phosphorylation from P_i, the enzyme was incubated with P_i and the reaction was started with Mg^{2+} alone or with Mg^{2+} and

Na^+ or K^+. With Mg^{2+} alone, phosphorylation was slow; addition of Na^+ inhibited phosphorylation, and addition of K^+ stimulated it (Fig. 8). It turned out that the phosphoenzyme formed in the presence of K^+ exchanged its phosphate group with P_i much more rapidly than did that formed in the absence of K^+ (see also Fig. 9). This rapid exchange was taken as an identifying characteristic of K^+-complexed phosphoenzyme or $K \cdot E_2$-P [5]. However, phosphoenzyme from P_i with Mg^{2+} alone was insensitive to K^+ (Fig. 9) and therefore was not suitable for demonstrating reversal of the normal reaction.

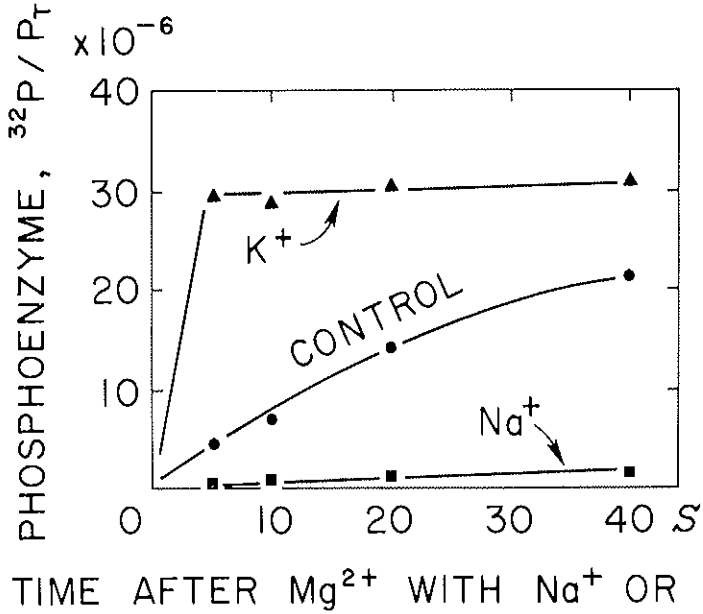

FIG. 8 — Phosphorylation from P_i of unwashed membranes. The membranes were incubated with 1 mM $^{32}P_i$ for 10 s before zero time. $[Mg^{2+}] = 2$ mM, $[Na^+] = [K^+] = 16$ mM. In the control Na^+ and K^+ were omitted. « P_T » refers to total phosphorus as an estimate of the amount of membranes in the mixture. Maximal phosphorylation was not estimated but was about 100 to 200 pmoles ^{32}P/μmole P_T as judged from other experiments.

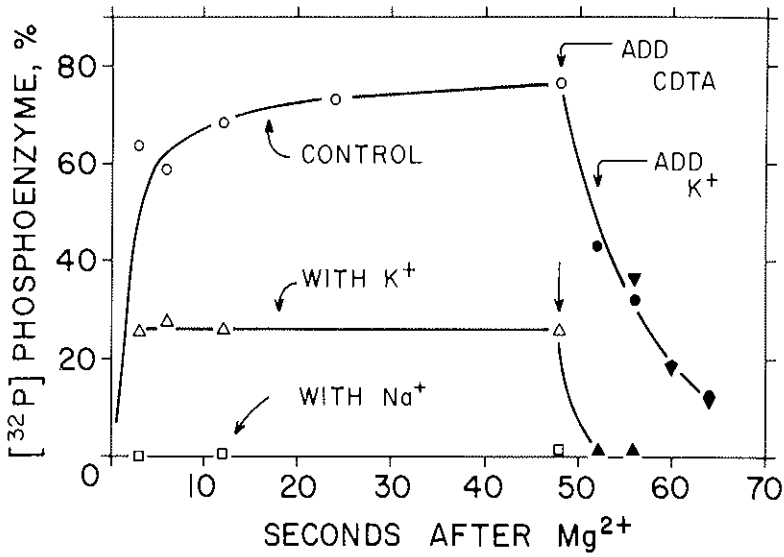

FIG. 9 — Phosphorylation from P_i of washed membranes. Membranes were washed in 1 mM $MgCl_2$ at neutral pH for 30 to 60 minutes by centrifugation and resuspension. Na^+ and K^+ were present with the enzyme before P_i was added except in the control. $[P_i] = 1$ mM, $[Mg^{2+}] = 2$ mM, $[Na^+] = [K^+] = 16$ mM, [CDTA] = 20 mM. Reproduced from [5].

We called this reactive state « insensitive phosphoenzyme » since it was also insensitive to ADP [5].

In Fig. 9 it is significant that formation of insensitive phosphoenzyme was more extensive than in Fig. 8 and was also disphasic. The significant difference in the procedure is that in Fig. 9 the membranes were washed once in 1 mM $MgC1_2$ at 0° and neutral pH by centrifugation and resuspension. After one wash the $MgCl_2$ was removed and the experiment was performed. In the experiment of Fig. 8 the membranes were not washed. Since this washing increased the yield of phosphoenzyme, we continued the procedure in subsequent experiments. It probably transforms E_1 into E_2.

Sensitive phosphoenzyme from ATP is slowly converted into insensitive phosphoenzyme due to an attack by Mg^{2+} [5]. Perhaps the insensitive phosphoenzyme in Fig. 9 really began as sensitive phosphoenzyme and then was attacked by the Mg^{2+}. Experiments at short times were indecisive. In order to try a different ligand, washed membranes were incubated with P_i and the reaction was started with Fe^{2+}. After 3 seconds three-fourths of the resulting phosphoenzyme was sensitive to K^+ (Fig. 10). It appeared that formation of sensitive phospho-

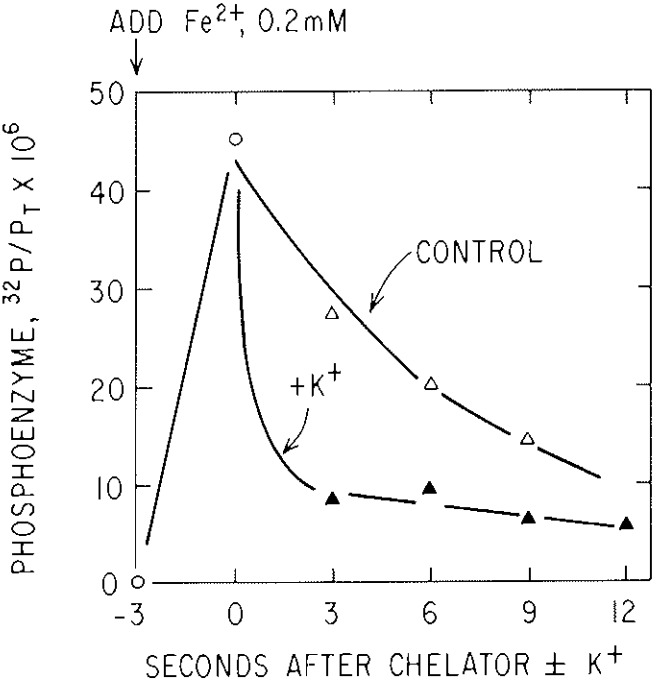

FIG. 10 — Formation of K^+-sensitive phosphoenzyme directly from P_i with Fe^{2+} in place of Mg^{2+}. The initial mixture contained 2.3 mg of protein of washed membranes, 20 μmoles of imidazole glyclyglycine and 0.1 μmole of CDTA in 0.8 ml. At 12 s before zero time 1 μmole of $^{32}P_i$ in 0.1 ml was added. At 3 s before zero time, 0.3 μmoles of $FeCl_2$ in 0.1 ml were added, ○. At zero time 15 μmoles of $(Tris)_3CDTA$ without, △, or with, ▲, 8 μmoles of KCl were added in 0.1 ml.

enzyme was taking place and that the problem was to protect it from a secondary effect of Mg^{2+}.

Na^+ protects sensitive phosphoenzyme (made from ATP) from attack by Mg^{2+} [5] but it also inhibits phosphorylation from P_i. In order to estimate the rate of development of the inhibitory effect of Na^+ in the washed membranes, Na^+ was added at various times. The fast and slow components of phosphorylation (Fig. 9) responded to inhibition by Na^+ at different rates. The slow component was inhibited immediately (as in Fig. 8) but the fast component was inhibited relatively slowly. Inhibition required more than 5 seconds and less than 10 minutes (Fig. 11). Six experiments showed that the

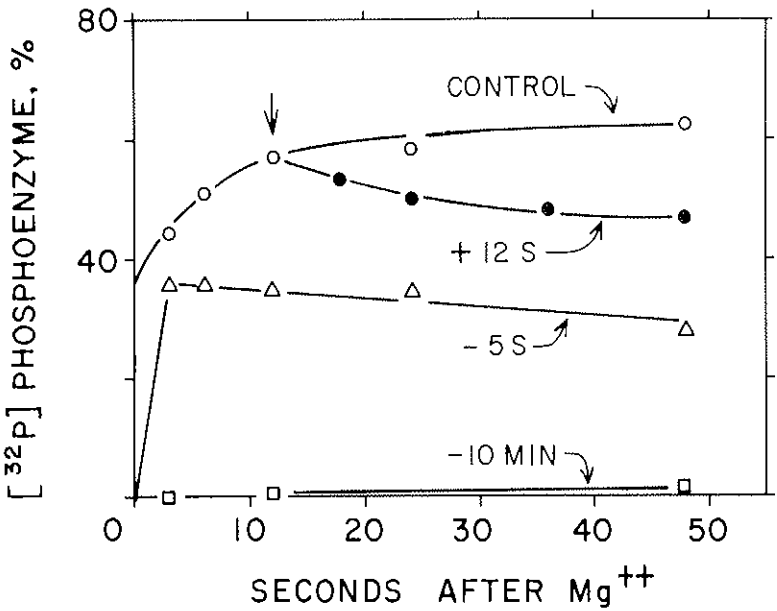

FIG. 11 — Slow inhibition by Na^+ of rapid component of phosphorylation from P_i of washed membranes. P_i at 1 mM was added to washed membranes in 0.1 mM CDTA 5 s before the reaction was started by addition of 2 mM $MgCl_2$. NaCl at 16 mM was added at the times shown before (−) or after (+) zero time.

apparent monomolecular rate constant for inhibition by NaCl was between 0.05 and 0.16s^{-1}. It was not very consistent. The relative slowness of the inhibitory action of Na^+ on washed enzyme permitted its use to protect sensitive phosphoenzyme from Mg^{2+}.

In order to estimate the stability of sensitive phosphoenzyme under these conditions, phosphoenzyme was treated with CDTA and K^+ and the fraction sensitive to K^+ was estimated at 4, 12, and 48 seconds. Sensitive phosphoenzyme was practically intact at 4 seconds (Fig. 12).

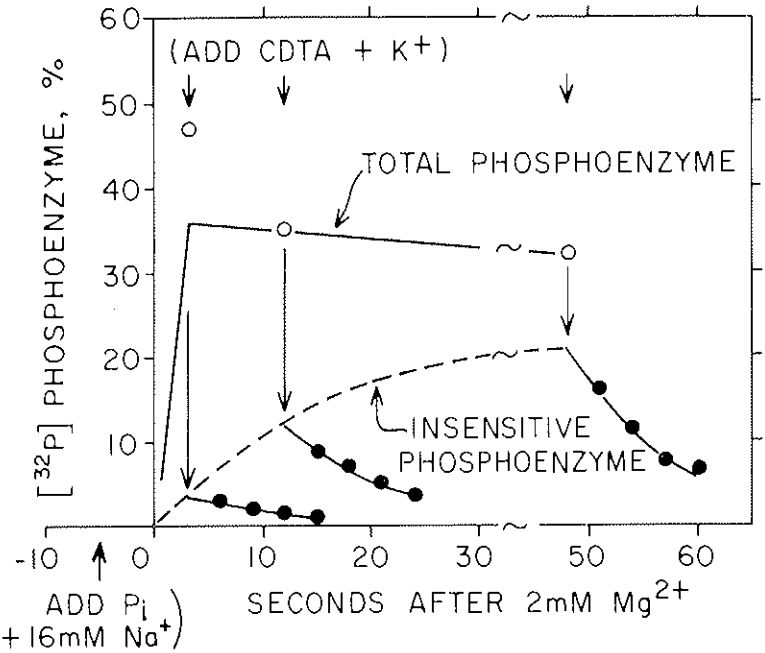

FIG. 12 — Stability of K^+-sensitive phosphoenzyme formed from P_i with Mg^{2+} in the presence of Na^+ added just before phosphorylation. To washed membranes in 0.1 mM CDTA there were added 1 mM $^{32}P_i$ simultaneously with 16 mM NaCl. Phosphorylation was started 5 s later with 2 mM $MgCl_2$ at zero time. At 3, 12, or 48 s 18 mM CDTA with 15 mM KCl was added to phosphoenzyme, ○, as shown by the arrows, ↓, in order to estimate the amount of insensitive phophoenzyme, ●.

In order to compare sensitive phosphoenzyme prepared in this way from P_i with sensitive phosphoenzyme prepared from ATP, the two phosphorylation procedures were applied to the same enzyme preparation. The rate of dephosphorylation of sensitive phosphoenzyme in the presence of various concentrations of K^+ or its congeners was estimated. Sensitive phosphoenzyme from both sources, [^{32}P]ATP or $^{32}P_i$, was identical. This preparation of sensitive phosphoenzyme from P_i was therefore suitable as a starting material for attempting reversal of the reaction.

Synthesis of ATP.

In order to synthesize ATP, sensitive phosphoenzyme was prepared as in Fig. 12. Four seconds later ADP and excess CDTA were added without or with supplemental Na^+. The purpose of the CDTA was to trap all the Mg^{2+} which might be formed and to protect the ATP from enzymatic attack. It also inhibited adenylate kinase. At 17 mM Na^+ the phosphoenzyme disappeared in almost the usual manner and no ATP appeared. At 176 mM Na^+ the rate of disappearance of the phosphoenzyme was more rapid and a small amount of ATP appeared slowly and then remained constant. About 20% of the initial amount of phosphoenzyme appeared to have transfered its phosphate to ADP. Later experiments showed that CDTA was not necessary in this reaction system. It did stabilize the ATP but reduced the yield by about 25% [7]. Stabilization was more important than maximal yield so that CDTA was used in later experiments.

Clearly a high concentration of Na^+ was important for synthesis. In further experiments the concentration of Na^+ was varied (Fig. 13) and the apparent monomolecular rate constant for transphosphorylation from the phosphoenzyme to ADP (at a saturating concentration) was estimated (Fig. 14). Fitting a Hill Equation to the data shown gave a maximal rate constant of 1.04 s^{-1}, a dissociation constant of 0.50 M, and an exponent,

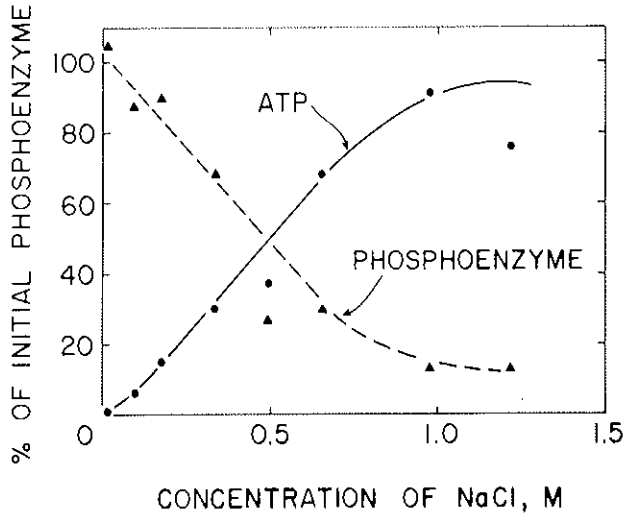

FIG. 13 — Synthesis of ATP from K^+-sensitive phosphoenzyme. In a volume of 0.25 ml, 3.9 mg of washed membrane protein was incubated with 5 μmoles of imidazole glyclyglycine, 10 nmoles onf $(Tris)_3CDTA$ and 0.5 μmoles of $^{32}P_i$ at pH 7.5 and 0°. To start phosphorylation 0.25 μmoles of $MgCl_2$ and 8 μmoles of NaCl were added in 0.05 ml. To start ATP synthesis 4 seconds later 10 μmoles of $(Tris)_3CDTA$ and 0.5 μmole of ADP with various quantities of NaCl were added in 0.2 ml to produce the final concentrations indicated on the horizontal axis. The reaction was stopped with acid 2 seconds later to obtain the data shown. Control points were also taken 80 seconds later at concentrations of Na^+ at or above 176 mM. At this time no phosphoenzyme remained and the amount of [^{32}P]ATP was between 51 and 95% of the initial amount of phosphoenzyme, which was 212 pmoles. The yield of ATP increased progressively with the concentration of Na^+. Reproduced from [9].

« n », of 2.6. These values cannot be taken too seriously since elimination of the last point on the right changed them to 1.24 s^{-1}, 0.61 M and 2.1, respectively. It also improved the fit between the data and the equation. In any case, it seems clear that the affinity for Na^+ was very low and that there was positive cooperativity between the sodium ions. This is appropriate for a stoichiometry of Na^+ to ATP greater than 1 (Fig. 1).

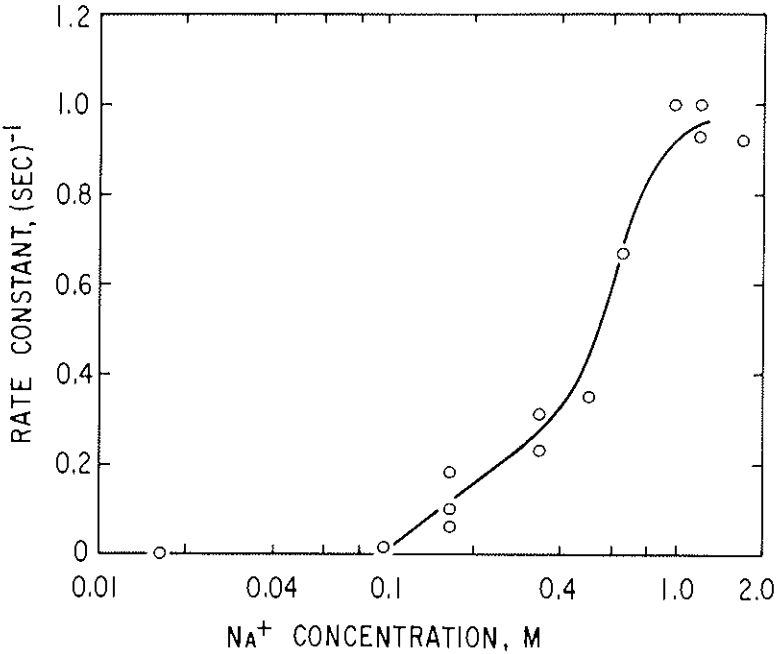

FIG. 14 — Apparent monomolecular rate constant for transphosphorylation from K^+-sensitive phosphoenzyme to ADP as a function of Na^+ concentration. Reproduced from [7].

Control Experiments.

ATP was not synthesized from K^+-complexed phosphoenzyme nor in the presence of the specific cardioactive steroid inhibitor, ouabain [7, 8]. ATP was not synthesized when the order of addition of ligands was changed. In a reaction system with 160 mM Na^+ and 1 mM ADP in which 22% of the initial amount of phosphoenzyme was converted to ATP, neither phosphoenzyme nor ATP appeared when the Na^+ or the ADP was added to the enzyme about 10 minutes before P_i was added. When K^+ or its congeners, Li^+, NH_4^+,

Rb^+, Cs^+, or Tl^+, were added to phosphoenzyme together with 160 mM Na^+ at concentrations which were estimated (from other experiments) to produce equivalent acceleration of dephosphorylation of K^+-sensitive phosphoenzyme, then approximately equivalent partial inhibition of ATP synthesis was obtained in each case [8]. Consequently, ATP synthesis appears to depend on competition between Na^+ and K^+ for occupancy of sensitive phosphoenzyme. If Na^+ predominates, sensitive phosphoenzyme can become E_1-P and transfer its phosphate group to ATP. If K^+ predominates, K^+-complexed E_2-P is formed, which is unresponsive to ADP.

Net synthesis of ATP was demonstrated in an experiment in which the amount of [^{32}P]ATP produced was 3- to 5-fold greater than the amount of unlabeled ATP added as a contaminant of the ADP [7]. Addition of unlabeled ATP did not affect the reaction [7]. Release of free ATP from the enzyme was shown in experiments in which excess dephosphoenzyme or hexokinase and glucose were added after the addition of ADP [7].

Was a Concentration Gradient of Na^+ Across the Membrane Necessary for Synthesis of ATP?

Suppose that fragments of plasma membranes spontaneously resealed to form tight vesicles. Addition of a high concentration of NaCl to the otside of such a vesicle cold produce a concentration gradient of Na^+, at least transiently. Such a gradient could be a source of energy. It seems clear that Na^+ must go on the enzyme at a high concentration in order to synthesize ATP, but is it necessary that it also come off again at a low concentration into a different solution on the opposite face of the membrane?

In order to see if there were vesicles in the membrane preparation, an electron microscopic picture was made. It showed membrane fragments with broken edges and also many apparently closed vesicles (Fig. 15). In order to make a

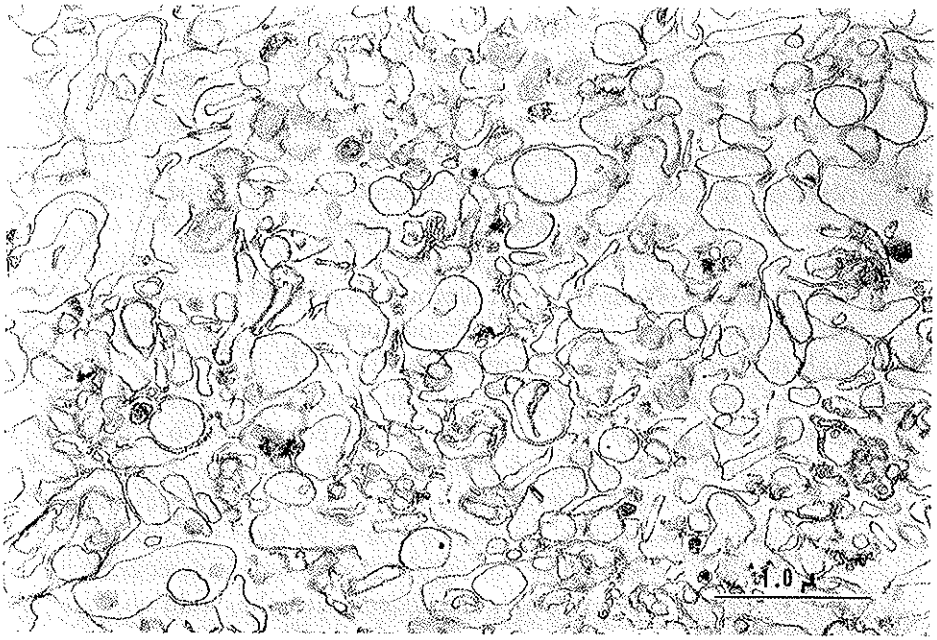

FIG. 15 — Electron micrograph of the membrane preparation used in the synthesis of ATP. This photograph was provided through the courtesy of Dr. SIDNEY FLEISCHER.

functional test, a leak-producing agent, gramicidin, was added to the membranes before synthesis of ATP. It had little effect [7]. Another leak-producing agent, Lubrol WX, reduced the rate of dephosphorylation of the phosphoenzyme and the rate of synthesis of ATP but did not affect the ratio of ATP recovered to phosphoenzyme split [7].

A test for a gradient was made in a different way. The site from which Na^+ would come off during the synthesis of ATP is the same site at which it goes on to catalyze phosphorylation of the enzyme from ATP. If there is a delay between the time of addition of Na^+ and the time at which it reaches this active site, there should be a retardation in the rate of phosphorylation

from ATP upon addition of a rate-limiting concentration of Na^+. An experiment with this membrane preparation showed no retardation [7]. Therefore, Na^+ could get as easily to the high-affinity site at which it catalyzes the forward reaction as to the low-affinity site at which it catalyzes the reverse reaction. (The $K_{0,5}$ for Na^+ for phosphorylation from ATP is about 1 mM.

Three tests for a gradient failed to produce positive results. It is more difficult to be confident of the absence of a gradient than of its presence, but at present is seems reasonable to assume that there was no gradient and that a gradient is not necessary.

Discussion

Interaction Free Energy of Ligand Binding in the Synthesis of ATP

In order to think about a relationship between binding of Na^+ and the energy level of the phosphoenzyme, consider a simple model first [13]. Let a protein have two ligands, A and B, and bind one molecule of each, separately or together. For the separate complexes let the dissociation constants be K_a and K_b respectively. Let there be an interaction between the ligands so that the dissociation constant for A to bind to the free protein, K_a, is different from that for A to bind to the complex of the protein with B, namely K_{ab}. That is $K_a \neq K_{ab}$. Writing out the equilibria shows that $K_a/K_{ab}=K_b/K_{ba}$ (Equation i). That is, whatever the change in the dissociation constant for A produced by the binding of B, there is a corresponding change in the dissociation constant for B produced by the binding of A. This relationship can be expressed more elegantly as an interaction free energy of ligand binding [13]. The free energy of dissociation is $\Delta G^\circ = -RT1nK$ (Equation ii). From this equation the free energy under standard conditions can be

defined for each reaction in the equilibria above. After substitution of the appropriate varieties of Equation ii into Equation i the following relationship emerges: $\Delta G^{o}_{a} - \Delta G^{o}_{ab} = \Delta G^{o}_{b} - \Delta G^{o}_{ba} = \Delta G^{o}_{i}$, where ΔG^{o}_{i} is the *interaction free energy*. This is the same for the effect of B on A as for the effect of A on B.

Application of these considerations to the synthesis of ATP by Na, K-ATPase leads to the idea that the interaction free energy for the effect of phosphorylation on the binding of Na^{+} should be the same as that for the effect of the binding of Na^{+} on the free energy of hydrolysis of the phosphate group of the enzyme. At this stage in the investigation the data are so scanty and so indirect that there is no question of testing the hypothesis quantitatively. The question is only whether the data can be maneuvered into tolerating the hypothesis [7].

For dissociation of Na^{+} from the phosphoenzyme, the $K_{0,5}$ is about 0.6 M at 0° according to Fig. 14, giving a ΔG^{o} of + 0.8 Kcal per 3 Na^{+} (compare Fig. 1 for the stoichiometry). For dissociation of Na^{+} from the dephosphoenzyme the $K_{0,5}$ is about 0.6 mM as judged from the half-maximal concentration for inhibition of phosphorylation from P_i [12]. The corresponding ΔG^{o} is + 12 Kcal per 3 Na^{+}. The interaction free energy is therefore about 11 Kcal per 3 Na^{+}. Phosphorylation from P_i is half-maximal at about 0.1 mM for insensitive phosphoenzyme (unpublished experiments). The free energy of hydrolysis of the phosphate group is therefore about + 5 Kcal/mol. The free energy of hydrolysis of E_1-P can be estimated in a partially poisoned form of the enzyme in which it is stable. The ratio of the concentration of ADP to that of ATP at half-maximal phosphorylation was 10 [12]. Consequently the magnitude of the free energy of hydrolysis is probably somewhat less than that of the terminal phosphate group of ATP, namely about − 6 Kcal/mole. The interaction free energy is therefore again about 11 Kcal per mole. These relationships are illustrated in Fig. 16. It is thus possible to

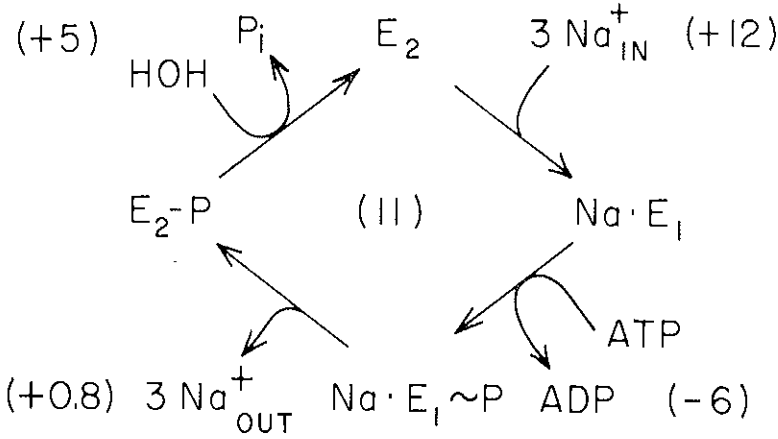

FIG. 16 — Scheme of possible free energies of ligand binding of Na^+ and the phosphate group on the enzyme in Na, K-transport ATPase. The assignment of the high affinity site to intracellular Na^+ and the low affinity site to extracellular Na^+ is discussed in the text. The stoichiometry is that of Fig. 1. The symbols are those of Fig. 2. The numbers in parenthesis in the corners are estimates of the free energy of dissociation of Na^+ or of hydrolysis of the phosphate group in kcal/mole at 0°. The number in the center is the interaction free energy. Details are given in the text and in [7]. Reproduced from [14].

consider interaction free energy of ligand binding as a possible feature of the mechanism of Na, K-ATPase.

Sidedness of the Actions of Na^+

In the scheme of Fig. 16 the dephosphoenzyme has a much greater affinity for Na^+($K_{0.5}$=0.6 mM) than does the phosphoenzyme ($K_{0.5}$=0.6 M). In broken membrane preparations it is likely that the pump itself retains its sidedness even though the vesicles in which it is located are too leaky to retain concentration gradients or any evidence of transport activity. Evidence for retention of sidedness comes from recent reconstitu-

tion experiments in which purified Na, K-ATPase showed transport activity after reconstitution into tight vesicles [15, 16]. The high and low affinity sites can be assigned to the inner and outer faces of the membrane respectively to correspond to the relative affinities of the Na, K-pump in human erythrocytes for intracellular and extracellular Na^+. The best estimate of the affinities is probably found under conditions of reversible in $\rightleftharpoons$ out exchange of Na^+. This exchange requires the absence of extracellular K^+ and the presence of both ATP and ADP intracellularly [3]. Under these conditions at 37° the $K_{0.5}$ for Na^+ inside is about 0.7 mM and that for Na^+ outside is about 120 mM [17]. These conditions probably provide concentrations near to equilibrium for 3 steps in the

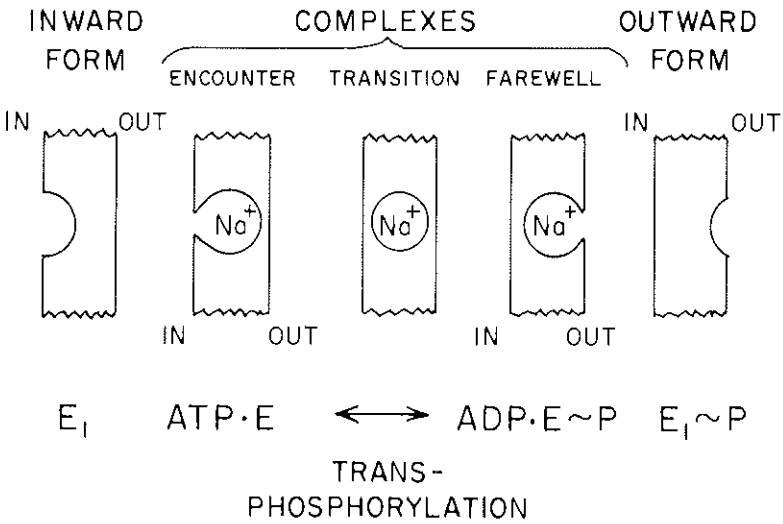

FIG. 17 — Diagram of a hypothesis that transition states for ion translocation and those for corresponding ion-activated transphosphorylation reactions are the same. Reactions with respect to Na^+ are in the *upper panel* and reactions with respect to K^+ are in the *lower panel*. The implied transition from E_2 to E_1 is probably not by way of the free enzyme as the diagram suggests (compare Fig. 2). Stoichiometry is not specified and Mg^{2+} is not shown. Reproduced from [14].

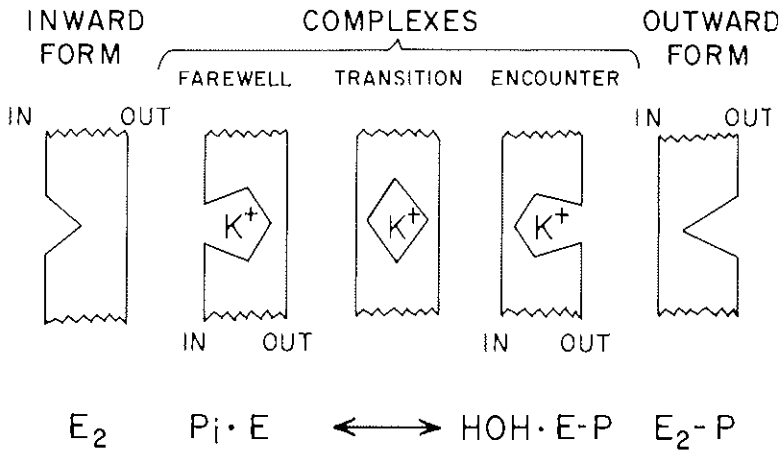

FIG. 17a — Lower panel.

scheme of Fig. 16 with hydrolysis of the phosphoenzyme as the rate-limiting step due to the absence of extracellular K^+.

It is therefore reasonable at this stage to assign the high affinity site to intracellular Na^+ and the low affinity site to extracellular Na^+. This assignment implies in turn that phosphorylation controls the sidedness of accessibility of Na^+ to the active site at which it is catalytically active. According to this hypothesis only intracellular Na^+ has access to the active site in the dephosphoenzyme and only extracellular Na^+ has access in the phosphoenzyme. It is assumed here that the Na^+ which catalyzes transphosphorylation in the ATPase is the same Na^+ which experiences translocation by the pump. This assumption is based on the ionic specificity of the partial reactions of this system. In these Na^+ is unique. On the other hand K^+ has congeners, Li^+, NH_4^+, Rb^+, Cs^+, and Tl^+ (although Li^+ occasionally fails to qualify for membership in certain respects) [4].

The control of sidedness by phosphorylation suggests

further that transphosphorylation and translocation share a common transition state. This hypothesis illustrated in Fig. 17. A nice feature of the hypothesis is that it is as applicable to K^+ as to Na^+.

ACKNOWLEDGEMENT

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DISCUSSION

Chairman: Prof. R. D. KEYNES

BAKER

The system you are describing in which a very high concentration of sodium is needed presumably on the outside of the membrane and a smaller amount at the inside to get reversal is, I imagine, the biochemical correlate of what we physiologists describe as sodium-sodium exchange. Would you like to comment on this relationship?

POST

Yes, indeed, I didn't bring out the point that this assignment of sidedness for the actions of the different concentrations of sodium is based on the in-out exchange experiments for sodium in squid-axon and in the erythrocyte. These in-out exchange experiments require the absence of extra-cellular potassium and the presence of intra-cellular ADP, as well as ATP. The half-maximum concentration for Na^+ in erythrocyte was about 0.7 millimolar inside and outside it was about .12 molar. In the squid-axons sea water allows outside concentrations up to almost half molar. Dr. BAKER pointed out to me the other day that there is no saturation for that exchange, with respect to extracellular sodium concentration, that maximal is out of sight — which fits very well with the requirements for the high concentration of sodium for ATP synthesis and is incompatible with the low concentration for phosphorylation from ATP.

BAKER

So you would say you have evidence for the correctness of a suggestion that we made a long time ago (BAKER et al., J. Physiol. 200, 459-496) that the sodium-sodium exchange in squid-axons probably involves the alternate breakdown and resynthesis of ATP.

POST

Yes, indeed, specifically the model in Fig. 15 C in that paper.

BOYER

These are indeed elegant experiments and I accept the conclusions as you presented them. About the concentration of ATP that might be in your system, you very nicely took care of any ATP in commercial ADP, but is there any need to worry about adenylate kinase making some ATP for you in the experiment?

POST

No, the high concentration of CDTA (cyclohexylenediamine tetraacetic acid) chelates all the free magnesium ion and prevents the action of adenylate kinase. The magnesium ion required by the sodium, potassium ATPase probably remains on it until after the phosphate has come off.

MACROBBIE

In your model of aqueous pockets opening and closing to allow transfer through the membrane, would you envisage that other substances could also be transported, and would you see this as

specific, or simply a little pocket of aqueous phase, with its contents going through?

POST

I see the sodium site as extremely specific since only sodium will catalyse phosphorylation from ATP and since, as has been shown in the cell preparations, net outward transport is specific for sodium.

MACROBBIE

But you've actually got a little pocket — I mean you've got an aqueous pocket, whose accessibility to the solutions is changing — would you see anything else possibly going with that?

POST

Well, you can imagine the terminal phosphate of ATP, if you wish, with the free oxygens around it and little pockets for the sodiums. When the phosphate comes on the enzyme each one of those oxygens fits exactly around one of the sodiums and this provides the specificity for sodium binding and trans-phosphorylation. But I tried that fantasy out on a phosphate chemist and he wouldn't have anything to do with it. It was originally suggested by Dr. MARTIROSOV some years ago.

BOYER

There is another objection to such a possibility. This concerns your stoichiometry, because you would need three phosphates to take care of three sodiums, but you only have one phosphate for three sodiums.

POST

The phosphate has three available oxygens — there is one oxygen which connects to the ADP.

BOYER

You were going to bind one sodium to each phosphate oxygen?

POST

Yes.

HASSELBACH

I just want to indicate that the scheme you presented is nearly identical to that I showed for the calcium transport system.

POST

I see. Replace the calcium by sodium? Yes, there is a very striking similarity, and I think there is also evidence for an E2 as distinct from an E1 in your enzyme.

ELECTROLYTE FLUXES AND ENERGY COUPLING IN PLANT CELLS

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Botany School, University of Cambridge
Cambridge - U.K.

The aim of this paper is to consider the existence of processes of active ion transport in plant cells, the information available on the nature of the coupling between such processes and energy-yielding sequences of metabolism, and the light such information throws on the mechanisms of the transport processes.

Active transport processes

There are three major transport processes in plant cells, which are together responsible for the maintenance of suitable ionic conditions in the cytoplasm for metabolism to proceed, but are also involved in the maintenance of the solute content of the central sap cavity characteristic of mature plant cells. The three transport systems are concerned with potassium-sodium regulation, particularly in the cytoplasm, with pH regulation in the cytoplasm, and with the net salt accumulation responsible for the very high solute content of the vacuole which occupies some 90 % of the cell volume. Fig. 1 shows the pattern of active transport processes indicated by the

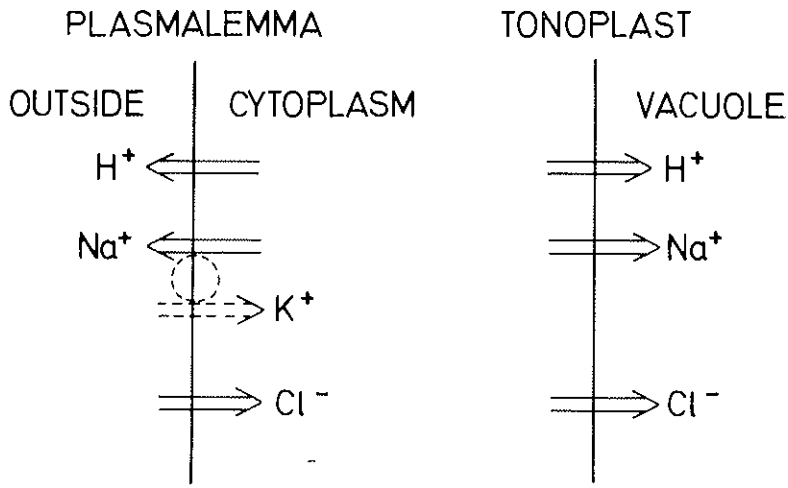

FIG. 1 — Pattern of active transport processes in plant cells suggested from measurements of ion concentrations, potentials, and fluxes.

existence of maintained electrochemical potential gradients in many plant cells, deduced from measurements of ion concentrations and potentials.

Potassium-sodium regulation

In a very wide range of plant cells the level of cytoplasmic sodium is maintained low by an active extrusion of sodium from the cell at the plasmalemma. This may or may not be sensitive to ouabain, and may or may not be coupled to potassium uptake, depending on the cell considered. In conditions of low external potassium, where the passive driving force provided by the negative potential in the cytoplasm could achieve only fairly low levels of potassium in the cell, then the observed level of K in the cell is generally above its equilibrium level, and an active uptake of potassium is inferred. In several

giant algal cells, where vacuolar and cytoplasmic concentrations may be determined separately, there is evidence also of active transfer of sodium from cytoplasm to vacuole. The net result is the maintenance of a very high K/Na ratio in the cytoplasm, a characteristic which seems to be of general occurrence of all cells, plant, animal or procaryotic. The detailed results on a range of plant cells have been reviewed previously (MAC ROBBIE, 1970, 1975), and will not be further considered here.

Active H^+ extrusion

The importance of processes of active extrusion of H^+ ions from plant cells has been recognised only fairly recently, although the pump is capable of very high flux rates, considerably in excess of those of the other transport systems recognised in the cell. There is evidence of two kinds for active proton extrusion at the plasmalemma in a range of plant cells — firstly, from direct observation of acidification of the surrounding medium, against an opposing gradient of electrochemical potential of H^+, and secondly, indirect evidence from anomalies in the electrical behaviour which could be explained by the electrogenic extrusion of cations. Thus many cells have membrane potentials more negative than can be accounted for as diffusion potentials controlled by any of the major ions present, and which are largely and rapidly affected by metabolic inhibition. The best characterised of such systems is in *Neurospora crassa* (SLAYMAN, 1965, 1970; SLAYMAN, LU and SHANE, 1970), but there is evidence of electrogenic proton extrusion at the plasmalemma in a number of both higher plant cells and giant algal cells (see for example, HIGINBOTHAM, GRAVES and DAVIS, 1970; and review by SLAYMAN, 1974). In giant algal cells KITASATO (1968) suggested that H^+ movements, and an active H^+ extrusion, were important in control of the membrane potential of *Nitella clavata.* Since then, the hypothesis has been considerably extended by SPANSWICK (1972, 1973, 1974),

who postulates a voltage-dependent active proton extrusion pump as the primary charge-carrying system in the plasmalemma, and whose work has done much to establish its properties. The primary role of this pump is taken to be the maintenance of a constant cytoplasmic pH, in the face of considerable generation of H^+ in metabolism in most conditions of growth, but secondary roles for the proton gradients created by its activity include co-transport systems, with H^+, for the uptake of other solutes against their electrochemical potential gradients. These include sugars and amino acids in yeast (EDDY and NOWACKI, 1971; SEASTON, INKSON and EDDY, 1973), sugars in *Chlorella* (KOMOR, 1973; KOMOR and TANNER, 1974 a, b), glucose, sucrose and amino acids in *Avena* coleoptiles (ETHERTON and NUOVO, 1974), and the mechanism is almost certainly more widespread than is yet recognised. Again the best characterised of such systems is the co-transport of glucose and H^+ in *Neurospora* (SLAYMAN and SLAYMAN, 1974). The central role of proton transport in the ionic regulation in plant cells is stressed by RAVEN and SMITH (1973, 1974); SMITH and RAVEN (1974 a), and reviews dealing with the H^+ pump in plant cells include those by HIGINBOTHAM and ANDERSON (1974), SLAYMAN (1974) and MACROBBIE (1975 a).

The question of H^+ transport at the tonoplast of plant cells has received little attention, but H^+ is unlikely to be in equilibrium at the tonoplast either, and it seems likely that transfers of H^+ at that membrane also are concerned with the regulation of cytoplasmic pH. For example, in giant algal cells the tonoplast potential is small (vacuole + 17 mV or so, with respected to the cytoplasm), yet the cytoplasm is frequently maintained at a considerably higher pH than in the vacuole. Thus in *Chara corallina,* in normal pond water, in light, with an external pH of 6, the cytoplasmic pH was about 7.7, but the vacuolar pH was only about 5.4 (WALKER and SMITH, 1975). In higher plant cells also, there is evidence that conditions associated with H^+ movements at the plasmalemma also involve movements at the tonoplast. Nevertheless there

is no experimental information on the nature of the processes by which H^+ is transferred (directly or indirectly) from cytoplasm to vacuole, against its electrochemical potential gradient.

Net salt accumulation

Net salt accumulation is the most striking characteristic of vacuolate plant cells, and the accumulation of salt in the large central vacuole is responsible for a large fraction of the osmotic pressure by which cell turgor is maintained. Two forms of salt accumulation are seen. In the presence of an anion and a cation both of which can be absorbed from the outside solution (for example K^+ and Cl^-), both are accumulated in the vacuole. But in the presence of an anion which cannot be absorbed, such as sulphate, we see instead K^+/H^+ exchange at the plasmalemma, and transfer of K^+ with an internally synthesised organic acid anion, such as malate, to the vacuole. This is also seen when the anion absorbed at the plasmalemma is subsequently metaholised (nitrate or bicarbonate).

In the condition with a non-absorbed anion outside, it is suggested that the primary electrogenic H^+ extrusion provides an electrical driving force for K^+ entry, and leaves the cytoplasm alkaline. The high pH stimulates the activity of phosphoenolpyruvate carboxylase (both hecause the enzyme has an alkaline pH optimum, and because it uses HCO_3^- as its substrate), and the oxalacetate so formed is then converted to malate by malic dehydrogenase (HIATT, 1967; HIATT and HENDRICKS, 1967; JACOBY and LATIES, 1971; reviews by SMITH and RAVEN, 1974a, and MACROBBIE, 1975a). If this sequence is to work then it is essential that both K^+ and malate are removed to the vacuole, to maintain a driving force for entry of K^+, and to avoid the inhibition of PEP carboxylase by malate as shown by TING (1968). This scheme is shown in Fig. 2a.

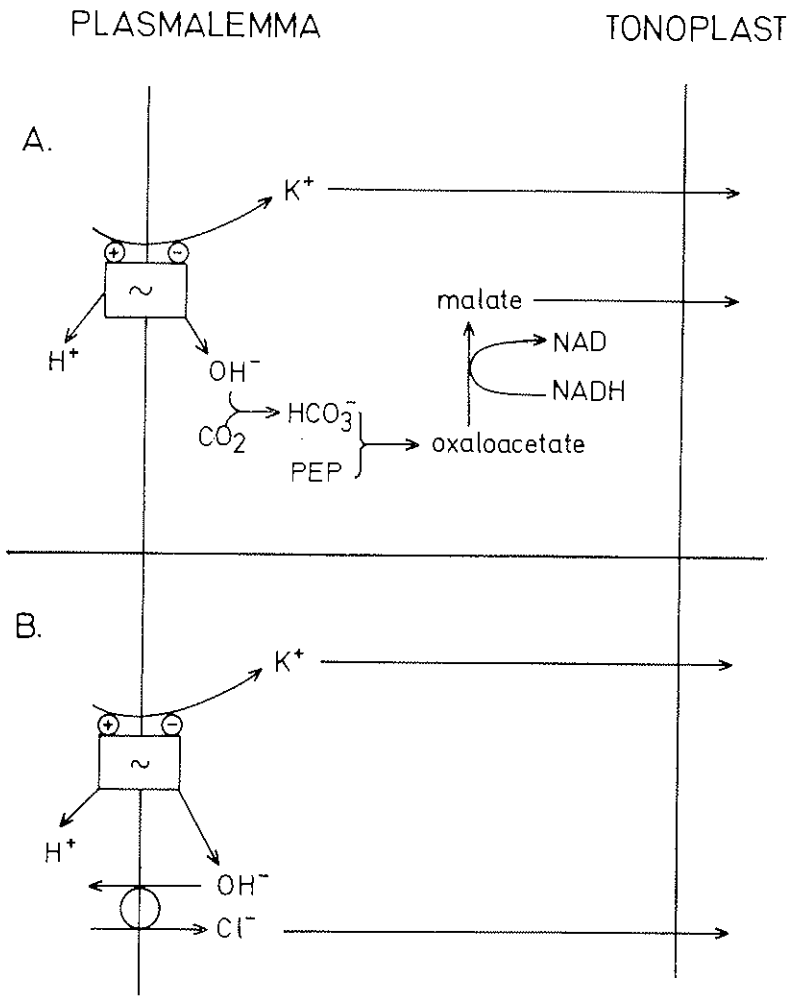

FIG. 2 — Schemes for net salt accumulation in plant cells. - A. Accumulation of inorganic cations from the solution with internally synthesised organic acid anions. - B. Accumulation of both cations and anions from the solution.

One of the current hypotheses for the other form of net salt accumulation, of KCl in the vacuole, is similar in form, being a revival by SMITH (1970) of older concepts of salt uptake as a double ion exchange process, of K^+ for H^+ and Cl^- for OH^-. Smith suggested that the cytoplasmic alkalinity produced by H^+ excretion created the driving force for Cl^- entry via the Cl^-/OH^- exchange process. The processes involved in salt accumulation on this hypothesis are shown in Fig. 2b. Again it is important to note that if Smith's hypothesis is correct, of Cl^- entry driven by the pH gradient set up by the primary proton extrusion, then two things follow. One is that the concentration gradient of Cl^- which can be sustained across the plasmalemma is limited by the pH gradient established across the membrane by the proton pump, unless the Cl^-/OH^- exchange is given an additional energy linkage of some kind. The second point is that continued operation of this mechanism for transfer of K^+ and Cl^- across the plasmalemma is entirely dependent on their removal from the cytoplasm, by transfer to the vacuole. SPANSWICK (1972) suggested that the H^+ pump will polarise the membrane, setting up both the electrogenic component of the membrane potential and a ΔpH across the membrane, until the energy gradient for further H^+ extrusion is equal to the free energy available from the energy-yielding side of the coupled process. At this point the pump will stall. In this state the charge transfer by the pump will be balanced by the net passive flux of ions, primarily by entry of K^+. In the scheme of Fig. 2b, there will be a net influx of KCl, but as K^+ and Cl^- build up in the cytoplasm the gradients of potential and pH required to allow further entry (of K^+ by passive diffusion down the electrical gradient, and Cl^- by exchange for OH^-), will increase steadily. In due course both processes will stall, and continued entry of salt is only possible if the levels of K^+ and Cl^- in the cytoplasm are kept down by the subsequent transfer to the vacuole. Hence net salt accumulation will be limited by the processes of transfer to the

vacuole, and there will inevitably be a degree of linkage between plasmalemma fluxes and vacuolar transfers. It is therefore unfortunate that we know so little of the processes by which ions are transferred to the vacuole.

Energy coupling of transport processes

Uphill transport of ions at the plasmalemma is dependent on some product or consequence of one of the energy-yielding sequences in cell metabolism, i.e. on some part or product of photosynthesis or respiration. We may ask first what is the agent by which energy is transferred from chloroplasts or mitochondria to the pumps in the plasmalemma, and second, what manner of molecular processes occur within that membrane. In green cells ion fluxes are strongly light-dependent, a property which offers considerable experimental advantages in attempts to clarify the nature of the energy coupling. As I have argued in the previous section, transport from cytoplasm to vacuole will inevitably affect the plasmalemma transport processes. In consequence, it is important to try to measure plasmalemma fluxes, separate from any interference from tonoplast processes, although this is difficult in higher plant cells. These difficulties have been considered a number of times (Cram and Laties, 1971; MacRobbie, 1971a), and will not be discussed in this paper. But because of such problems, the giant algal cells have provided favourite experimental material, in which cytoplasm and vacuole can be separated, plasmalemma fluxes can be accurately measured, and some attempt can be made to measure also the tonoplast fluxes.

Three fresh-water giant algal cells have been studied in enough detail to allow some conclusions to be drawn on the question of the energy coupling of the ion fluxes. These are *Nitella translucens, Chara corallina,* and *Hydrodictyon africanum*; *Nitella* and *Hydrodictyon* have all four active fluxes, H^+ efflux, Na^+ efflux, K^+ influx and Cl^- influx, whereas *Chara*

lacks an active influx of K^+ but has the other three. All these fluxes are light-dependent but to varying degrees, with ratios of fluxes in light to dark of 2-20. (Full references to this work are given in MACROBBIE, 1970, 1975a.) A good deal of work has been aimed at establishing the nature of the link between ion transport and photosynthesis, by the use of conditions or inhibitors which allow some, but not all, of the partial reactions of photosynthesis to proceed.

For the purpose of this discussion a summary of current views of photosynthetic electron flow is required. Recent relevant reviews include those of WITT (1971) and TREBST (1974). Two types of electron flow are postulated:

a) *non-cyclic electron flow,* driven by two photosystems (PS1 and PS2) in series, yielding both NADPH and ATP as useful products, and evolving also oxygen. Non-cyclic flow will not work in far red light ($>$ 700 nm in green algae or higher plants), since PS2 cuts off at shorter wavelengths than PS1. Non-cyclic flow is also inhibited by low concentrations of DCMU (dichlorophenyl-dimethyl urea) which blocks electron flow from PS2;

b) *cyclic electron flow,* in which electron flow in a closed redox chain is also coupled to phosphorylation. This can be driven by PS1 alone, hence it will work in the far red, and it is insensitive to low concentrations of DCMU. The only product is ATP, since no reducing equivalents can be withdrawn from the cyclic path. Both types of phosphorylation are sensitive to uncoupling agents, such as CCCP, or to energy transfer inhibitors.

Proton movements at the chloroplast membranes are involved in these redox systems, and in the associated phosphorylations, and the evidence for the high energy state in chloroplasts being a combination of membrane potential and pH gradient across the thylakoid membrane is strong (summarised in WITT, 1971 and TREBST, 1974, among other reviews). Light-induced pH changes in the phases of intact chloroplasts

were measured by HELDT et al (1973); with a pH 7.6 outside, the pH inside the thylakoids in light was 5.61, while that in the stroma was 7.87; in the dark there was no pH gradient across the thylakoid membrane. Thus in light there is a large movement of H^+ from stroma into the thylakoids, and Mg^{2+} is most likely counter ion. WALKER (1973) has discussed the role of these changes in the pH and $[Mg^{2+}]$ in the stroma in the regulation of photosynthetic carbon fixation, by activation of key enzymes in the carbon cycle, including rihulose diphosphate carboxylase. There is also some evidence for light-induced H^+ movement across the chloroplast envelope, from stroma to cytoplasm (HEBER and KRAUSE, 1971; HELDT et al, 1973; WALKER, 1974). LIN and NOBEL (1971) reported large movements of Mg^{2+} into chloroplasts in light. Thus their measurements suggested that, in light, Mg^{2+} moved into the stroma both from the thylakoids and across the envelope from the cytoplasm. What is interesting for the present discussion is that the movement may also result in considerable changes in cytoplasmic $[Mg^{+2}]$ in light, which might have drastic effects on a number of other processes, including other ion transport systems. Lin and Nobel suggested that the chloroplast concentration of Mg^{2+} increased in light by about 9 mM. In Characean cells, for example, where the chloroplasts occupy about half the total cytoplasmic volume, and the concentration of Mg^{2+} in the flowing cytoplasm is only 3.6 mM (WILLIAMSON, 1975) very large light-induced changes would be expected. If Ca^{2+} movements were also involved further regulatory effects might be expected. Suggestions that effects of this kind might be important were made by a number of authors in the discussion to a recent symposium (ZIMMERMANN and DAINTY, 1974; p. 246). Thus light-induced cytoplasmic changes may be concerned in the regulation and triggering of light-dependent plasmalemma transport processes, as well as in the provision of their energy supply.

Three possibilities for the energetic coupling of ion transport processes in the plasmalemma to chloroplast metabolism have

been considered. The two direct ones are by the provision of either ATP or reducing power from the choloroplast, to drive ATP-ase- or redox-associated transport systems in the plasmalemma. The third possibility is that some of the ion transport processes in the plasmalemma are the secondary consequences of the light-activation and/or energisation of primary active proton extrusion at that membrane, with the generation of a pH gradient across it.

Experimental observations in giant algal cells

The evidence suggests that ATP provides the energy source for cation transport in the plasmalemma, both for K/Na regulation and for proton extrusion. In *Nitella translucens* and *Hydrocictyon africanum* the active Na^+ efflux and K^+ efflux are sensitive to uncouplers, or to energy transfer inhibitors, but not to low concentrations of DCMU, and will work in far-red light, when only cyclic phosphorylation can function. (*Nitella*: MACROBBIE, 1965, 1966; *Hydrodictyon*: RAVEN, 1967, 1968, 1969a, b, 1971). The light induced hyperpolarisation of the membrane potential and decrease in resistance, taken to be indicators of the operation of the proton pump, were shown by SPANSWICK (1972, 1974) to occur in far-red light, or in the presence of low concentrations of DCMU, but not in the presence of CCCP.

The strongest evidence for ATP-dependence of the cation transport is in *Hydrodictyon*, where RAVEN (1967) showed that Na^+ efflux and K^+ efflux had an increased quantum efficiency in the far-red ("red-rise"), indicating a process which can be supported by cyclic flow in PS1 alone. (In contrast, processes which depend on the operation of both photosystems, such as CO_2 fixation show a " red-drop " in quantum efficiency).

Very extensive studies using inhibitors on a variety of transport processes and CO_2 fixation in *Hydrodictyon* (RAVEN, 1969b, 1971) reinforce this conclusion, that ATP synthesis in

cyclic phosphorylation can drive a range of cytoplasmic processes in that cell, including Na and K transport at the plasmalemma. Supporting evidence in *Nitella* comes from similar studies on phosphate uptake and glucose uptake (SMITH, 1966, 1967), again making it clear that cyclic phosphorylation can power transport at the plasmalemma.

In contrast, the energy linkage of chloride transport in *Nitella* and *Hydrodictyon* seems to be quite different. Chloride transport in these cells is light-dependent, but will not work in conditions of cylcic electron flow, in far-red light or in the presence of DCMU MACROBBIE, 1965, 1966; RAVEN, 1967, 1968). It would seem therefore that chloride transport in these cells is not ATP-dependent, and this is consistent with the observations that the chloride influx is relatively insensitive to uncouplers.

The situation in *Chara corallina* is less clear-cut. Fluxes are light-dependent, but SMITH and RAVEN (1974b) found no evidence that cyclic phosphorylation could drive any transport process in this cell. They concluded that if cyclic phosphorylation exists in *Chara* it is unable to supply ATP for processes outside the chloroplast, for reasons which are not yet clear. As a consequence the possibility of distinguishing between ATP and some other form of energy coupling is not available in *Chara*.

Mechanisms of cation transport processes

Where a ouabain-sensitive sodium and potassium transport exists, with a degree of coupling between K and Na, it is assumed that it shares the same mechanism as the K/Na pump of animal cells (recently reviewed by SKOU, 1974); this covers the transport in *Nitella* and *Hydrodictyon*, but in *Chara* the sodium efflux is not ouabain-sensitive, and is not linked to potassium influx. In higher plant cells active sodium extrusion is sometimes ouabain-sensitive (carot: CRAM, 1968; *Elodea*

in the dark, but not in the light: JESCHKE, 1970a). In other tissues sodium efflux may be insensitive to ouabain, yet linked to potassium influx (barley roots: PITMAN and SADDLER, 1967; JESCHKE, 1970b, 1973). Hence the relations between the cation transport systems in different plant cells are uncertain. There is however no work in plant cells which contributes to our understainding of the mechanism of such transport systems; so far work has been concerned solely with establishing the existence or non-existence of the processes.

The active proton extrusion seems from SPANSWICK's work (1974) to be the result of a membrane ATP-ase system, perhaps similar in mechanism to those found in coupling membranes of chloroplasts, mitochondria, bacteria. Spanswick suggested that the pump would work until stalled by the energy gradient, until the electrochemical potential increase of the transported H^+ is equal to the free energy change of the chemical reaction involving the non-transported reactants. The recent measurements of cytoplasmic pH in *Chara* under different conditions seem to support this (WALKER and SMITH, 1975). The summed energy gradient for H^+ transport at the plasmalemma, calculated from the measured pH and potential gradients, over the range of external pH 5-8 in the light and 5-7 in the dark, was such as to balance the free energy of hydrolysis of ATP under cytoplasmic conditions, with a stoichiometry of $2H^+$ per ATP. Hence it does appear that the gradients are such as could be produced by a proton-pumping ATP-ase reaction of this stoichiometry.

Mechanism of chloride transport

The mechanism of the chloride transport is rather more obscure than that of the cation transports, since it is neither clear what the energy source is, nor what kind of pump is involved. In the only instances in which any clear distinctions can be drawn between possible energy sources, it is clear that

ATP does not directly provide the energy for Cl^- transport, i.e. provision of ATP is not a sufficient condition for energising the pump.

Two other possibilities remain. One is that the energy for chloride transport in the plasmalemma is in fact provided by the export of reducing power from the chloroplast — by one of the indirect shuttle systems by which NADPH in the chloroplast can give rise to NADH (or other reduced compound) in the cytoplasm. Such shuttles have been reviewed recently by HEBER (1974) and by WALKER (1974). This possibility, of a redox-driven pump, leaves open the question of what kind of pump it may be, whether a salt pump, an anion exchange pump (or an electrogenic Cl^- transport, though other evidence is against this).

The other possibility is that, as SMITH (1970) suggests, the Cl^- influx is a Cl^-/OH^- exchange pump, driven by the alkalinity created in the cytoplasm by the light-activated proton extrusion. The alternative form of this hypothesis is that H^+ secreted by the proton pump accumulates to some extent at some site in the membrane, allowing the inward partitioning of undissociated HCl through the lipid (SPEAR, BARR and BARR, 1969). In favour of these hypotheses are the observations that Cl^- influx is inhibited at high external pH, and is stimulated by ammonium ions or imidazole, particularly in the dark, treatment which (it is assumed) will increase the internal pH (SMITH, 1970, 1972; JAYASURIYA, unpublished observations). Smith found that pretreatment of *Chara* cells at high pH, in the dark, put them in a state in which their ability to take up Cl^- in the dark is higher than after pretreatment at low pH. Jayasuriya found that pretreatment with ammonium ions also had marked stimulatory effects. Thus it seems that Cl^- influx is sensitive to both internal and external pH, and RAVEN (1974) argued that the slow response of Cl^- influx in *Hydrodictyon* to light/dark changes could reflect the slow decay of a pH gradient. That this implies the operation of a Cl^-/OH^-

exchange is less clear, but there is indirect evidence in favour, at least in higher plant cells. This includes the evidence already discussed that many plant cells acidify the medium in sulphate solutions but not in chloride solutions (for example, JACKSON and ADAMS, 1963; HIATT, 1967; HIATT and HENDRICKS, 1967; PITMAN, 1970). It also includes the demonstration by CRAM (1974) that external chloride suppresses malate synthesis in barley roots and carrot, for which the simplest explanation is that Cl^-/OH^- exchange lowers the cytoplasmic pH. However it seems unlikely that the pH gradient across the plasmalemma can be large enough to provide the sole driving force for Cl^- entry under all conditions. WALKER and SMITH (1975) argue that the measured gradient in *Chara* would be adequate with a pH in the cell wall of 5.5, but not at higher external pH. In *Nitella*, with higher measured values of cytoplasmic chloride (65-87 mM; SPANSWICK and WILLIAMS, 1964; HOPE, SIMPSON and WALKER, 1966), the cytoplasmic pH would have to be very alkaline indeed. There are difficulties therefore in postulating that the OH^- gradient provides the energy for Cl^- entry. It was argued by SPEAR, BARR and BARR (1969) that the proton pump released its protons at some inaccessible site within the membane, allowing considerable build-up of H^+ there. While this increases the concentration of undissociated HCl at this site, it would seem to require some sort of chloride ionophore to allow access of Cl^- to the site. JUNGE, AUSLÄNDER and ECKHOF (1974) have argued that the redox reactions associated with proton transport in the thylakoid membranes are shielded from the outer phase by a diffusion barrier, and that a reservoir of H^+ exists within the membrane on the outer side, which is refilled only slowly from the outer medium. To aid chloride transport in the plasmalemma a diffusion barrier on the side to which H^+ is delivered would be required, rather than on the donating side, but the principle might be similar.

The other difficulty with the hypothesis that chloride transport is a secondary consequence of the primary proton extrusion

is that the light dependence and inhibitor characteristics of the two processes seem to be different. Cyclic electron flow in the chloroplast can support the proton pump at the plasmalemma, but under these conditions chloride influx is inhibited. Either some other energy input is required, or there is some feature of the conditions for export of ATP from the chloroplast, in conditions of cyclic electron flow, which inhibits chloride influx. The shuttle systems proposed for the export of ATP, and reducing power, from chloroplasts have been reviewed recently by HEBER (1974) and by WALKER (1974). They involve two exchange systems in the inner membrane of the chloroplast envelope — the phosphate translocator and the dicarboxylate translocator (HELDT, SAUER and RAPLEY, 1972; WERDAN and HELDT, 1972; HEBER and KRAUSE, 1971). The phosphate translocator can carry PGA (3-phosphoglycerate), GAP (glyceraldehyde-3-phosphate), DHAP (dihydroxyacetone phosphate), and P_i (inorganic phosphate), in an exchange process, and the dicarboxylate translocator facilitates the exchange of a number of dicarboxylic acids, including malate, oxalacetate (OAA) and aspartate. Indirect export of ATP is achieved by the transfer out of the chloroplast of DHAP fomed by the light-dependent reduction of PGA by NADP-linked triose phosphate dehydrogenase; the subsequent oxidation of DHAP to PGA in the cytoplasm will generate both ATP and NADH. The reducing equivalents can then be returned to the chloroplasts by the combined operation of cytoplasmic NAD-linked malic dehydrogenase producing malate from OAA, the malate/OAA shuttle in the chloroplast envelope, the chloroplastic NADP-linked malic dehydrogenase regenerating OAA. Under conditions in which CO_2 is available, and non-cyclic flow possible, there is no need to return the carbon in PGA to the chloroplast, and the phosphate translocator may simply catalyse the export of DHAP in exchange for P_i. Under cyclic conditions it is necessary to return both the carbon of PGA and the reducing equivalents to the chloroplast, relying on light energy to supply only the

ATP required. The combined operation of DHAP/PGA and OAA/malate shuttles are suggested. There will be competition between P_i and PGA for entry into the chloroplast, in exchange for the DHAP coming out, and the import of PGA is favoured by low external pH and high internal pH (hence it is light-stimulated whereas the other phosphate translocations are not). WERDAN and HELDT (1972) suggested co-transport of H^+ with PGA^{3-}, or HEBER (1974) suggests that PGA^{2-} is in fact the species translocated. At the usual cytoplasmic pH there will be little PGA^{2-} to compete with P_i^{2-} for entry, and hence the import of PGA may be very sensitive to cytoplasmic pH. If the light-dependent movement of H^+ out of the chloroplast, and Mg^{2+} into the chloroplast, did reflect the operation of yet another ATP-driven proton pump then this might contribute to the inward movement of PGA, but its operation (triggered by the continued build up of chloroplast ATP in these conditions) might lower the cytoplasmic pH, and hence tend to reduce the chloride influx. Alternatively the action of 3-phosphoglycerate phosphatase in the cytoplasm (RANDALL, TOLBERT and GREMEL, 1971; TOLBERT, 1973) might produce glycerate, which could enter the chloroplast, but this would also lower the cytoplasmic pH. Glycerate is readily labelled in photosynthesising cells, and RANDALL et al. (1971) suggest that it may be involved in some carbon and/or ionic transport system between chloroplast and cytosol. Hence although there is ample evidence that PGA can very readily enter the chloroplasts by the shuttle system, and that the indirect export of ATP by the combined shuttles can be made to work in the proper experimental conditions *in vitro*, the particular processes involved *in vivo* will depend on the products generated in the light reactions. Indirect export of ATP clearly does occur under cyclic conditions in at least some cells (but not in *Chara*). But in cyclic conditions the chloroplast ATP/ADP is much higher and the NADPH/NADP is much lower than in non-cyclic flow, and the shuttles are not working under the same relative driving

forces. It is possible therefore that the conditions for the operation of indirect export of ATP in cyclic conditions are such as to inhibit chloride influx, perhaps by a change of cytoplasmic pH. This is speculative but testable. A further difficulty is of course the means of generating cytoplasmic ATP in CO_2-free conditions. Since CO_2 may not be zero within the cell, even when the medium is flushed with CO_2-free air, as CO_2 will be produced in photorespiration, some degree of carbon recycling may occur. Under these conditions both phosphoglycollate and PGA are produced in the chloroplast. In photorespiration it is suggested (review by TOLBERT, 1973) that glycollate is exported from the chloroplast, and may be converted to photorespiratory CO_2 and glycerate, which could be re-imported to the chloroplast, where it can be phosphorylated to PGA. In the conversion of glycollate to glycerate and CO_2, with the consumption of NADH, there will be a net uptake of H^+ ($2H^+$ per glycerate), hence in conditions where CO_2 is low but not zero it may be possible to have the shuttles going in conditions in which Cl^- uptake will still take place. But some experimental measurements of the effects on cytoplasmic pH of changes in the pattern of photosynthetic metabolism seem to be required.

The alternative explanation for the inability of cyclic phosphorylation to drive chloride influx is, of course, that a further energy input is required, in the form of reducing power, driving some sort of redox chain in the plasmalemma. It might still be easiest to imagine this as a Cl^-/OH^- exchange process, for energetic reasons. Although the possible span $NADH/O_2$ has a very large free energy change, it is unlikely that any individual redox reaction responsible for a charge separation in the membrane has a span equivalent to more than about 200 mV. While this would be adequate to drive chloride against the concentration gradient involved it is not enough to drive it against both that and the electrical potential gradient of up to 200 mV. Hence a neutral chloride pump seems more likely, either of the form Cl^-/OH^- exchange or as a salt uptake process.

From this lengthy discussion it is clear that we do not yet understand the nature of the chloride transport, or its energy coupling. What might be stressed again is that the plasmalemma influx must inevitably be linked to the processes of transfer to the vacuole, and in some cells (such as *Chara*) the energy barrier for this transport of chloride is also considerable. Very little is known of this, but the process has curious properties; these include a close link with the plasmalemma influx, and an absolute lack of discrimination between chloride and bromide (MAC ROBBIE, 1969, 1971b, 1975a, b). It has been argued that the last feature is unexpected for any active transport process for single ions, and that instead a process of salt transfer to the vacuole is involved, perhaps by the formation of salt-filled vesicles from the endoplasmic reticulum, and their discharge to the main vacuole. The interpretation remains speculative but what is important is that understanding of the process of salt accumulation in plant cells must encompass not only the initial entry at the plasmalemma, but also the process of transfer from cytoplasm to vacuole. Vacuolar transfer should now be measured under the same range of conditions as have been used in the studies of the initial uptake to the cytoplasm, to test the closeness of the apparent link between the two, to establish whether the link is in the kinetics of the two processes, reflects similar energy coupling, or is inherent in the mechanism.

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DISCUSSION

Chairman: Prof. R. D. KEYNES

KEYNES

It seems to me that when we are talking about animal cells we know about sodium potassium ATP-ase but there are several other ion transport processes which are totally neglected, usually because we just don't have any inhibitor to block them with, which makes them very hard to characterize. One of the most important ones, I feel, is chloride transport in animal cells, for an active transport of chloride undoubtedly does take place in some cases. Are there any parallels that could be drawn with the chloride system in plants? There is no evidence in animal cells that chloride active transport involves an ATPase, but I thought that there is some evidence for a chloride activated ATP-ase in plants.

MACROBBIE

There is evidence in plants, in a rather different situation, in halophytic salt-glands where there is a direct extrusion of chloride in an electrogenic process, with sodium then following, that a Cl -ATP-ase is involved. This is an inducible protein, as shown by HILL's work on *Limonium*. If *Limonium* is grown in the absence of chloride there is no chloride ATP-ase activity or practically none; if the plant is then given high Na Cl the enzyme is induced, and salt be excreted. This is, however, an output from specialized cells, rather than the primary process of salt uptake. But if the

animal chloride pump is in fact a dual exchange system, including a chloride-bicarbonate or a chloride-hydroxyl exchange, then there might be some similarity.

KEYNES

Of course, the animal system is certainly tied up with bicarbonate transport somehow or other, because the standard inhibitors of the process are carbonic anhydrase inhibitors, but what the relationship is between the action of that carbonic anhydrase inhibition and its effect on chloride transport or bicarbonate transport is not known. Do carbonic anhydrase inhibitors have any effect in plants?

MACROBBIE

There is one report in *Chlorella* that there is inhibition of chloride transport by Diamox. I've tried it in *Nitella* and it didn't do anything. I'd like to try it again to make quite sure, but it didn't in the first experiment.

KEYNES

There's another parallel one might explore, which is that in insects there is an electrogenic potassium pumping mechanism which again doesn't seem to be an ATP-ase system. But that doesn't really tie up with what you said, because you were getting the potassium transport down the electrical gradient rather than a potassium flux generating the electrical gradient, which seems to be what happens in insects.

MACROBBIE

No. In plant cells potassium influx is either coupled to sodium

efflux, probably by a sodium-potassium ATP-ase, or it is down hill, down the electrical gradient.

LIQUORI

It seems to me that the ATP pump creates the gradients while other pumps which are, essentially physico/chemical pumps use the gradient. I think that what you say about the chloride may be an example. The pH gradient can be used by the physico/chemical pump to pump against its owen gradient, say water, chloride, and so on. So we probably have at least two different mechanisms: what I would call a biochemical pump which requires an enzyme system and a physico/chemical pump which also requires conformational transitions, but no enzyme system.

MACROBBIE

But either the cells create a bigger pH gradient than is actually measured or the chloride influx does in fact need an extra energy input, perhaps from an associated redox reaction. It may be a chloride/hydroxyl change but driven by an extra energy input by reducing power, a redox system at the plasmalemma.

BAKER

Have you actually measured the cytoplasmic pH using a pH-sensitive electrode?

MACROBBIE

Well, it has recently been measured by SPANSWICK, in *Nitella*. The problem is that the cell wall is too tough for pH microelectrodes. He has made some measurements recently, but not very many.

He gets figures of 7.7 or so for the cytoplasmic pH. There is also work by WALKER and SMITH, who used DMO distribution, and again got some values for cytoplasmic and vacuolar pH. I think in a year's time there will be a good many more measurements, and I think that probably a perfusion system, where there is not the problem of penetrating the cell wall and wrecking the electrode, is probably the answer.

DE DUVE

What are the main cations of the vacuole?

MACROBBIE

In the giant cells 100 mM potassium and 50 mM sodium and some calcium.

DE DUVE

There are no organic cations?

MACROBIE

Not in the giant cells. There are organic anions in most higher plant cells, but under most conditions there are no organic cations. I think if you grow them in unusual conditions you may find organic cations, but normally no.

DE DUVE

The reason I am asking this question is that one can cause tremendous vacuolation of animal cells by exposing them to weak organic bases. The vacuoles form from lysosomes, which accumulate large amounts of the protonated form of the bases thanks to their

low internal pH. The bases probably enter the lysosomes in unprotonated form and become protonated inside the lysosomes. The amounts involved are so large that we believe that the phenomenon must be powered by a proton pump. I noticed that you put a proton pump on the vacuole membrane. I understand that there are some reasons to believe that the vacuole of plant cells may be related to the lysosome system of animal cells. Possession of a proton pump might be another similarity between the two structures.

MACROBBIE

Yes, in higher plant cells there is evidence that the vacuole may be related to the lysosome system, although nothing has been done with the giant algal cells. Ammonium ions can accumulate to high levels in the vacuole, presumably by entry in the unprotonated form and protonation inside, producing alkalinity in the vacuole. But it then seems that the cell can regulate its pH, that it can compensate, within reason, for changes produced in its pH. The stimulation of chloride transport by the presence of a weak base outside is fairly short term. After about an hour or so the high influx declines, presumably as the cell pH falls again.

TEORELL

May I ask you whether a water transport in the *Nitella* cells is visible? I think it was BRADY in California, who did some investigations on that: he recorded some water movement accompanying the action potentials, do you recall that? Would you kindly comment on what happens during the « firing » of an action potential. I repeat the fact mentioned by the speaker that *Nitella* has a large action potential, up to several hundred millivolts, conveniently slow (about one second) for recording.

MACROBBIE

There is a chloride loss during the action potential. The biggest

gradient in the cell is of chloride which has been pumped in, and when fired, the plasmalemma seems to go specifically permeable to chloride. As the membrane potential changes by chloride loss, potassium is then no longer in equilibrium, and also moves out. There is therefore a net loss of KCl, a small loss of water with it, and a small fall in hydrostatic pressure associated with the passage of the action potential. The streaming also stops at the peak of the action potential, which may reflect a change in calcium concentration within the cytoplasm, perhaps because there is also some calcium influx.

STOECKENIUS

To go back to the proton-gradient: is it possible that the pH actually occuring in the wall is lower than in the external fluid rather than in the membrane as you have suggested; and that therefore the actual gradient across the cell membrane is larger than what you find by measuring the pH in the medium?

MACROBBIE

The pH should be lower in the wall, in fact, because the wall is a very strong Donnan system with a very high fixed negative charge. The intact cell will excrete protons over more or less the whole length, hence the pH outside is lower than that of bulk medium. In the presence of bicarbonate there are specific spots on the *Nitella* cell surface where there is net hydroxyl extrusion, with formation of alkaline bands in the wall, where precipitation of calcium carbonate occurs. This seems to be specifically associated with bicarbonate uptake probably by a bicarbonate-hydroxyl exchange process. However, any build of protons, and of the HCl product, to allow HCl to partition inwards against a higher chloride concentration gradient would, I think, have to be in the membrane — to make sure that HCl did not simply leak away, but moved into the cell.

ELECTROPHYSIOLOGICAL ASPECTS OF ENERGY TRANSFER IN THE PLASMA MEMBRANE OF NEUROSPORA

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INTRODUCTION.

It is now fairly clear that « active » transport systems can be divided into three distinct categories, with respect to the means by which metabolic energy is supplied to them. These three categories are outlined in Table 1. 1) In *Redox transport systems*, the energy for moving ions across membranes comes directly from oxido-reduction reactions. For the examples which are best understood, such as respiration-dependent movement of protons through mitochondrial and bacterial membranes, the redox catalysts themselves appear to comprise the transport systems; and they are arranged so that reducing equivalents (H^o) can traverse the membrane in one direction, but only the separated electrons (e^-) pass in the other direction. Protons left behind constitute the « transported » H^+ ions.

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TABLE I — *Distribution of Types of "Active" Transport Systems*

Type of System	Redox	Driven by covalent bond energy	Ion-dependent cotransport
Animal cell membranes	—	ATP-driven Na^+/K^+ exchange (Na^+/K^+-ATPase) ATP-driven Ca^{++} transport (sarcoplasmic reticulum)	Na^+-dependent cotransport of sugars amino acids and Ca^{++}
Plant and Fungal plasma membranes	—	ATP-driven K^+ or Na^+ transport(?) ATP-driven H^+ efflux	H^+-dependent cotransport of sugars and amino acids OH^--dependent Cl^- transport (?)
Bacterial plasma membranes	H^+/ε^- separation by electron transfer system	[ATP-driven H^+ efflux (H^+-ATPase) H^+-driven ATP synthesis] ATP-driven amino acid transport (periplasmic binding proteins) Group translocation (phosphotransferase)	H^+-dependent cotransport of sugars and amino acids
Mitochondrial and Chloroplast membranes	H^+/ε^- separation by electron transfer system	[ATP-driven H^+ efflux H^+-driven ATP synthesis]	Generalized anion exchange OH^-/anion exchange H^+-dependent anion exchange

2) In the classical *ATPase systems*, the energy from hydrolysis of covalent bonds is converted into transmembrane solute gradients; the best known cases are the Na^+/K^+ – ATPase of animal cell membranes, Ca^{++} – ATPase of the sarcoplasmic reticulum, and the H^+ – ATPase of the mitochondrial inner membrane. 3) In *ion-dependent cotransport systems* (or countertransport systems), uphill transport of one substance is coupled to the downhill movement of another substance, as has been described for Na^+ – dependent transport of sugars or amino acids in epithelial membranes and H^+ – dependent transport of the same substrates in microbial membranes.

Transport systems in all three of these categories can function, at least under restricted circumstances, as charge-transfer (electrogenic) mechanisms, thereby interacting with the electric field through biological membranes. This fact is of fundamental importance in bioenergetics, since the difference of electric potential across a membrane can thereby become an alternative to phosphate anhydride bonds as a generalized mechanism for energy transfer. It is also significant for excitable membranes, since polarization due to electrogenic pumps may directly influence the ability of such tissues to respond to external stimuli. Finally, it is methodologically important, because measurements of the transmembrane current and potential produced by electrogenic transport systems can reveal a great deal about the transport mechanisms themselves.

Classically, application of electrophysiological methods to the study of active transport processes has been restricted largely to specialized secretory tissues: frog skin, gastric mucosa, intestinal mucosa, salt glands in certain marine plants, etc., or to a few nerve and muscle preparations. The former have the disadvantage of complexity; they are multiple membrane systems always containing more tban one type of cell membrane, usually containing more than one type of cell, and being further complicated by paracellular, as well as transcellular, transport pathways. Nerve and muscle preparations, on the other hand,

have the disadvantage that electrogenicity is generally a transient property of the transport systems, conspicuous and easily measured only briefly during recovery from a metabolic shock. The system which I wish to describe, that of the plasma membrane of *Neurospora*, has none of these disadvantages. It consists of a single kind of membrane in a homogeneous population of cells, and it possesses both an ATP-dependent electrogenic ion pump and at least one ion-dependent cotransport system which may be considered electrogenic under steady-state conditions. [The membrane potential of the organism can be determined readily with a conventional capillary microelectrode, and membrane resistance and current flows can be estimated, despite the rather complicated branched configuration of the hyphal filaments, from measurements employing 3-4 microelectrodes].

The Primary H^+ Pump.

The most salient physiological feature of the *Neurospora* membrane is the large potential difference which it sustains under normal circumstances: we have observed membrane potentials up to − 280 mV, (cell interior negative), with the usual values lying in the range − 170 to − 230 mV. Such values are found with hyphae bathed in buffer solutions (compositions listed in Table II) containing 25 meq/liter of univalent cations (K^+, Na^+, and NH_4^+, 1 mM free Ca^{++}, and usually 1 % glucose at pH 5.8. The intracellular concentration of potassium is about 200 mmoles/kg cell water; that of sodium, 20 mM; that of ammonium is not really known, but for osmotic reasons is unlikely to exceed 100 mM; and the intracellular pH is about 6.4 [Ref. 15]. Hence, even with the most favorable assumptions, the ion diffusion potentials for these cations could account for only about 100 mV of the total membrane potential. Furthermore, the membrane potential of *Neurospora*

bas proved to be only slightly sensitive to variations in either internal or external concentrations of the common cations, and it is quite indifferent to the choice of anions in the bathing medium [10, 11].

Direct evidence for the involvement of an electrogenic ion pump in generating the membrane potential of *Neurospora* came from the observation that addition and removal of respiratory inhibitors rapidly switches off and on a large fraction of the resting membrane potential. This phenomenon is illu-

TABLE II — *Buffers used for electrophysiology of* Neurospora

Buffer solution	A	B	C
Cations			
K^+	25	25	10.7
Na^+	—	—	7.3
NH^+_4	—	—	7.3
Ca^{++}	1.0	1.0	3.0
Mg^{++}	—	—	0.2
Anions			
Cl^-	2.0	2.0	6.0
NO^-_3	—	—	7.3
SO^-_4	—	—	0.2
DMG	20	—	—
Citrate	—	—	2.4
Phosphate	—	23	10.7
Glucose	55	55	—

All three solutions have a final pH of 5.8. Solution C is a diluted growth medium, and also contains added heavy metal trace elements and biotin. DMG = 3,3-dimethylglutaric acid. Concentration given in mM.

strated in Fig. 1. [Here, and elsewhere throughout the paper, membrane potentials are reported for the cell interior with respect to an extracellular reference, and are plotted downward according to the usual electrophysiological convention. Depolarization is therefore indicated by an upward shift of the traces.] The two traces represent two independent hyphae observed simultaneously in a preparation to which 3 mM KCN was added. Depolarization was complete within about 45 sec. for both hyphae, and after a small rebound the membrane potentials stabilized at − 50 to − 60 mV. The initial phase of repolarization upon washout of cyanide occurred nearly as rapidly as the depolarization, but complete recovery was obtained somewhat more slowly than was the stable level of inhibition.

On some occasions the single « spike » of overshoot during recovery is extended into overt oscillations (indicated very weakly in the righthand portion of the lower trace). Very clear and reproducible oscillations of the membrane potential at the *onset* of cyanide blockade can be produced by using a strain (*poky f*) which contains a secondary, cyanide-insensitive respi-

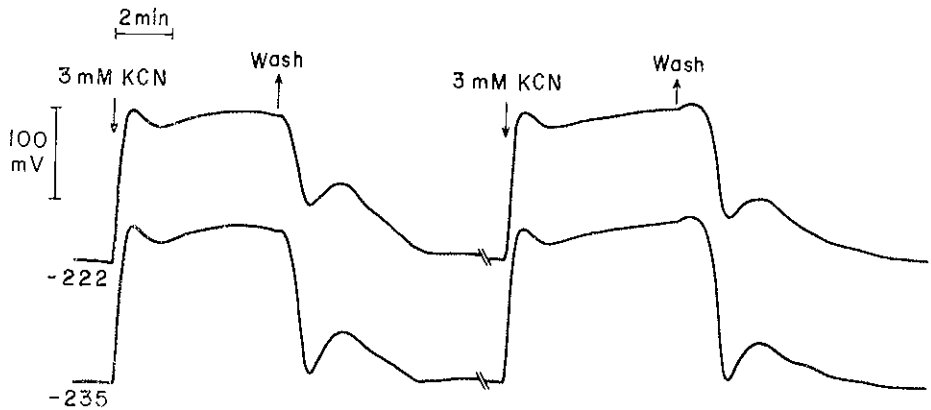

FIG. 1 — Reversible depolarization of the *Neurospora* plasma membrane by cyanide. Simultaneous records from two independent hyphae in the same preparation; 7 minutes omitted at the break in the records. Numbers at the left below the traces are the control membrane potentials. Buffer solution A; ambient temperature (ca. 24°C). Records inverted from Fig. 1, Ref. 18.

ratory pathway. Switching of the major electron flow from one respiratory pathway to the other leads to damped oscillations of the type illustrated in Fig. 2. In many respects these oscillations resemble the oscillations of H^+ transport, volume flow, and potassium transport which have frequently been observed in mitochondria during rapid metabolic shifts. In the case of the *Neurospora* plasma membrane, the oscillations appear to arise from the action of a metabolic feedback control system which regulates the membrane electrogenic pump, as

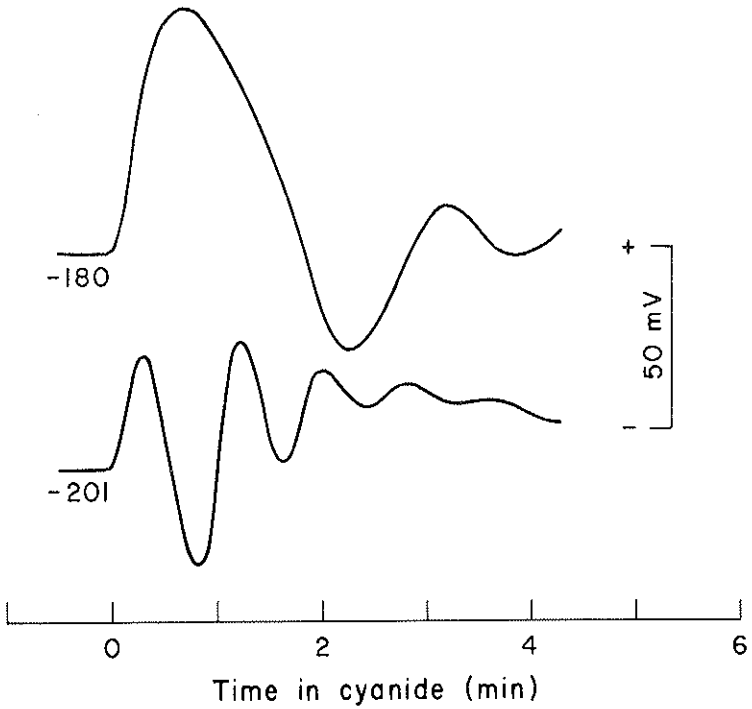

FIG. 2 — Oscillations of membrane potential in *Neurospora* (*poky f* strain) inhibited with cyanide. Upper trace: buffer B; 0.3 mM KCN added at zero time. Lower trace: buffer A; 1 mM KCN. The different frequencies and amplitudes represent normal variation from one preparation to the next, and are not functions of the buffer or the inhibitor concentration. Records from Fig. 3, Ref. 4.

well as a variety of other energy-consuming processes in the cells [4, 17].

Under the somewhat simpler conditions of cyanide depolarization in wild-type *Neurospora*, it is possible to demonstrate that the electrogenic pump is not driven by energy from respiratory electron transfer directly, since the decay of membrane potential occurs significantly more slowly than the cessation of mitochondrial electron transfer [14]. Some kind of energy reservoir must be interposed between the respiratory process and the electrogenic pump, and time course studies – shown in Fig. 3 – strongly suggest that the reservoir is ATP. In the upper part of the figure we have plotted the ATP content of *Neurospora*, assayed on cell extracts with firefly luciferase, at intervals following injection of 10 mM potassium cyanide into cell suspensions. Three different temperatures are shown, and the time constants for decay are 5.7 sec, 11.3 sec and 33.3 sec, respectively, at 25°C, 15.2°, and 6.8°. In the lower half of the figure are shown the corresponding time courses for initial depolarization with cyanide, each point-plot representing the time-average potential for 8-23 trials on 8 or more hyphae. The solid curves are simply taken from the ATP data, turned over, and transposed along the ordinate. The coincidence between the ATP curves and the voltage plots is almost exact, except for an initial brief period – 4 sec at the highest temperature, and 15 sec at the lowest – during which the change in membrane potential lags behind the change in ATP.

If this lag is taken as a manifestation of pump saturation by its substrate, ATP, then the time courses for ATP and voltage can be very well reconciled by a modified Michaelis equation with the following values (at 25°C):

$$V_m = V_o + \frac{V_{pm} \cdot [ATP]_i}{K_{1/2} + [ATP]_i} = -7 + \frac{-311 \cdot [ATP]_i}{2 + [ATP]_i},$$

where the voltage parameters are given in mV, and the $K_{1/2}$ in mM [13]. Of course, the ATP and voltage curves could also

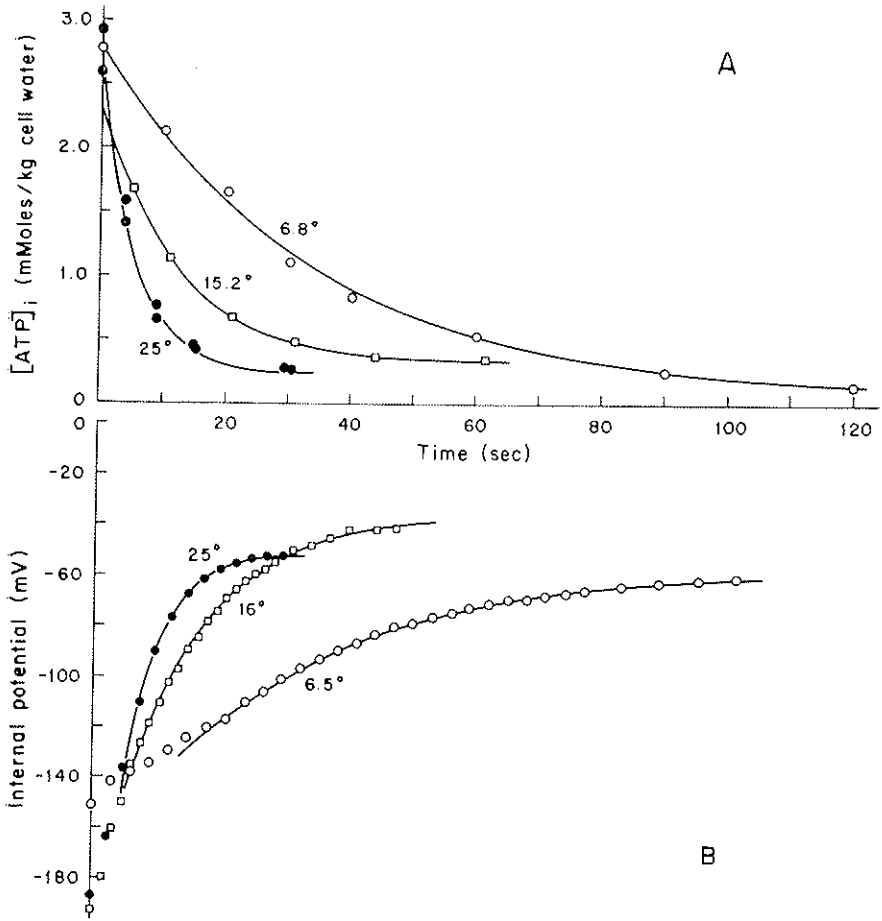

FIG. 3 — Time courses for decay of ATP (A) and membrane potential (B) at the onset of cyanide inhibition (wild-type *Neurospora*). Points plotted in A represent ATP values assayed by firefly luciferase on triplicate samples. The equation $[ATP]_i = a_1 + a_2e^{-kt}$ has been fitted to the data, by the method of least-square, to yield the solid curves (see text for parameters). Points plotted in B represent time averaged membrane potentials for 8-23 trials; solid curves are the ATP curves inverted and transposed. Buffer solution A; 10 mM KCN (neutralized) added at zero time in A; 25 mM KCN, in B. The plots at 15.2-16°C have been displaced slightly along the ordinate to avoid overlap. Data from Ref. 13.

be reconciled by a model in which an intermediate compartment (unidentified substrate) lies between ATP and the electrogenic pump, but simple forms of that model are quantitatively less satisfactory than is the enzyme-kinetic model [13].

If a metabolic inhibitor, or specific pump inhibitor, could be found which affected only the electrogenic pump and no other transport channels in the plasma membrane, the electrical characteristics of the pump could be defined unequivocally. But since no such ideal inhibitor has yet been identified, we have been forced to extract the pump characteristics by somewhat arbitrary means. Of all the metabolic inhibitors tested, cyanide seems to be the simplest, and its effects on current flow through the plasma membrane of *Neurospora* - over a wide range of voltages - are illustrated in Fig. 4 (light curves). In the absence of inhibitor, the resting membrane potential in this case was - 167 mV, and the slope conductance of the membrane (tangent in Fig. 4) was 145 μmhos/cm^2, corresponding to a slope resistance of 6900 ohm/cm^2. In order to clamp the membrane potential at - 167 mV in the presence of 1 mM KCN, we needed to pass an inward current of 4.7 nA between an intracellular microelectrode and a distant reference electrode in the bathing medium. When the transmission cable characteristics of the *Neurospora* hypha were taken into account (1), the actual membrane current density in the vicinity of the microelectrodes worked out to be 18.3 μA/cm^2; and the slope conductance had increased 50%, to 220 μmhos/cm^2. The resting potential in the presence of cyanide was - 56 mV, while the depolarizing current required to produce this membrane potential without cyanide was 10.4 μA/cm^2. By subtracting the currents on the « cyanide » curve from those on the « control » curve over the whole range of membrane potentials, we obtain a difference curve (not shown) which extrapolates to zero in the neighbor-

(1) C. L. SLAYMAN, U.-P. HANSEN and D. G. GRADMANN, manuscript in preparation.

hood of +50 mV, and becomes steadily larger at increasingly negative membrane potentials (see legend to Fig. 4).

A current-voltage curve (I-V curve) of such shape would be quite surprising for an electrogenic pump. From a variety of theoretical considerations, we would expect pump I-V curves to be sigmoid, with a region of voltage-dependence sandwiched between two regions (the voltage extremes) of saturation. The difference curve obtained from Fig. 4 shows no sign of saturation, even at quite large negative membrane potentials, but can be made consistent with a saturated pump, if we assume that cyanide itself introduces a leakage pathway into the membrane.

A tentative analysis of this kind is given by the dashed line and the heavy curve in Fig. 4. The dashed line represents a conductance of 50 μmhos/cm², with an equilibrium potential of zero mV, presumed to arise from the presence of 1 mM cyanide; and when that line is added to the difference curve, control minus cyanide, the heavy curve is obtained, which has been designated « i_{pump} ». Obviously, there is no unique way in which to carry out this analysis; the shape of the i_{pump} – curve depends on both the steepness and the equilibrium potential chosen for the leakage curve. What should be emphasized, however, is that by supposing a very simple kind of leakage curve we obtain a pump I-V function that is roughly constant, and independent of voltage over the testable range. While tbe properties of the cyanide-induced leak remain to be verified by other methods, the conclusion about the I-V curve of the electrogenic pump itself was reached previously from experiments of quite different strategy carried out on the *poky f* strain of *Neurospora* [12]. In the jargon of electric circuits, then, this electrogenic pump appears to function as a current source, capable of driving the same fixed current through the membrane at all voltages from near zero to about – 240 mV.

In the example of Fig. 4, the current driven by the electrogenic pump is 8-10 μA/cm², amounting to a unidirectional ion

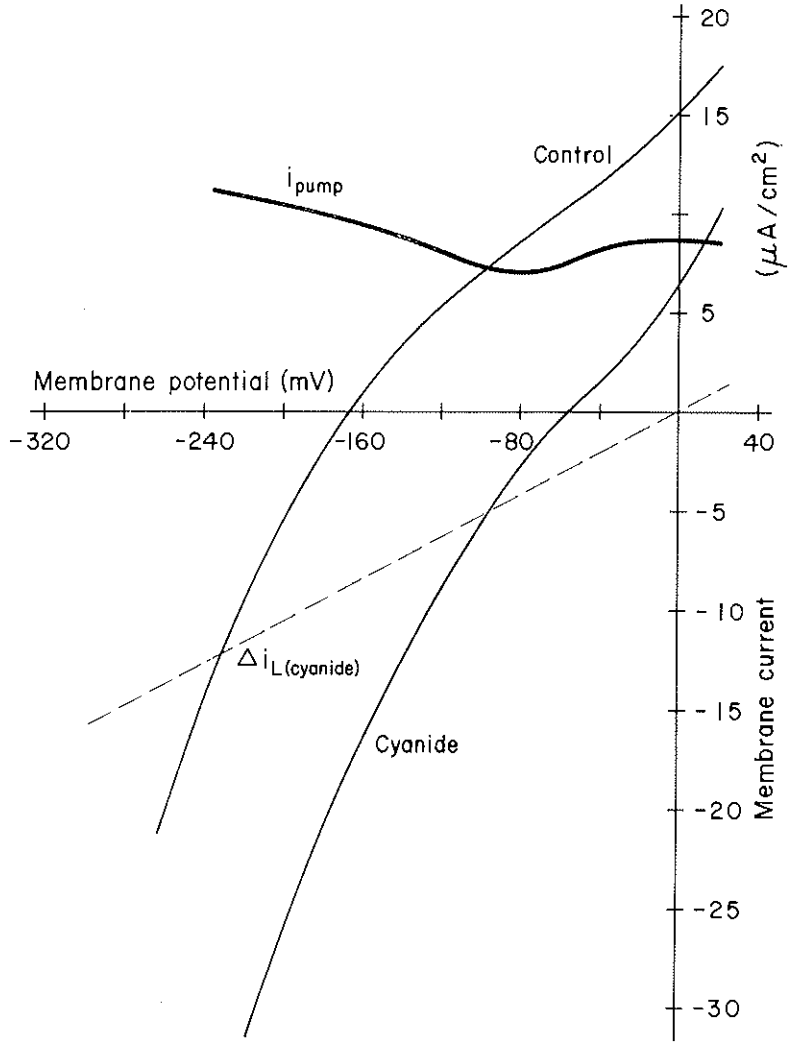

FIG. 4 — Cyanide effect on the membrane current-voltage curve in *Neurospora*. Measurements were made following the insertion of three microelectrodes into the same hypha: one to pass current, and two to measure voltage displacements in adjacent cells. The membrane current density near the current-passing electrode was computed using a lumped cable theory for non-linear networks ([1]). The two light, solid curves represent the computed I-V curves for the whole membrane. The difference curve, control minus cyanide (not shown) would run diagonally upward to the left, intersecting the ordinate at 8.7 $\mu A/cm^2$. It has been split here into a linear component which we suppose represents membrane leakage current (i_L) introduced by cyanide, and a near-constant component, which may represent the electrogenic pump (i_{pump}). Buffer solution A; 1 mM KCN.

flux of 80-100 pmoles/cm^2.sec. [This flux is only about half of that previously calculated for *Neurospora* [12, 13]. The discrepancy may arise partly from the relatively low resting potential (- 167 mV) in the example of Figure 4, but comes largely from the non-linearity of the membrane I-V curve. Previous calculations had assumed a fixed slope conductance at all membrane potentials]. A variety of indirect evidence has led to the notion that the requisite current is carried outward through the pump by hydrogen ions. Unfortunately, this notion cannot be tested rigorously in intact cells, since *unidirectional* proton fluxes cannot be measured meaningfully; but some comparisons with measured *net* bydrogen ion fluxes are illuminating. Under steady-state conditions at high extracellular pH (8 and above), the apparent net efflux of hydrogen ions from *Neurosphora* averages 20-40 mmoles/kg cell water.min, which would convert to 100-200 pmoles/cm^2.sec in the large hyphae used for electrical measurements. This is certainly large enough to accommodate the predicted pump flux. However, at the more normal pH of 5.8, net efflux of hydrogen ions is only about 20 pmoles/cm^2.sec, so that at least 75-80% of the proton flux driven through the electrogenic pump must be returned to the cell interior as an inward current of protons. At first sight, this conclusion seems energetically implausible: why should the organism expend metabolic energy simply to circulate hydrogen ions? This question prompted us to examine the « chemiosmotic » notion that uptake of certain metabolic substrates by *Neurospora* might be linked, via cotransport systems, to an inflow of hydrogen ions [18].

H^+-glucose cotransport.

From the point of view of electrophysiology, the most propitious transport system to examine for evidence of ion-coupling appeared to be the active transport system for glucose uptake

(System II) in *Neurospora.* This system supports fluxes of about 9 mmoles/kg cell water.min - faster than any other transport system in *Neurospora* except for the electrogenic H^+ pump and the Na^+/K^+ exchange system. Furthermore, synthesis of System II is known to be repressed under normal conditions, but to be derepressed by an hour or two of carbon starvation, so that comparisons between cells lacking the system and cells possessing it can readily be made.

In control experiments, addition of glucose to cultures deprived for 10-15 minutes had very little effect on the electrical properties of the hyphae. But addition of glucose after a 3-hour period of deprivation (room temperature, ca. 24°C) produced records of the type illustrated in Fig. 5 (upper). In both traces, 1 mM glucose was added at the abrupt vertical shift; and depolarization occurred with a time-constant of 0.5-1.0 sec, about 10-fold faster than depolarization with cyanide. Peak depolarizations in the cases shown in Fig. 5 were 153 mV (upper trace) and 138 mV (2nd trace). When glucose is flushed from the chamber at or before the point of maximal depolarization, recovery of voltage follows a time-course very similar to that for recovery from cyanide inhibition (see Fig. 1), and is probably rate-limited by the washout characteristics of the chamber. However, when glucose is maintained in the chamher for a minute or two, the membrane potential undergoes partial spontaneous recovery, with a time-constant of roughly 30 sec; subsequent removal of glucose produces a rather slow recovery, which is not rate-limited by the washout characterictics of the chamber. Qualitatively similar events are observed with the non-metabolized analogue 3-0-methyl glucose (Fig. 5, lower), but the absolute magnitude of depolarization is about 20% smaller in that case.

The magnitude of peak depolarization has been found to vary with sugar concentration, along the curve shown in Fig. 6 (for glucose). That curve can readily be described by a Michaelis equation, with a voltage asymptote of - 169 mV (in

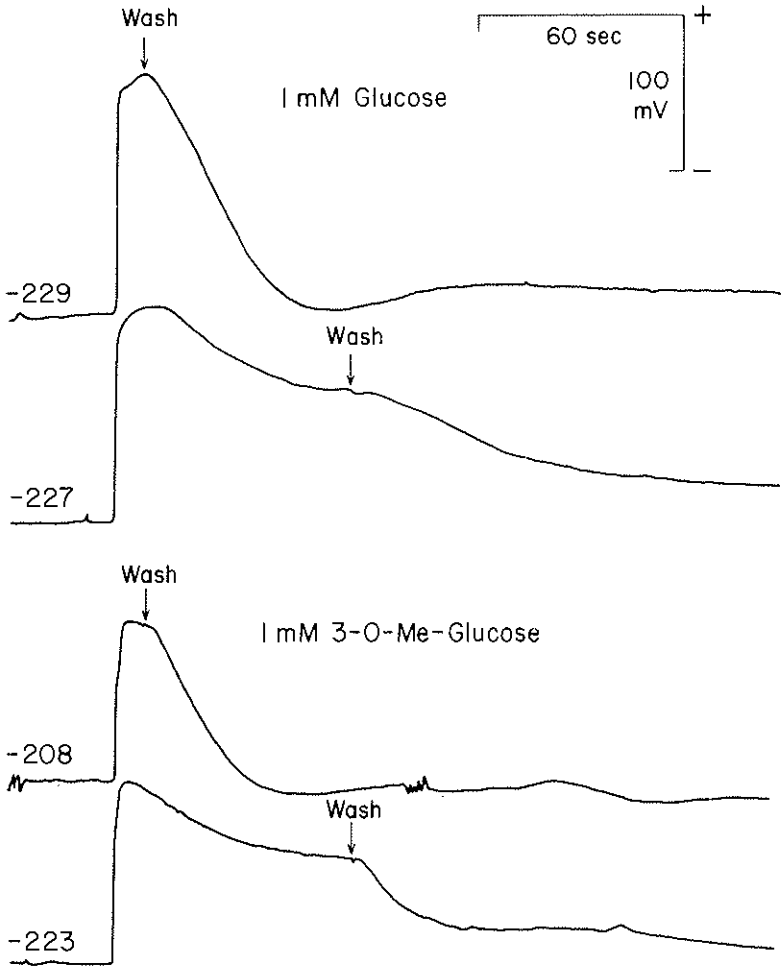

FIG. 5 — Depolarization of carbon-starved hyphae by glucose and 3-0-methyl-glucose. Preparations incubated in sugar-free growth medium for 3 hours before tests; sugar added just before the abrupt vertical (depolarizing) shift in the traces. Buffer solution C.

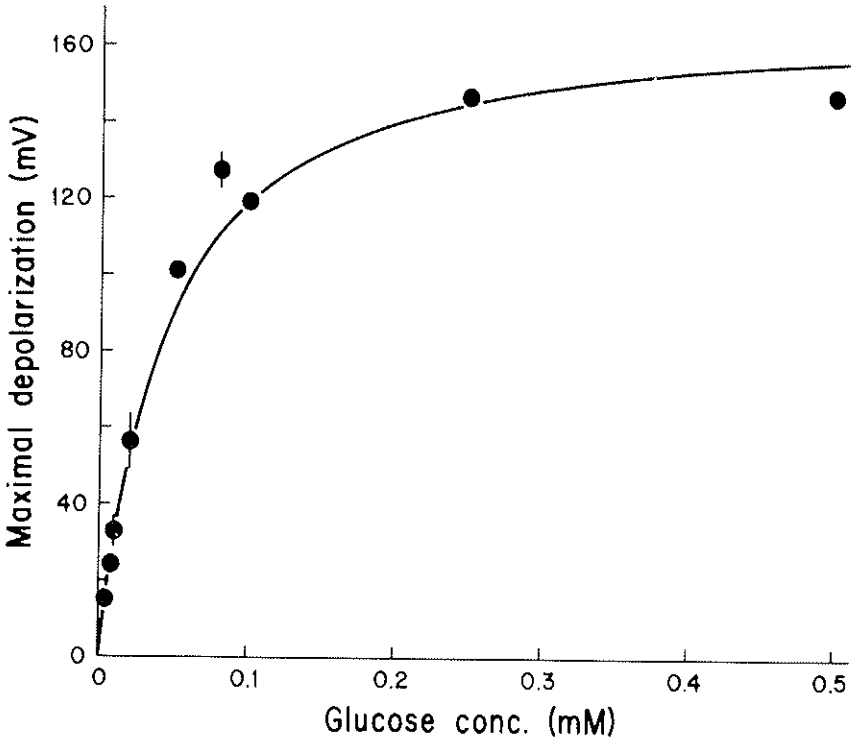

FIG. 6 — Peak depolarization of carbon-starved hyphae as a function of the glucose concentration. Conditions as in Fig. 5. Points give the average values (± 1 S.E.M.) for four determinations on two hyphae. The solid curve was drawn according to a Michaelis equation, fitted to the data by the method of least squares (see text for parameters). Fig. modified from Ref. 16.

this particular experiment) and a $K_{1/2}$ of 42 μM. The latter value is in the range, 30-50 μM, given in the literature for the $K_{1/2}$ of glucose uptake by System II [9].

We do not yet understand the cause of the spontaneous repolarization (nor of the subsequent slow recovery after wash-out of glucose), but similar behavior has been observed in

comparable experiments on ileal mucosa [8] and on oat coleoptile cells [1]. It seems to be correlated in *Neurospora* with the disappearance of a *measurable* H^+ influx, found during the initial minute or two of sugar uptake [16].

Of more interest in the present context is the detailed current-voltage relationship for the glucose transport system. One such curve is derived in Fig. 7. In this case the resting membrane potential without glucose was - 171 mV, and the slope conductance was 32 μmhos/cm², about 25% of that observed on normal, unstarved hyphae of *Neurospora*. The steady potential measured in the presence of glucose (at 1-2 min; i.e., after the spontaneous partial recovery had occurred) was - 117 mV, and the membrane current required to repolarize the membrane to - 171 mV was 1.6 μA/cm². In fact, over the entire range of voltages which could usefully be tested in this experiment — from +40 to - 250 mV — the apparent current through the glucose transport system lay between 1.6 and 2.1 μA/cm², so that this cotransport system seems, even more clearly than does the primary electrogenic pump, to be a current-source device. It acts, of course, to depolarize (rather than hyperpolarize) the membrane. The current of 1.6 μA/cm² should represent an ion influx of 16 pmoles/cm²/sec, but the glucose influx of 9 mmoles/kg cell water/min (see above), would correspond to 45 pmoles/cm²/sec. The obvious implication of this 3-fold discrepancy is that coupling between glucose entry and proton entry is incomplete after the initial period of spontaneous repolarization, but assignment of a definite stoichiometry requires a survey of many more current-voltage curves than we presently have.

The question of stoichiometry, and also that of the current-source property of System II, are somewhat complicated by the fact that during repeated trials with glucose the " control " and " glucose " I-V curves tend to converge in the vicinity of zero membrane potential, as illustrated in Fig. 8. Because it is difficult to see how glucose would induce a membrane leak-other than through the cotransport system, and also because curves

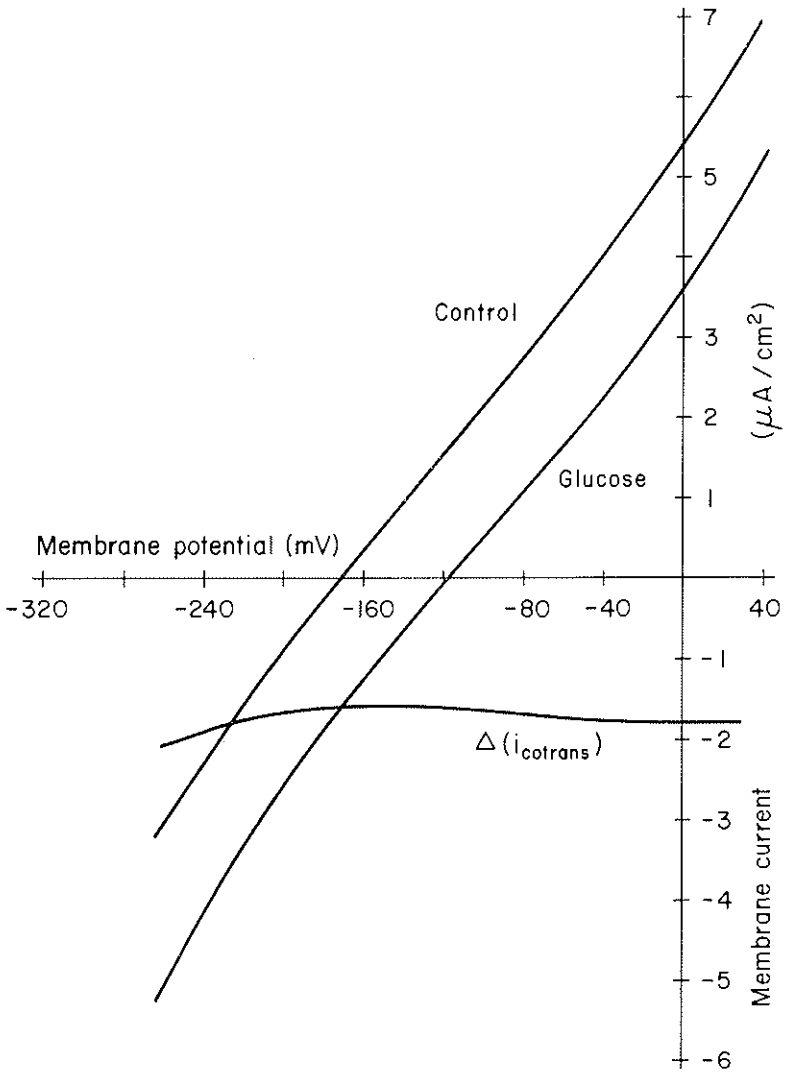

FIG. 7 — Glucose effect on the membrane current-voltage curve in *Neurospora*. First trial with glucose, after 4 hours carbon starvation. I-V curve for the glucose cotransport system ($i_{cotrans}$) was obtained as the difference (glucose minus control). Buffer solution C; methods as in Fig. 4.

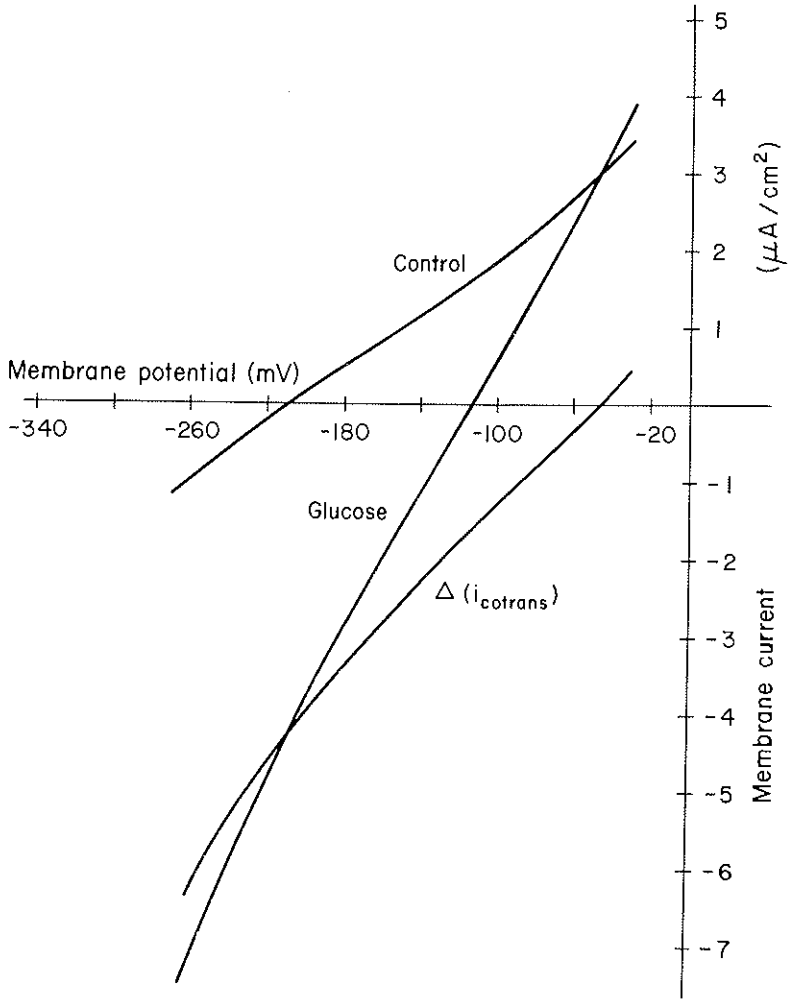

FIG. 8 — Glucose effect on the membrane current-voltage curve in *Neurospora*. A second trial with glucose, after 4 hours carbon starvation. Different preparation from Fig. 7, but methods identical.

of this sort have not been observed on first trials after carbon starvation, it seems inappropriate to introduce a new leakage channel (as in Fig. 4) into the interpretation. Rather it is more plausible to regard the sloping I-V curve for the cotransport system as arising from physiological changes, perhaps intracellular accumulation of sugar, accompanying sustained glucose uptake. This interpretation is presently being examined in experiments with non-metabolized analogues of glucose.

Discussion

The ensemble of results described above, and especially the current-voltage curves presented in Figs. 4, 7 and 8, are summarized in the membrane equivalent circuit shown in Fig. 9. The basic shape of the normal membrane I-V relationship is presumed to arise from a variable diffusion resistance (R_L), which may be associated with a small E.M.F. (E_L). The primary H^+ extrusion pump, in the left-hand limb of the

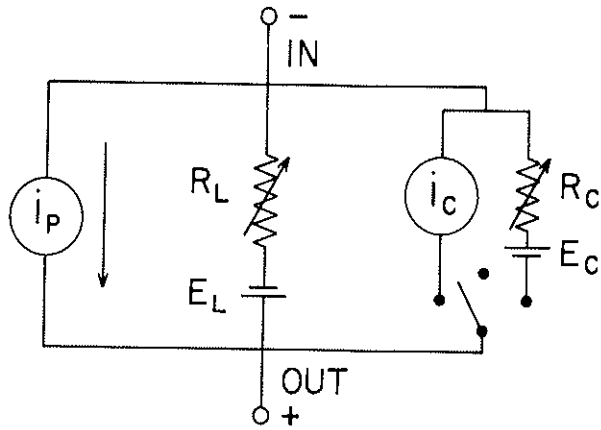

FIG. 9 — A tentative equivalent circuit for the *Neurospora* plasma membrane. Synopsis of Figs. 4, 7 and 8.

circuit, drives a constant current (i_P) outward, and that current returns through the membrane via the leakage resistance in normal cells, so that the sum $E_L + i_P R_L$ amounts to about − 200 mV. During carbon starvation, at least three changes occur: 1) R_L diminishes several-fold (conductance, 1/R, went from 145 μmhos/cm² in Fig. 4 to 32 μmbos/cm² in Fig. 7); 2) i_P diminishes by roughly the same amount, though usually the resultant membrane potential is slightly higher than before starvation; and 3) glucose transport system II, shown in the right-hand limb of the circuit, is synthesized. When glucose is added back to the carbon-starved cells, it and an H^+ current flow inward through System II: either in a voltage-independent mode (i_G), presumably when the inward glucose gradient is very large; or in a voltage-dependent mode (E_G, R_G), probably when free glucose has begun to accumulate intracellularly.

The finding that an electrogenic cotransport system can function either in a voltage-dependent or a voltage-independent mode complements the picture already drawn in the literature [3, 5, 7, 12, 19] for ATP-dependent electrogenic pumps. At the molecular level the issue raised by the two modes of operation is simply which transfer rates in the overall transport cycle are limiting: the membrane transit steps? or the interfacial processes of association and dissociation between the transported ions and the membrane "carriers"? Only the transit reactions, not the interfacial reactions, should be sensitive to tbe transmembrane voltage (although tbe interfacial reactions might well be influenced by changes in the membrane surface charge). In general membrane potential will interact in a complex manner with substrate levels and with tbe intrinsic rate constants around the transport cycle. However, for any one set of rate constants and substrate levels, the total I-V curve for an electrogenic pump can be expected to be sigmoid in shape [2, 6], with a region of voltage-dependence merging into positive and negative regions of voltage-independence.

The point at which such a curve intersects the voltage axis defines the equilibrium potential (E_P or E_G, in Fig. 9) for the

transport mechanism. For the primary H^+ pump in *Neurospora,* E_P must lie substantially negative to -240 mV, and other experimental data [12] indicate that it is negative to -300 mV. Since the free energy of hydrolysis of ATP under normal conditions in *Neurospora* is near 520 mV [13], and the normal H^+ gradient is slightly inward [15], it would appear that only one proton, not two, can be extruded for each ATP molecule hydrolyzed. For the H^+-glucose cotransport system, Fig. 7 indicates E_G to be substantially positive to $+40$ mV. Though no attempt has been made to estimate the free glucose concentration in carbon-starved cells, 1 μM is probably not too low. That would give an equilibrium potential (external glucose = 1 mM) near $+175$ mV, for a stoichiometry of 1 proton: 1 glucose. In Fig. 8, the apparent value of E_G is -47 mV, which would imply an internal glucose concentration near 10 mM. We regard that figure as too high, given the overall circumstances of the experiment, and analysis of further experiments is clearly necessary. In principle all of these calculation based on the electrical data can be checked against measured substrate gradients, and for that purpose tests are in progress with non-metabolized glucose analogues and with extremes of extracellular and intracellular pH.

ACKNOWLEDGEMENTS

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DISCUSSION

Chairman: Prof. R. D. KEYNES

KEYNES

Thank you very much. One thing that bothers me is this: surely if you observe in effect a constant current generator, there must be some potential where the chemistry that's driving it fails to provide sufficient energy. I can't remember what the free energy of ATP hydrolysis is in your preparation.

SLAYMAN

The free energy of ATP hydrolysis under conditions of these experiments is between 500 and 600 millivolts. We see the constant current generator out to something like 300 millivolts. We don't know how fast the curve bends over, but it must bend and intersect the axis at some point. That is, there must be a potential at which current can be driven through this pump. By rough extrapolation of the curve I showed that the potential is large enough to make it unlikely that two protons are transported per ATP split. Probably the stoichiometry is 1:1. I regret that conclusion because I would like to think that the system is more comparable to the mitochondria membrane. But that is the result.

TEORELL

Do you think that constant current sources could occur giving

rise to local currents in the cell membranes. Which paths is the current going? If you have a current source the current has to short circuit itself somehow.

SLAYMAN

In the absence of a demonstrable cotransport system we must have a leak of some sort. In the equivalent circuit for the membrane I must put a variable resistance which I assume gives the shape of the normal current-voltage curve. Now, you ask me whether, when the cotransport system isn't operating, the pump/leak must be wasting a lot of energy. Yes, it does seem to be. I don't know how to account for that except to say that I think the normal energy-dependent K^+-K^+ exchange which goes on in these cells occurs by an H^+—dependent cotransport system too, and I've lumped that into the resistance. We haven't yet investigated it, though.

BOYER

I have a question about that very nice correlation you showed between the ATP level and the potential. Do you get the same correlation if you calculate on the basis of the ratio of ATP, ADP to Pi, that is the phosphate potential?

SLAYMAN

We haven't done the calculation that way, but the phospate concentration itself is high and the ADP concentration is controlled by myokinase under the conditions of the experiment. Therefore ADP and phosphate don't change very much.

IV

MEMBRANE THERMODYNAMICS AND TRANSPORT

ENERGY TRANSDUCTIONS IN BIOLOGICAL SYSTEMS

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INTRODUCTION

The useful energy conversions in biological processes, other than the conversion of energy to heat, may be separated into two types, depending on whether one or more than one form of energy is involved. The simplest type, involving only one form of energy, is the chemical syntheses based on energy obtained from molecular rearrangements. The second type involves such changes as chemical bond energy to energy of concentration gradients, light energy to chemical bond energy, or chemical bond energy to contraction or motion. They may be grouped as biological energy transductions.

A relatively satisfactory understanding has been achieved for the energy conversions involving only molecular rearrangements. In these processes, energy from the bonds and structure of one molecule is used to make the bonds and structure of another molecule in solution. The synthesis of ATP by glycolysis or fermentation is the example par excellence. Here by means of relatively well understood catalytic events, inorganic phosphate, P_i, is taken up then added to adenosine diphosphate, ADP, to form adenosine triphosphate, ATP. Such a process will serve all the energy needs of anaerobic microorganisms or

even of anaerobic muscle. Similar covalent interconversions also occur in chemical syntheses coupled to ATP cleavage, as in the use of ATP to make peptide bonds in protein synthesis, to deposit storage glycogen, or to form nucleoside triphosphates for DNA and RNA synthesis. In all such conversions, the form of energy is the same, namely that inherent in the covalent structure of the molecules concerned. The conversions take place in one phase as defined by molecules in aqueous solution.

In contrast, most biological energy transductions take place in two phase systems, as exemplified by a muscle fiber and the surrounding solution, or by a cellular membrane involved in active transport of ions. The processes that involve or appear to involve energy transductions are at present quite inadequately understood at the molecular level. Important examples of such energy transductions of concern to us here may be grouped according to the source of energy used as follows:

ATP-driven transductions
  Active transport of ions and other solutes.
  Movement, including muscle contraction.

Oxidatively-driven transductions
  Oxidative phosphorylation
  Active transport of ions and other solutes
  Movement

Light-driven transductions
  Photophosphorylation
  Active transport

Most of these energy transductions have heen recognized for a number of years. The recognition that oxidations may drive active transport [1] or motion [2] in bacteria, without the intervening formation of ATP, are more recent. In some of the processes mentioned above, more than one energy transduction appears to occur, as will be amplified in discussion of oxidative and of photophosphorylation.

Protein Conformation Changes in Energy Transductions

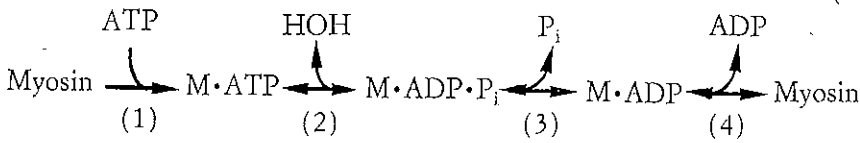
For the transductions of oxidative and photophosphorylation, of muscle contraction or movement and of active transport, there is emerging evidence that energy-linked protein conformational changes may play a vital role in energy use. Such energy-linked protein conformational changes must be distinguished from the conformational changes that undoubtedly accompany at least to some extent all enzyme catalyzed processes. In energy transductions linked to conformation change, the energy derived from change at one location of the molecule is transmitted through the molecule to another location where it is used. The vast strides in protein-structure function relationships give ample foundation for such possibilities. An elegant example of energy transmission between subunits of a protein molecule is the change in affinity of oxygen for one iron of hemoglobin by oxygen binding at another iron. Other examples are shown by regulatory enzymes such as aspartate transcarbamylase, where binding of an effector molecule on one subunit changes the free energy for binding of a substrate at the catalytic site on another subunit. This regulatory behavior of hemoglobin and aspartate transcarbamylase does not perform a function of useful energy conversion such as occurs in synthesis of ATP, contraction, etc. But the properties of these and other proteins give a firm base to the possibility that energy-linked conformational changes of proteins could play a vital role in biological processes.

I have discussed aspects of conformational coupling in biological energy transductions elsewhere [3, 4]. At this meeting, I will discuss principally energy use for ATP synthesis by oxidative phosphorylation and use of ATP energy for active transport, together with a brief mention of ATP use in muscle contraction. Some facets of the oxygen exchanges and catalytic events occurring with the muscle myosin system will be discussed first as these are better understood and serve as a base for considerations with mitochondria.

Oxygen Exchanges and Catalytic Events with Myosin

For some years myosin has been known to catalyze exchanges of phosphate oxygens with water. The observed $P_i \rightleftharpoons HOH$ exchanges are of two types, designated as the « intermediate » and the « medium » $P_i \rightleftharpoons HOH$ exchanges [5, 6]. The intermediate exchange occurs with the bound P_i formed from ATP. The medium exchange occurs with P_i of the reaction medium. Measurement of the total incorporation of ^{18}O from $H^{18}OH$ into P_i during the hydrolysis of ATP gives a measure of the sum of the medium and intermediate exchange. Measurement of the loss of ^{18}O from added ^{18}O-P_i gives a measure of the medium exchange. The reaction sequence and exchanges are depicted by Equation 1:

(1)



During the continued hydrolysis of ATP, reversal of step 2 prior to P_i release will give rise to the intermediate $P_i \rightleftharpoons HOH$ exchange. A reversal of steps 2 and 3 will give rise to the medium $P_i \rightleftharpoons HOH$ exchange.

Recent experiments by BAGSHAW and TRENTHAM at the University of Bristol [7], in my laboratory at UCLA [8], and cooperative experiments between both laboratories [9] have given important experimental evidence for the above explanations for the medium and intermediate exchanges. Both exchanges result from dynamic reversal of the cleavage of ATP at the catalytic site. It is also relevant to our discussions here that measurements in our laboratory [10] and that of MANNHERZ and GOODY [11] have shown that the free energy of binding of ATP by myosin is very large. ATP dissociates very slowly from myosin·ATP, but addition of actin greatly accelerates this dissociation. Present information from a variety

of sources suggests that in muscle contraction, protein conformational changes accompanying the binding and reversible cleavage of ATP at the catalytic site are used to drive the actin filament when actin combines with the energized myosin and favors product dissociation [See 4, 7]. A sequence for contraction cycle depicting these events is indicated in Fig. 1.

ATP Synthesis by Mitochondria

For over thirty years the formation of ATP coupled to oxidations in mitochondria has been recognized as the principal way that aerobic organisms get useful energy from ingested foods. An understanding of how this oxidative phosphorylation occurs has eluded the efforts of many. A logical approach

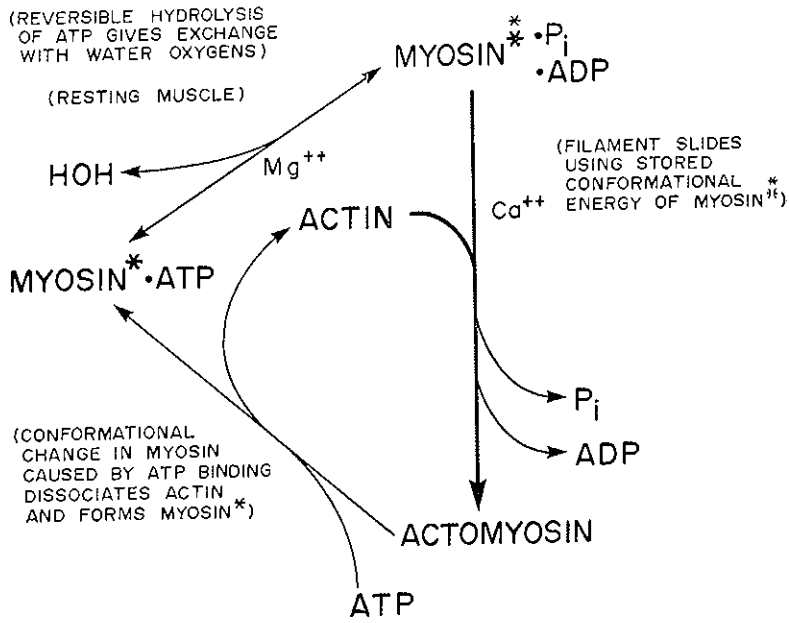


FIG. 1 — The steps of ATP cleavage in the contraction cycle.

has been to look for steps in the process akin to those of the energy changes in the molecular rearrangements of the substrate level phosphorylations. But such searches have not uncovered any covalent phosphorylated intermediates or precursors. Something different appears to be involved. There has been developed increasing recognition that oxidations give rise to some type of energized state of the membrane and that this is used for ATP synthesis. The overall process may be separated into three steps, as indicated by Fig. 2.

From important observations of many laboratories, structurally and functionally separate protein complexes that carry out the oxidations and the phosphorylation to make ATP have been recognized. When these protein complexes are separated from the mitochondrion, the oxidative complexes oxidize substrates without phosphorylation and the phosphorylation complex behaves as an ATPase. But in the mitochondrion the

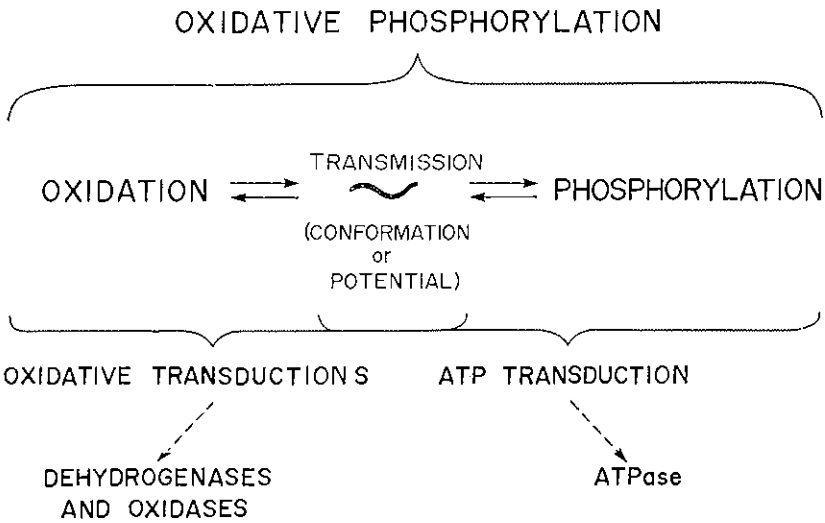


FIG. 2 — The three steps and the energy transductions of oxidative phosphorylation.

energy released in the oxidations is in some manner captured by the membrane or the proteins therein, and is transmitted to the phosphorylation complex to drive the hydrolysis of ATP backward.

A very prominent impact on the field has arisen from the suggestion developed by PETER MITCHELL that membrane potential or proton grandients might serve for transmission of energy from the oxidation complexes [11]. An alternative is that the oxidations produce energy-linked conformational changes that are transmitted through an interlocking protein network in the membrane to the phosphorylation complexes [3, 4]. But the principal purpose of this paper is not to focus on how the energy is transmitted, but on how it is used after arrival at the phosphorylation complex to make ATP.

In a 1973 paper from my laboratory [12], a new concept of energy use in oxidative phosphorylation was suggested. This was based on the demonstration of the uncoupler insentivity of the prominent exchange of phosphate oxygens with water ($P_i \rightleftharpoons HOH$ exchange) catalyzed by mitochondria and on the rapid formation of bound ^{32}P-ATP from $^{32}P_i$ at the phosphorylation catalytic site. The results pointed to a prominent use of energy not to bring about the covalent structure of ATP, but to cause release of ATP formed at the catalytic site by simple reversal of hydrolysis. Our results at that stage allowed interpretations as indicated in Fig. 3A.

The above-mentioned and subsequent experiments in our laboratory are based on the assumption that the oxygen exchanges accompanying oxidative phosphorylation result from dynamic reversal of formation of ATP from ADP and P_i at the catalytic site. If this explanation is correct, the measurement of the relative rates of exchanges, particularly the oxygen exchanges, provides a powerful tool for probing energy inputs at the phosphorylation site. Such an explanation of the oxygen exchanges has considerable experimental support, but is not at this stage proven. We are currently studying the exchange

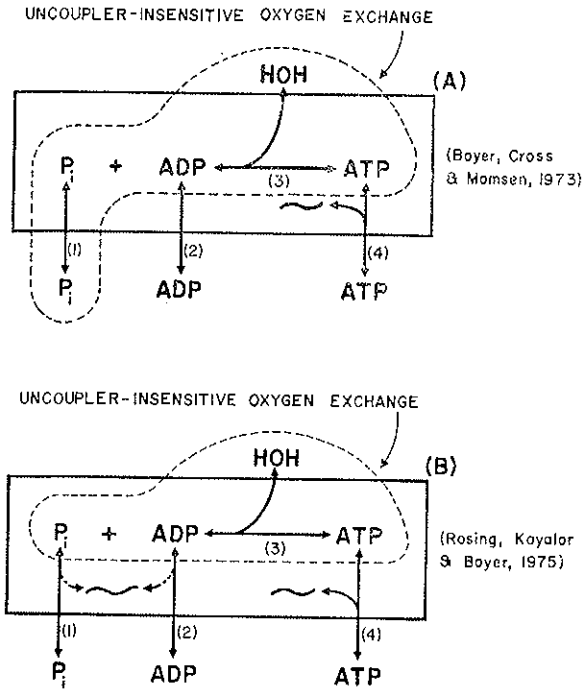


FIG. 3 — Steps of energy input and uncoupler sensitivity in oxidative phosphorylation: (A) as based on 1973 data; (B) a more recent refinement.

mechanism in mitochondria by rapid mixing and quenching experiments. As mentioned above, there is evidence for an analogous explanation for the oxygen exchanges of myosin ATPase.

In a continuation of isotopic probes of mitochondrial ATP synthesis, CROSS and BOYER noted a marked sensitivity of the $P_i \rightleftharpoons HOH$ catalyzed by submitochondrial particles exchange to addition of hexokinase-glucose [13]. Fig. 4 gives some of their results. Although no ATP but only ADP and P_i were added to reaction mixtures, the presence of adenylate kinase

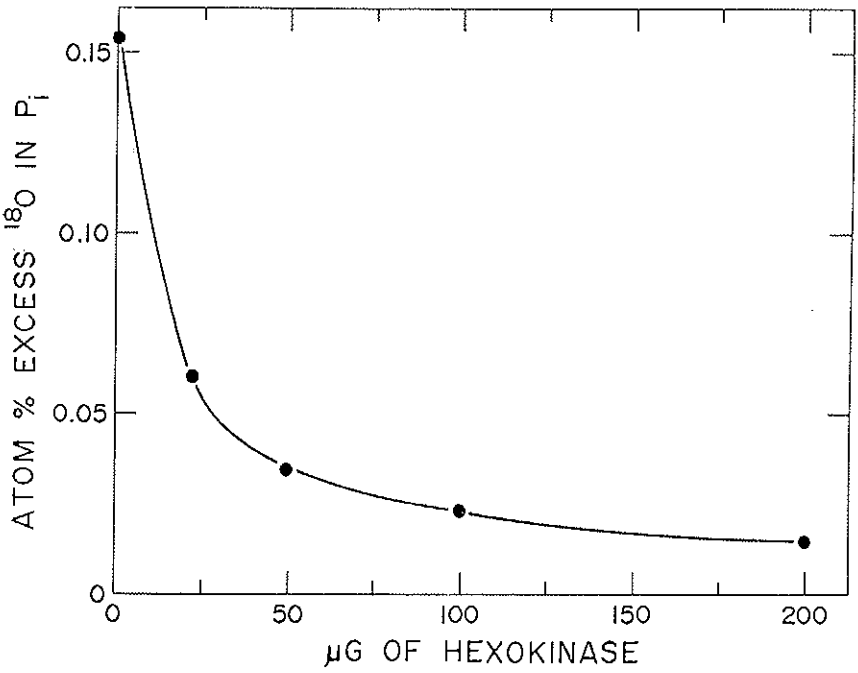


FIG. 4 — Sensitivity of the $P_i \rightleftharpoons HOH$ exchange catalyzed by submitochandrial particles to addition of hexokinase plus glucose (from CROSS and BOYER).

activity in the submitochondrial particles can make ATP and AMP from the ADP. The results thus suggested that presence of ADP and P_i alone, without some energization by either ATP cleavage of oxidations, was insufficient for occurrence of a prominent $P_i \rightleftharpoons HOH$ exchange. More recent experiments by Dr. Jan Rosing in my laboratory have shown that if the conversion of ADP to ATP is nearly completely blocked and 2,4-dinitrophenol is present, the otherwise very rapid $P_i \rightleftharpoons HOH$ exchange is reduced to near zero. A logical interpretation is that energy input is occurring at another step or steps in the catalytic sequence, in addition to the ATP release step.

Evidence for Energy Input for P_i Binding

To probe further the nature of the energy input steps, Jan Rosing and Celik Kayalar have more recently been studying the effect of uncouplers on the two components of the $P_i \rightleftharpoons HOH$ exchange. Measurements have been made of the intermediate and medium exchanges and of their sensitivity to uncouplers of oxidative phosphorylation during the cleavage of ATP by submitochondrial particles. The results show that the medium $P_i \rightleftharpoons HOH$ exchange, along with the $P_i \rightleftharpoons ATP$ and the $ATP \rightleftharpoons HOH$ exchanges are strongly inhibited by the uncoupler, but the intermediate $P_i \rightleftharpoons HOH$ exchange persists. These findings have the striking implication that it is the step in which bound ADP and P_i combine to form bound ATP and water that has the least dependency on energy input. They further suggest that another prominent point of energy input in the oxidative phosphorylation cycle occurs in the binding of P_i or ADP or both. The present interpretation of these findings is given in Fig. 3b. The covalent interconversion of the bound substrates is depicted as the step with the least sensitivity to uncouplers. Reactant binding and product release appear to be the chief points of energy input in the formation of ATP. Experiments in progress are directed toward finding if the binding of P_i or ADP or both is primarily affected.

The Molecular Mechanism of Energy Use in Oxidative Phosphorylation

An attractive suggestion at this stage is that the energy-requiring increased affinity of reactant P_i and/or ADP and the decreased affinity for ATP are caused by energy-linked protein conformational changes. If this concept proves to be true, it is evident that a formidable task for future researches is to define that molecular nature of the conformational changes. The problem is akin to that of describing the conformational changes in muscle and, as will be developed later, in the trans-

port ATPases. It is made even more difficult by the multitude of components present in the mitochondrial inner membrane extremely complex. For some years most of our probes of conformational events are likely to be indirect, and the more useful approaches will likely be those that experimentally assess whether indeed energy-linked protein conformational changes provide the mechanism for ATP synthesis in oxidative phosphorylation.

A conformational mechanism of ATP formation at the phosphorylation site as suggested above is in sharp contrast to the « chemiosmotic » mechanism put forth by MITCHELL. He recently published a somewhat more detailed suggestion of a chemiosmotic mechanism in which a flow of protons through the catalytic site of the ATPase is purportedly linked to the formation of ATP from ADP and P_i [14]. I have called attention to deficiencies in this chemiosmotic scheme [15] and these criticisms have been inadequately rebutted by MITCHELL [16]. In addition to the theoretical objections to Mitchell's chemiosmotic formation of ATP, there is now added an experimental objection. The more recent findings of Rosing and Kayalar in my laboratory provide experimental evidence against the chemiosmotic scheme of Mitchell, or any other scheme where the principal energy input serves to make the covalent structure of ATP. Our data point to energy input in the reactant binding and product release steps, not the chemical step of forming ATP from ADP and P_i.

It must be emphasized that an invalidation of Mitchell's chemiosmotic mode of formation of ATP does not mean that his suggestion for transmission of energy from oxidations to phosphorylation by a membrane potential gradient is similarly invalid. Indeed for me, and I expect for many other, the recognition of a chemically and physically satisfying means of use of a membrane potential for ATP synthesis makes more acceptable the possibility that membrane potential may serve for energy transmission in oxidative phosphorylation. A mem-

brane potential can quite logically cause protein conformational change, and this, as noted, can be linked to ATP synthesis. Charged group movement under influence of a potential could result in small but vital protein conformational changes. A scheme for how a membrane potential and movement of protons across a membrane might be coupled to ATP synthesis is depicted in Fig. 5. The scheme is shown in the direction of ATP synthesis. Operation in the reverse direction would result in a transport of protons across the membrane coupled to ATP cleavage. For simplicity, only movement of one group and one proton is depicted for each ATP cleaved. The protein subunits involved could have two or more sites that move to account for observed stoichiometries.

Various bases for and features of the scheme are developed more fully elsewhere [17]. The important point is that transduction of energy from a membrane potential to energy of protein conformation might be a process of far-reaching significance.

The Use of Energy by Transport ATPases.

More pertinent to the main theme of this meeting is the use of energy by the membrane bound ATPases that pump ions across membranes against a concentration gradient, commonly called the transport ATPases. The two most studied examples are the Na^+, K^+-ATPase of the microsomes and the Ca^{++}, Mg^{++}-ATPase of the sarcoplasmic reticulum.

The transport ATPases differ from those involved in oxidative and photophosphorylation in that a covalent acyl phosphate intermediate participates in the reaction. As demonstrated by recent studies in POST'S laboratory [18] and in mine [19], the phosphoryl group is attached to the side chain carboxyl of an aspartyl residue. Among the important characteristics of the transport ATPases that have been established is the existence of two different forms of the phosphorylated en-

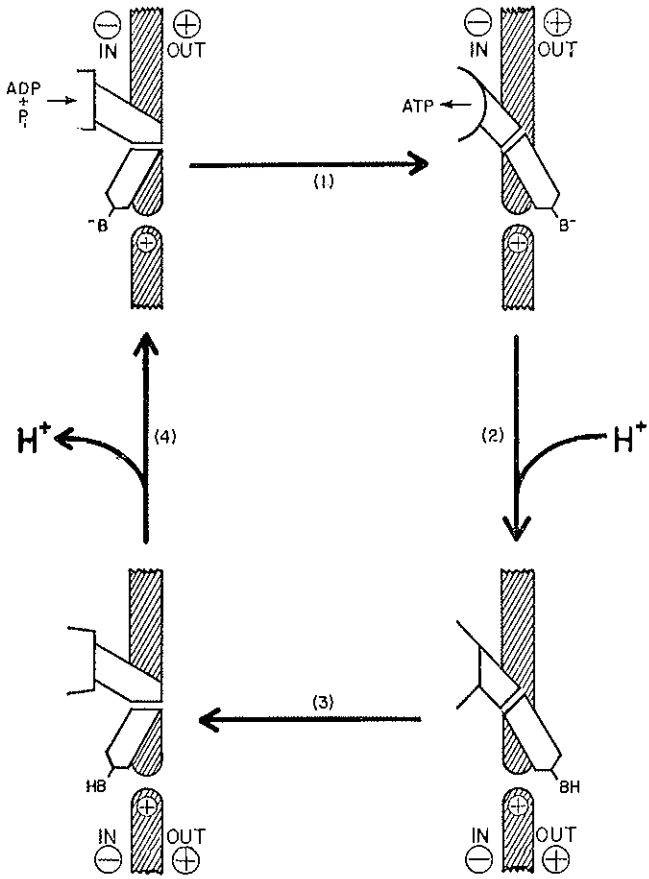


FIG. 5 — A scheme illustrating how a membrane potential and proton movement might be coupled to synthesis or cleavage of ATP. (The arrows indicate the direction of changes when ATP is formed by energy from a membrane potential or pH gradient. Operation in the reverse direction would pump protons coupled to ATP cleavage.)

zyme, one in which the carboxyl group can be phosphorylated by P_i with little free energy change, the other in which the carboxyl group is phosphorylated by ATP with little free energy change [see 20-26]. Obviously a major free energy change must be associated with the interconversion of the two enzyme forms. These and other findings have led to formulations of the transport cycle as depicted in Fig. 6. Again, the evidence suggests that energy-linked protein conformational change plays a key role in the process.

There are other features of the transport ATPases that are related to our observations with muscle and with mitochondria. An important consideration is how the acyl phosphate group is formed from P_i. One way that has considerable support from known biochemical mechanisms is the phosphorolysis of an acyl-X group, e.g., and acyl-S or acyl amidazole as depicted by Eq. 2. If the inorganic phosphate oxygens were labeled with

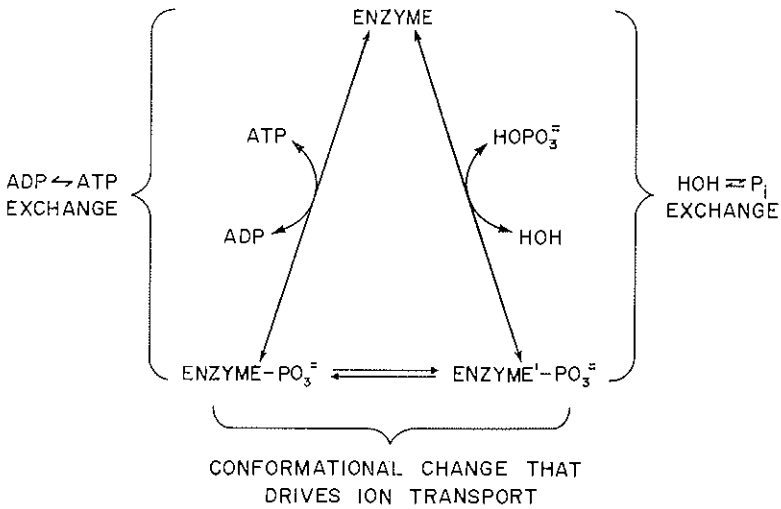


FIG. 6 — Major steps in the transport ATPase cycle.

^{18}O, as depicted by the filled ⊘ atoms, such a reaction would give an acyl phosphate

$$\text{Protein-C(=O)-X} + \text{H}\oslash\text{P}\oslash_3^{=} \longrightarrow \text{Protein-C(=O)-}\oslash\text{-P}\oslash_3^{=} \quad (2)$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \text{XH}$$

where the C-O-P bridge oxygen was furnished by inorganic phosphate. Conversely, in a reaction for which there is little biochemical analogy, the acyl phosphate might be formed through displacement of a phosphate oxygen by the carboxylate, as depicted by Eq. 3. For this type of reaction, the C-O-P bridge oxygen would be furnished by the carboxylate group.

$$\text{Protein-C(=O)-O}^{-} + \text{H}\oslash\text{P}\oslash_3^{=} \longrightarrow \text{Protein-C(=O)-O-P}\oslash_3^{=} + \text{H}\oslash\text{H} \quad (3)$$

This is as we have observed experimentally [27]. Thus in the transport ATPases, the enzyme active site can favor the formation of an otherwise thermodynamically unlikely formation of a " high-energy " acyl phosphate group. This is akin to the ability of the catalytic site of myosin and of the mitochondrial membrane to favor formation of a bound ATP from ADP and P_i.

There is also another similarity. The dynamic reversal of the formation of bound ATP appears to be the base of the rapid $P_i \rightleftharpoons HOH$ exchange catalyzed by myosin and by mitochondria. Similarly, the transport ATPases under conditions that favor acyl phosphate formation from P_i have been found to catalyze a rapid $P_i \rightleftharpoons HOH$ exchange [24, 25]. This exchange may likewise occur by the dynamic reversal of the formation of a " high-energy " phosphate compound, namely the protein acyl phosphate.

There is another aspect of the action of the transport ATPases that needs mention before consideration of how conformational changes might drive active transport. On the basis of present information I regard it likely that the energetic coupling takes place within the membrane and is not dependent on or in equilibrium with membrane potential. This is in contrast to the behavior of bacterial membranes where a potential across the membrane has been demonstrated to drive transport [see 28, 29]. The membrane of aerobic *E. coli* combines the capacities for oxidative formation of ATP and for ATP driven or oxidatively driven active transport [1, 30]. In contrast, tbe membrane of the microsomal ATPase of kidney or nerve or of the sarcoplasmic reticulum is more specialized. It is designed to accept ATP from solution to drive transport. As such, the transport reactions, unlike those of aerobic *E. coli*, [1, 31] should not be sensitive to the classical uncouplers of oxidation phosphorylation, except as they may interfere with the ATP source. Only limited information appears to be available in this regard. Na^+ transport of the giant squid axon is insensitive to 2,4-dinitrophenol [32], as is the oxygen exchange catalyzed by sarcoplasmic reticulum vesicles [25].

Because the transport ATPases can move two or more cations per ATP hydrolyzed, schemes for direct coupling of the transported ion to the P_i or ADP released from ATP, or other similar suggestions, do not seem likely. But one can readily visualize a conformational coupling akin to that suggested for oxidative phosphorylation, namely that the cleavage of ATP is linked to conformational changes that modify the affinity of ions for binding sites. For the active transport, this has to be correlated with alternate exposure to the binding sites to opposite sites of the membrane. Again, the requisite conformational changes could be small, both to cause the changes in affinity and the changes in access to solvent water from one side of entry to the other in transport pore. The phosphory-

lated carboxyl group could be serving a function akin to that of the negatively charged group suggested in Fig. 6 for oxidative phosphorylation; this is that the group is forced to change position as part of a vital energy-linked conformational change. This conformational change is transmitted to the cation binding site for the requisite change in binding affinity for ions and change in access to water penetrating the transport pore. Such a suggestion again is in distinct contrast to Mitchell's extension of his chemiosmotic mechanism to transport ATPases [33].

Concluding Remarks

It is clear that satisfying molecular explanations of biological energy transductions remains a task for the future. But it is encouraging that researches with three quite different energy-transducing systems, that for muscle, for oxidative phosphorylation and for transport ATPases, point to common mechanistic features. This is that in all the processes, energy-linked protein conformational changes may play a vital role. When confronted with the problem of transducing energy to make or to use the ATP molecule, Nature appears to be using a similar mechanism. The ATP when cleaved to ADP and P_i may be used to cause vital protein conformational changes that modify affinity of solutes and cause movement of key groups in transport ATPases or entire molecular assemblies in muscle contraction. Conversely, in oxidative phosphorylation energy-linked conformational changes may drive ATP synthesis by increasing the binding of ADP and P_i and decreasing the binding of ATP at the catalytic site.

ACKNOWLEDGEMENTS

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DISCUSSION

Chairman: Prof. J. M. RITCHIE

MONNIER

The concept of ATP requiring energy to be driven into the membrane is perfectly reasonable because ATP is a highly hydrophilic molecule and if it has to be transferred, of course, across a lipidic component of the membrane, it requires plenty of energy. Thus, this aspect of the matter is quite compatible with what you said in your presentation.

BOYER

If removal of ATP from the site required passage through a hydrophobic area, this would, as you note, increase the energy requirement. It is quite plausible for an enzyme active site with tight binding of ATP and low water activity to favour the formation of an ATP once you can get hold of enough ADP and Pi, but then it is going to be hard to release the ATP once formed.

MONNIER

Perhaps the whole complex would become rather lipophilic and it would get into the membrane.

HASSELBACH

I want to ask you about the role of calcium in your scheme for muscle contraction.

BOYER

The role of calcium in the muscle scheme is not an area to which we have contributed.

HASSELBACH

I think you can just omit calcium from the scheme.

BOYER

No, not when you get the power stroke in the muscle. As you know, calcium greatly accelerates the rate of ADP and Pi release from the myosin. But, the whole cycle would work even in the absence of calcium, as far as I know, because calcium serves for the regulation of the system. The manner in which Ca^{++} regulates this system is complex and the slide was quite incomplete with regard to the Ca^{++} effect. As you know, the troponin-tropomyosin system is involved. But the important point is that the conformational energy obtained from ATP binding and cleavage cannot be used for the combination with actin and the power stroke without promotion of ADP and Pi release.

ELECTRICAL EXCITATION IN LIPID BILAYERS AND CELL MEMBRANES

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Introduction

Several compounds of fungal or bacterical origin (EIM, alamethicin, monazomycin, DJ400B) can be incorporated into planar lipid bilayers where they form molecular channels and generate voltage dependent ion conductances and action potentials [1-3]. When studied by voltage clamp [4], the kinetic and steady state characteristics of these conductance changes are in every respect identical to those found in excitable cell membranes and their major aspects can be quantitatively described by the HODGKIN-HUXLEY equations [5-]. Thus, the steady state conductance is an exponential function of the membrane potential, the conductance rises with a sigmoid time course, and the time constants of the conductance changes go through a maximum as a function of the potential (see fig. 1). The conductances also show inactivation as seen in the sodium channels of nerve and the potassium channels of muscle (fig. 2). In addition, there appear for particular pulsing sequences cer-

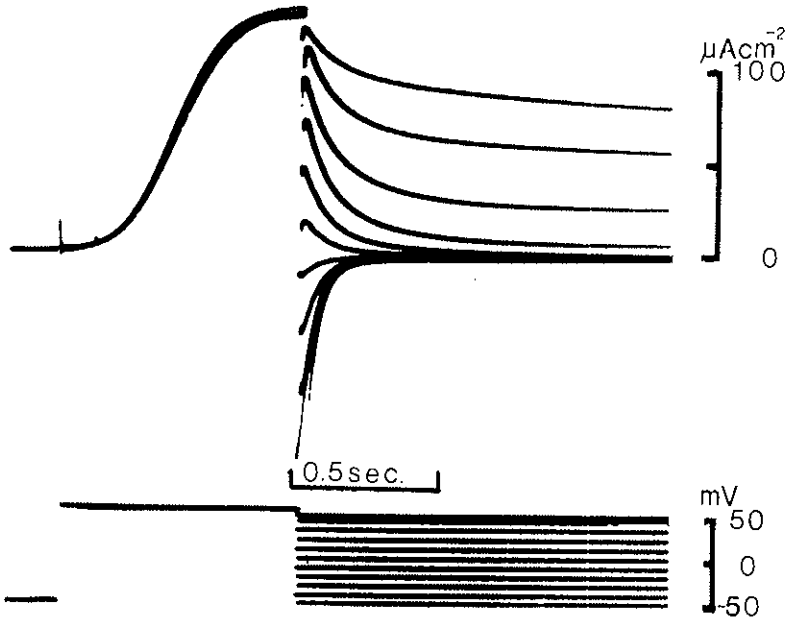

FIG. 1 — Membrane currents (upper record) in response to applied potential steps (lower record) from a bilayer in the presence of 10^{-6}M monazomycin (one side) and 0.01M KCl (both sides). Eleven records are superimposed. The voltage steps were applied at 20 sec intervals. During each oscilloscope sweep the voltage was first stepped from a holding potential of −50mV to a potential of 70mV, and then reduced to different levels. During the 70mV step the current rises along an S shape curve. It then decays exponentially to the level corresponding to the lowered potential. The time constants of the decays show a maximum near 35mV (third trace from top in the current records).

tain kinetic transients that cannot be accounted for by the HODGKIN-HUXLEY equations but are also seen in identical form in nerve.

Because the kinetics are identical in all excitable cell membranes and in these bilayers, it is conceivable that in spite of tbe diverse chemical nature of the channel forming molecules in the bilayers and the differing ion selectivities in the cellular

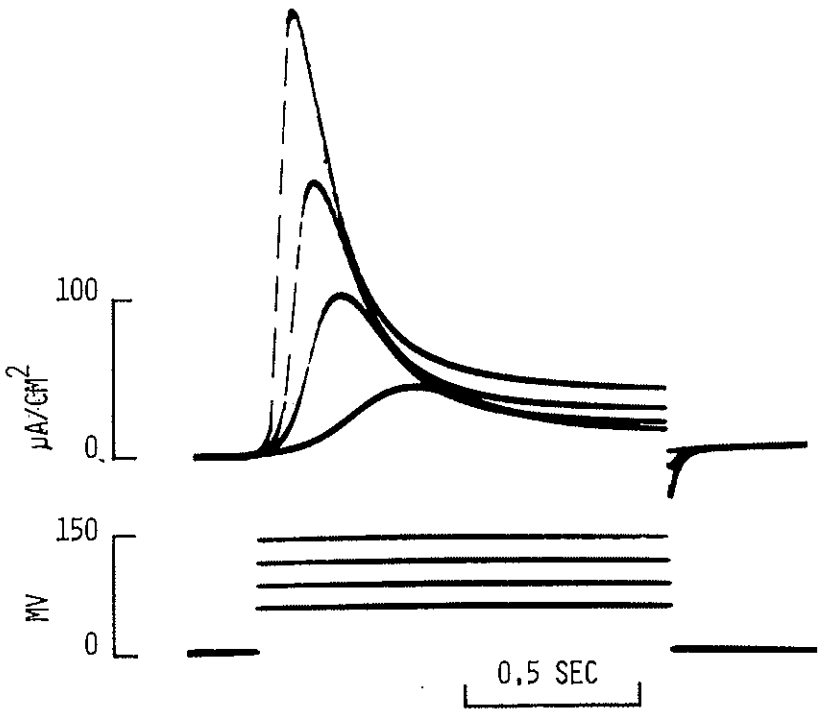

FIG. 2 — Membrane currents (upper record) in response to applied potential steps (lower record) from a lecithin bilayer in the presence of 10^{-5}M monazomycin at a temperature of 38°C. At this concentration of monazomycin and at the elevated temperature, the currents show an early maximum and subsequent decline, i.e., inactivation. The magnitude of inactivation, i.e., the ratio of the steady state to the peak conductance is variable and depends on the nature and concentration of the translocator, the lipid composition and external factors such as the ionic composition of the aqueous phase and the temperature.

systems, the mechanism by which the membrane opens and closes for the flow of ions is essentially the same in all cases and that conclusions drawn from a study of the excitable bilayers may be applicable to the cellular systems.

Aggregation as a gating mechanism in excitable bilayers

The sigmoid conductance rise and the exponential dependence of the steady state conductance on the voltage suggest that the gating process is a cooperative phenomenon. Possible molecular models can be divided into two classes. The first involves field-induced intramolecular rearrangements within a preformed channel structure. In this case the kinetics would result from internal cooperative or sequential interactions, such as a helix-coil transition or allosteric interactions between channel subunits as they occur in regulatory enzymes. Whereas this mechanism is quite plausible, a choice among the many conceivable models is at the present state of knowledge rather arbitrary.

In the second class of mechanisms, the membrane potential controls the aggregation of non-conducting channel precursors into a functional channel.

Obviously one cannot decide between these alternatives on the basis of current-voltage data alone and very little additional information is available for the cellular systems. The situation is different in the bilayers. The chemical structure of alamethicin and DJ400B are known and the experimental control of the lipid composition and of the translocator concentration, orientation and chemical nature provide data not yet available for the biological membranes.

Among these data are two crucial observations suggesting almost unequivocally that at least in the bilayers the gating proceeds by the latter alternative. The first is the exponential dependence of the steady state conductance on the translocator concentration. This conductance-concentration function is a variable. It depends on experimental conditions and on the channel former and power functions with exponents between 2 and 10 have been observed indicating that more than one molecule is involved in the formation of the channel.

The second observation concerns the relation between the translocator concentration and the time constants of the voltage

dependent conductance changes. In every case studied so far the time constants measured at the same voltage decrease with increasing concentration as expected if during the gating process several translocator molecules aggregate into an open channel. An example is shown in fig. 3.

Based on these data, a detailed molecular model of the gating process has been proposed [4, 10, 11]. Its critical features are best illustrated by the structure of DJ400B (fig. 4) and the scheme of fig. 5.

They can be summarized as follows:

1) The channel forming part of the translocator molecule must be about 30Å long in order to span the bilayer hydrocarbon region.

2) It must contain at one end a hydrophilic group which can anchor that part of the molecule at the lipid-water interface.

3) The opposite end must either contain a charged group or the molecule as a whole should have a large dipole moment.

4) The part of the molecule which is to enter the hydrocarbon region must contain several polar groups facing to one side; the other side should be predominantly hydrophobic.

When these conditions are fulfilled, the gating is assumed to proceed in the following way:

At rest, i. e., at a potential where the membrane conductance is low, the molecules lie flat on the membrane surface. An applied field of appropriate sign pulls the charged end through the membrane towards the other side such that the molecules now span the membrane. Aggregation of the monomers by lateral diffusion leads to the formation of dimers, trimers, etc. Monomers and dimers do not form an open channel but higher oligomers do. In the open channels, the polar groups face towards the center forming a hydrophilic lining, while the nonpolar sides of the translocator face outward interacting with the hydrocarbon chains. When the potential is removed the

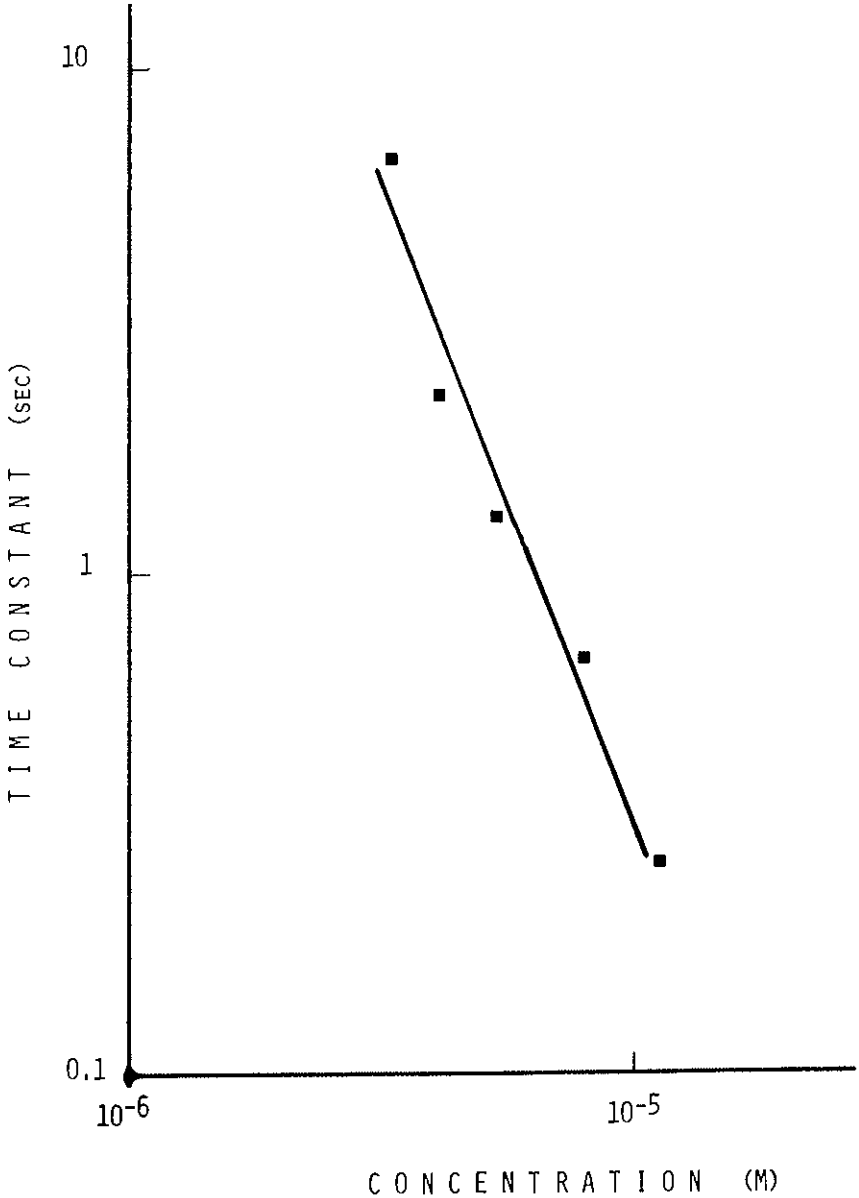

FIG. 3 — The relation between the concentration of monazomycin in the aqueous phase and the time constant of the conductance rise in response to a 100mV potential step. The data were obtained from a lecithin bilayer.

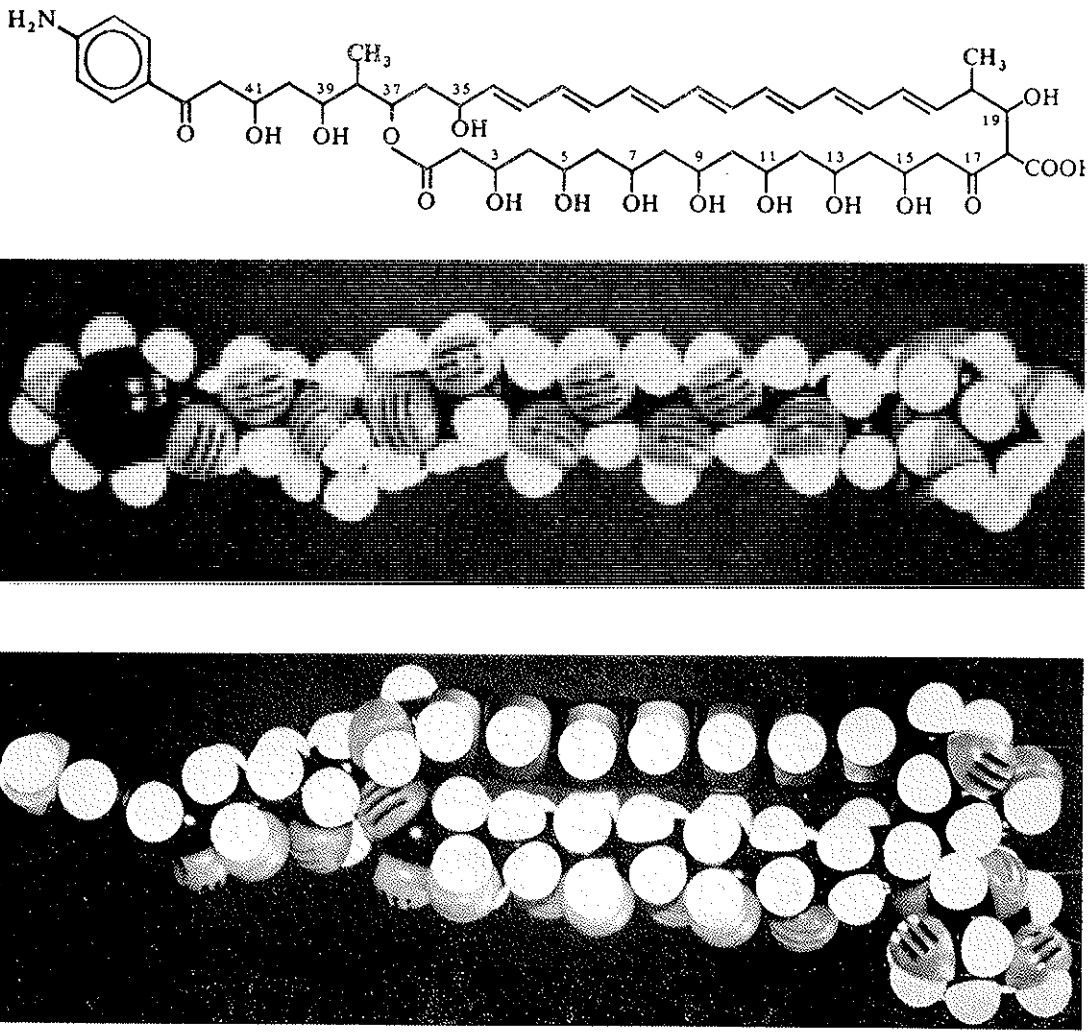

FIG. 4 — The structure and a molecular model of the cyclic polyene DJ400B. The formula (top) is that of the aglycone. The position of the sugar in the model is hypothetical but in agreement with the usual position of this residue in other polyenes. From reference [4].

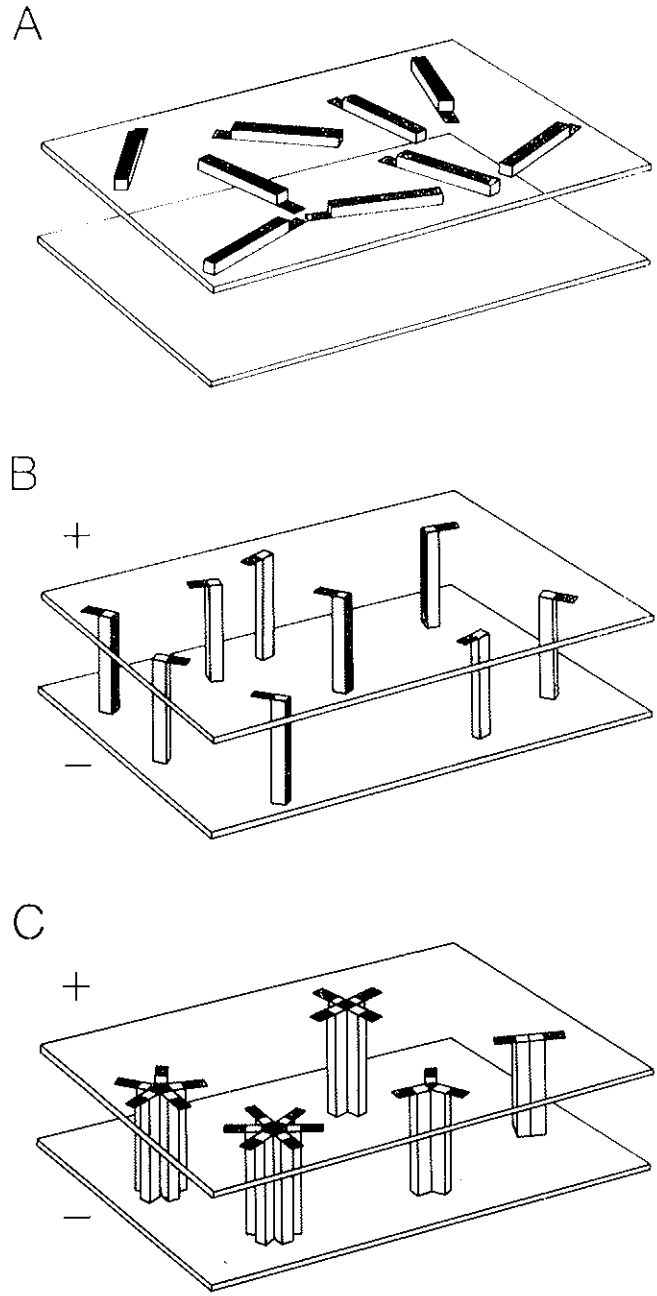

FIG. 5 — A model of the excitation process as postulated for the bilayers. (A) At rest, the translocator molecules lie flat on the membrane surface. The extension at one end of the molecule represents a water soluble anchor group. (B) An applied field acting on a gating charge at the other end of the molecules has pulled them into the membrane towards the trans surface. (C) Lateral diffusion within the membrane leads to aggregation of the monomers into oligomers. Trimers, and higher oligomers form a central opening acting as a channel for the flow of ions. From reference [10].

molecules return to their original position at the membrane surface. The charge or dipole moment represents the gating charge and its movement in and out of the membrane would generate a small transient displacement current which is also predicted by the H & H theory and has recently been observed in nerve [30].

The structural requirements listed above can be realized by a number of different molecular compounds, and the cyclic polyenes are particularly suited to form this type of " barrel stave " channel. Peptide chains, either singly or as hairpin loops can also organize in this way, and a specific configuration based on a cyclic secondary structure has been proposed for alamethicin, in which the peptide chain is arranged into an ellipsoid with one side in β configuration its peptide carbonyls forming the hydrophilic channel lining, the other side forming a hydrophohic α helix (see fig. 6). The polar group attaching the molecule to the membrane surface consists of two glutamyl residues located at one end. Although alamethicin is now known to be a linear peptide [12], this proposed hairpin loop configuration could still be correct. It any case the basic aspects of the model would not change significantly if only part of the alamethicin molecule would extend through the membrane as a straight chain.

The model is also not restricted to small molecular entities. Peptide chains or loops, extending from a large protein might be inserted by the field into the hydrocarbon region. The open channels are then formed by the association of chains helonging to neighboring proteins.

Mathematical description of the gating process

The mathematical representation of the proposed gating process is very similar to the H & H equations. The insertion

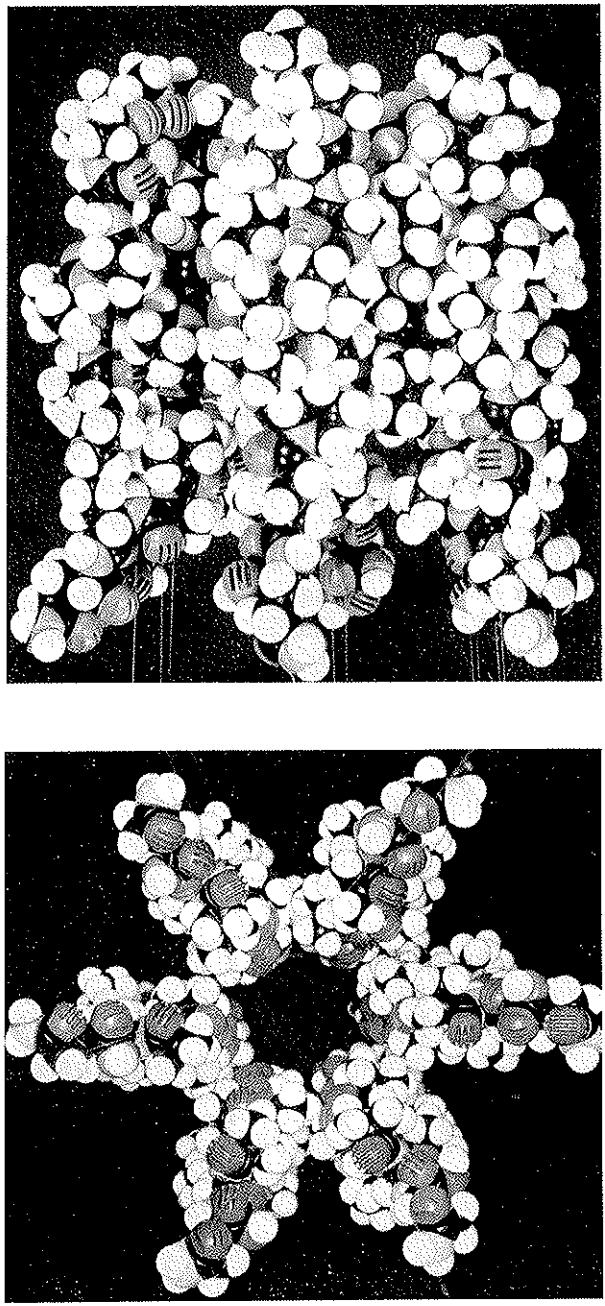

FIG. 6 — A hypothetical molecular model of the hexameric channel formed by the aggregation of 6 alamethicin molecules. The alamethicin is folded into a hairpin loop with one side in β configuration, the other containing a segment of α helix turns. A: side view. B: top view. From reference [10].

step represents the voltage-induced tilting of the translocators into the membrane:

$$P_0 \underset{k_{10}}{\overset{k_{01}}{\rightleftharpoons}} P_1 \tag{1}$$

where P_0 represents the concentration of monomers at the surface, P_1 that of inserted monomers. The aggregation of the inserted monomers is assumed to proceed by the linear addition of monomers to other n mers.

Thus

$$P_1 + P_n \underset{k_{n+1,n}}{\overset{k_{n,n+1}}{\rightleftharpoons}} P_{n+1} \tag{2}$$

$$P_1 + P_{m-1} \underset{k_{m,m-1}}{\overset{k_{m-1,m}}{\rightleftharpoons}} P_m \tag{3}$$

P_n varies from P_1 to P_m and represents the concentration of monomers, dimers, trimers, etc. P_m is the largest stable oligomer. The total translocator concentration,

$$C_1 = P_0 + \sum_{n=1}^{m} nP_n \tag{4}$$

In the simplest case, the aggregation rate constants are determined only by the molecular interactions between monomers, oligomers, lipids, water and ions in the channel and by the lateral diffusion rates of the inserted monomers and oligomers and are in first approximation not dependent on the membrane potential. They are of the general form:

$$k_n = A \exp(-E_n/RT) \tag{5}$$

where the constant A expresses the diffusionally controlled encounter frequencies and the steric factors determining the reaction probabilities. E is the activation energy. Under certain conditions the aggregation rate constants can become voltage dependent perhaps due to a voltage induced lateral phase separation between lipids and translocator molecules. This has several distinct kinetic consequences which have been discussed elsewhere [13].

Because monomers and dimers are assumed to be nonconductive, the membrane conductance is proportional to the sum of the individual conductances of the higher oligomers. Numerical solutions of the differential rate equations describing this reaction scheme [13] allow the calculation of the membrane conductance as a function of voltage and time [4, 10, 11], and the results reproduce the basic H & H kinetics as well as the dependence of the gating time constants and steady state conductances on the translocator concentration. In addition, they also account for the kinetic peculiarities mentioned aboce which are not part of the H & H scheme [4]. Such features appear very prominently in the bilayer systems under particular voltage pulsing sequences and have also recently been observed in nerve [28].

Inactivation

The H & H analysis requires separate equations for the description of inactivation and it has been generally assumed tbat inactivation is caused by an additional process not coupled to the activation, i. e., the opening of the channels. Recent data from squid and myxicola axons suggest that activation and inactivation are sequentially coupled processes, and appropriate modifications of the H & H equations have developed to take this fact into account [14, 15].

In the aggregation model the inactivation follows directly and without ancillary assumptions as an inherent property of

the reaction, provided the rate constants are such that the nonconductive oligomers, e. g., dimers or trimers have a lower free energy and/or are formed at a slower rate than the higher, conducting oligomers [4, 10]. When under these conditions, the concentration of inserted monomers is suddenly increased by an applied voltage, the reaction proceeds initially very fast towards the formation of higher oligomers, which consequently decay back to dimers and trimers. Thus in the inactivated state the majority of the translocators is in the form of these lower non-conducting aggregates. Quantitative calculations of this process have shown that it can generate not only the classical H & H phenomena related to inactivation but also those phenomena that made it necessary to modify the H & H equations such as the variable degree of inactivation, the shifts of the inactivation curve along the voltage axis as a function of different test potentials and the delayed onset of inactivation. In this model, inactivation is nothing else but a polymerization overshoot [16], a phenomenon well known for linear polymerizations such as that of tobacco mosaic virus coat protein [17]. Of course, there may exist other mechanisms resulting in inactivation such as the transfer of monomers to the opposite membrane surface or the blocking of an open channel by peptide chains or lipids and the fact that the aggregation mechanism can show inactivation does not prove that nerve or bilayers inactivate by this mechanism.

Single channel studies

In the bilayers it is possible to study the current fluctuations associated with the opening and closing of single channels. Because its channels are relatively large, alamethicin is particularly suited for such experiments. The results provide additional support for the aggregation model [13, 18, 19], and the multi-channel kinetics agree with the single channel conductance fluctuations and their statistical behavior.

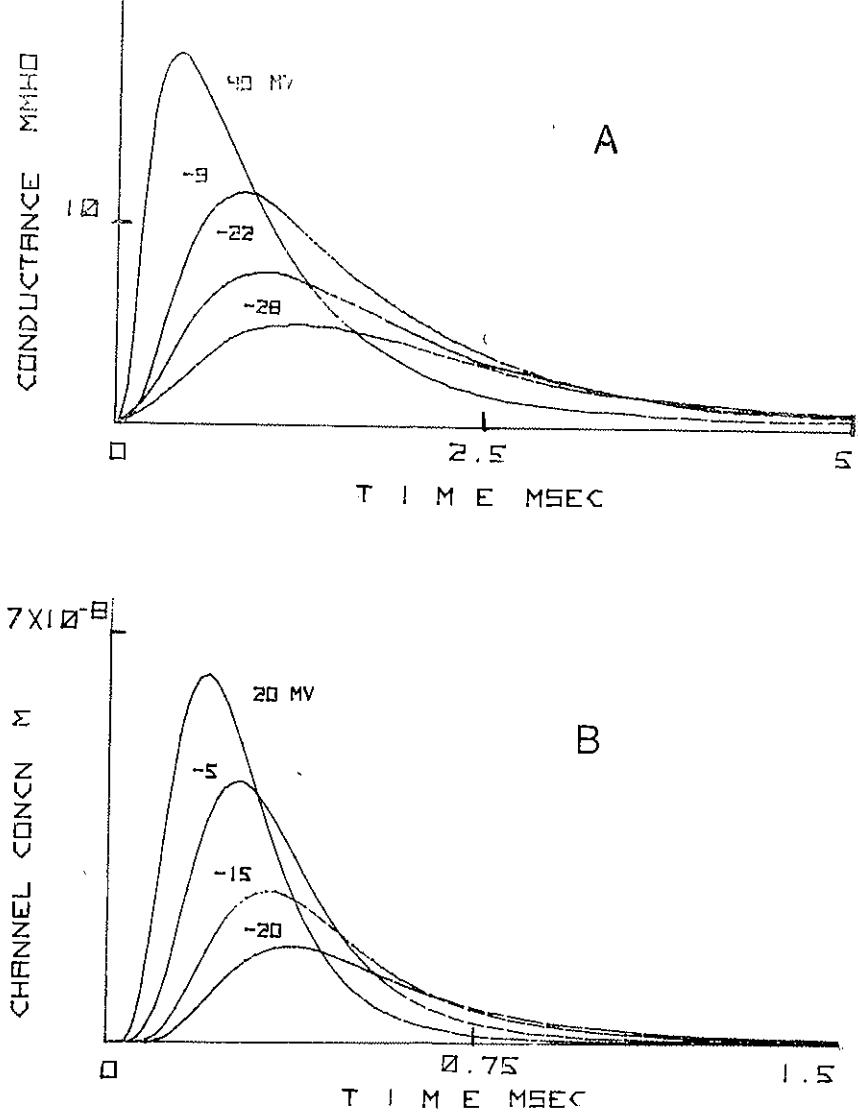

FIG. 7 — Inactivation in nerve (A) and in the aggregation model (B). The curves in A were plotted from data in ref. [9] and show the time course of the sodium conductance in the squid axon in response to voltage steps from a holding potential of −60mV to the different potentials indicated above each curve. The curves in B represent the concentration of hexameric channels as a function of time in response to voltage steps from a holding potential of −58mV to the indicated values. They were obtained from numerical solutions of the differential rate equations describing the aggregation scheme (1)-(4) assuming that only the hexamers form a conductive channel and that they are the largest possible oligomers. The activation energies, E, of the rate constants (equation 5) had tre following values in Kcal/mol: $E_{01} = .9$; $E_{10} = 2.1$; $E_{12} = 0$; $E_{21} = 3.2$; $E_{23} - E_{65} = 0$. All preexponential factors (A) were 2×10^4 and C = 1M. With this set of rate constants, the dimers have the lowest free energy and in the inactivated state most of the inserted monomers have formed non-conducting dimers. From reference [4].

1) At a sufficiently large voltage, the conductance fluctuates between 5-6 well-defined levels (see fig. 8).

2) The fluctuations occur sequentially between adjacent levels, never involving more than one level.

3) The spacing between the levels, i. e., the amplitude of the conductance steps increases in a systematic manner from the lower to the higher levels.

4) The frequencies of the transition from one level to the next follow a Poisson distribution.

5) The average life times of the different levels have an approximate Gaussian distribution. The distribution maximum shifts from lower to higher levels with increasing membrane potential.

6) The individual transition rates, defined as the number of transitions from a given level to the next per unit life time of that level, depend on the voltage in the sense that the ratio of the upward transition rates to the downward transition rates increases with increasing voltage.

7) The individual transition frequencies increase with increasing voltage.

8) The transition frequencies at a fixed potential increase with increasing concentrations of alamethicin.

9) The lifetime distribution of the levels measured at a fixed potential is shifted to higher levels with increasing alamethicin concentrations.

10) The transition frequencies are a steep inverse function of the lipid viscosity.

The conductance values can he derived quantitatively from the hypothetical channel dimensions and the increase of the level spacing is a direct consequence of the postulated channel enlargement by monomer interposition (see fig. 9). The interlevel transition rates appear to he of first order because the

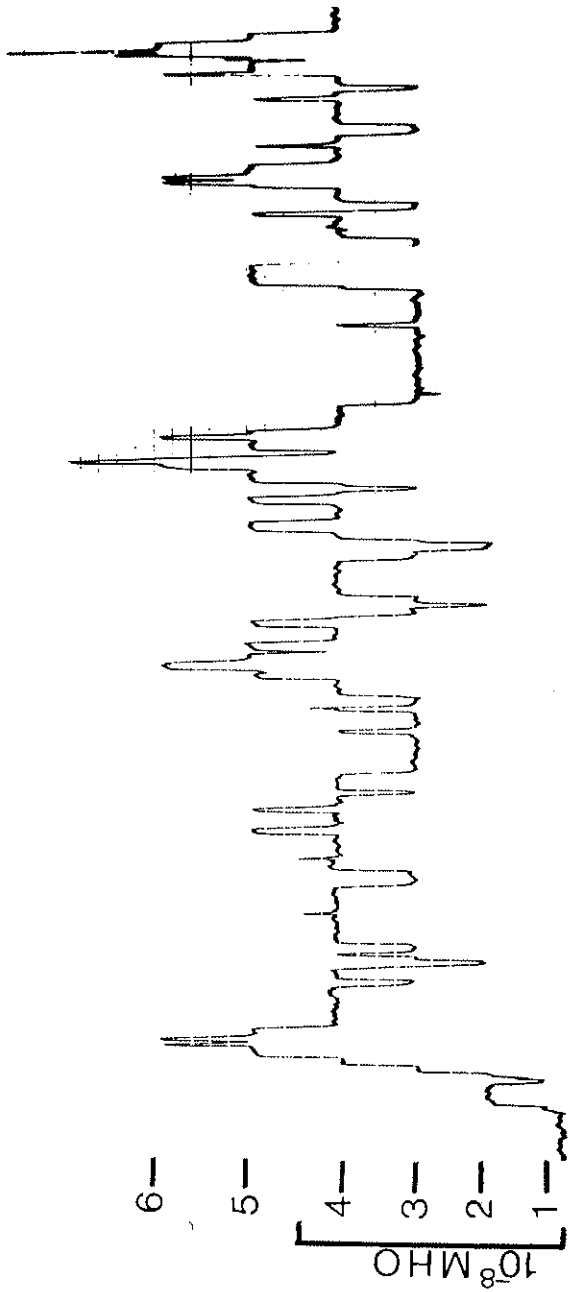

FIG. 8 — Single-channel conductance fluctuations from a lecithin bilayer in the presence of 10^{-7} M alamethicin and 5M KCl. A potential of 200mV was applied to a 10 µm diameter membrane patch, isolated by pressing a small glass capillary against a performed bilayer. The numbers indicate the different conductance levels.

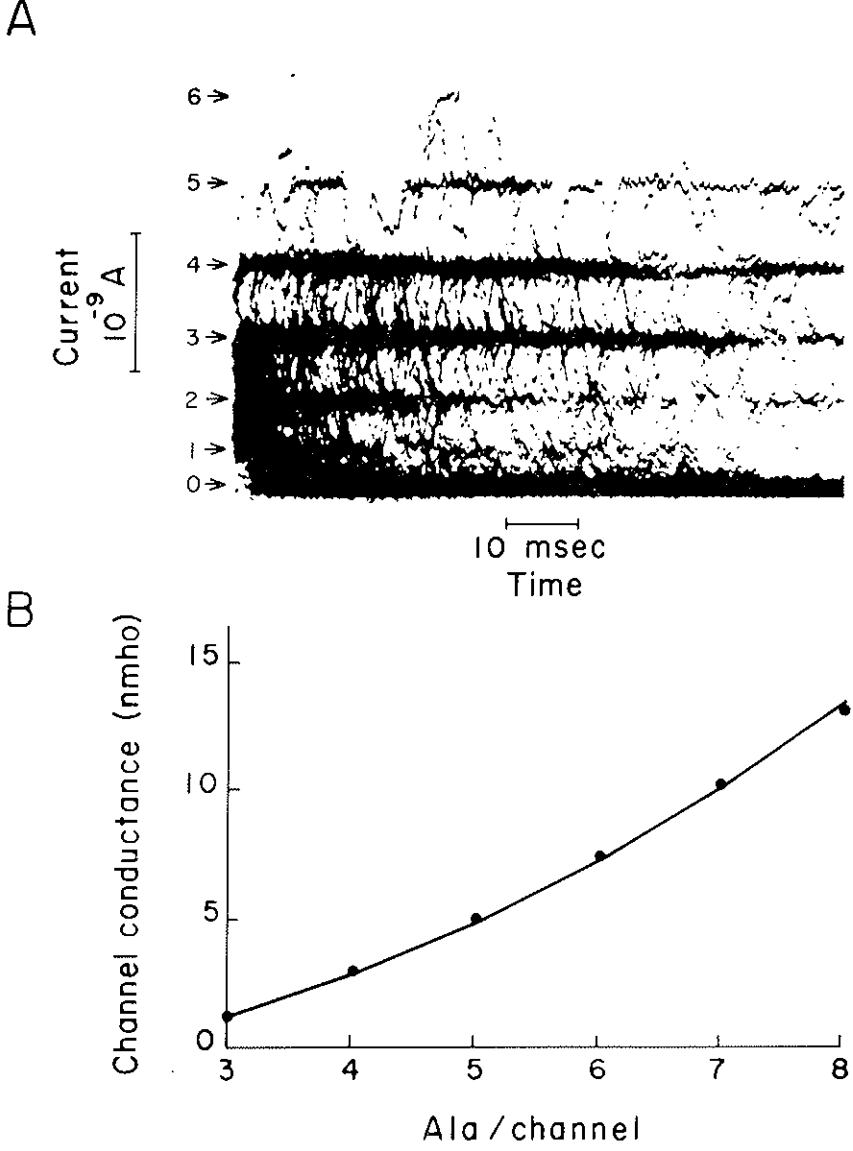

FIG. 9 — (A) Current fluctuations through a lipid bilayer membrane (glycerol monolcate) in the presence of 10^{-8} mol/l alamethicin. The membrane potential was clamped at 210mV. The aqueous phase contained 2M KCl. Many sweeps were superimposed on the screen of a storage oscilloscope, each sweep being triggered by the leading edge of a current transition. The base-line corresponds to the conductance of an unmodified bilayer. Six different current levels can be distinguished (arrows) and their relative probability of occurrence can be estimated from the intensity of the baseline traces. The 3rd and 4th levels are the most probable. The spacing between the levels increases with the current. From reference [18]. (B) Correlation between the conductance levels calculated from (A) and the conductance of hypothetical channels formed by the aggregation of 3 to 8 alamethicin molecules. The points represent the conductance levels and are obtained from the current levels in (A). From reference [10].

concentration of monomers is much higher than that of the open channels and thus stays constant during the observation time, as long as the potential is not changed.

The dependence of the level distribution and of the transition frequencies on the alamethicin concentration is particularly significant because it agrees with the concentration dependence of the multi-channel conductance and gating time constants and strongly supports the contention that the molecular process responsible for the conductance change involves the aggregation of several alamethicin molecules.

In particular, it is difficult to reconcile these observations with a mechanism in which oligomeric channel precursors are first formed at the membrane surface and then transformed by the voltage into an open channel. In this case neither the multichannel gating time constants nor the single channel fluctuation frequencies would be concentration dependent.

Application to cell membrane excitability

In the bilayers the aggregation mechanism is well supported by chemical, kinetic and single channel data.

In the cellular systems, chemical data such as the effects of the translocator concentration on the steady state and kinetic conductance parameters or single channel gating statistics are lacking and the validity of the model rests primarily on the similarities between the conductance — voltage — time relations in both systems. As shown in table 1, these similarities are far-reaching and extend beyond the classical H & H features. Nevertheless, because the arguments rest mainly on the kinetic similarities no definitive conclusions can be drawn at this time.

Even within the framework of the aggregation scheme there are certain features pertaining to the cellular channels which necessitate special consideration and perhaps modification of the minimal scheme.

Most of the cellular channels show a high degree of cation

TABLE 1

Comparative Features	*Observed in:* Bilayer Translocator	Bilayer Ref.	Nerve Ref.	*Accounted for by:* Aggregation Model	Hodgkin-Huxley Equations
A) *Constant current data*					
1. Salt gradients generate resting potentials	E, A, M, D	1-3	5		
2. Delayed rectification	E, A, M, D	2	5	Yes	Yes
3. Two channels with different ion selectivities generate action potentials under constant current stimulation	E, A	1,2	5	Yes	Yes
4. Action potentals are blocked by local and general anesthetics	E, A	3	21		
B) *Voltage clamp data*					
Steady State					
5. The conductance-voltage curve is exponential, (4-10 mV/e fold increase)	E, A, M, D	1	5-9	Yes	Yes
6. Strong rectification	E, A, M, D	1	5-9	Yes	Yes
7. Conductance - voltage curve saturates at high potentials					
Kinetics (I) (Hodgkin & Huxley)	E, A, M	4	5-9	Yes	Yes
8. Delayed currents	E, A, M, D	3,10,13,22	5-8	Yes	Yes
9. Delay increases with hyperpolarization	A, M	23	24	Yes	Yes
10. Time constants go through maximum as a function of voltage	E, A, M	4	5-8	Yes	Yes

TABLE 1 (*continuation*).

Comparative Features	*Observed in:* Bilayer Trans-locator	Bilayer Ref.	Nerve Ref.	*Accounted for by:* Aggrega-tion Model	Hodgkin-Huxley Equations
11. Salt gradients lead to negative resistance	E, A, M	1	25	Yes	Yes
12. Inactivation	E, A, M	4	5-8	Yes	Yes
13. Inactivation time constants go through maximum	A, M	23	5-8	Yes	Yes
14. Peak conductances drop at high potentials	A, M	23	15	Yes	Yes
15. Inactivation continues after short pulse	A, M	23	8	Yes	Yes
16. Inactivation is variable	A, M	23	26	Yes	Yes
Kinetics (II) (Non-Hodgkin-Huxley)					
17. "On" and "Off" time constants depend on holding potential	A, M	4	27	Yes	No
18. Time constants for "off" depend on conductance level	A, M	4	29	Yes	No
19. Conductance continues to rise after short "on" pulse	A, M	4	28	Yes	No
20 "On" time constants after brief "off" pulse depend on duration of "off" pulse	E, A, M, D	4,10	28	Yes	No
21. Conductance continues to fall after brief "off" pulse	A, M	4	?	Yes	No

TABLE 1 (*continuation*).

Comparative Features	*Observed in:* Bilayer Translocator	Bilayer Ref.	Nerve Ref.	*Accounted for by:* Aggregation Model	Hodgkin-Huxley Equations
22. High "on" pulses from "on" state lead to transient conductance decrease	A, M	13	28	Yes	No
23 "Off" pulses from "on" state lead to transient conductance increase	A, M	4,10,13	?	Yes	No
24. High "on" pulses lead to channel inversion	A, M	13	?	Yes	No
25. Time constants of inverted channels depend on duration and potential of "on" prepulse	A, M	13	?	Yes	No
26. Inactivation measured by steady state/peak ratio differs from inactivation measured by prepulse	A, M	23	15	Yes	No
27. Hoyt shift	?		14	Yes	No
28. Onset of inactivation delayed	A, M		15	Yes	No
29 Time constants of "off" gating currents depend on conductance	?		30	Yes	No
30 Gating currents decrease during inactivation	?		29	Yes	No
31. Time constants depend exponentially on translocator concentration	E, A, M	4	?	Yes	No
32. Conductance at a fixed potential depends exponentially on translocator concentration	E, A, M	1,4	?	Yes	No

TABLE 1 (*continuation*).

Comparative Features	*Observed in:* Bilayer Trans-locator	Bilayer Ref.	Nerve Ref.	*Accounted for by:* Aggrega-tion Model	Hodgkin-Huxley Equations
33. Time constants depend on lipid fluidity	A, M	4,10		Yes	?
34. Conductance-voltage curve is shifted along Voltage axis by:					
a) Surface potential	E, A, M	2	31	Yes	Yes
b) Lipid composition	E, A, M	2,3	?	Yes	?
c) Translocator type	E, A, M, D	2,3,4	?	Yes	?
d) Local anesthetics	E, A, M	2,3	21	Yes	Yes
35. Instantaneous conductance of open channel is a hyperbolic sine function of the potential	E, A, M	23	28		
36. Gating kinetics is independent of channel ion selectivity	E, A	1-3	32		
37. Ion selectivity of open channel can be altered by addition of water soluble compounds	E, A	2,3	?		

Comparison between electrokinetic features in excitable bilayers and nerve membranes.

The letters in the translocator column indicate with which translocator the phenomenon has been observed. E = EIM, A = alamethicin, M = monazomycin, D = DJ400B. Omission of a letter means that the phenomenon has not been studied or has not been observed with that compound. A question mark indicates insufficient data. The nerve data were obtained from squid, myxicola or frog. Many of these phenomena have also been observed with membranes from other excitable cells such as muscle and algae.

selectivity, whereas the simple bilayer gating systems do not. It has generally been recognized that the selectivity is determined only by part of the channel structure acting as a series selectivity filter, and that this part is not involved in the gating action. This view seems justified especially for the sodium channels where the conductance is blocked by tetrodotoxin without effect on the gating currents. The selectivity filters may either be a permanent part of the channel or they may exist as separate entities at the membrane surface with which the gating structures link up after their insertion.

One example of a truly separate filter molecule is provided by the selectivity inversion of EIM and alamethicin channels by externally applied protamine which changes the predominant cation permeability into an anion permeability without affecting the gating kinetics [2-3]. If there exists such a series selectivity filter, its inherent conductance might be lower than that of the gating part of the channel. As a consequence the conductance would not increase stepwise with increasing diameter of the gating part caused hy addition of monomers, but would instead have only two states; open or closed. Thus the suggestion based on noise data that the gating mechanism in nerve operates by a two state mechanism [33] does not exclude an aggregation process.

It is most likely that the sodium and potassium channels are built from larger elements than alamethicin or monazomycin, and that they bear more resemhlance to EIM with dispersive molecular weights in the range from 6,000-10,000. But size alone does not exclude an aggregation mechanism. Membrane proteins can diffuse rapidly within the lipid bilayer matrix, and aggregation by lateral diffusion plays a role in immunological phenomena.

The short time constants of the conductance changes also present no prohlem, especially if the gating structures are already pre-aggregated in patches at the membrane surface so that rotation or diffusion over a very short distance suffices to organize the inserted components into an open channel. Quanti-

tative estimates of diffusion - limited reaction rates give time constants comparable to those observed for the sodium system, provided the surface concentration of the channel precursors is in the order 10^{12}-10^{13} molecules cm^{-2} [4, 13].

One can also easily envision more complex molecular versions of the aggregating structures leading to somewhat different kinetic schemes. For example, as mentioned above, several peptide chains or loops attached to, and extending from a larger protein body might he inserted by the field and aggregate with chains attached to neighboring proteins, forming channels [4]. In this case, the aggregation leads to an extended net of coupled channels and the statistics of this process are somewhat different from the completely uncoupled scheme assumed above. In another model the voltage-induced aggregation between proteins extending normally through the membrane would impose intramolecular stresses which then would open an intramolecular channel.

If the aggregation takes place between components of a larger molecular assembly and not between freely diffusing entities, the mechanism takes on more of the characteristics of the original HODGKIN-HUXLEY scheme and the kinetics consequently lose some or all of the state dependent features discussed above. The same holds true for a model in which sequential intramolecular rearrangements within a fixed channel structure lead to the conductance changes. In such models, the inactivation is no longer an inherent property of the gating mechanism but instead requires the ad hoc assumption of a separate physical process.

At the current state of knowledge, the speculative nature of such assumptions provides little incentive for extensive kinetic calculations and the few that have been attempted have now yet been tested against the full range of experimental observations. This is not to mean that these models can be excluded, and it may very well turn out that the cellular exci-

tability mechanisms do not involve an aggregation process but represent instead voltage-induced configurational changes within a preformed channel structure, in which case the similarities between the electrokinetics in bilayers and cells are a remarkable but, nevertheless, fortuitous coincidence.

ACKNOWLEDGEMENT

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DISCUSSION

Chairman: Prof. J. M. RITCHIE

MONNIER

The main question remains what are the translocators or channel-forming molecules in real membranes. Many features of excitability and ionic selectivity are observed in membranes made of very different lipid derivatives. Thus, we might perhaps imagine that there are channel lining or channel forming molecules, perhaps some very simple molecules. For instance, oleic acid molecules in small numbers coming from hydrolysis of phospholipids could aggregate themselves. The electric fields might play a part in this orienting and channel forming process, which might look as a linear polymerization of acidic groups.

MUELLER

This is quite possible. As a matter of fact certain lipid membranes show just by themselves a very high potassium over sodium selectivity. The selective permeability has all the properties of the leak conductance of nerve membranes.

KEDEM

You have mentioned the overshoots and referred to some apparently well known phenomena, but normally an overshoot requires

at least two simultaneous processes. A single process just does not overshoot its own target — the equilibrium.

MUELLER

Well, it just turns out that this one does. Polymerization overshoots have been experimentally observed and theoretically studied in the polymerization of TMV virus coat protein and also in other systems.

BAKER

Could you go on a little bit further? What sort of ratio of your reacting particles do you need to the ones you assume to be in the channels, to see this inactivation phenomena? Do you need a large excess of the particles?

MUELLER

No, you really don't. Almost all of the monomers can transiently appear as conducting oligomers.

BAKER

If your hypothesis is correct, it would have considerable implication for people studying gating currents. Have you got any evidence for gating currents in your system?

MUELLER

Unfortunately we cannot measure gating currents because we do not have specific poisons to cut off the conductance. However,

what we are now trying to do is to measure the insertion and tilting off the translocators optically by the change of fluorescent polarization.

DE CARVALHO

You mentioned that you need to bind one cation to the molecule in order to produce the gating effect. Is this of the same species of the ion being transported?

MUELLER

Yes, but the gating ion is apparently bound to the alametricin and does not carry any current.

DE CARVALHO

When you explain step changes in conductance, how do you differentiate the change in the degree of polimerization of the « channel » from the formation of new channels?

MUELLER

The conductance fluctuations always appear in bunches. The conductance steps always increase in a typical fashion. When a second channel appears its conductance steps are overlaid on those of the first channel and can he distinguished by their size.

RITCHIE

Thank you very much Dr. MUELLER. Let me congratulate you on both being lucid and concise. The next speaker is going to be Dr. BOYER. He is going to talk about energy conversion and transport phenomena.

THERMODYNAMICS OF NERVOUS CONDUCTION

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During the passage of action potential along a nerve a complex set of rapid changes in its temperature occurs. These changes are extremely small, being measured in μ°C; and highly sensitive recording equipment is required to detect them. The earliest attempts to study this heat (HELMHOLTZ, 1848) were unsuccessful because of the lack of sufficiently sensitive recording equipment. However, in the years 1925-1935 it became clear that each impulse (in crab nerve) leads to an increase of about 2 μ°C (see FENG, 1936; HILL, 1965). Reexamining tbis question in 1958, ABBOTT, HILL and HOWARTH made tbe exciting discovery that this initial heat production in crab nerve is really much larger: in fact, the early rise in temperature is about 10 μ°C, but the phase of heat production is followed almost immediately by a phase of re-absorption of heat. The rise of 2 μ°C observed in the earlier experiments reflects tberefore only a *net initial heat*, which is the difference between the *positive initial heat* and the *negative initial heat*.

The main reason for the failure to observe the two phases of initial heat earlier is that the instruments necessary for recording such a small change in temperature are necessarily rather slow. In thermal experiments on nerve, the rise in temperature is measured by a thermopile (Fig. 1) whose output

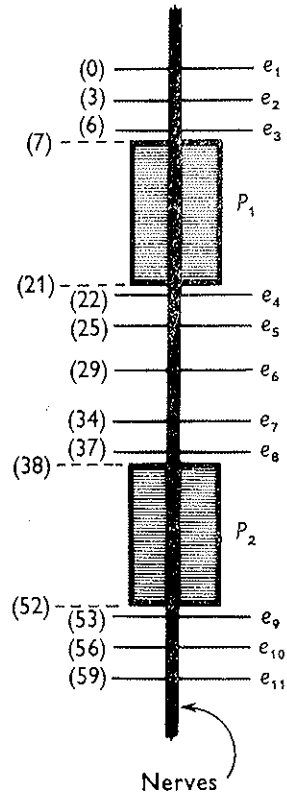

FIG. 1 — Diagram of a typical thermopile. P_1 and P_2 are two elements of the pile, each containing one hundred palladium-gold/iron thermocouples whose « hot » or active junctions lie along the centre line of pile (under « nerves ») and whose « cold » or reference junctions are in close contact with a large mass of aluminium and epoxy resin at the sides of the pile. e_1-e_{11} are 11 platinum electrodes. The numbers in brackets indicate distances in mm from e_1. Taken from HOWARTH *et al.* (1968).

is fed into a sensitive galvanometer/photocell amplifier (HILL, 1948; HOWARTH, KEYNES, RITCHIE and VON MURALT, 1975). Although with this system it is possible to achieve the sensitivity required, very fast changes in temperature cannot be recorded; for the inertia of the system is such that the response to sudden step increase in temperature of the nerve (by passing a 20 msec rectangular pulse of current between electrodes e_I and e_{II}, for example) takes 60 msec to reach 75% of its final maximum value and 80 msec to reach 90% of this value (heating control, Fig. 3). This slowness is why most experiments on nerve heat are done at low temperatures — to slow the biological responses sufficiently to match the slowness of the recording system.

The rabbit vagus nerve is particularly suitable for thermal experiments since it consists of a large number of extremely small non-myelinated fibres (average diameter 0.7 μm) and, in consequence, has a large area of axonal membrane per unit weigbt of tissue (6000 cm^2/g wet; KEYNES and RITCHIE, 1965). The walking leg nerve of the crab, used by ABBOTT *et al.* (1958), has a similar large surface/volume relation, but the spread of fibre diameters in the population is much greater; interpretation of the compound thermal responses of the crab nerve in terms of the events occurring at the nerve membrane is therefore more difficult than in the rabbit nerve. Figure 2 shows a typical record of the initial heat production in desheathed rabbit vagus nerve at about 5°C. Soon after the stimulus, the temperature rises rapidly to a maximum value of ahout 5 μ°C and then rapidly declines (HOWARTH, KEYNES and RITCHIE, 1968). That this decline in nerve temperature is due to an active re-absorption of heat becomes clear (Fig. 2) when the heating control is scaled so that its early rising phase corresponds with the early rising phase of the thermal record. Had only positive initial heat been produced, the thermal record must necessarily continue to rise, at least as fast as the heating control. The fact that it deviates

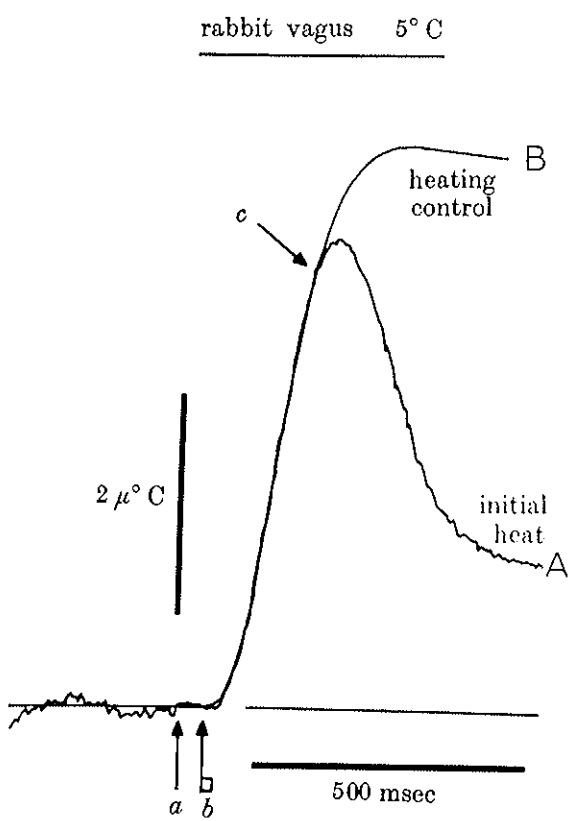

FIG. 2 — Curve A: the changes in temperature of the non-myelinated fibres of a rabbit desheathed vagus nerve at 4.2°C in response to a single maximal shock 2 msec in duration applied at *a*. The record is the result of averaging 20 records electronically. Curve B: the rise in temperature of the same nerve, after it had been rendered inexcitable by isotonic potassium chloride, in response to a block of heat (twenty-one 200 μsec pulses of current applied between the two ends of the nerve at 1 msec intervals) beginning at *b*. The amplification for B was adjusted to make the early part of the rising phase of B coincide with the early part of curve A. Taken from HOWARTH *et al.* (1968).

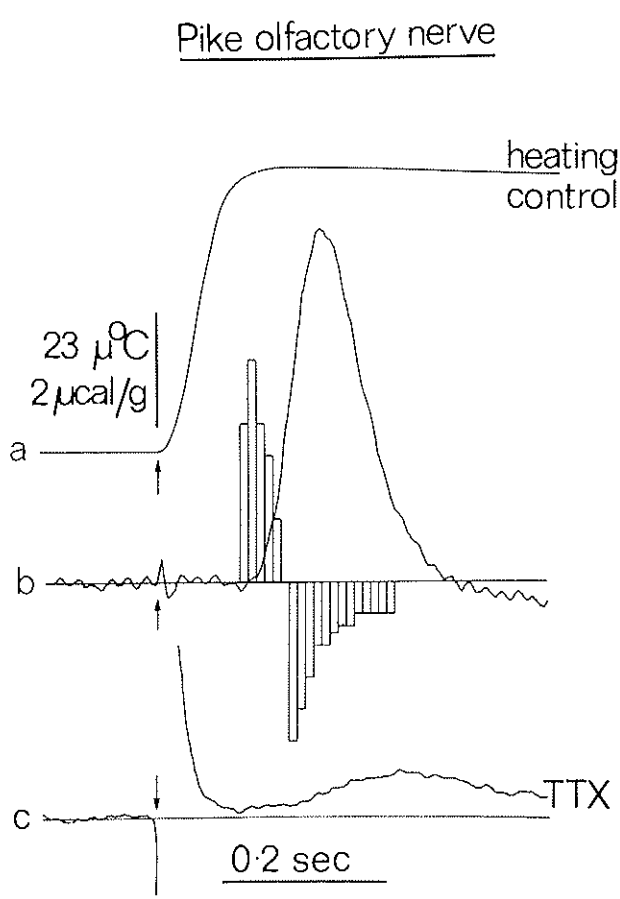

FIG. 3 — The initial heat production of the non-myelinated fibres of the pike olfactory nerve at 0°C. Record *a* is the « heating control » and is the rise in temperature produced in the nerve by a 65 msec pulse of heat (injected by passing current from one end to the other) starting at the arrow and applied after the nerve had been rendered inexcitable by tetrodotoxin (1 μM). Record *b* is the average of fifty thermal responses to single maximal stimuli applied at the arrow. The record has been corrected for stimulus heating by subtraction of record *c*. Superimposed on the thermal response is the result of a heat block analysis, which indicated a positive heat production of 85.5 μcal/g. impulse and a negative heat of 94.5 μcal/g. impulse. The vertical calibration represents 23 μ°C for the thermal phase, and 2 μcal/g. impulse for the heat block analysis. The horizontal calibration is 200 msec. Record *c* is the average of fifty responses to single shocks applied after the nerve had been rendered inexcitable by tetrodotoxin (1 μM). Taken from HOWARTH et al. (1975).

from the heating control (falling below it at arrow *c*) indicates that heat must have been re-absorbed at this time. Figure 3*b* shows the result of a similar experiment on the pike olfactory nerve. This nerve (SOWTON, 1900) like the similar garfish olfactory nerve consists of even smaller fibres than those in the rabbit vagus (average diameter 0.2-0.3 μm) and has an area of axonal membrane of about 41,000 cm^2/g wet (GASSER, 1956; EASTON, 1971; VON MURALT, WEIBEL and HOWARTH, 1975). The temperature change with stimulation, which again consists of a rapid rise and subsequent rapid fall in temperature, is thus a good deal larger than in rabbit nerve. Such experiments, which have now been done on crab (ABBOTT *et al.* 1958), lobster (ABBOTT, HOWARTH and RITCHIE, 1965), rabbit (HOWARTH *et al.* 1968), and pike (HOWARTH *et al.* 1975) non-myelinated nerve fibres, clearly establish the diphasic nature of the initial heat production in nerve.

THE SIZE OF THE INITIAL HEAT

The thermal records in Figs. 2 and 3*b* are usually analyzed by the method of heat block analysis (see HILL, 1965; HOWARTH *et al.* 1968), the result of one such an analysis being shown in Fig. 3 superimposed on the thermal record. Each heat block indicates the appropriate factor by which the heating control must be multiplied, and then added or substracted at appropriate times, to produce a thermal response that would mimic the actual response of the thermal record. In the rabbit vagus nerve at 5°C the positive initial heat observed on the basis of such analyses is 7.2 μcal/g.impulse; and the negative initial heat is 4.9 μcal/g.impulse. In the pike nerve at 0°, presumably because of the larger area of membrane per gram of tissue, the corresponding heats are much larger, being 40.9 μcal/g.impulse and 44.2 μcal/g.impulse for the positive and

negative heats respectively. It should be noted that the net initial heat in rabbit nerve is positive (2.4 μcal/g.impulse) whereas in the pike olfactory nerve it is negative (- 4.7 μcal/g.impulse).

The two phases of initial heat production, positive and negative, are thus not exactly equal. Therefore, if the preparation is not to get progressively, warmer or colder with stimulation (depending on whether the net initial heat is positive or negative) there must be a third slower phase, namely a *late initial heat*. This late initial heat, however, seems too small and too slowly evolved to be distinguished separately in the analyses, particularly as it occurs at the same time as the recovery heat begins.

Because the thermopile has a finite length (4-28 mm long), and because of the temporal dispersion of conduction velocities in the fibres of differents sizes, the true initial heats are greater than those observed. This is because the negative heats in more quickly conducting fibres may be cancelled by positive heats in more slowly conducting fibres. Correction for this « overlap » effect (ABBOTT *et al.* 1958; HOWARTH *et al.* 1968; HOWARTH *et al.* 1975) requires that the positive and negative initial heats be multiplied by a factor which may be as large as 3-5. Such a correction indicates that the true positive and negative heats in the rabbit vagus nerve at about 5°C are 24.5 and 22.1 μcal/g.impulse respectively; the corresponding values in the pike olfactory nerve at 0° are 62 and 67 μcal/g.impulse respectively. Referred to the axonal membrane, these values correspond with values for the positive initial heat of 2-4 ncal/cm^2. The results for rahbit and pike nerves together with those on crab nerve are summarized in Table 1. In lobster nerve (ABBOTT *et al.* 1965) again the initial heat is clearly diphasic; but the absolute values are too fragmentry for inclusion in the table.

TABLE 1

Nerve preparation *	rabbit vagus (5°C)		pike olfactory (0°C)		crab walking leg (0°C)	
	observed	corrected	observed	corrected	observed	corrected
Maximum rise in temperature (μ°C)	7.0	—	40.9	—	(8)	—
Positive initial heat (μcal/g. impulse)	7.2	24.5	44.2	62	8.8	14
Negative initial heat (μcal/g. impulse)	4.9	22.1	48.9	67	6.8	12
Net initial heat (μcal/g. impulse)	2.4	2.4	—4.7	—4.7	2.0	2.0
Positive initial heat per unit area membrane (ncal/cm²)	—	4.1	—	1.6	—	1.8

* Values are taken from: rabbit, HOWARTH *et al.* 1968; pike, HOWARTH *et al* 1975; crab, ABBOTT *et al.* (1958) (except for maximum temperature rise which is taken from ABBOTT *et al.* 1965).

THE TIMING OF THE POSITIVE AND NEGATIVE INITIAL HEATS

Because of temporal dispersion and the relatively long length of the thermopiles, the thermal records are more drawn out in time than would be records of the action potential recorded from a single fibre at a single point. However, when these factors are allowed for, it becomes clear (HOWARTH *et al.* 1968) that the positive initial heat is evolved during the phase of depolarization of the action potential while the negative initial heat occurs during the phase of repolarization. Qualitative support for this conclusion comes from experiments with procedures that alter the shape of the action potential. For example, removal of potassium from the medium bathing rabbit non-myelinated fibres causes the phase of repolarization of the

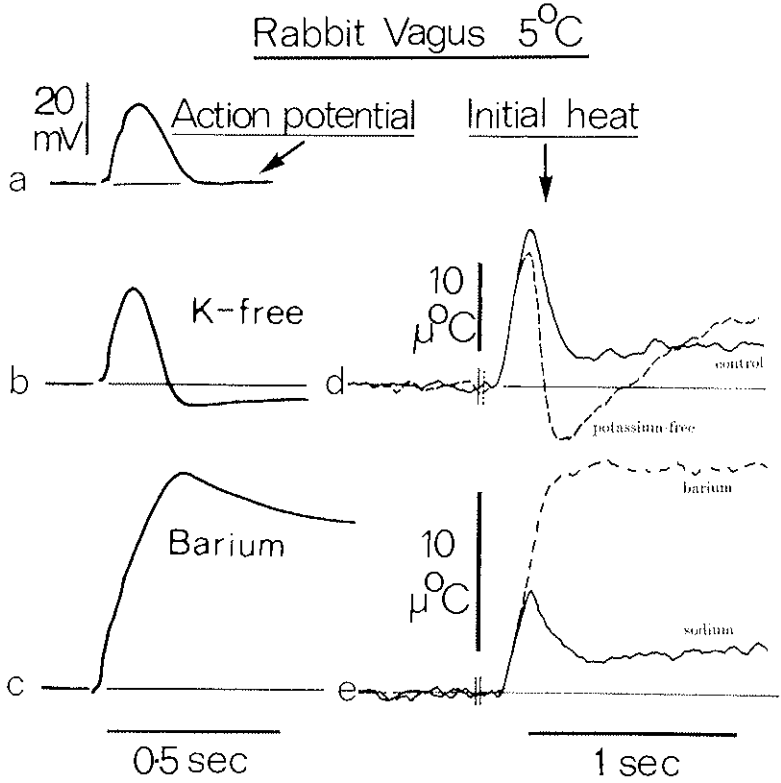

FIG. 4 — The effect of potassium-free solution and of barium on the electrical (left-hand records) and thermal (right-hand records) responses of the non-myelinated fibres of the rabbit desheathed vagus nerve at 5-6°C. Records *a*, *b* and *c* are records of the compound action potential. Record *a* was taken before, and record *b* after, the preparation had soaked in potassium-free Locke solution. Record *c* was taken from another preparation, whose action potential was initially similar to that shown in record *a*, after it had soaked in barium-Locke solution (in which 70.2 mM barium chloride had replaced 92.4 mM sodium chloride). Records *d* and *e* are records of the initial heat production. The interrupted lines show these thermal responses after the preparation had soaked in potassium-free Locke solution (record *d*) and barium-Locke solution (record *e*). The continuous lines in records *d* and *e* show the control responses in each preparation in ordinary Locke solution. Taken from HOWARTH *et al.* (1968).

action potential to overshoot (Fig. 4*b*), while replacement of most of the sodium chloride of the Locke solution by barium chloride leads to very long drawn out action potentials (Fig. 4*c*). These changes in the electrical responses are matched by corresponding changes in the thermal responses. Thus, the negative heat is enhanced in potassium-free solutions whereas it is almost abolished in the barium-Locke solution. It is interesting that in this latter situation, where there is little or no negative heat and hence no overlap, the measured positive heat is about three times the value measured in the normal nerve, in good agreement with the value of 3-5 for the overlap factor calculated for this nerve at this temperature (HOWARTH *et al.* 1968).

ORIGIN OF HEAT

Heats of mixing.

During the passage of an action potential the fibre loses potassium into, and gains sodium from, the bathing medium. The sodium ion are the first to move, from a sodium-rich solution into a potassium-rich solution, whereas the potassium ions move later, from a potassium-rich to a sodium-rich medium. Do the two phases of initial heat production, therefore, just reflect the heats of mixing accompanying the two phases of this interchange? In crab nerve, ABBOTT *et al.* (1958) concluded that a substantial part of the initial heat might indeed be derived from the interchange of sodium and potassium. However, in rabbit nerve and in pike nerve this possibility seems unlikely. Taking the potassium loss per impulse as an index of the cationic exchange, HOWARTH *et al.* (1968) calculated that the initial heat production in rabbit non-myelinated nerve fibres corresponds with a value of about 2000 cal/mole; the corresponding value in pike non-myelinated nerve fibres (HOWARTH *et al.* 1975) is about 1300 cal/mole. Since the heats of mixing of equal volumes of dilute salt solutions of sodium chloride, potassium chloride, and lithium chloride are

all small, ranging from −8 to +4 cal/mole (HOWARTH *et al.* 1968) it seems unlikely that the heats of mixing can make any substantial contribution to the initial heat.

Free energy changes.

The most obvious candidate for the source of the initial heat in nerve is clearly the free energy ($\triangle F$) which is stored in the membrane capacity (C) and which would be dissipated as heat when this capacity is depolarized during the action potential to give the positive initial heat. There would also be heat dissipated as a result of the continued flow of ionic currents down the electrochemical gradients across the two sides of the nerve membrane during the action potential; hut analogous to the expansion of a gas such heat would be exactly compensated for by a warming of the solutions providing the ions. During repolarization the same amount of the energy ($1/2\ CV^2$) must be returned to the membrane. And if it comes from the themal energy of the ions in solution there must be a corresponding cooling of the solution, to yield the negative initial heat. This energy amounts to $1/2\ CV^2$, where V is the voltage to which the membrane is polarized.

In crab nerve, ABBOTT *et al.* (1958) calculated that although the time relations relative to the action potential seem to be quite wrong, the heats derived from the free energy changes are of the right order of size. However, in both rabbit and pike nerves (HOWARTH *et al.* 1968, 1975) even the most generous estimates of the free energy stored in the membrane capacity seem to be well below the observed heat changes, accounting for less than one-third of the heat actually measured and an even smaller fraction of the value for the positive heat after correction for overlap. For example, in rabbit nerve the observed heat is about 25 μcal/g.impulse. However, with a membrane capacity of $1\mu F/cm^2$, an axonal area of 6000 cm^2/g, and a resting potential

of 40 mV, $\triangle F$ is calculated to be only 1.2 μcal/g.impulse. Even if an improbably high value of 80 mV (which is near the potassium equilibrium potential) is assumed, the value of $\triangle F$ is still only 4.7 μcal/g.impulse. In pike nerve a value much greater than 40 mV is unreasonable: this is the value of the action potential in the similar garfish olfactory nerve (RITCHIE and STRAUB, 1975) and also the value of the potassium equilibrium potential in the pike nerve (from the chemical data of HOWARTH *et al.* 1975). For the pike, therefore, with a membrane capacity of 1 μF/cm² and an axonal area of 41,000 cm²/g, a free energy change of 8.0 μcal/g.impulse would be expected. This is only about an eighth of the heat observed experimentally, which is 62 μcal/g.impulse.

These calculations were, however, based on plausible estimates of the resting membrane potential, and using this resting potential is incorrect. For it is now generally accepted that there are fixed charges at the surface of biological membranes whose density may be quite different on the two sides of the membrane, so that the potential across the dielectric itself may differ appreciably from the potential that exists between the bulk solutions on either side of the membrane and wbich is the potential measured by conventional electrophysiological recording electrodes. The earlier calculation of ABBOTT *et al.* (1958) and of HOWARTH *et al.* (1968), which were based on the potential between tho two bulk solutions and not on the potential across the dielectric, clearly have to be modified. The experiments of CHANDLER, HODGKIN and MEVES (1965) and of COHEN, HILLE, HEYNES, LANDOWNE and ROJAS (1971) suggest that the distribution of fixed charges on the two sides of the membrane may be sufficiently asymmetrical to make the inner surface 70 mV more negative than the outer surface even when the net potential difference between the bulk phases is zero. Figure 5, for example, shows how the net PD across the membrane may reverse from v_1 to v_2, yielding on the basis of the earlier calculations a difference in the free energy levels in the two states,

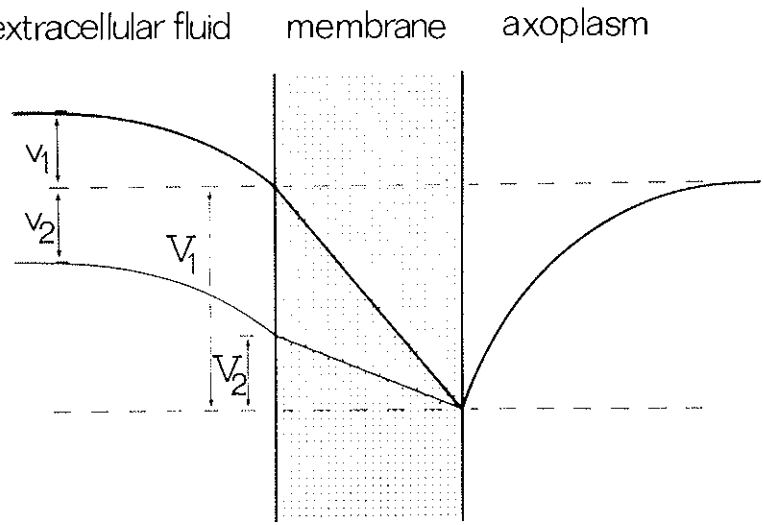

FIG. 5 — Hypothetical diagram of the effect of fixed negative charges in the nerve membrane on the electric fields across the membrane. The lines depict the potential profile in the immediate vicinity of the membrane on either side. A linear drop in potential across the membrane is assumed. An asymmetry in the fixed charge distribution is also postulated: when the potential difference between the bulk of the solutions on either side is zero, the axoplasmic side remains 70 mV more negative than the extracellular side of the membrane. v_1 and v_2 are potentials of the extracellular fluid referred to the potential of the axoplasm. V_1 and V_2 are potentials referred to the inner surface of the nerve membrane. The figure also illustrates that even although the recorded membrane potential may reverse (going from v_1 to v_2) the potential across the dielectric (V_2) need not.

i.e. $1/2\ C(v_1^2 - v_2^2)$, of about zero if v_2 is approximately equal to $-v_1$. The actual voltage across the dielectric, however, falls from V_1 to V_2. The true free energy change, $1/2\ C\ (V_1^2 - V_2^2)$, clearly differs substantially from the apparent change erroneously calculated as $1/2\ C\ (v_1^2 - v_2^2)$. In pike nerve, for example, if the net PD changes from -40 mV to zero, the free energy released calculated on the basis of the resting potential would be about 80 μcal/g. But if there were a 70 mV

asymmetry, so that the potential across the dielectric actually goes from − 110 to − 70 mV, as determined for squid axons by COHEN *et al.* (1971), the corresponding calculation would suggest a much increased heat, 36 μcal/g, which would still not be quite enough to account for the initial positive heat (Table 1).

If the same values of the asymmetry potential used above for the pike nerve were applied to the rabbit vagus, which has less than a sixth as much membrane per gram, a free energy change of 7.6 μcal/g.impulse would be expected. Clearly this is still so much smaller than the observed positive initial heat, which is 24.5 μcal/g.impulse (HOWARTH *et al.* 1968), that it is still necessary to invoke some additional contribution to the nerve heat.

Entropy changes.

On general grounds the extremely large changes in voltage gradient associated with the action potential, amounting to about 10^5 V/cm across the nerve membrane, would be expected to be accompanied by some molecular rearrangement, and hence some change in entropy, of the phospholipid nerve membrane. Such changes in entropy ought in principle be easy to detect for they should be reflected in a temperature-dependence of the dielectric constant (ε) of the membrane material. Quantitatively, these changes in entropy with depolarization must lead (GURNEY, 1962; HOWARTH *et al.* 1968; RITCHIE, 1973) to an extra evolution of, or absorption of, heat equal to

$$(T/\varepsilon)\cdot(d\varepsilon/dT)\cdot\Delta F$$

depending on whether $d\varepsilon/dT$ is positive or negative (ΔF being the free energy change). Figure 6, which is based on FRÖLICH (1958), shows how the dielectric constant of a material in general varies with temperature. At extremely low tempera-

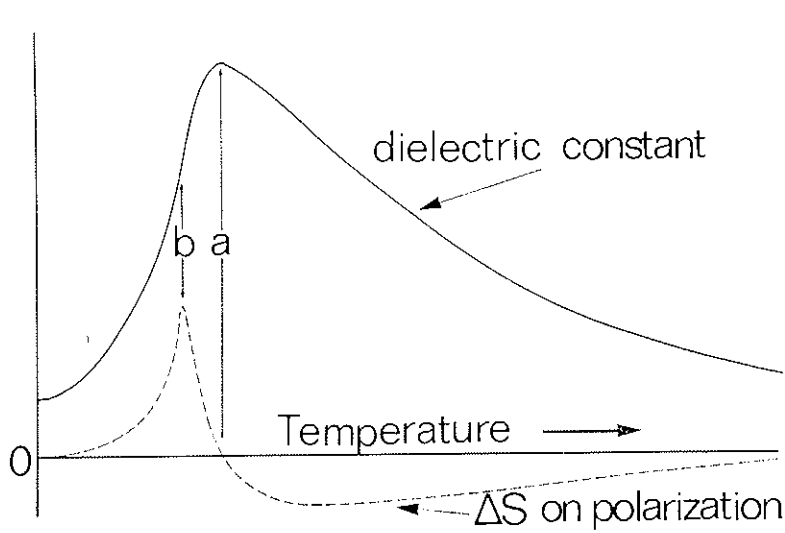

FIG. 6 — The variation of dielectric constant, ε, with temperature. The origin is at zero degrees absolute. Also shown (- - - - -) are the predicted entropy changes (ΔS) produced by polarizing a condenser using the material as dielectric (increase upwards). The arrow *a* marks the peak of the curve and indicates where the *amount* of heat released on depolarization changes from positive to negative. The arrow *b* marks the point of inflection of the rising phase of the curve and indicates where the *temperature coefficient* of the heat released on depolarization changes from positive to negative. Taken from FRÖLICH (1958).

tures near ahsolute zero, while the material remains highly crystalline or highly ordered, $d\varepsilon/dT$ is positive (left-hand side); for most common materials at more usual temperatures, however, $d\varepsilon/dT$ is negative (right-hand side).

What contribution can a change in entropy make to the initial heat production? Reference to Fig. 6 illustrates that for materials operating to the left of a critical temperature (arrow *a*) $d\varepsilon/dT$ is positive so that removal of an electric field necessarily leads to an increase in molecular ordering (with a decrease in entropy) and hence an evolution of heat. Restoration of the electrical field would lead to a corresponding

absorption of heat. The question thus becomes: Where on the ε/T curve do nerve membranes normally operate? This question has been answered for squid giant axon. Measurements of the temperature dependence of the membrane capacity show that the temperature coefficient is positive (TAYLOR and CHANDLER, 1962; PALTI and ADELMAN, 1969). This means that the entropy factor will modify the measured initial heat in nerve in the right way, namely to increase it by a factor of 2-4 (see RITCHIE, 1973 for more details).

For rabbit nerve the entropy factor alone (i.e. ignoring fixed charge effects) would raise the expected positive initial heat to 3.6 - 6.0 μcal/g.impulse for a potential change of 40 mV: for a potential change of 80 mV the corresponding value would be 14.1 - 23.5 μcal/g.impulse. For pike nerve, where the potential change cannot be more than 40 mV, the expected positive initial heat would be about 24 - 40 μcal/g. impulse. It seems therefore that (except when the improbably high value of 80 mV is used for the rabbit nerve) the entropy effect by itself is insufficient to account for the discrepancy between the observed initial heat (25 μcal/g.impulse for the rabbit nerve and 62 μcal/g.impulse for the pike nerve) and that calculated on the basis of the free energy change. It is necessary therefore to invoke both the entropy factor and the effect of surface charge described above. The relevant values are summarised in Table 2.

THE EFFECT OF TEMPERATURE

Since both the membrane capacity (TAYLOR and CHANDLER, 1962; PALTI and ADELMAN, 1969, 1969) and the membrane potential (HODGKIN and KATZ, 1949) depend on temperature, both the free energy change on depolarization and the change in entropy would be expected to be temperature-dependent. Although it is not possible to measure the heat production in squid giant axon, it has been calculated (RITCHIE, 1973) that

TABLE 2

Nerve preparation (*)	Rabbit vagus nerve	Pike olfactory nerve
Observed enthalpy change (μcal/g. impulse)	24.5	62
Free energy change, $\Psi = 0$mV (μcal/g. impulse)	1.2 (4.7)	8
Free energy change, $\Psi = -70$mV (μcal/g. impulse)	5.2 (21)	36
Free energy plus entropy change, $\Psi = 0$mV (μcal/g. impulse)	3.6-6.0 (14-24)	24-40

(*) Rabbit, HOWARTH *et al.* (1968); Pike, HOWARTH *et al.* (1975).
The calculated free energy and entropy changes are based on a resting potential of −40mV (and those within parentheses on a resting potential of −80mV). The value for Ψ is the difference between the surface potential on the two sides of the membrane.

the positive initial heat in this axon should decrease when the temperature is raised from 5 to 15°C (by a factor of 0.44). This theoretical prediction for squid nerve agrees well with the experimental observation in rabbit nerve, that increasing the temperature 10°C reduces the positive heat (by a factor of 0.54).

However, the situation is complex (Fig. 6). To get heat in excess of that simply predicted by the free energy changes one clearly must be operating to the left of arrow *a*. If the operating temperature is quite far to the left (in fact to the left of the point of inflexion in the ε/T curve, i.e. arrow *b*) the entropy contribution will increase with increasing temperature. On the other hand, if the operating temperature is between arrows *a* and *b*, the contribution from entropy will decrease with decreasing temperature. That these considerations are relevant is suggested by the findings that whereas in rabbit nerve the positive initial heat decreases with de-

creasing temperature, in the pike nerve the reverse is true: in pike nerve a rise of 10°C (from 0°C to 10°C) increases the positive initial heat by a factor of 1.86 (HOWARTH *et al.* 1975).

It has been calculated (RITCHIE, 1973) on the basis of electrical measurements that in squid giant axons whereas increasing the temperature by 10°C, from 5°C to 15°C would decrease the positive initial heat by a factor of 2.25, further increments of 10°C would reduce it by smaller factors, of 1.60 (15-25°C) and 1.04 (25-35°C). It would clearly be of great interest to test this theoretical prediction, that the temperature-dependence of the initial heat is itself temperature-dependent. Such experiments are as yet unfortunately not possible on squid giant axons. But they could easily be done on pike or garfish olfactory nerves.

How Many Phases of Initial Heat are There?

Both the original condenser theory (ABBOTT *et al.* 1958) and its modification by HOWARTH *et al.* (1968) to include an entropy term might under some circumstances require that the two phases of initial heat originally described by ABBOTT *et al.* (1958) and by HOWARTH *et al.* (1968) be replaced by four phases: a first positive phase due to an evolution of heat as the resting potential falls to zero; a first negative phase caused by a reabsorption of heat as the membrane potential overshoots or reverses to the peak of the action potential; a second positive phase as heat is evolved when the membrane potential returns to zero again; and finally, a second negative phase as heat is reabsorbed when the membrane repolarizes to the resting potential again. Although there is an isolated report of four phases of initial heat production (ABBOTT, HOWARTH and MATSUMOTO, 1970), in practice only two phases of heat production are ever required in the heat block analyses of thermal records (crab: ABBOTT *et al.* 1958; lobster: ABBOTT *et al.* 1965; rabbit: HOWARTH *et al.* 1968; pike: HOWARTH *et al.* 1975). The simplest explanation is

probably that the true potential across the membrane dielectric does not reverse, even although the potential between the bulk solutions does, because of the presence of fixed charges in the membrane (Fig. 5). Thus, even if the membrane potential recorded by conventional electrodes reversed from − 40 to + 40 mV, the potential across the dielectric might not, merely changing from − 110 to − 30 mV. In this case, clearly the initial heat production would be expected to be diphasic and not quadriphasic.

The experiments of CHANDLER *et al.* (1965) and COHEN *et al.* (1971) argue strongly for the presence of a potential, of about − 70 mV in squid giant axons, across the membrane dielectric caused by the asymmetrical distribution of fixed charges. It seems highly likely, therefore, that the argument presented above prevails, and that there are indeed only two phases of initial heat. However, even if four phases were present, it is doubtful wbether the present equipment could resolve them unless they were larger and better separated in time than seems likely. HOWARTH *et al.* (1975) constructed synthetic thermal records from the heating control using a sequence of heat blocks that they chose to be *tri*phasic. However, they showed it was relatively easy to analyze the resultant records in terms of just a single phase of heat production. Thus, since a known triphasic record can be analyzed in terms of a single phase, a quadriphasic record might easily be erroneously analyzed in terms of two phases. What the analysis of HOWARTH *et al.* (1975) means is that in the light of current speculation on surface charge, and the presently available slow recording equipment, there is neither the theoretical expectation of four phases of initial heat nor the practical ability to resolve them even if they existed.

MYELINATED FIBRES

On general grounds the initial heat production, reflecting changes in the free energy and entropy of a membrane when

the potential across it alters, would be expected to depend on the amount of excitable membrane present in the tissue. This is why small non-myelinated fibres have been used almost exclusively to study the initial heat: the smaller the diameter of a cylindrical axon the greater is its surface/volume ratio. Each gram of rabbit vagus nerve contains 6000 cm^2 excitable membrane (KEYNES and RITCHIE, 1965). The corresponding values for garfish and for pike olfactory nerves are 65,000 and 41,000 cm^2/g respectively (EASTON, 1971; VON MURALT *et al.* 1975). For crab nerve Table 3 of ABBOTT *et al.* (1958) gives a value of 1.12×10^4 cm^2/g uncorrected for extracellular space. With an extracellular volume of 0.31 (KEYNES, RITCHIE and ROJAS, 1971), the practical value would be about 8000 cm^2/g wet. Myelinated fibres, on the other hand, have, at the most generous estimate, an area of exposed nodal membrane of less than 4 cm^2/g (ABBOTT *et al.* 1958). The positive initial heat, therefore, if it were proportional to the relative amount of membrane in the preparation should (on the basis of the findings in the rabbit vagus) be about $24.5 \times 4/6000$ μcal/g.impulse, i.e. about 0.02 μcal/g.impulse.

It is difficult to determine experimentally a reliable value for the positive initial heat in myelinated fibres to compare with this predicted value. Because of the rapidity of the electrical response in myelinated fibres the recorded early positive heat might reflect only the net initial heat, and at best would provide just a lower limit for the positive initial heat. Even so, the experimental values, at first sight, seem disproportionately high: ABBOTT *et al.* (1958) found a value of 0.3 μcal/g.impulse for the *net* initial heat, and KEYNES and RITCHIE (1970) found a value of 0.8 μcal/g.impulse, for the early positive heat, in frog myelinated nerve. This latter experimental value, which must still be smaller than the true positive initial heat, is fifty times that predicted above.

However, there need be no discrepancy between the observed heats in myelinated and non-myelinated fibres. For

the membrane capacity of myelinated fibre may be considerably greater than the 1 $\mu F/cm^2$ assumed for non-myelinated nerve (e.g. by: HOWARTH *et al.* 1968; HOWARTH *et al.* 1975). Indeed, TASAKI (1955) has shown that the capacity of a single frog node is about 1.5 pF, which is in parallel with a capacity of 3-4 pF in the myelin sheath. This latter capacity is, like the nodal capacity, discharged and recharged during tbe action potential (HODLER, STÄMPFLI and TASAKI, 1952). The total capacity thus about 8 times that calculated for a node composed of 60 μm^2 membrane (see ABBOTT *et al.* 1958) with a capacitance of 1 $\mu F/cm^2$ (i.e. 0.6 pF per node). Furthermore, the action potential in myelinated fibres, which is over 100 mV (STÄMPFLI, 1954), may be a good deal larger than in non-myelinated nerve where it has been postulated to be perhaps as low as 40 mV (RITCHIE and STRAUB, 1975). Since the voltage (V) appears in both the free energy and entropy terms as V^2, the heat changes in the myelinated fibres might be predicted to be greater than those in non-myelinated fibres by a further factor of about 6. Expressed per unit area of nodal membrane, threfore, the positive initial heat in myelinated nerves might be expected to be up to fifty as large as that in non-myelinated nerve. That this factor is in such remarkably good agreement with experimental findings is probably fortuitous: nevertheless, it is clear that there is no evidence that the positive initial heats in myelinated and non-myelinated fibres are in any way discrepant.

NET INITIAL HEAT AND RECOVERY HEAT PRODUCTION

Following the early initial heat production in nerve there is a long period (measured in minutes) during which heat is produced at a relatively small rate. In the nonmyelinated fibres of the rabbit vagus nerve the average heat evolved in the 200 seconds after a period of brief stimulation is about

100 μcal/g.impulse (HOWARTH *et al.* 1968). This heat production agrees well quantitatively with predictions from measurements of the oxygen consumption (RITCHIE, 1973). The late heat production is in fact the recovery heat production associated with the metabolic processes involved in restoring the ionic gradients that have been disturbed by the passage of the impulse.

Although the total recovery heat is not large, its rate of production is probably greatest soon after the action potential and it might well contribute to the initial heat production. That it does in fact do so is indicated by experiments in which the sodium of Locke solution has been replaced by lithium. The action potential is relatively unaffected by this procedure; but the lithium that enters the fibres during the early phase of the action potential is not subsequently pumped out. There is, therefore, no enhancement of the activity of the sodium pump, and consequently no extra metabolism. Since the action potential is more or less normal in lithium-Locke solution, it is not surprising that the initial heat production (Fig. 7) is not much affected by the replacement procedure: the positive initial heat, for example, is found to be reduced by only 20% and the negative initial heat increased by about the same small amount. However, consistent with the absence of stimulation of the recovery mechanism there is a marked reduction in the late heat that is prominent for several seconds after the impulse in ordinary Locke solution.

A small fraction of the net initial heat that is recorded after the initial rapid rise and fall in nerve temperature can thus be attributed to the early onset of recovery heat. However, a major, but volatile, contribution seems clearly to be determined by the potential to which the membrane recovers immediately after the action potential. Conditions that favour the appearance of a negative afterpotential (increasing temperature, barium, repetitive activity) increase the net heat. On

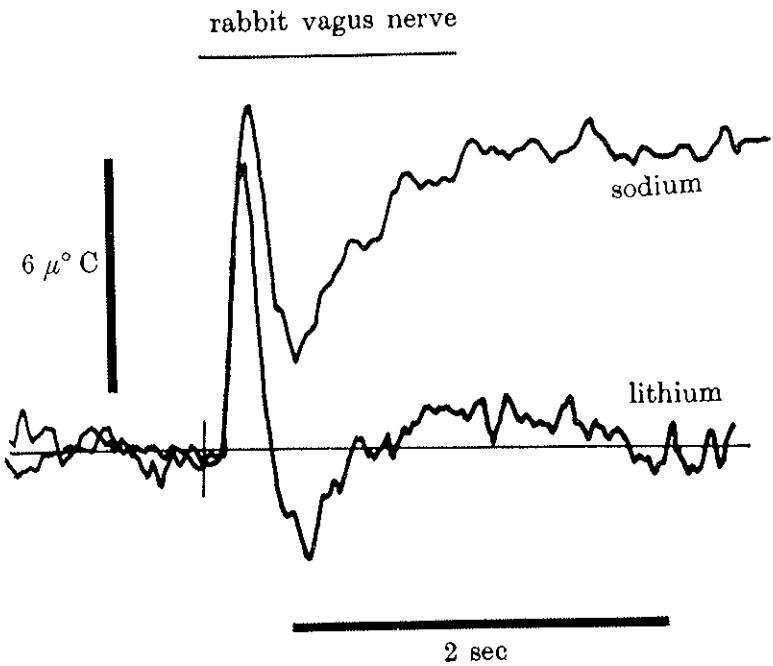

FIG. 7 — The effect of lithium on the initial heat production of the non-myelinated fibres of a rabbit desheathed vagus nerve at about 5°C. The « lithium » record was taken after equilibration in a solution in which all the NaCl had been replaced by LiCl. Taken from HOWARTH et al. (1968).

the other hand, procedures that favour the appearance of a positive afterpotential (cooling to O°C, potassium-free solutions) reduce the net heat and may in fact change it from being positive to being negative (HOWARTH *et al.* 1968; 1975). There seems little reason therefore to relate the neat heat to any heat of ionic interchange during the action potential, or to believe that it is in any way directly related to the process of excitation.

Conclusions

It has become clear in the last twenty years that small but definite changes in nerve temperature accompany the nervous impulse. These temperature changes are closely related in time to the various phases of the electrical impulse; and the free energy and entropy changes in the membrane (with its asymmetrical distribution of fixed charges) are clearly sufficient to account for them. The original hope in such studies was that the origin of this heat might provide some insight into the mechanism of excitation. However, the above discussion shows that most of the heat seems to be derived from thermodynamic changes in the phospholipid of the membrane generally, and that the thermal studies provide little information on the elements critical for excitability, the sodium channels. The heat production, however, does seem to have provided useful information on the general structural nature of the nerve membrane. For it has been found necessary to suppose that the entropy of the nerve membrane increases when it is polarized, i.e. the membrane becomes thermodynamically more ordered on depolarization. Such behavior is consistent with the results of optical experiments on nerve (Cohen, Hille and Keynes, 1970) and of measurements of the temperature dependence of the membrane capacity (Taylor and Chandler, 1962; Palti and Adelman, 1969). It is, indeed, characteristic of the behavior of a crystalline material, and confirms that the nerve membrane is, despite the fluidity of its interior, a highly ordered structure.

The lack of insight into the mechanism of excitation provided by these studies clearly reflects the sparseness of distribution of the elements critical for excitation on the nerve membrane. Recent studies with tritium-labelled tetrodotoxin and saxitoxin, two toxins that bind specifically to sodium channels, have shown that each square micron of membrane contains only 27 sodium channels in rabbit nerve and about 6

in garfish olfactory nerves. The latter value corresponds with one sodium channel for about every 300,000 phospholipid molecules (RITCHIE, 1975). The thermal changes in nerve are barely greater than the random thermal changes occurring in the tissue and recording apparatus, which is why, even with the highly sensitive thermopiles, signal averaging techniques are necessary to detect them. The recent work on toxin binding to axonal membranes (COLQUHOUN, HENDERSON and RITCHIE, 1972; HENDERSON, RITCHIE and STRICHARTZ, 1973) makes it clear that the sodium channels are so sparsely distributed that any thermal events directly and specifically associated with them are probably several orders of magnitude too small to be recorded with the methods available.

ACKNOWLEDGMENT

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DISCUSSION

Chairman: Prof. J. M. RITCHIE

MEARES

May I ask one question about the electrical energy storage and its dissipation? If the potential that you use is that recorded with microlectrodes situated well outside the membrane then it appears to me, as an outsider to this field, that you have got two double-layer capacities together with a membrane capacity in series. The potential drops across the double layers would be something like the Donnan potentials on each side of the membrane, and then you have, additionally, part of the potential difference across the membrane.

RITCHIE

I agree with your comment on potentials measured with conventional electrodes. I had hoped to have made it clear that this is not the potential we used in calculating the free energy changes.

MEARES

Energy is associated with the double layers, and the double layer structure must change when the action potential occurs. Do you suppose that the dissipation of that energy is not observed in the temperature changes because it is lost immediately to the surroundings?

RITCHIE

It is certainly true that energy is stored in the double layer, additional to that stored in the membrane dielectric. However there is no evidence that the fixed charge distribution on the two sides of the membrane changes during the action potential. There would thus be no change in the double layer free energies, or any contribution from them to the initial heat.

MONNIER

I have been very interested by a paper of yours in which you showed the importance of the small increment of dielectric constant of the nerve membrane with temperature. You showed that taking into account this dielectric constant change with temperature permits a more exact balance between absorbed and liberated heat. We have found that our artificial membranes also increase their capacity with temperature. This is a paradox. The dielectric constant of all insulators decreases with temperature. Perhaps we could imagine an explanation of this paradoxical increase of dielectric constant with temperature. Assuming that the membrane contains a mixture of lipids of gradual softening with temperature. As more material is melted the dielectric constant increases. We made a dielectric study of such mixtures.

RITCHIE

An increase in dielectric constant with temperature is in fact characteristic of a crystalline, highly ordered, material. In this respect the one thing that has emerged from our thermal studies has been a confirmation of something that is already generally believed, namely that the nerve membrane, despite the fluidity of its interior, is still a highly ordered structure.

VII

ARTIFICIAL MEMBRANES: THERMODYNAMICS AND TRANSPORT

INTERPRETATION AND PREDICTION OF THE TRANSPORT PROPERTIES OF CHARGED MEMBRANES USING IRREVERSIBLE THERMODYNAMICS

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The discipline of irriversible thermodynamics provides a precise mathematical description of the processes of transport and diffusion in membrane systems. Its application to membrane processes is a natural development of the basic theory of Onsager and has been developed by STAVERMAN, KEDEM, KATCHALSKY, CAPLAN, MEARES and others in an extensive and expanding literature [1].

The present discussion will be confined to ion exchange polymer membranes under isothermal conditions and excludes the possibility of chemical reaction within the membrane. The simplest practical membrane system consists of a monofunctional exchanger in equilibrium with an aqueous binary electrolyte, [1, 2]. The membrane contains four chemical species, counterion, 1; co-ion, 2; and water, 3. The fourth component is the membrane matrix, which carries fixed ionogenic groups. For the purposes of a thermodynamic description the matrix of polymer is considered as species 4 and the number of moles of 4 is taken to equal that of the fixed ionic groups on the matrix. Species 4 may therefore be considered to consist of

the fixed charge and adjacent polymer segments, which together constitute the repeat units of the matrix. It is therefore possible that kinetic coupling interactions for 4 will include not only the contribution of fixed charge, but also specific polymer effects if such exist. This is an important qualification to the description of species 4 as simply an ion, although it appears from earlier studies [2, 3, 4] that such effects are small, at least in certain polystyrene sulphonic acid membranes.

For slow processes, under conditions not far from equilibrium, the flows of membrane species, $\overline{\overline{J}}_i$, may be related by linear phenomenological equations to the thermodynamic forces in the system. These forces, $\overline{\overline{X}}_i$, are defined by the negative gradients of electrochemical potential within the membrane. The dissipation function Φ is defined as the sum of products of flows and conjugate forces eqn. (1)

$$\Phi = \sum_{i=1}^{4} \overline{\overline{J}}_i \overline{\overline{X}}_i \geqslant 0 \qquad (1)$$

From the Gibbs-Duhem equation it is easily shown that $\sum_{i=1}^{4} \overline{\overline{C}}_i \overline{\overline{X}}_i = 0$ where $\overline{\overline{C}}_i$ is the concentration of i in the membrane, usually in moles cm^{-3} of total membrane volume. The four forces in eqn. (1) are therefore not independent. One may be eliminated. For practical purposes the force on the matrix, $\overline{\overline{X}}_4$, is usually chosen, since the dissipation function may then be defined in terms of the experimentally measured flows, $\overline{\overline{J}}_i^4$, relative to the stationary membrane, eqn. (2).

$$\Phi = \overline{\overline{J}}_1^4 \overline{\overline{X}}_1 + \overline{\overline{J}}_2^4 \overline{\overline{X}}_2 + \overline{\overline{J}}_3^4 \overline{\overline{X}}_3 \geqslant 0 \qquad (2)$$

It is pertinent to note that in studies of electrolyte solutions wbich are formally similar the *natural* frame of reference is stationary solvent. In a meaningful comparison of the transport properties of electrolytes and membranes it will be necessary

to use a common frame of reference and transport parameters such as $\bar{R}_{ik}$ (below), which are independent of frame of reference.

In eqn. (2) flows and forces are independent and, subject to the usual restrictions, linear phenomenological equations may be written in terms of mobility, ($\bar{\ell}_{ik}$), or frictional, ($\bar{R}_{ik}$) coefficients, eqns. (3) and (4).

$$\bar{J}_i^4 = \sum_{k=1}^{3} \bar{\ell}_{ik}\bar{X}_k \qquad i = 1, 2, 3 \qquad (3)$$

or

$$\bar{X}_i = \sum_{k=1}^{3} \bar{R}_{ik}\bar{J}_k^4 \qquad i = 1, 2, 3 \qquad (4)$$

The Onsager reciprocal relations, (O.R.R.), require $\bar{\ell}_{ik} = \bar{\ell}_{ik}$ and $\bar{R}_{ik} = \bar{R}_{ki}$, reducing the number of coefficients to describe all isothermal vectorial transport properties to six.

Although it is more convenient to express measured transport properties such as conductivity in terms of mobility coefficients, (Appendix), frictional coefficients are to be preferred for interpretation. Unlike mobility coefficients they are independent of the frame of reference for flows and frictional coefficients between mobile species and the matrix-4 may be obtained from the summations eqn. (5) [5].

$$\sum_{i=1}^{4} \bar{C}_i\bar{R}_{ik} = 0 \qquad k = 1, 2, 3, 4 \qquad (5)$$

Useful information may be obtained about the relative importance of frictional interactions using the SPIEGLER, [6] frictional coefficient, ($-\bar{C}_i\bar{R}_{ki}$), commonly given the symbol X_{ki} [6] or $\bar{f}_{ki}$, [7] which is the frictional coefficient between one mole of k and those i in unit volume of the environment.

It has the advantage of being analogous to the coefficient of kinetic friction in mechanical terms and represents the frictional force between i and k, when the relative velocity $(V_i - V_k)$ is unity, eqn. (6)

$$\bar{X}_i = \sum_{k=i} \bar{f}_{ik} (V_i - V_k) \qquad i = 1, 2, 3 \qquad (6)$$

From these analyses the relative importance of frictional contributions to measured transport properties may be assessed and the effect of salt uptake, water content, and capacity may force between *i* and *k*, when the relative velocity $(V_i - V_k)$ is be determined. In this way a more fundamental evaluation of the source of functional properties of membranes may be obtained and within the scope of a specific series of experiments on a given membrane, or series of related membranes, more detailed predictions of, for example, concentration profiles or composite membrane properties may be obtained.

Irreversible thermodynamics is a macroscopic discipline. Transport coefficients, obtained experimentally, cannot be calculated from molecular theory and the physical parameters of the system. The only exceptions appear to be very dilute electrolyte solutions in the range of the Onsager limiting law for electrical conductance [8].

The interpretation, and even the prediction of the membrane properties might be advanced if suitable and accessible analogue systems might be found.

The analogy between transport in an aqueous electrolyte and in a charged membrane might be considered. It has proved useful in earlier interpretation of thermodynamic problems such as selectivity [9]. The first choice for a model would fall most naturally on the polyelectrolyte salt solution analogous to the crosslinked polyelectrolyte gel, which constitutes the membrane. Imbibed electrolyte in the membrane would require the model to be a ternary electrolyte and transport could be compared at equal molalities in membrane and model. There are

however insufficient data on the transport properties of polyelectrolyte solutions and their ternary mixtures with simple salts to allow meaningful calculations at this time. The attraction of such models may stimulate such work and provided the model polyelectrolyte does not undergo conformational or other changes, which are not allowed in the crosslinked membrane, the model should be excellent.

For the time being, therefore, model systems must remain more modest and this discussion will adopt a limiting or extreme position by assuming that the properties of an ion exchange polymer membrane are similar to simple aqueous electrolyte. Much of the discussion will concern the observed properties of AMF C60 polystyrene sulphonic acid membranes in the sodium form containing sodium chloride as invading salt. As a first step towards an analogue system it is proposed that the sodium polystyrene sulpbonic acid membrane may be modelled by an equimolal solution of sodium chloride. Although the theories developed are applied to several membrane systems and to various ionic forms with apparent success, the salt model calculation presented below was developed primarily from observations on tbe C60 systems, although it is to be hoped that the results and conclusions drawn will have more general application to the field of membrane transport and gel permeation.

The AMF C60 membrane (American Machine and Foundry Co.) is prepared from low-density polyethylene, containing 35% styrene and 2% divinylbenzene. The sulphonating agent is oleum. Using the method of ARNOLD and KOCH [10], an expanded form C60E was prepared by immersing the membrane in water at 95°C for thirty minutes. The physical properties of the normal, C60N, and expanded, C60E, membranes in solutions of sodium cbloride in the range 0.1-2.0M are given in Table 1 [3]. The more expanded membrane takes up more salt, $\bar{C}_2$, at each concentration and salt uptake ranges from 0.2% - 22% (of the total capacity) for the system. The results of electrical and isotopic diffusional studies are shown in

TABLE 1 — *Physical characteristics of AMF C60 membranes* [3].

C60N: Dry weight of leached membrane disc in Na form = 0.2345 g, Ion-exchange capacity = 1.57 mequiv. g^{-1} dry membrane.

ext. soln. NaCl/M	wet wt./g	% water w.r.t. dry wt.	diameter/ cm	thickness/ cm	volume/ cm^3	$\overline{C}_1$	$\overline{C}_2$	$\overline{C}_3$
						mequiv. cm^{-3}		
0.1	0.3579	52.6	3.698	0.0335	0.360	0.980	0.0024	19.03
0.5	0.3449	46.7	3.655	0.0314	0.329	1.104	0.0360	18.48
1.0	0.3320	40.8	3.611	0.0308	0.315	1.209	0.0946	16.86
2.0	0.3176	33.7	3.551	0.0299	0.296	1.419	0.2312	14.89

C60E: Dry weight of leached membrane disc in Na form = 0.2249 g, Ion-exchange capacity = 1.70 mequiv. g^{-1} dry membrane.

ext. soln. NaCl/M	wet wt./g	% water w.r.t. dry wt.	diameter/ cm	thickness/ cm	volume/ cm^3	$\overline{C}_1$	$\overline{C}_2$	$\overline{C}_3$
						mequiv. cm^{-3}		
0.1	0.3998	77.7	3.847	0.0333	0.387	0.960	0.0052	25.07
0.5	0.3807	68.6	3.754	0.0321	0.355	1.112	0.0717	24.11
1.0	0.3629	59.8	3.697	0.0317	0.340	1.270	0.1832	21.94
2.0	0.3470	50.5	3.616	0.0308	0.316	1.621	0.4533	19.95

Figs. (1-4). The flows of ions and water are larger in the more open structure of the expanded membrane and in general decrease in both as the membrane shrinks in more concentrated solution in part at least due to the increased obstruction of the polymeric matrix as diffusional pathways become more tortuous and the fractional pore volume decreases. In electrical conductivity, Fig. 1, there is the added effect of increased salt uptake, which should increase conductivity. These two opposing effects almost cancel for C60N above 0.5M NaCl (ext), but increased salt uptake in the C60E membrane more than balances the effect of increased tortuosity and, at higher concentrations, specific conductivity increases. Isotopic diffusional coefficients for counterion, co-ion and water (using tritiated water) show similar diversity between C60N and C60E and there is little to suggest the common source of these membranes at this stage, Fig. 2. When scaled by the tortuosity factor, θ_m, (the value calculated by MEARES [11] for *path length* tortuosity) the two sets of data for C60N and C60E effectively coincide and are close to the corresponding value for sodium [12], chloride [12] and tritiated water [22] in equimolal solutions of aqueous sodium chloride, Fig. 3a. (Since θ_m is used outwith the terms of reference of MEARES' derivation [11] it is to be noted that using PRAGER'S estimate, θ_p [14], (which is obtained from a more generalised treatment), alternative values, $D_{ii}\theta_p$, are some 15% lower), Fig. 3b. In either case these purely geometric scaling factors bring C60N and C60E data into correspondence and to good agreement with equimolal sodium chloride. Values for sodium counterion, $\bar{D}_{II}\theta$, are lower than for sodium chloride and this point can be considered when isotope-isotope effects are discussed in later sections.

Electro-osmotic transference numbers, t_3, defined by eqn. (7), measure the number of moles of water transferred across the membrane by one Faraday of electricity.

$$t_3 = \bar{J}_3{}^4F/I \qquad (7)$$
$$I = \text{amp cm}^{-2}$$

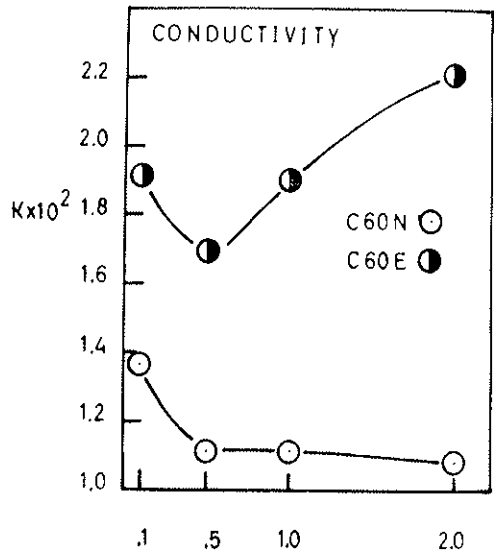

FIG. 1 — Specific conductivities (⊙, C60N; ◑, C60E) as functions of external concentration of salt [3].

Experimental values range from 15.77 for C60E (0.1M) to 5.48 for C60N (2.0M), Fig. 4. At corresponding external salt concentrations the transference numbers for C60E are greater than for C60N, but plotted against molality of electrolyte in the membrane, both sets combine in a single curve, Fig. 5. Transference numbers are independent of tortuosity, since they are defined by the ratio of flows, but are, in this system, dependent on total membrane molality and not the proportion of free salt. SPIEGLER [6] has shown that a large number of exchangers obey the empirical equation $t_3 = \beta \bar{C}_3/\bar{C}_1$, where $\beta \approx 0.5$. Fig. 6, shows that the ratio t_3/t_1 is a linear function of the concentration ratio $\bar{C}_3/\bar{C}_1$ and has slope 0.576. Once more the properties of normal and expanded membranes are shown to obey a common law and it is of interest to examine the significance of this relationship.

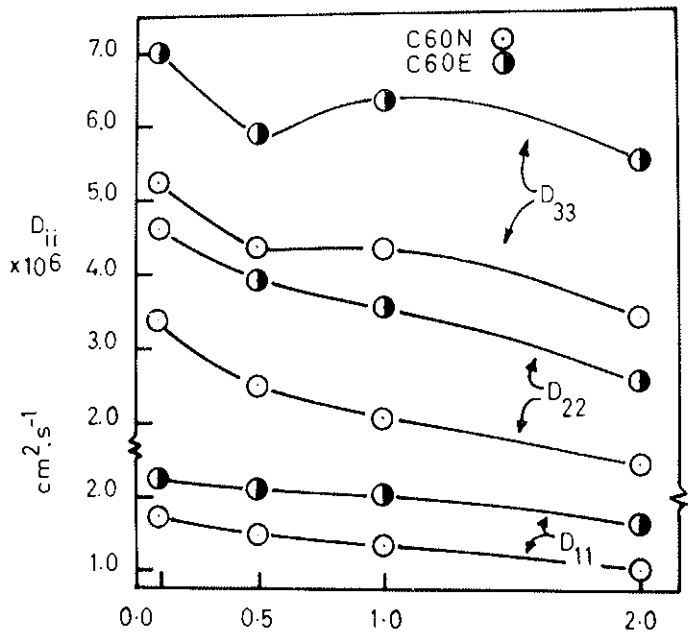

FIG. 2 — Variation of self-diffusion coefficients for water, $\bar{D}_{33}$; co-ion, $\bar{D}_{22}$; and counterion, $\bar{D}_{11}$ as functions of external salt concentration. ⊙ and ◑, denote C60N and C60E membranes respectively [3].

The definitions of t_3 and t_1 as $J_3{}^4F/I$ and $Z_1J_1{}^4F/I$ respectively may be expanded in terms of concentrations $\bar{C}_i$ and velocities relative to the fixed membrane, $V_i{}^4$. Since $\bar{J}_i = \bar{C}_iV_i{}^4$

$$t_3/t_1 = \bar{C}_3V_3{}^4/Z_1\bar{C}_1V_1{}^4 \qquad (8)$$

where Z_i is the signed valency of the counterion 1. The ratio $V_3{}^4/V_1{}^4$ is therefore a constant (0.576) for both membranes under all conditions studied and in agreement with the results quoted by SPIEGLER, where, in general counterion transport numbers might be expected to be close to unity. To examine

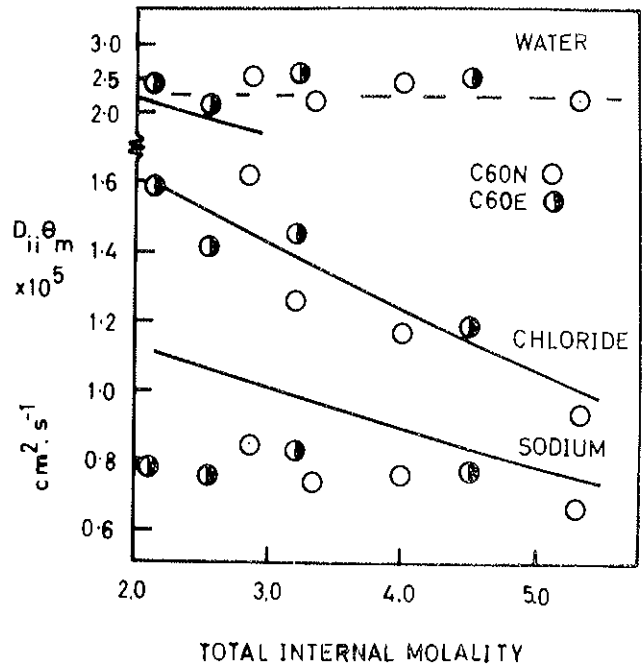

FIG. 3a — Tortuosity corrected diffusion coefficient $\bar{D}_{ii}\theta_m$, compared with those of water, chloride and sodium ions in equimolal solutions of sodium chloride; solid lines. ⊙ and ◑ denote C60N and C60E membranes respectively.

the analogy with aqueous solution the velocities V_i^4 may be converted to a solvent-fixed frame of reference V_i^3. Then

$$V_3^4/V_1^4 = -V_4^3/(V_1^3 - V_4^3) = 0.576$$

and so the ratio of the velocities of sulphonate-matrix to sodium, $-V^4_3/V_1^3 = 1.36$. This shows that these two ions have similar velocities relative to water solvent. The sulphonate-matrix ion has therefore much in common with a simple order destroying anion (such as nitrate or chloride) which have somewhat larger mobilities than sodium in aqueous solutions in

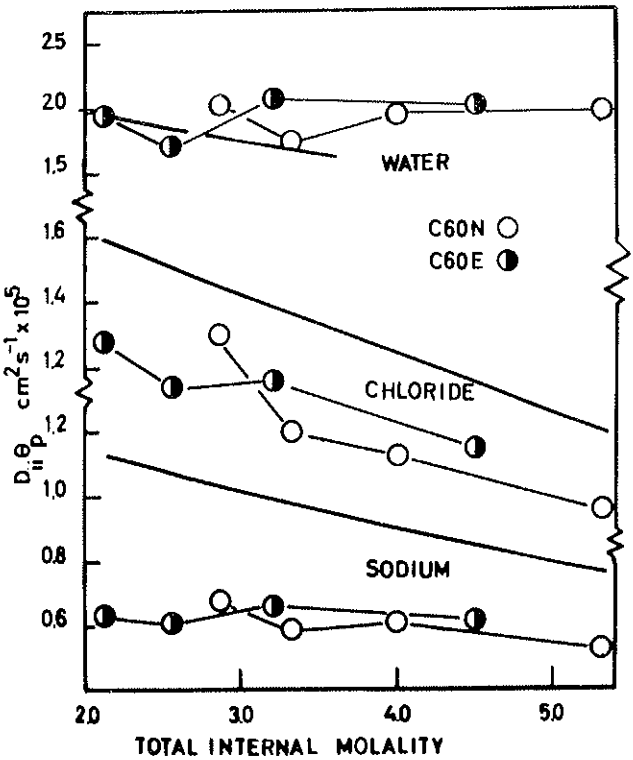

FIG. 3b — Data as for Figure 3a but using Prager's tortuosity coefficient, θ_p.

the same concentration range as internal electrolyte concentrations in the membranes. In concentrated sodium chloride the mobility ratio $-V^3_{Cl}/V^3_{Na} = 1.70$. The fact that increasing concentrations of salt in the membranes, up to 22%, has no effect upon the mobility ratio is fair indication that, from a kinetic standpoint, sulphonate and chloride are broadly similar.

The linear relationship between t_3 and $\bar{C}_3/C_1$, Fig. 6, is not confined to C60 membranes, more recent studies with the chloride form of a quaternary ammonium (anion) exchange membrane [15] show similar linearity, Fig. 7. In that case the

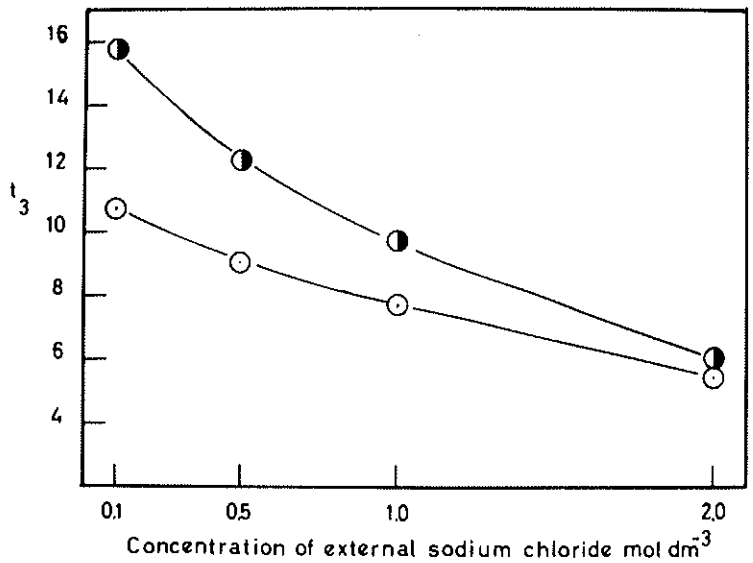

FIG. 4 — Electro-osmotic transference number, t_3, for C60N, ⊙, and C60E, ◑, membranes as functions of external sodium chloride concentrations, mol dm^{-3}.

slope is < 0.5 (0.37_4) indicating that on a solvent-fixed frame of reference the velocity (mobility) of the quaternary ammonium group would be less than for chloride (counterion), as might be expected.

Using frictional coefficients an equivalent interpretation may be obtained. It can be shown easily that the slope of the t_3 against $\bar{C}_3/\bar{C}_1$ is a function of the ion-water functional coefficients of counterion, $\bar{f}_{13}$, and fixed charge, $\bar{f}_{43}$, eqn. (9).

$$\frac{Z_1 t_3}{t_1} = \frac{\bar{C}_3}{\bar{C}_1} \frac{\bar{f}_{13}}{\bar{f}_{13}+\bar{f}_{43}}, \qquad (t_1 = 1) \qquad (9)$$

This equation is strictly applicable to a salt-free membrane in which the counterion transport number is unity. [This con-

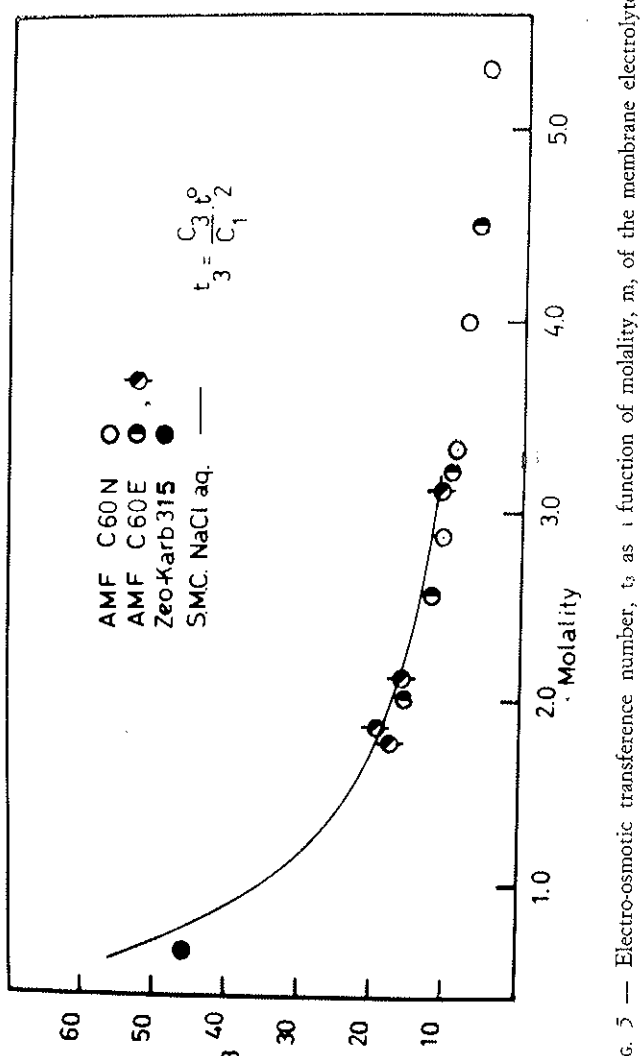

FIG. 5 — Electro-osmotic transference number, t_3 as a function of molality, m, of the membrane electrolyte.

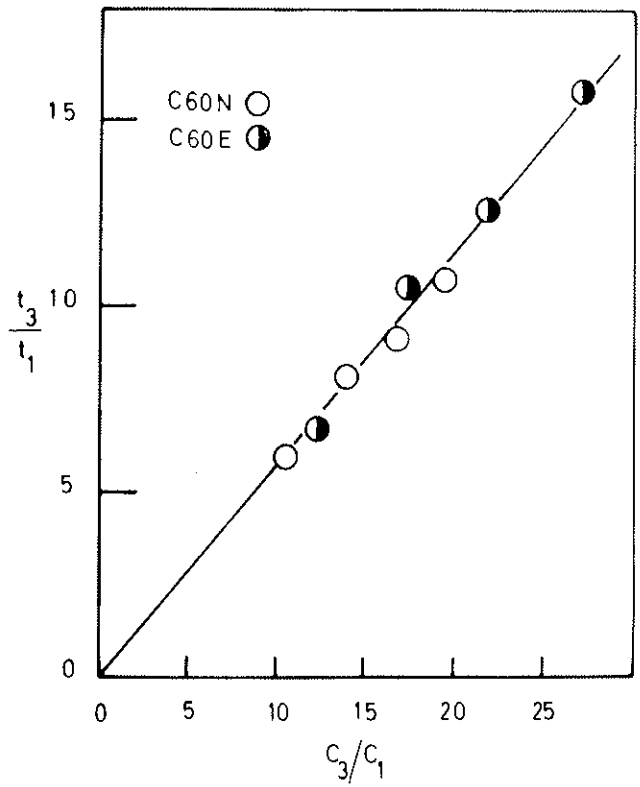

FIG. 6 — t_3/t_1 against $\overline{C}_3/\overline{C}_1$: ○, C60N; ◑, C60E [3].

dition is met effectively by the C60 membranes in O.1M solution and by the quaternary ammonium exchanger [15]]. For the C60 (sulphonate) membranes a slope of greater than 0.5 indicates that the sodium friction with water, $\overline{f}_{13}$, is larger than for sulphonate, $\overline{f}_{43}$. Again the membrane ions behave in the manner expected from a knowledge of simple electrolytes [16], with the larger sulphonate ion, 4, having the lower water friction. The transport number of anion 4 on a solvent fixed frame of reference (in a binary electrolyte or a salt-free mem-

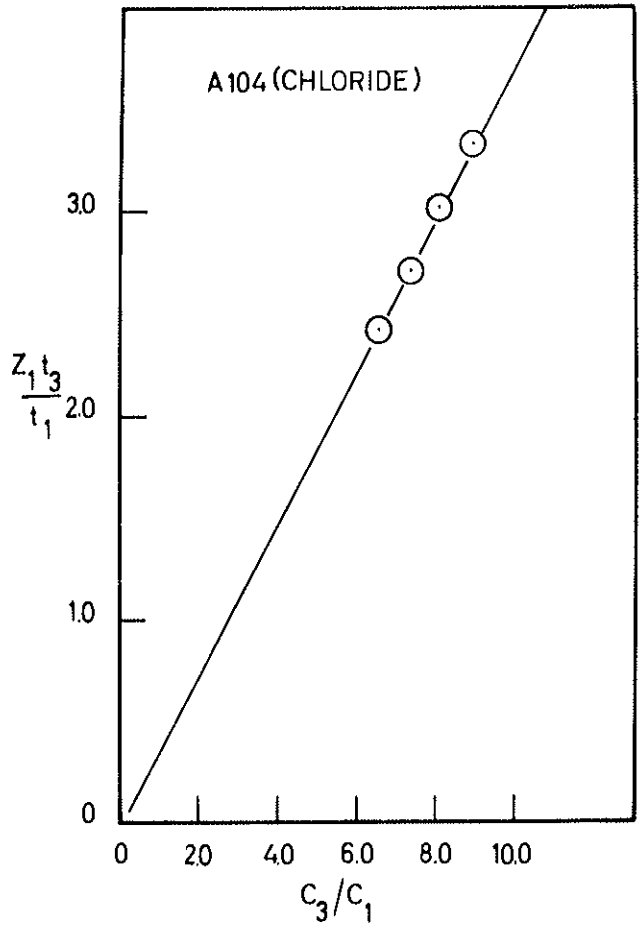

FIG. 7 — $Z_1 t_3/t_1$ against $\overline{C}_3/\overline{C}_1$ for the anion exchanger AMF A104 in the chloride form, reference [15].

brane), t^3_4, is given by eqn. (10), for a 1:1 salt (both ions univalent) [17].

$$t^3_4 = f_{13}/(f_{13}+f_{43}) \qquad (10)$$

Anion transport numbers are available for many simple electrolyte solutions at molal concentrations equal to those of component ions in a test membrane. We may therefore compare observed electro-osmotic transference numbers, t_3, for a membrane with those predicted from aqueous electrolyte transport numbers of a suitable series of salts. [By changing the frame of reference of the electrolyte data to an anion-fixed frame of reference a transference number for water is defined, eqn. (9) (10)].

The results are shown in Fig. 8, where experimental transference numbers for C60 membranes (in various univalent forms) are compared with those calculated using the equivalent aqueous chloride electrolytes as models. In all cases experimental data was obtained with dilute external electrolyte, (0.1M), when the exchanger would be effectively salt-free using a C60N membrane. Sodium data is, as presented in Fig. 6. It is observed that there is excellent agreement between the results for the hydrogen form and for hydrochloric acid. For the alkali chlorides the salt model calculation underestimates transference numbers by between 5 and 15%. The exceptions are for the rubidium and caesium forms in which the observed membrane data are anomalously high and close to the limiting value ($t_3 = \bar{\bar{C}}_3/\bar{C}_1$, Fig. 8) (*).

For the C60 membranes there is therefore good evidence that frictional interaction between sulphonate-membrane and water,

(*) This slope of unity is the maximum possible, for then $\bar{r}_{13} >> \bar{r}_{43}$. There is effectively no friction between matrix fixed charge and water and consequently complete coupling between the counterion under an electrical force and the water. Alternatively, $-V_3'/V_1'$ cannot be larger than unity since the water, 3, cannot migrate faster than the ion, 1, which causes its motion by coupling.

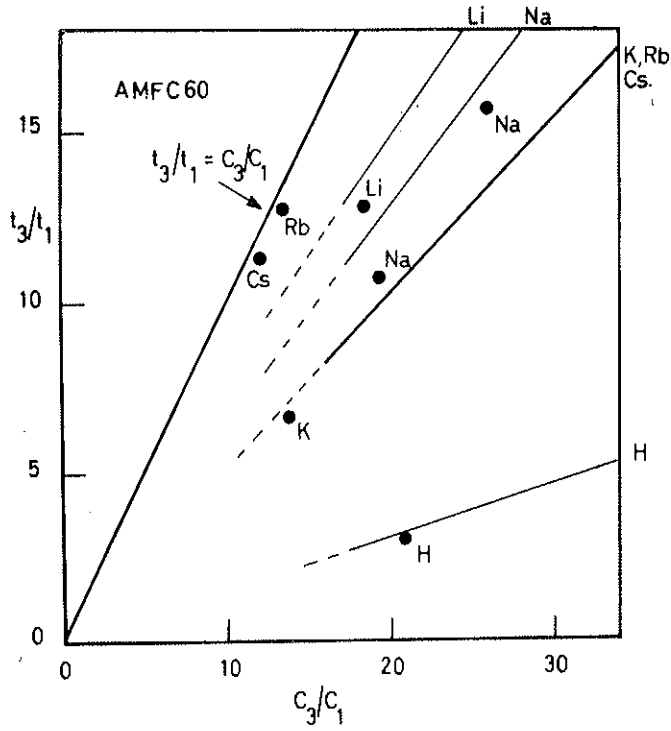

FIG. 8 — Electro-osmotic transference numbers for ionic forms of C60E. Solid lines represent predicted behaviour, from the S.M.C., circles experimental points for ionic forms.

$\bar{f}_{43}$, are similar to those of chloride ion in simple aqueous electrolytes. The basic difference appears to be that the sulphonate has a relatively larger friction with water. The C60 membranes are based upon polystyrene sulphonic acid in which the styrene is effectively electron-donating to the substituted sulphonate. It was therefore of interest to examine another sulphonate membrane in which the polymeric backbone would be electron withdrawing. This would lower the electron density on the sulphonate and render it more solvent-order destroying in the GURNEY

sense [16]. It might then be expected that $\bar{f}_{43}$ would now be reduced and the comparison with chloride salts be more exact. The Nafion membrane (Du Pont) was chosen. This is a perfluorosulphonic acid membrane with exceptional chemical and thermal stability. The electron withdrawing power of the adjacent fluorides on fully substituted methylene groups will reduce the effective change on the ($-SO_3^-$) functional group, below.

$$[-(CF_2CF_2-)_x\ (CF_2-CF)_y-]_n$$
$$[OCF_2CF-]_z\ OCF_2CF_2-SO_3^-$$
$$CF_3$$

Two Nafion membranes were studies [18], which differed slightly in dry weight capacity and consequently swelled to different degrees in the same external electrolyte. In each case transference numbers were measured in 0.1M external electrolyte when the membranes contained effectively no invading electrolyte. The results are shown in Fig. 9. As predicted, above, the Nafion membranes are much more accurately modelled by the chloride electrolyte analogues and in all cases [except caesium, once more] the two Nafion membranes have the expected linear relationship between t_3 and $\bar{C}_3/\bar{C}_1$ with slopes corresponding to the transport number of chloride, t^3_{Cl}, relative to water which is the usual reference for solution studies. The basic similarities between membrane and solution have been established, but, before expanding and developing a purely salt model calculation, it is useful to consider the application methods for predicting ternary solution data for electrolytes to the membrane problem. Two application will be considered. The effect of salt uptake on membrane performance and the electrical conductivity of mixed ionic forms.

Calculation of Membrane Properties for C60E (1.0) using Ternary Solution Analogues: Irreversible thermodynamic analyses for C60N and C60E membranes have been obtained in

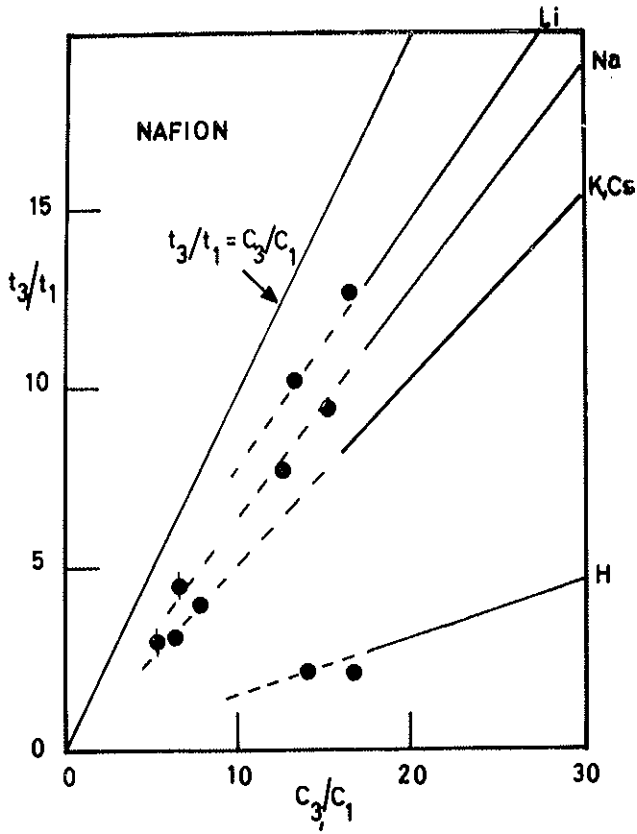

FIG. 9 — Electro-osmotic data for the perfluorosulphonic acid (Nafion) exchangers. Two membranes, differing in capacity and swelling, were studied in lithium, sodium, potassium, caesium and hydrogen form (Only the caesium forms deviate significantly from the salt model, ●).

0.1 and 1.0M sodium chloride solutions. The methods used for the evaluation of frictional and mobility coefficients are described in detail in the original papers [2, 4]. The C60E membrane in 1.0M sodium chloride, (C60E (1.0)), has a sulphonate-matrix concentration of 2.75 molal and total ionic molality 3.21. The C60N (0.1) membrane is almost salt free (0.2%) and has molality 2.87. The two membranes have therefore almost equal molalities of sulphonate and C60E (1.0) may be considered to be equivalent to C60N (0.1) with added sodium chloride. It is therefore useful to consider C60E (1.0) as a ternary electrolyte of sodium sulphonate and sodium chloride and apply the empirical methods developed by MILLER [19a], which have been used successfully to calculate the transport properties of a ternary electrolyte solution from a knowledge of the solvent-fixed mobility coefficients of the binary electrolyte components. Appendix. To apply this method to membrane calculations, solvent-fixed coefficients $\bar{L}_{ik}$, are obtained from the experimental values of $\bar{\ell}_{ik}$ for C60N (0.1). These coefficients refer to the bulk membrane and include tortuosity effects and are therefore not compatible with the solvent fixed mobility coefficients for aqueous sodium chloride. To convert membrane $\bar{L}_{ik}$ to solvent-fixed coefficients, which are representative of the aqueous or pore volume of the membrane the scaling factor θ/ν must be used, where ν is the fractional pore volume of the exchanger. (The scaling factor is dealt with more explicitly on the section devoted to salt model calculations, below).

$\bar{L}_{ik}\theta/\nu$ are therefore equivalent to mobility coefficients for a non-tortuous membrane. Once obtained for C60N (0.1), these may be combined with corresponding mobility coefficients for aqueous sodium chloride at 3.21m [19b] in the LN calculation [19a, 4]. The resulting coefficients may then be corrected for tortuosity and pore volume corresponding to those of the C60E (1.0) membrane and converted to a membrane or 4-fixed frame of reference for comparison. The results of these calculations are shown in Table 2, where calculated $\bar{\ell}_{ik}$ coefficients

TABLE 2 — *Membrane-fixed Mobility Coefficients for C60E in 1.0 molar Sodium Chloride (25°C).* ($\bar{C}_2/\bar{C}_{Total} = 0.144$).

	$\bar{\ell}_{11}$	$\bar{\ell}_{12}$	$\bar{\ell}_{13}$	$\bar{\ell}_{22}$	$\bar{\ell}_{23}$	$\bar{\ell}_{33}$
			x 10^{12} (mole^2 J^{-1} cm^{-1} s^{-1})			
Method						
Exptal						
(a)	2.09	0.210	32.0	0.372	12.3	1010
(b)	2.09	0.199	30.0	0.361	10.0	1076
Ternary Model						
(m)	2.24	0.33	25.3	0.31	7.1	695
(p)	2.41	0.32	26.7	0.40	7.7	735
S.M.C.						
(s)	2.40	0.29	28.6	0.29	5.1	570
(m)	2.48	0.30	29.6	0.30	5.3	591
(p)	3.33	0.41	39.6	0.41	7.1	792

Assumptions made in the evaluation of parameters from experimental data [4]

(a) $$\frac{\bar{\ell}_{23}}{C_2C_3} = \frac{2\bar{\ell}_{33}}{\bar{C}_3^2} - \frac{\bar{\ell}_{13}}{\bar{C}_1\bar{C}_3}, \text{ and}$$

(b) $R_{22}^* = 0$

may be compared with experimentally derived data. The ternary-model calculations have been made using both MEARES and PRAGER tortuosity corrections in the manner described above. Both sets of calculated $\bar{\ell}_{ik}$ results are in good agreement, and show the same trends and magnitudes as the experimentally derived data with average discrepancy of $\approx 20\%$ between observed and calculated, $\bar{\ell}_{ik}$, coefficients. Calculated and observed conductivities transport and transference numbers are given in Table 3.

TABLE 3 — *Observed and Predicted Transport Measurements for C60E (1.0).*

		Specific conductivity $\bar{k} \times 10^2$ ohm^{-1} cm^{-1}	t_1	t_3
Observed		1.90	0.92	9.70
Ternary Model	(m)	1.80	1.00	9.53
	(p)	1.94	0.98	9.13
S.M.C.	(s)	1.96		
	(m)	2.03	1.00	11.16
	(p)	2.71		
		Salt model calculation		

Again very reasonable agreement is obtained, and it would appear that this empirical method of MILLER may be used with success to predict membrane performance. The method appears however to over-estimate coupling between sodium and chloride, $\bar{\ell}_{12}$, resulting in low values of t_2, the co-ion transport number, and to consistently under-estimate the direct mobility for water $\bar{\ell}_{33}$.

Conductivity of mixed ionic forms: In recent studies the irreversible thermodynamic parameters for the calcium form of the C60E membrane have been obtained with external solution 0.05M calcium chloride at 298.15K [18]. The conductivities of the pure ionic forms and of mixed calcium and sodium forms have also been measured, Fig. 10. The membrane may be considered as effectively salt free in the external solutions of mixed $CaCl_2$/NaCl which have constant equivalent concentration 0.1N. The sample of C60E membrane is sufficiently similar to that studied earlier [2] to allow use of

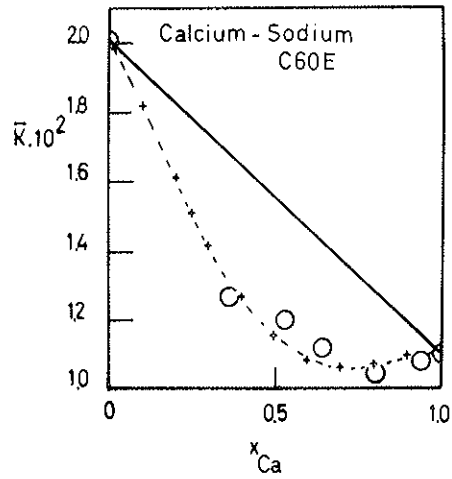

FIG. 10 — Specific conductivities of mixed sodium/ calcium forms of C60E, external solution 0.05N in aqueous NaCl/$CaCl_2$. ([units] $\bar{k}$, ohm^{-1} cm^{-1}; x_{Ca}, equivalent fraction of calcium.)

mobility coefficients from that study. There is little change in water content on converting the sodium form of C60E into the calcium form and so we may neglect changes in conductivity due to that source (to a first degree). The problem may now be attempted using MILLER's LN approximations for ternary solution. In terms of solvent-fixed mobility coefficients the specific conductivity of a mixed a, b form, $\bar{\varkappa}_{ab}$, may be represented by eqn. (11).

$$\bar{k}_{ab} = (Z_a^2\bar{L}_{aa} + Z_b^2\bar{L}_{bb} + Z_4^2\bar{L}_{44} + 2(Z_aZ_b\bar{L}_{ab} + Z_aZ_4\bar{L}_{a4} + Z_bZ_4\bar{L}_{b4}))\,F^2 \qquad (11)$$

Using MILLER's method eqn. (11) becomes eqn. (12).

$$\bar{k}_{ab} = ((\bar{x}_a\bar{k}_a + \bar{x}_b\bar{k}_b) + 2Z_aZ_b\bar{L}_{ab})\,F^2 \qquad (12)$$

where $\bar{L}_{ab}$ is the coupling coefficient between a and b in the

mixed ionic form with composition, $\bar{x}_a$ (equivalent fraction). $\bar{L}_{ab}$ may be calculated by MILLER's assumption that

$$\bar{L}_{ab} = -(\bar{x}_a \bar{x}_b)^{3/2} \sqrt{\bar{L}_{a4} \bar{L}_{b4}}, \ \bar{N} = \text{constant}$$

where $\bar{L}_{a4}$ and $\bar{L}_{b4}$ are the coupling coefficients between counterion and matrix in the pure a and pure b forms respectively. The results of this calculation are given in Fig. 10. Excellent agreement between observed and calculated conductivities is obtained. This analysis shows that there will be negative deviations from the linear relationship $(\bar{x}_a \bar{k}_a + \bar{x}_b \bar{k}_b)$ due solely to coupling between sodium and calcium ions. In electrolyte solutions such $\bar{L}_{ab}$ coefficients between ions of like charge are always negative.

Salt Model Calculations: To provide an easily accessible model independent of membrane transport measurements the method of calculation is adopted based upon the properties of a single electrolyte for which a complete irreversible thermodynamic analysis is available at concentrations which include the total ionic molality of the exchanger. From the experimental exidence cited above on the C60 membrane systems, there is a similarity between the function of sulphonate-matrix fixed charge in the membrane and free chloride ion in solution. This similarity suggests that chloride and sulphonate have broadly similar kinetic behaviour. Since the co-ion in these experimental studies was also chloride, a model of the exchanger may be conceived, in which chloride of two "sorts" is present; chloride, species 2, co-ion in the membrane and chloride, species 4, which is fixed relative to the membrane matrix. The relationship of these two forms is taken to be that between chemically identical, but physically distinguishable isotopes. This postulate allows a precise definition of the frictional and mobility coefficients of the ternary solution and, in particular, the absolute magnitudes of frictional coefficients $\bar{R}_{2i}$ and $\bar{R}_{4i}$, (i = 1, 2, 3, 4), the frictional coefficients between

co-ion and fixed charge and the other components of the system. Implicit in this model is the assumption that the polymer matrix has no chemical influence upon the transport process, other than by its presence, to obstruct diffusional pathways in the membrane phase and render them tortuous. Major deviations between the predictions of the model and observed transport parameters can therefore be considered as indications of specific polymer effects and the possibility, with certain ions, that sulphonate and chloride may have quite different complexing or ion pairing characteristics would obviously undermine the model. It might also be expected that dilute, homogeneous membranes or membranes with significant voids would have local ionic distributions which were grossly different from those in simple aqueous solution of equal molality, and consequently the processes of transport would be polymer dependent and specific processes such as polymer chain diffusion or pseudo-mosaic effects would be significant.

If, however, the aqueous and ion-permeable regions of the membrane constitute an essentially homogeneous phase, it is conceivable that the distribution of charges might approximate to those in an aqueous electrolyte solution and in this range a salt model would predict membrane properties, which were of the correct magnitude.

The choice of a salt model based upon a ternary isotopic solution allows precise evaluation of model coefficients. The theory of isotopic diffusion and the identification of isotope-isotope coefficients has been developed by LAITY [20] and by KEDEM and ESSIG [21]. The shortened theoretical treatment given below owes much to these papers and is developed primarily to express frictional interaction in the isotopic ternary solution in terms of those of the parent binary electrolyte and the isotopic diffusion coefficient for co-ion.

Since the analysis requires a change of frame of reference from solvent, (the normal frame for discussion of solutions), to ion-4-fixed in the membrane model, a development is pre-

sented using frictional coefficients, R_{ik}, which are independent of frame of reference.

The phenomenological equations for a binary electrolyte (1, 2) are given in matrix form by eqn. (13), in which 1 represents the counterion and 2, co-ion.

$$\begin{vmatrix} X_1 \\ X_2 \end{vmatrix} = \begin{vmatrix} R_{11} & R_{12} \\ R_{21} & R_{22} \end{vmatrix} \begin{vmatrix} J_1^3 \\ J_2^3 \end{vmatrix} \qquad (13)$$

The flows of counterion, J_1^3 and co-ion J_2^3 are given on a solvent fixed frame of reference. Thermodynamic forces on counterion and co-ion, defined by the negative gradients of chemical potential are given by X_1 and X_2 respectively. In the ternary isotopic solution of equal concentration, some of co-ion 2 is replaced by co-ion 4, which is chemically identical in all respects, such that the total co-ion concentration in the binary is the sum of the concentration of 2 and 4 in the ternary, eqn. (14)

$$C_2 = c_2 + c_4 \qquad (14)$$

The phenomenological equations for this ternary solution are given in eqn. (15) in which forces, frictional coefficients and flows are represented by lower case symbols, x_i, r_{ik}, and j_i^3 respectively.

$$\begin{vmatrix} x_1 \\ x_2 \\ x_4 \end{vmatrix} = \begin{vmatrix} r_{11} & r_{12} & r_{14} \\ r_{21} & r_{22} & r_{24} \\ r_{41} & r_{42} & r_{44} \end{vmatrix} \begin{vmatrix} j_1^3 \\ j_2^3 \\ j_4^3 \end{vmatrix} \qquad (15)$$

For eqns. (13) and (15) the ONSAGER reciprocal relations, (O.R.R.) will be assumed, such that $R_{ik} = R_{ki}$ and $r_{ik} = r_{ki}$ for all i and k. (Extensions to relationships proved between R – and r – coefficients, due to these identities, will be represented by (+O.R.R.)).

In a comparison of a binary solution, eqn. (13), and the

same solution in which an isotopic form of 2 is present, eqn. (15), the following identities exist, eqns. (16), (17) and (18);

$$J_1^3 = j_1^3;\ J_2^3 = j_2^3 + j_4^3;\ X_1 = x_1 \qquad (16), (17), (18)$$

and from the GIBBS-DUHEM relationship for forces in both systems, eqn. (19), is obtained.

$$C_2X_2 = c_2x_2 + c_4x_4 \qquad (19)$$

Under conditions for isotopic diffusion of co-ion, eqn. (15), $x_1 = 0 = j_1^3$ and from eqns. (16), (17) and (18),

$$r_{12} = r_{14} \quad (+\text{O.R.R.})$$

Comparison of X_1 and x_1 in experiments without isotopic forces shows,

$$R_{11} = r_{11} \text{ and } R_{12} = r_{12} = r_{14} \quad (+\text{O.R.R.})$$

The coefficients r_{22}, r_{24}, (r_{11}), and r_{44} of eqn. (15) cannot be determined solely by comparison of coefficients in eqn. (13) but are related to the isotopic diffusion coefficient for co-ion, D_{22} (and D_{44}).

If a purely electrical force is applied to both solutions,

$$X_2 = x_2 = x_4 = Z_C F\ (-\text{grad}\ \psi) \qquad (20)$$

where $Z_C = Z_2 = Z_4$, is the co-ion valency (including sign), and grad ψ, the local gradient of electrical potential. Comparison of expansions for X_2 and x_2 (and x_4) give the equalities of eqns. (21) and (22).

$$R_{22}J_2^3 = r_{22}j_2^3 + r_{24}j_4^3 \qquad (21)$$

and

$$R_{22}J_2^3 = r_{42}j_2^3 + r_{44}j_4^3 \qquad (22)$$

Since co-ions 2 and 4 are chemically identical, they will have the same electrochemical mobility, V, ($cm.s^{-1}$) under unit electrical potential gradient. Since $J = CV$,

$$C_2R_{22} = c_2r_{22} + c_4r_{24} = c_2r_{42} + c_4r_{44} \qquad (23)$$

Under conditions for isotopic diffusion of co-ion, eqn. (15), the total force and total flow of co-ion are separately zero and from eqns. (19) and (17)

$$c_2x_2 = -c_4x_4 \text{ and } j_2^3 = -j_4^3 \qquad (24)$$

It is easily shown that the isotopic flows obey Fick's Law and that the isotopic diffusion coefficients of 2 and 4 are equal, D_{22} and D_{44} respectively, eqns. (25).

$$D_{22} = \frac{RT}{c_2(r_{22} - r_{24})} = \frac{RT}{c_4(r_{44} - r_{42})} \qquad (25)$$

From eqn. (23) these diffusion coefficients may be expressed in terms of the direct frictional coefficient of co-ion in the binary, R_{22}, and the frictional coefficient between isotopes 2 and 4 in the ternary $r_{24}(r_{42})$, eqn. (26), so that

$$D_{22} = D_{44} = \frac{RT}{C_2(R_{22} - r_{24})} \qquad (26)$$

The relative concentrations of co-ion isotopes, c_2 and c_4, are not required in this equation and the isotope-isotope frictional coefficient r_{24} (r_{42}), may be obtained directly from the self- or isotopic diffusion coefficient of the co-ion in solution and the corresponding values of R_{22} and C_2 in the binary.

From eqns. (23) and (26) explicit expressions for the fric-

tional coefficients r_{11} (r_{42}), r_{22} and r_{44} of eqn. (15) are obtained: eqns. (27), (28), (29)

$$r_{24} = r_{42} = R_{22} - \frac{RT}{C_2 D_{22}} \tag{27}$$

$$r_{22} = R_{22} + \frac{c_4}{c_2} \frac{RT}{C_2 D_{22}} \tag{28}$$

and $$r_{44} = R_{22} + \frac{c_2}{c_4} \frac{RT}{C_2 D_{22}} \tag{29}$$

These last two coefficients, r_{22} and r_{44}, depend upon the relative proportions of isotopes 2 and 4 in the solution.

From the identities

$$\sum_{i=1}^{3} C_i R_{ik} = 0 \qquad k = 1, 2, 3$$

and

$$\sum_{i=1}^{4} c_i r_{ik} = 0 \qquad k = 1, 2, 3, 4$$

applied to the phenomenological equations (13) and (15) respectively, it is easily shown that the ion-to-water frictional coefficients in the isotopic ternary are equal to those in the parent binary, eqn. (30).

$$R_{13} = r_{13} \text{ and } R_{23} = r_{23} = r_{43}; \ (+\text{O.R.R.}) \tag{30}$$

consequently $R_{33} = r_{33}$

Phenomenological equations relative to ion 4: Eqn. (15) may be taken as a model for the exchanger membrane in which ion 4 represents the fixed charge on the polymer and 2, the co-ion imbibed by the membrane from the external solution. Membrane flows are measured relative to the stationary matrix

and therefore relative to the ion, species 4. On a "4-fixed" frame of reference the phenomenological equations of eqn. (15) become eqn. (31)

$$\begin{vmatrix} x_1 \\ x_2 \\ x_3 \end{vmatrix} = \begin{vmatrix} r_{11} & r_{12} & r_{13} \\ r_{21} & r_{22} & r_{23} \\ r_{31} & r_{32} & r_{33} \end{vmatrix} \begin{vmatrix} j_1^4 \\ j_2^4 \\ j_3^4 \end{vmatrix} \qquad (31)$$

The flows j_i^4 are the flows of ions 1, 2, and water, 3, relative to the fixed anion 4, where $j_i^4 = (j_i^3 - \frac{c_i}{c_4} j_4^3)$. All frictional coefficients, r_{ik}, are defined by the binary, with the exception of r_{22}, which, from eqn. (28), is dependent on the isotopic diffusion coefficient D_{22} and the concentration ratio, c_4/c_2. Thus the direct coefficient r_{22} is strongly dependent on the concentration of salt in the membrane and increases as co-ion uptake c_2 diminishes; a feature observed in experimental studies of ion exchange membranes [2].

Scaling factors for transport parameters, relating to those of the solution model to practical membrane values.

Eqn. (31) is a model of a non-tortuous membrane in which no account has been taken of the presence of polymer. The model makes the explicit assumption that the membrane function is determined by the ionogenic fixed groups on the polymer, that these are similar to simple aqueous anions and that the polymer matrix has no influence on the movement of ions or water other than, by its presence, to restrict movement by constraining mobile species to tortuous diffusional pathways.

In common practice concentrations in the membrane are expressed in moles cm^{-3} of total membrane volume, $\bar{C}$, where $\bar{C} = \nu c$ and ν is the fractional aqueous volume (or pore-volume) of the membrane. Equally flow across the membrane is, in practice, referred to flow/unit area of exposed membrane,

$\bar{J}$ so that $\bar{J}=jv'$ where v' is the ratio of "pore" to geometric area at the membrane surface. The membrane may be defined as homogeneous in macroscopic terms if $v'=v$.

The presence of polymer in the membrane may be considered to increase the effective length of diffusional pathways across the membrane, such that a membrane of geometric thickness $\bar{d}$ may be considered to have an effective diffusional path length of $\bar{d}\theta$ which corresponds to a solution of path length d, where $\theta > 1$.

Fick's equation for isotopic diffusion of co-ion may be chosen to illustrate these scaling effects, eqn. (32),

$$j_2 = -D_{22}\Delta c_2/d \tag{32}$$

becomes

$$\frac{\bar{J}_2}{v'} = \frac{-(D_{22})}{\theta}\,\frac{(\Delta\bar{C}_2)}{\bar{d}}\,\frac{1}{v} \tag{33}$$

and if $v'=v$, as in a homogeneous membrane

$$\bar{J}_2 = \frac{-D_{22}}{\theta}\,\frac{(\Delta\bar{C}_2)}{\bar{d}} \tag{34}$$

so that

$$D_{22}/\theta = \bar{D}_{22} \tag{35}$$

The diffusion coefficient of co-ion in the tortuous membrane $D_{22}=D_{22}/\theta$ using this salt model calculation and so is smaller than in free solution. Using barred symbols to represent the membrane,

$$D_{22} = \frac{RT}{\bar{C}_2(\bar{R}_{22} - \bar{R}_{22}^*)} \tag{36}$$

and consequently

$$\bar{R}_{22} = \frac{r_{22}\theta}{v} \text{ and } \bar{R}_{22}^* = \frac{r_{22}^*\theta}{v},$$

where 2* is the isotopic form of 2 used in membrane co-ion

diffusion (it is easily shown that $r_{22}^* = r_{24}$). Since this analysis may be applied to the forces and flows in the phenomenological eqns. (31), it is generally true that,

$$\bar{R}_{ik} = r_{ik} \frac{\theta}{\nu} \tag{37}$$

or in inverse form as mobility coefficients ℓ_{ik};

$$\bar{\ell}_{ik} = \ell_{ik} \frac{\nu}{\theta} \tag{38}$$

The value of the tortuosity coefficient calculated by theoretical estimations is dependent upon the statistical model of the exchanger phase chosen and values from independent theoretical models may not be consistent. In earlier papers PRAGER's estimate of the tortuosity factor θ_p and MEARES' value of the *path* tortuosity θ_m have been used [2, 3, 4].

The salt model calculation, S.M.C., however defines θ as $D_{22}/\bar{D}_{22}$ and as θ_s, will be used in the comparisons of experimental membrane and S.M.C. parameters given below.

An estimate of the frictional coefficients for an experimental membrane may therefore be obtained from a knowledge of its physical dimensions, the concentrations of ions and water in the membrane, and the co-ion diffusion coefficient. Before making comparisons of this sort it is useful to summarise the predicted correspondence between membrane, $\bar{R}_{ik}$, and solution frictional parameters, eqn. (39), which is represented conveniently in matrix form,

$$\begin{vmatrix} \bar{R}_{11} & \bar{R}_{12} & \bar{R}_{13} & \bar{R}_{14} \\ \bar{R}_{21} & \bar{R}_{22} & \bar{R}_{23} & \bar{R}_{24} \\ \bar{R}_{31} & \bar{R}_{32} & \bar{R}_{33} & \bar{R}_{34} \\ \bar{R}_{41} & \bar{R}_{42} & \bar{R}_{43} & \bar{R}_{44} \end{vmatrix} = \begin{vmatrix} R_{11} & R_{12} & R_{13} & R_{12} \\ R_{21} & r_{22} & R_{23} & r_{24} \\ R_{31} & R_{32} & R_{33} & R_{32} \\ R_{21} & r_{42} & R_{23} & r_{44} \end{vmatrix} \times \frac{\theta}{\nu} \tag{39}$$

"Membrane" (S.M.C.) Solution

where r_{22}, r_{44} and r_{24} ($=r_{42}$) are defined by eqns. (28) (29) and (27) respectively.

Application of the S.M.C. Before making a detailed comparison between this simple model calculation and the observed properties of membranes, it is of interest to note that the model predicts that a value of $\bar{R}_{22}$ may be obtained directly from the isotopic diffusion coefficient of co-ion in the membrane $\bar{D}_{22}$ provided the ratio of fixed charge to co-ion concentrations is large. For most binary electrolytes the function RT/C_2D_{22} is of the same order of magnitude as R_{22}. For example in aqueous sodium chloride at 3M the function is some 20% smaller than R_{22} [22]. If the co-ion to sulphonate friction in the experimental membrane is, even approximately, equal to r_{24} of the salt model calculation,

$$\bar{\bar{R}}_{22} \sim RT/(\bar{\bar{C}}_2\bar{\bar{D}}_{22}) \qquad (40)$$

Again within the limits of applicability of the model the error in using eqn. (40) would be approximately $-0.2\,x\%$ when the ratio of $\bar{C}_2/\bar{C}_4 \times 100$ is x in the membrane, (at these molalities).

There are certain consequences of the isotope model presented. The first is that since co-ion and matrix fixed charge 4 are taken as chemically identical, the transport number of co-ion, t_2, will he defined as zero. The change of frame of reference to memhrane or 4-fixed automatically requires co-ion to be stationary relative to 4 in an electrical experiment. Equally the value of the electro-osmotic transference number t_3 will be identical to that obtained by considering the flow of water relative to all co-ions, fixed, in the binary, so that

$$t_3 = \frac{-C_3}{Z_2C_2} \qquad t_2{}^3 = \frac{C_3t_2{}^3}{Z_1C_1}$$

where $t_2{}^3$ is the transport number for co-ion in the binary solution. The specific conductivity k is independent of the frame

of reference chosen for the measurement and so the S.M.C. predicts the specific conductivity of the membrane $\bar{k}$ by eqn. (41)

$$\bar{k} = k \frac{\nu}{\theta} \tag{41}$$

For these parameters it is therefore sufficient to know the co-ion transport number relative to water, t_2^3, and the specific conductivity of the model binary $\varkappa$ to obtain predicted membrane parameters.

Observed and Calculated Membrane Parameters: The frictional coefficients for the binary model electrolyte, sodium chloride, were obtained from MILLER'S tabulated data [19b] and isotopic diffusion coefficients, for chloride co-ion, obtained by MILLS [12]. Since the molarity of species, relative to unit volume of aqueous pore solution, is not defined unequivocally, this concentration was estimated by assuming the ratio of molarity to molality in the membrane to be the same as in equimolal aqueous sodium chloride at 25°C.

Frictional coefficients obtained from experimental data and from the Salt Model Calculation, (S.M.C. are given in Table 4. Tortuosity corrections, θ/ν, have been estimated, using the ratio $D_{22}/\bar{D}_{22}$, as suggested by the model, and those of MEARES and PRAGER in the manner discussed above. These three methods, designated (s), (m) and (p) respectively are shown in all tabulated data.

The agreement between calculated and experimental coefficients, $\bar{R}_{jk}$, is in general very good, with calculated values following in detail the trends and magnitudes found from data derived from experimental measurements [2, 4]. There is particularly good correspondence between calculated and experimental values of $\bar{R}_{22}$ and $\bar{R}_{44}$, which largely justifies the basic assumption of the S.M.C.; that aqueous chloride and sulpbonate-matrix anions have similar kinetic characteristics. For both normal and expanded membranes the salt uptake is small and $\bar{R}_{44}$ is given to a good approximation by tbe solution coef-

TABLE 4 — *Comparison of salt model calculations (S.M.C.) with experimental values of R-coefficients for C60N and C60E. Membranes in sodium chloride solutions.* (0.1M) *at* 25°C.

C60N	Method	$\overline{R}_{22}$	$\overline{R}_{11}$	$\overline{R}_{44}$	R_{22}*	$\overline{R}_{33}$	$-\overline{R}_{12}$	$-\overline{R}_{14}$	$-\overline{R}_{13}$	$-\overline{R}_{23}$	$-\overline{R}_{34}$
		J cm s mole$^{-2} \times 10^{-12}$									
	Exptal.	349	1.01	0.88	—	0.0028	−1.57(?)	0.442	0.030	0.144	0.023
S.M.C.	(s)	294	1.30	0.82	0.82	0.0045	0.204	0.204	0.057	0.032	0.032
	(m)	329	1.46	0.92	0.92	0.0050	0.229	0.229	0.064	0.036	0.036
	(p)	260	1.15	0.72	0.72	0.0040	0.181	0.181	0.050	0.028	0.028
S.M.C. (uncorrected)		24.76	0.1101	0.0691	0.68895	0.000386	0.01725	0.01725	0.0048	0.00267	0.00267

m_1 m_2 m_4, the molalities of sodium, chloride and sulphonate were 2.87, 7.08×10^{-3} and 2.863 respectively, and the concentration ratio $m_4/m_2 = 404.38$

C60E	Method	R_{22}	R_{11}	R_{44}	R_{22}*	R_{33}	$-R_{12}$	$-R_{14}$	$-R_{13}$	$-R_{23}$	$-R_{34}$
	Exptal.	102	0.83	0.602	—	0.0013	−1.65(?)	0.283	0.0218	0.102	0.0128
S.M.C.	(s)	103	0.88	0.619	0.619	0.0019	0.149	0.149	0.0320	0.0181	0.0181
	(m)	103	0.98	0.621	0.621	0.0019	0.149	0.149	0.0320	0.0181	0.0181
	(p)	82.9	0.79	0.501	0.501	0.0015	0.120	0.120	0.0258	0.0145	0.0145
S.M.C. (uncorrected)		14.024	0.134	0.0848	0.0848	0.000261	0.0203	0.0203	0.00436	0.00247	0.00247

m_1, m_2 and m_4 are 2.12$_5$ 11.49×10^{-3}, and respectively and $m_4/m_2 = 183.49$

In salt model calculations, (S.M.C.), (s), (m), (p) refer to scaling factors θ/ν calculated using θ_s (experimentals), θ_m, and θ_p respectively.

* R_{22} the aqueous binary frictional coefficient, for chloride, is retained for comparison with $\overline{R}_{44}$.

ficient $R_{22}/(\theta/v)$, Table 4, eqn. (29). Equally, the very large values for $\bar{R}_{22}$ are explained by the dominant contribution of the concentration ratio, c_4/c_2 in eqn. (28). The lower value of $\bar{R}_{22}$ in the expanded membrane, C60E (0.1) is caused primarily by the greater uptake of co-ion, $\bar{C}_2$, since the value of the direct coefficient R_{22} in the model solution, is largely unaffected by the change in concentration from 2.87 m in C60N (0.1) to 2.13 m in C60E (0.1).

The direct frictional coefficients for sodium and water, $\bar{R}_{11}$ and $\bar{R}_{33}$, are somewhat over-estimated by the S.M.C. particularly in the more concentrated normal membrane, C60N (0.1). Ion-to-water coefficients $\bar{R}_{13}$ and $\bar{R}_{34}$ indicate that sodium and sulphonate-matrix have similar water interactions to sodium and chloride in the model. Since $\bar{f}_{i3}$ $(i = 1, 4)$ is defined as $-\bar{C}_3\bar{R}_{i3}$, this similarity has been confirmed earlier from analysis of electro-osmosis experiments, eqn. (9).

S.M.C. calculations under estimate the resistance coefficient $\bar{R}_{14}$, Table 4, and this has a marked effect upon the sources of friction contributing to electrical conductivity, Table 5, $(\bar{f}_{14} = -\bar{C}_4\bar{R}_{14})$. Using a frictional analysis the specific resistance, $\bar{\rho}$, of a membrane (containing no invading electrolyte) is represented hy eqn. (42) [17]

$$\bar{\rho} = \frac{1}{\bar{k}} = \frac{1}{Z_4\ \bar{C}_4 F^2}\left[f_{14} + \left(\frac{\bar{f}_{13} + \bar{f}_{43}}{\bar{f}_{13}\bar{f}_{43}} \right) \right] \qquad (42)$$

units, $\bar{\rho}$, ohm cm

A comparison of the percentage contribution of the ion-matrix and ion-water contributions, shows, that in the C60 membranes, the electrical resistance is dominated by the friction between counterion and fixed charge, which amounts to some 60% of the total frictional term. Solution data for lithium, sodium and caesium chlorides [16] are presented at roughly

equivalent molalities, m. It is therefore obvious that although the salt model is particularly useful for ion-water predictions, it also indicates that counterion-fixed charge interactions are more important in these membranes than in analogous solutions.

As the total concentration of electrolyte is decreased f_{14} becomes smaller and ultimately at infinite dilution becomes zero. The chloride and iodide forms of the anion exchanger (A104) are also presented in Table 5. These have very much larger internal molalities and, in consequence, no solution analogue can be found. It is, however, interesting to observe that the counterion-fixed charge friction, $\bar{f}_{14}$, now dominates the specific resistance, $\bar{\rho}$, and amounts to 80% for the chloride-form and 98% for the iodide-form. In this latter, the exchanger is approaching total dehydration with no more than four water molecules to each fixed charge iodide, pair. The interionic interactions become extremely large and other evidence suggests strong ion pairing may occur.

Mobility coefficients, $\bar{\ell}_{ik}$, for this system are shown in Table 6. The model, by assuming identical co-ion and fixed charge restricts the co-ion transport number (calculated) to zero so that for a 1:1 salt, $\bar{\ell}_{12} = \bar{\ell}_{22}$ in the S.M.C. calculation, eqn. (A3). Once more the agreement between experimental and calculated coefficients is good and tortuosity corrections (s) and (m) superior to the PRAGER estimate, (p).

The experimental value of $\bar{\ell}_{12}$ is small and cannot be calculated with confidence for these memhranes which have such low co-ion uptake. Good agreement between calculated and observed coefficients, $\bar{\ell}_{22}$, indicate that the coupling coefficient $\bar{\ell}_{12}$, in the membrane, must be small. The most serious disagreement is observed for values of $\bar{\ell}_{33}$, the direct mobility of water. The S.M.C. underestimates this major parameter. In consequence, water flow, for example, osmotic flow in eqn. (A6) which depends largely on the magnitude of $\bar{\ell}_{33}$ is underestimated, Table 7.

In earlier papers [2, 4] approximations were made which

TABLE 5 — *Frictional Contributions to Conductivity (eqn. 42).*

	m	$\bar{f}_{14}$ %	$\bar{f}_{13}\bar{f}_{43}/(\bar{f}_{13}+\bar{f}_{43})$ %	$\bar{f}_{13}/(\bar{f}_{13}+\bar{f}_{43})$
Na-form C60E(0.1)	2.18	62	38	0.57
Na-form C60N(0.1)	2.78	63	37	0.57
Cl-form A104(0.1)	6.24	80	20	0.37
I-form A104(0.1)	14.18	98	2	0.54
Solution				
LiCl	3.20	32	68	0.74
NaCl	3.20	35	65	0.64
CsCl	3.45	41	59	0.51

Where $\bar{f}_{i4} = -\bar{C}_i\bar{R}_{i4}$ and $\bar{f}_{i3} = -\bar{C}_i\bar{R}_{i3}$, i = 1,4, units: Js $cm^{-2}mol^{-1}$. Solution data from reference [19b] for lithium and sodium chlorides, reference [16] for caesium chloride.

involved the neglect of certain coefficients. In particular neglect of co-ion-to-water coupling $\bar{\ell}_{23}$ (such that $\bar{\ell}_{23} >> \bar{\ell}_{13}$) was found to be valid. The S.M.C. justifies this assumption for these membranes, since, by calculation, $\bar{\ell}_{23}$ is less than one per cent of the value of ℓ_{13} and appears only in the expression for electro-osmotic transference number, t_3, eqn. (A4) (which can normally be measured only to an accuracy $\pm$ 1%).

For the expanded membrane C60E (1.0) in which salt uptake is some 14%, neglect of this coefficient is no longer justified, Table 2. The S.M.C. is included in Table 2, where calculated coefficients may be compared with experimental and ternary-model coefficients. Once more the agreement is good, but $\bar{\ell}_{33}$ (and $\bar{\ell}_{23}$) are again underestimated by both calculation models.

Prediction of Experimental Measurements: Measured and predicted transport properties are given in Tables (3) and (7).

TABLE 6 — *Mobility coefficients, $\bar{\ell}_{ik}$, for C60N and C60E membranes in sodium chloride solutions.* (0.1M) and 25°C.

Method		$\bar{\ell}_{11}$	$\bar{\ell}_{12}$	$\bar{\ell}_{22}$	$\bar{\ell}_{13}$	$\bar{\ell}_{23}$	$\bar{t}_{33}$
		mole² J⁻¹s⁻¹cm⁻¹ x 10^{12}					
C60N		1.47	—	0.0029	16.0	0.159	547
S.M.C.	(s)	1.65	0.0034	0.0034	20.7	0.065	475
	(m)	1.49	0.0031	0.0031	18.6	0.059	427
	(p)	1.84	0.0039	0.0039	23.6	0.075	540
S.M.C. (Uncorrected)		19.9	0.0405	0.0405	247.6	0.784	5673
C60E		2.05	—	0.0103	33.6	1.07	1930
S.M.C.	(s)	2.25	0.0099	0.0099	33.3	0.257	1152
	(m)	2.24	0.0099	0.0099	33.2	0.256	1149
	(p)	2.78	0.0122	0.0122	46.4	0.328	1425
S.M.C. (Uncorrected)		16.14	0.0722	0.0722	274.3	1.88	8423

In both sets the specific conductivity is estimated and particularly good agreement obtained with (s) and (m) tortuosity corrections. Electro-osmotic transference numbers calculated by the salt model are some 10-15% too large, but, since these are calculated from the transport number of co-ion, t_2[3], in the binary model, they do not take account of co-ion movement in the membrane which will tend to reduce electro-osmotic flow. In Table (7) both salt and osmotic flows across the membrane were measured when a concentration gradient 0.15/0.05 m was maintained across the membrane [2]. For the S.M.C. these

TABLE 7 — *S.M.C. Predictions for membranes in 0.1 M sodium chloride.*

Membrane	Specific Conductivity $\bar{k} \times 10^2$ ohm^{-1}cm^{-1}	t_1	t_3	J_s* mole $\times 10^{10}$	J_w* cm^{-2} s^{-1} $\times 10^7$
C60N					
obs.	1.37	0.998	10.75	3.90	0.65
S.M.C. (s)	1.55			4.90	0.43
(m)	1.39	1.00	12.47	4.40	0.39
(p)	1.70			5.60	0.49
C60E					
obs.	1.92	0.995	15.77	12.2	1.65
S.M.C. (s)	2.06			15.2	1.40
(m)	2.06	1.00	16.68	15.2	1.40
(p)	2.57			19.2	1.73

* J_s and J_w are flows of salt and osmotic flows of water observed when a concentration gradient 0.15/0.05 M salt is maintained across the membrane [2].

flows, given in eqns. (A5) and (A6), become, for a univalent form,

$$J_s = (\bar{\ell}_{22})X_{12} + (\bar{\ell}_{23})X_3 \tag{43}$$

and

$$J_w = (\bar{\ell}_{32})X_{12} + (\bar{\ell}_{33} - t_3^2\alpha)X_3 \tag{44}$$

Salt and water flows calculated by the S.M.C. are respectively higher and lower than observed. (In each case the error is $\approx 20\%$). The lower value of J_w, eqn. (44), is due primarily to underestimated $\bar{\ell}_{33}$, and over-estimated t_3, in the dominant second term of that equation. Salt flow, J_s, is overestimated because the coupling coefficient, $\bar{\ell}_{12}$, is overestimated, but in both cases the agreement may be said to be remarkable when

it is considered that only concentrations of the species in the membrane phase are required for the model calculation.

Isotope-isotope friction: At the basis of the model is the requirement that co-ion diffusion in membrane and model electrolyte differ solely due to tortuosity effects. Consequently $\bar{R}_{22}$ and $\bar{R}_{22}^*$ in the membrane are very similar to solution values. The agreement between S.M.C. and experimental frictional coefficients largely justifies this assumption. It is of interest however to examine isotope-isotope friction for counterion and for tritiated water, $\bar{R}_{11}^*$ and $\bar{R}_{33}^*$ respectively. Since eqn. (26) may be written for any isotopic species, R_{11}^* may be calculated from tbe self-diffusion coefficient $\bar{D}_{11}$ and estimated values of R_{11}, (which are in good agreement with the S.M.C., Table 4). From this membrane data it is easily shown that isotopic friction between counterions, $\bar{R}_{11}^*$, is negative for all membranes studied [2, 4, 1b]. For all ionic solutions for which data is available, ion-to-ion isotopic friction R_{ii}^* is positive [22]. The source of this particular effect is unknown but it is not to be expected, since even for non isotopic species i and k, R_{ik} is positive where i and k are ions of like-charge [18] (*).

Few data are available for isotopic diffusion coefficients of water in concentrated aqueous electrolytes. The data of BRUN [13] using tritiated water in sodium and potassium chlorides and of ANDERSON and PATERSON [22] for caesium chloride were combined with frictional coefficients from MILLER [19b] and from DUNSMORE et al [16] to provide SPIEGLER frictional coefficients f_{33}^*, $(-C_3R_{33}^*)$, eqn. (45).

$$D_{33} = RT/(C_3R_{33} - C_3R_3^*{}_3)$$
$$= RT/(f_{31} + f_{32} + f_3^*{}_3) \qquad (45)$$

for electrolyte 1,2

(*) *Footnote*: For this reason comparisons between $\bar{D}_{11}$ 0 and D_{11} of the S.M.C. are poor, Figure (3a, b), while $\bar{R}_{11}$ predicted by tbe S.M.C. is in rather better agreement with experimental, Table 4.

An equivalent expression is obtained for membrane diffusion,

$$\overline{D}_{33} = RT/(\overline{f}_{31} + (\overline{f}_{32}) + \overline{f}_{34} + \overline{f}_3{}^*{}_3)$$

in which water to co-ion friction, $\overline{f}_{32}$, is negligible if $\overline{C}_2$ is small, which is the case when DONNAN exclusion is effective.

In pure water C_3R_{33} is zero and so $D^o{}_{33} = RT/f_3{}^*{}_3 = 11.09.10^8$ Js cm^{-2}mol^{-1} (using MILLS' value for $D^o{}_{33}$ of $2.236.10^{-5}$cm^{-2}s^{-1} for tritiated water diffusion [23], Table 8. The effect of concentrated salt solutions upon $f_3{}^*{}_3$ is relatively small. Results for lithium and sodium chlorides and for potassium and caesium chloride show that $f_3{}^*{}_3$ will increase in the presence of solvent order producing cations, (Li^+, Na^+), and decrease when the cation is order-destroying (K^+, CS^+). In all cases the effects are small, Table 8 [22]. Data for C60 membranes show that the tortuosity corrected $f_3{}^*{}_3/\theta_p$ are similar to the alkali chlorides, (e.g. NaCℓ KCℓ) and this general similarity may be extended to A104 quaternary ammonium exchanger [15] although internal molalities are much greater. It appears therefore that the solvation effects of ions in concentrated solutions of electrolytes or in charged membranes cause only minor variations in water-to-water friction, $f_3{}^*{}_3$.

The contribution of water to ion friction to D_{33}, $(f_{31}+f_{43})$, is small amounting to only some 10-15% of the total friction for all systems (except lithium chloride), at approximately 3.0 mol kg^{-1} concentration. In A104 membranes the proportion is larger $\sim$ 25%. Solution data indicate that $(f_{31}+f_{34})$ is effectively proportional to the total concentration of salt and so $(f_{31}+f_{43})/m$ almost independent of concentration for a given salt (once more lithium chloride, with its strongly solvated cation, is an exception) and typical of the electrolyte involved. Data for membranes, corrected for tortuosity, indicate, once more, that this contribution to $\overline{D}_{33}$ is similar to solution data with C60 membranes in the sodium form comparing well with sodium chloride both in terms of the percentage contributions of $(\overline{f}_{31}+\overline{f}_{43})$ and $\overline{f}_3{}^*{}_3$ to the total and in absolute terms.

TABLE 8 — *Frictional Contributions to water Diffusion.*

membrane	m	θ_p	$(\overline{f}_{31} + \overline{f}_{32} + \overline{f}_{33}^*)$ 10^{-7}	$(\overline{f}_{31} + \overline{f}_{34})$ %	$(\overline{f}_{33}^*)$ %	$\frac{\overline{f}_{31} + \overline{f}_{34}}{m\theta_p}$ $\times 10^{-6}$	$\frac{(\overline{f}_{33}^*)}{\theta_p}$ $\times 10^{-7}$
Na-C60E (0.1)	2.13	2.78	35.4	9.6	90.4	5.74	11.5
Na-C60N (0.1)	2.87	3.84	47.2	11.2	88.8	4.81	10.9
Cl-A104 (0.1)	6.24	9.09	105	25.5	74.5	4.72	8.6
Cl-A104 (1.0)	7.53	10.46	154	26.4	73.5	5.15	10.84
Solution							
LiCl	3.20	1.0	15.6	23.4	76.6	11.4	11.9
NaCl	3.20	1.0	14.1	16.5	83.5	7.3	11.8
KCi	3.31	1.0	11.2	13.0	87.0	4.4	9.7
CsCl	3.45	1.0	10.6	13.0	87.0	4.4	9.2
Water	—	1.0	11.1	0.0	100.0	0.0	11.1

$f_{3i} = -C_i R_{3i}$; Js cm^{-2}mol^{-1}

Hyperfiltration: A series of hyperfiltration experiments have been carried out by BURKE and PATERSON [25]. Calculated and observed desalination characteristics are shown in Table 9. Salt and water flows were calculated, using eqns. (A5) and (A6) using guessed values for product concentration, C_P. Since (J_s/J_w). C_3 (*) also defines the product concentration C_P, C_P guessed was varied until self consistent results were obtained. The results are given in Table 9 where observed performance may be compared with that calculated using $\bar{\ell}_{ik}$ coefficients obtained by independent experimental studies (2) and the model calculation. The former accurately reproduce membrane performance and justify the approximations used in these earlier studies [2]. The salt model estimates are in poorer agreement. Predicted flows are somewhat smaller than observed and salt rejection under estimated by some 10%. It is however significant that the general effect of expansion and concomitant increase in salt uptake will have little effect on rejection but will increase flows of salt and water by a factor of three: not a conclusion to be reached intuitively.

The examples above serve to indicate the degree to which predictions by this simple model are valid and so in turn the validity of the basic assumption that ion exchange polymers are similar to electrolyte solutions. The calculated results give good reason to believe that there are indeed close similarities and that specific polymer effects are rather fewer than might have been expected. Tortuosity correction remains a serious theoretical problem although the model defines θ_s, which may be superior to theoretical calculations of this parameter.

It is to be hoped that this method will be tested and expanded in future studies, both as a quick and simple metbod of estimating probable conductance or t_3 values in new membranes and for detailed comparison of transport coefficients, using familiar, if not fully understood references, ionic solutions.

(*) [Footnote]: C_3 is the molarity of water in the product solution and is required when J_s and J_w are defined in their usual units mole $cm^{-2}s^{-1}$.

TABLE 9 — *Hyperfiltration Results and Model Calculations*: Feed 0.1 M NaCl and pressure difference across the membrane 27.57×10^5 Nm^{-2}. (400 p.s.i.).

Membrane	Method	Salt flow $J_s \times 10^{10}$ mole $cm^{-2}\ s^{-1}$	Water flow $J_w \times 10^7$	Product C_p mole ℓ^{-1}	Rejection $\frac{C_f - C_p}{C_f}$ 100%
C60N	obs	(4.01)	4.80	0.042	58%
	$\bar{\ell}_{ik}$ (2)	4.12	5.17	0.044	56%
	S.M.C. (s)	3.70	3.40	0.060	40%
C60E	obs.	(16.00)	21.80	0.041	59%
	$\bar{\ell}_{ik}$ (2)	14.50	19.90	0.041	59%
	S.M.C. (s)	10.96	10.35	0.059	41%

Salt flow J_s was not measured directly but obtained from J_w and C_p.

APPENDIX

From the phenomenological equations, eqn. (3), measured transport parameters may be expressed in terms of mobility coefficients, $\bar{\ell}_{ik}$ [2].

Specific Conductivity, $\bar{k}$:

$$\bar{k} = [Z_1^2 \ell_{11} + Z_2^2 \bar{\ell}_{22} + Z_1 Z_2 (\bar{\ell}_{12} + \bar{\ell}_{21})] F^2 = \alpha F^2 \qquad (A1)$$

Transport and transference number for water:

$$t_1 = (Z_1^2 \bar{\ell}_{11} + Z_1 Z_2 \bar{\ell}_{12})/\alpha \qquad (A2)$$

$$t_2 = (Z_2^2 \bar{\ell}_{22} + Z_2 Z_1 \bar{\ell}_{21})/\alpha \qquad (A3)$$

$$t_3 = (Z_1 \bar{\ell}_{31} + Z_2 \bar{\ell}_{32})/\alpha \qquad (A4)$$

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Salt flow, $\overline{J}_s$, and osmotic flow of water, $\overline{J}_3$ under local gradients of chemical potential $\overline{X}_{12}$ and $\overline{X}_3$;

$$\overline{J}_s = \overline{\ell}_{ss}\overline{X}_{12} + \overline{\ell}_{sw}\overline{X}_3 \qquad (A5)$$

$$\overline{J}_3 = \overline{\ell}_{ws}\overline{X}_{12} + \overline{\ell}_{ww}\overline{X}_3 \qquad (A6)$$

where

$$\overline{\ell}_{ss} = -\frac{Z_1 Z_2}{r_1 r_2}\left(\frac{\overline{\ell}_{11}\,\overline{\ell}_{22} - \overline{\ell}_{12}\,\overline{\ell}_{21}}{\alpha}\right)$$

$$\overline{\ell}_{sw} = \overline{\ell}_{ws} = \left(\overline{\ell}_{13} - \frac{t_1 t_3 \alpha}{Z_1}\right) \cdot \frac{1}{\nu_1}$$

and $$\overline{\ell}_{ww} = \left(\overline{\ell}_{33} - \frac{t_3^2 \alpha}{Z_1}\right)$$

Z_1, Z_2 are the signed valencies of the salt ions and r_1, r_2 their stoichiometric coefficients in the salt molecule.

A summary of Miller's (LN) Ternary Calculation (19a).

Solvent fixed mobility coefficients, L_{ik}, for a ternary solution containing ions 1, 2 and 3 may be obtained from the corresponding solvent-fixed mobility coefficients of the binary solutions (1, 3) and (2, 3), obtained at an equivalent concentration, N, equal to that of the ternary solution, eqns. (A7)-(A10).

$$L_{ii} = x_i (L_{ii})_{i,N} \quad i = 1, 2 \qquad (A7)$$

$$L_{33} = x_1 (L_{33})_{1,N} + x_2 (L_{33})_{2,N} \qquad (A8)$$

$$L_{i3} = x_i (L_{i3})_{1,N} \quad i = 1, 2 \qquad (A9)$$

and

$$-L_{12} = x_1 x_2 (L_{13} L_{23})\,½ \qquad (A10)$$

$(L_{ik})_{jN}$ represents binary data, evaluated at an equivalent concentration, N. The equivalent fraction of ion i, x_i, is defined as $N_i/(N_1+N_2)$, i = 1, 2 in the ternary solution. For a membrane (1, 4) containing salt (1, 2) the (LN) approximation may be used after correction of membrane (solvent-fixed) coefficients of the salt free membrane for tortuosity:

$$\overline{L}_{ik} = L_{ik}\ \nu/0.$$

For a mixed ionic form (containing no imbided salt) 1 = a, 2 = b and 3 = fixed charge (4) of the membrane and eqn. (11) is obtained.

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DISCUSSION

Chairman: Prof. P. MEARES

TEORELL

I should like to emphasise the importance of this indeed beautiful paper, because it draws our attention to the necessity of looking into the driving forces of water, which are equally important in biology as the driving forces for ions. From this presentation we learn, if we didn't know it before, that there is an intimate coupling between the membrane matrix, the ions and the water flow. We have to remember that the biological membranes in all probability have fixed charges. These may arise from carboxyl groups and phosphoric acid residues, which are built into the proteins and the lipoids. In the future I think we have to readjust the focus on the ions in the nerve theories to include also the water movements. I think we should be much obliged to the theoretical membrane people for their emphasising the driving forces of water.

KEDEM

I would like to continue Prof. TEORELL's remarks in a somewhat more specific way. With respect to the very large amounts of water which are transported in the kidneys and in the intestines, a lot of evidence indicates that they are taken along by just this mechanism, frictional drag. The cell membrane of course does not have large

aqueous pores — that would be disastrous. The channels which are analogous to those in a swollen membrane are the channels formed by infoldings, like in the brushborder and the microvilli. It is possible to transport in this way isotonic solution simply by the frictional drag the chloride and the sodium exert on the water after they have passed the lipid membrane. The water then finds itself in an aqueous channel which is lined with charges. The analogy with ion-exchange membranes is thus closer than it might seem.

MACROBBIE

Could I ask how good you would expect the agreement to be if it wasn't sulphonate but one of the other possible fixed charged groups, such as phosphate or carboxyl? You looked at two types but would you expect the agreement to be a great deal worse if it were one of the other fixed charges, as it might be in a biological system?

PATERSON

Well. If it contained carboxylic acid groups, then I think we would have problems with ion-association or other effects. I think that then you would have to think very carefully about a new analysis, pH dependence would become very important because if you reduced your pH for example, then of course you would have two effects. You would not only lose your fixed charge but the membrane would also shrink. You would have to shift your whole philosophy very rapidly to a new problem, much more complicated but very interesting. Phosphate would present less of a problem.

MONNIER

I would like to ask what is the influence of temperature on this friction between water and ions? Is it important?

PATERSON

I have no direct experience of that in membranes but there is sufficient data for electrolyte solutions, which I hope you will allow me to use as an analogue. Ion-water frictions are temperature sensitive and particularly so for small cations, which are water-order producing. They are therefore much more sensitive to their solvent environment within the medium, and therefore much more sensitive to the temperature factor.

SPIEGLER

I believe Dr. PATERSON's analysis is of exceptional value from the practical point of view, because it sets limits on what one can do with the membrane. For instance, in electrodialysis one often meets the question: « Can I have a very conductive membrane because I want as little dissipation of the electrical energy as possible »? And yet a membrane which has very low electro-osmotic water transport. In electrodialysis one tries to remove ions through the membranes to produce fresh water and one doesn't want to lose the water at the same time. I think this type of analysis makes it possihle to answer such questions: What is compatible and what is not compatible from the practical point of view?

PATERSON

I have used it myself having found this and it is very convenient to predict what you might expect the transference number to be. Although you don't get it quite right you know where you are, I think that's important.

SPIEGLER

Now I have some questions too, and my main question concerns

the use of the tortuosity concept. First I would like to ask, do you use a tortuosity factor only for water or for all the ions too?

PATERSON

I have to cover myself, I suppose, by saying that the tortuosity problem is extremely long standing and as yet not fully solved. The short answer is yes, I do, I use the same tortuosity for all species. I assume that tortuosity is purely a geometric factor.

SPIEGLER

In my opinion this is where the problem lies. I did not perceive a tortuosity argument in one of your first explanations where you explained that the ratio of t_3/t_1 is roughly half of the ratio c_3/c_1. Doesn't one have to introduce a tortuosity concept there too? The other problem is the transport number of the co-ions. In one of your slides the chloride ion had a high transport number. This is natural because in a cation exchange membrane the positive counterions and the negative co-ions apparently don't move by exactly the same route. Now if you use the same tortuosity there, could you perhaps obtain a tortuosity for the chloride that is smaller than for water? This is really not in accordance with the definition of the tortuosity.

PATERSON

First of all the transference number for water is proportional to the ratio of frictional coefficients; $\frac{f_{13}}{f_{13} + f_{14}}$

If we multiply each coefficient by any factor the ratio will be unchanged. For transport number the same considerations apply but under more restricted conditions. The basic heterogeneity that

you are talking about would probably be more important under two conditions, one would be if you had large pores and had to go back to the electro-chemist's double layer concept. You would then have co-ions in the centre of the pores and they might then have a lower tortuosity. The second would be if you had membrane voids in which you would contain free salt and water: holes, if you like, within the membrane. The interesting thing is that a meaningful membrane model may be obtained by considering an homogeneous aqueous electrolyte and looking at flows relative to a fixed ion, provided the membrane to be modelled is not grossly inhomogeneous, at least as regards the environment of species in the aqueous phase. That is a distinct and clear assumption.

COMPUTER PREDICTION OF STATIONARY STATES OF MEMBRANES FROM DIFFERENTIAL PERMEABILITIES

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Abstract

Discussions of fluxes and forces across ion-exchange membranes have frequently been couched in the terms of non-equilibrium thermodynamics in its linear form. The value of many such treatments has been restricted because it is well known that the phenomenological coefficients, which describe the behaviour of such a membrane in a macroscopic experiment, are functions of the concentrations outside the membrane and of the concentration profiles within the membrane. As a result the empirical coefficients appear to be functions of the forces. This is in conflict with the requirements of linear theory.

Despite these limitations on the non-equilibrium approach as applied directly to global or macroscopic flux data, it has been shown that the local or microscopic behaviour of ion-exchange membranes obeys the linear approximation well over usefully wide ranges of the three gradient forces: activity, electric potential and pressure. By using an appropriate analysis of sufficient experimental data it has been found possible to

extract the local phenomenological permeability coefficients of a cation exchange membrane as functions of local composition. From these coefficients, resistance and friction coefficients have been calculated and they have revealed much concerning the mechanism of interaction between the various mobile ionic components, water and the membrane material.

It is desirable to be able to predict from the data on the concentration-dependent permeabilities how a macroscopic membrane of known dimensions would perform under a given set of external constraints e. g. when a predetermined current is passed through the membrane placed between particular external solutions as in electrodialysis.

The problem at once arises that the fluxes and profiles are strongly interdependent and mean permeabilities cannot be chosen on a simple basis. In order to overcome this difficulty a computational procedure has been developed which enables the final steady state under given constraints to be evaluated by an iterative procedure.

The membrane is regarded as a hypothetical series array of slices of equal thickness. The number of slices has to be chosen sufficiently large that the difference in composition across any single slice is small enough for its behaviour to be described by the local coefficients appropriate to the mean composition of the slice. The computation then adjusts the profiles across the whole membrane until each slice is in interfacial equilibrium with its neighbours and the imposed constraints are satisfied. The required fluxes and forces are then evaluated without difficulty. Useful and informative by-products from the computation are the steady profiles of the intensive variables in the membrane. These are not accessible to direct measurement.

The procedure has been tested by calculating the osmotic and salt diffusion fluxes and the membrane potentials when the membrane separates different solutions at zero current and pressure difference. Although the computation requires the inversion of a 60×60 square matrix in most cases, the results

have been found to agree satisfactorily with those obtained experimentally.

The procedure has then been used to generate fluxes when an electric current is passed between different solutions as in electrodialysis. The data show how the membrane properties in electrodialysis depend on the current density, external concentrations and membrane thickness in a more precise way than has been available hitherto. The membrane profiles are found to exibit a very pronounced dependence on the current density.

Introduction

Theoretical papers dealing with the non-equilibrium thermodynamics of membrane processes still greatly outnumber experimental studies in which the necessary phenomenological coefficients have been measured systematically. Nevertheless experimental evidence has now been accumulated which gives grounds for confidence in the basic concepts and suggests that the thermodynamic approach can play a useful role in the interpretation of membrane phenomena and in the design of membrane processes. This paper describes and demonstrates one method by which non-equilibrium thermodynamic coefficients can be used to obtain information on conditions in a membrane that are not accessible to direct observation. The coefficients are used also to predict fluxes and forces under constraints of practical interest in which precise measurements cannot easily be made.

The most completely developed versions of non-equilibrium thermodynamics are concerned with linear relations between the applied forces and the fluxes of mobile components [1]. Many definitions of fluxes and forces may be made subject only to the restriction that they be appropriately conjugated. In this paper isothermal fluxes across ionic membranes are considered under three forces. They are the differences of electric potential, hydrostatic pressure and concentration be-

tween the opposite faces of the membrane. In practice one has to work with finite differences in these intensive variables. Because the intrinsic permeability of the membrane material may be a function of these intensive variables, its properties may vary across the membrane from one face to the other. The extent of such variations will change when the forces are varied. Consequently, a superficial interpretation of membrane flux data may appear to indicate that the relation between fluxes and forces cannot be represented by a set of linear equations.

This difficulty has probably been the main reason for the slowness of the acquisition of satisfactory thermodynamic data on many membranes. It is necessary to collect sufficient experimental results to enable a local rather than a global view of the membrane to be taken. This is sometimes referred to as taking a continuous rather than a discontinuous approach but we prefer to call it a differential discontinuous approach because all information is derived from observations made on the phases external to the membrane [2]. Here however a start has been made on finding a connection between the continuous and differential discontinuous representations because information has been extracted on conditions existing within the continuous membrane phase.

Over the range of potentials explored in most investigations on membranes it appears that no significant non-linearity in behaviour arises directly from the dependence of the membrane properties on either the local value of the electric potential or on its gradient [3]. Only limited information is available on the effect of pressure on the membrane properties but it appears that certainly the pressure gradient and possibly also the absolute value of the pressure alters the permeability and causes a true non-linearity of behaviour with respect to the pressure force [3, 4]. The practical problem of dealing with this is not yet fully solved; one tries to work at small pressure gradients but extrapolation to zero gradient has not been seriously attempted.

That membrane permeability frequently varies markediy with the composition and concentration of the ambient solutions is a well documented fact. The effects appear to be due entirely to variation in properties with the local value of the concentration and not to its gradient. Thus a concentration difference across a membrane gives rise to fluxes which may not be directly proportional to the concentration difference (a global non-linearity) but the fluxes across any plane of constant composition caused by the concentration gradient are directly proportional to that gradient (local linearity is maintained). A method that involves making a series of flux measurements under appropriate concentration differences has been developed to deal with this global non-linearity [2, 5]. By using it a set of membrane permeabilities has been evaluated which refer to a well-defined composition i. e. to the limit of a vanishing concentration gradient across the membrane [3]. Phenomenological permeability coefficients derived in this way are used in the calculations to be described here.

Whereas the extraction of the phenomenological coefficients from experimental data involves a metbod of graphical differentiation, the prediction of fluxes under macroscopic forces requires the procedure to be reversed. Direct integration is not possible because the profiles of concentration versus distance in the membrane are initially unknown. An iterative procedure is therefore needed and is described in a later section.

The membrane as a series of slices

Although the actual concentration difference across a membrane, and consequently the variation in its properties from one side to the other, may be large the membrane can be viewed as a succession of plane, parallel slices across each of which tbe concentration difference is small. The transport properties of each slice may then be represented by values appropriate for the mean composition of the slice. For each

slice, linear relations may then be written between the fluxes and the forces.

Attention is restricted here to stationary states of flow so that the fluxes have the same values across every slice. Furthermore, since the interfaces between adjacent slices are entirely hypothetical, the intensive variables are continuous throughout a homogeneous membrane i. e. the exit face of the m – th slice is at equilibrium with the entry face of the (m + 1) – th slice. Provided the permeability coefficients of the membrane are known as functions of the concentration of the external solution and the overall boundary conditions in any system it is desired to explore are properly defined, the above requirements are sufficient to ensure a unique solution for the fluxes across the membrane and the profiles of the intensive variables within it. A convenient way of expressing the values of the intensive variables at the hypothetical interface between two slices is to state the values of these variables in a free aqueous solution which would be in equilibrium with the membrane material at that interface. Thus one expresses the conditions which would arise in a microscopic cavity created in the membrane in the plane of the interface. Because the membrane properties have been determined as functions of the external solution concentration, this procedure makes it straightforward to relate permeability to local conditions.

The procedure adopted here is the most obvious and also the simplest in computation. The membrane is treated as though it were divided into a set, usually twenty, of slices of equal thickness. The concentration difference across each of these slices is permitted to adjust itself in response to the overall boundary conditions across the membrane. This simple procedure has one disadvantage. Under some circumstances the concentration profile in the membrane may be far from linear and a major part of the concentration interval would be spanned by only a small number of slices. In such a case one would have to question the permissibility of using the linear equations to express the relations between fluxes and forces in the highly

non-uniform slices. Seen from this standpoint it would be better to sub-divide the membrane into slices across which the concentration differences were equal and to allow the thicknesses of the slices to be unequal. This procedure requires a more complex computation but we hope to study it in the future.

Development of theory

The system examined in this paper is an ion-exchange membrane (the data refer to a cation permeable membrane but the treatment is general) separating two solutions of the same single salt at the same temperature. The solutions may be at different concentrations and electric potentials. They may also be at different pressures but such cases are not included in the examples calculated here.

The formulation of fluxes and forces has been dealt with previously [2]. Essentially we have used the practical system of KEDEM and KATCHALSKY [6], derived from the work of STAVERMAN [7]. The deduction of the differential phenomenological coefficients owes much to the ideas of KEDEM and MICHAELI [8].

There are three independent particle fluxes viz. the two kinds of ions and water. The chosen practical fluxes are the cation flux density ϕ_1, the volume flux density ϕ_v and the electric current density i. The fluxes under infinitesimal forces will be written $\partial\phi_1$, $\partial\phi_v$ and ∂i. The forces conjugated with these infinitesimal fluxes are $\partial\pi/\upsilon_1 c_s$, $\partial(p - \pi)$ and ∂E respectively where υ_1 is the number of cations per mole of salt and c_s is the concentration of the solution which differs by a vanishing amount ∂c_s between opposite faces of the membrane thus creating an osmotic pressure difference $\partial\pi$. ∂p is the pressure difference and ∂E the electric potential difference measured with reversible anion-selective electrodes.

In order to avoid unnecessary negative signs, fluxes are

defined as positive in the direction opposite from that chosen to express increases in the intensive variables c_s, π, p and E. The linear flux equations can then be set down in the differential forms

$$\partial \phi_1 = \mathcal{L}_\pi \, (\partial \pi / \upsilon_1 c_s) + \mathcal{L}_{\pi p} \, \partial (p - \pi) + \mathcal{L}_{\pi E} \, \partial E \qquad (1)$$

$$\partial \phi_v = \mathcal{L}_{p\pi} \, (\partial \pi / \upsilon_1 c_s) + \mathcal{L}_p \, \partial (p - \pi) + \mathcal{L}_{pE} \, \partial E \qquad (2)$$

$$\partial i = \mathcal{L}_{E\pi} \, (\partial \pi / \upsilon_1 c_s) + \mathcal{L}_{Ep} \, \partial (p - \pi) + \mathcal{L}_E \, \partial E \qquad (3)$$

Here the coefficients $\mathcal{L}_\pi$, $\mathcal{L}_{\pi p}$ etc. are independent of the values of the fluxes and forces. They are functions of the membrane, temperature, salt and c_s only. They are inversely proportional to the thickness of the membrane d and, in previous publications, their values have been related to a standard membrane of thickness 1mm. In an actual membrane of thickness d their values are $\mathcal{L}_\pi/d$ etc. where d is the thickness in mm.

The membrane is now imagined to be composed of m equal slices of thickness d/m numbered 1 - - - k - - - m in the direction of positive fluxes. The flux equations for the k-th slice can be written in the form

$$\phi_1 = L_\pi (k) (\pi_{k-1} - \pi_k) / \upsilon_1 \overline{c_s} (k) + L_{\pi p} (k) (p_{k-1} - p_k - \pi_{k-1} + \pi_k) + L_{\pi E} (k) (E_{k-1} - E_k) \qquad (4)$$

$$\phi_v = L_{p\pi} (k) (\pi_{k-1} - \pi_k) / \upsilon_1 \overline{c_s} (k) + L_p (k) (p_{k-1} - p_k - \pi_{k-1} + \pi_k) + L_{pE} (k) (E_{k-1} - E_k) \qquad (5)$$

$$i = L_{E\pi} (k) (\pi_{k-1} - \pi_k) / \upsilon_1 \overline{c_s} (k) + L_{Ep} (k) (p_{k-1} - p_k - \pi_{k-1} + \pi_k) + L_E (k) (E_{k-1} - E_k) \qquad (6)$$

Here E_k, p_k, π_k and c_k refer to the values of E, p, π and c_s in the hypothetical solution in equilibrium with the membrane

at the interface between slices k and (k+1). $\overline{c_s}(k)$ is the mean of c_k and c_{k-1}.

The correct choice of mean concentration is not obvious. We have tested the log mean

$$\overline{c_s}(k) = (c_{k-1} - c_k)/(\ln c_{k-1} - \ln c_k) \tag{7}$$

and the arithmetic mean

$$\overline{\overline{c_s}}(k) = \tfrac{1}{2}(c_{k-1} + c_k) \tag{8}$$

and have found the latter to be preferable. This finding supports the observations of others [9].

It will be assumed that, provided $|c_{k-1} - c_k|$ is sufficiently small, the average conductance coefficients $L_\pi(k)$ etc. may be replaced by

$$L_\pi(k) = m \mathscr{L}_\pi \{\overline{c_s}(k)\}/d \tag{9}$$

where $\mathscr{L}_\pi \{\overline{c_s}(k)\}$ means the value of the differential coefficient $\mathscr{L}_\pi$ appropriate to concentration $\overline{c_s}(k)$.

The osmotic pressure difference $(\pi_{k-1} - \pi_k)$ may be written, with negligible error, in the form

$$\pi_{k-1} - \pi_k = \upsilon RT\,\overline{\overline{\theta}}(k)\,(c_{k-1} - c_k) \tag{10}$$

where υ is the total number of ions released per mole of salt and $\overline{\theta}(k)$ is the osmotic coefficient at concentration $\overline{\overline{c_s}}(k)$.

For the sake of compactness, three parameters λ_π, λ_p and λ_E are defined by

$$\lambda_\pi(k) = \upsilon RT\,\overline{\overline{\theta}}(k)\,[L_\pi(k)/\upsilon_1\overline{c_s}(k) - L_{\pi p}(k)] \tag{11}$$

$$\lambda_p(k) = \upsilon RT\,\overline{\overline{\theta}}(k)\,[L_{p\pi}(k)/\upsilon_1\overline{c_s}(k) - L_p(k)] \tag{12}$$

$$\lambda_E(k) = \upsilon RT\,\overline{\overline{\theta}}(k)\,[L_{E\pi}(k)/\upsilon_1\overline{c_s}(k) - L_{Ep}(k)] \tag{13}$$

In terms of these parameters the flux equations for a slice (4), (5) and (6) may be rearranged into the simpler expressions

$$\lambda_{\pi}(k)(c_{k-1} - c_k) + L_{\pi p}(k)(p_{k-1} - p_k) + \\ + L_{\pi E}(k)(E_{k-1} - E_k) - \phi_1 = 0 \qquad (14)$$

$$\lambda_{p}(k)(c_{k-1} - c_k) + L_{\cdot}(k)(p_{k-1} - p_k) + \\ + L_{pE}(k)(E_{k-1} - E_k) - \phi_v = 0 \qquad (15)$$

$$\lambda_{E}(k)(c_{k-1} - c_k) + L_{Ep}(k)(p_{k-1} - p_k) + \\ + L_{E}(k)(E_{k-1} - E_k) = i \qquad (16)$$

It should be noted that although in steady flow through a plane membrane, ϕ_1 and i are independent of k, the same is true of ϕ_v only in so far as the compressibility of the flowing medium may be ignored. The linear scheme of flux equations can be used over only moderate pressure intervals. In this range the error in assuming ϕ_v to be independent of k is in the region of tenths of one per cent and so is quite negligible.

Conditions in the solution on the exit side of the membrane are denoted by subscript m while at the entry face subscript $_0$ is used. In the conditions to be discussed here it will be assumed that c_0, c_m, p_0, p_m and E_0 are known and kept constant but no major difficulties are met in dealing with other conditions e. g. by setting c_m equal to ϕ_1/ϕ_m, as in reverse osmosis. The current i is a variable under the control of the investigator and this permits ϕ_1, ϕ_v and the potential difference $(E_m - E_0)$ in the configuration of electrodialysis to be studied as a function of i.

It can be seen that there are 3m equations like (14), (15) and (16) and 3m unknowns viz. c_1, c_2 --- c_{m-1}; p_1, p_2 --- --- p_{m-1}; E_1, E_2 --- E_m; ϕ_1 and ϕ_v. The problem to be solved may be set out in matrix form remembering that the equations concerning the first and last (m-th) slices are somewhat different

from those for each of the intermediate slices. For illustrative purposes, the matrix is written down in Table 1 for a membrane of five slices.

Computation

Programme development was started on Aberdeen University's English Electric System 4 computer but most of the computation was done on the CDC 7600 machine at the University of London.

The basic method of solution makes use of matrix inversion by a translation of CACM Algorithm 120: Matinv 2. The flow diagram of the main programme is shown in Figure 1.

The experimental data on the six phenomenological coefficients, $\mathcal{L}_p$ etc., and published data on the osmotic coefficients of the salt solutions [10], each as functions of the concentration c_s were fitted in advance to orthogonal polynomials. From these the appropriate values were interpolated as required during the progress of the iterative computation. The polynomial coefficients and arrays A and B, which arise from the recurrence relation between orthogonal polynomials, are read in Block 3.

Block 4 reads in control data on the situation to be studied and defines the ingoing side as the higher concentration side of the membrane. This condition is required in Blocks 19-22.

Blocks 7 and 8 set the starting condition as being a linear concentration gradient. (This is not a linear gradient in the membrane but a linear gradient in the hypothetical solutions in equilibrium at the slice interfaces.) The arithmetic mean of the interfacial concentrations is chosen here as determining the mean composition of each slice but the logarithmic or any other mean concentration may easily be substituted.

Block 9 interpolates the appropriate values of the coefficients for each slice from the information in Block 3 and these are scaled for the slice thickness in Block 10.

1 — *Conductance matrix for a membrane of five slices.*

$$
\begin{array}{cccccccccccccc|c|c|l}
0 & 0 & 0 & -L_{\pi p}(1) & 0 & 0 & 0 & -L_{\pi E}(1) & 0 & 0 & 0 & 0 & -1 & 0 & c_1 & & -\lambda_{\pi}(1)c_o - L_{\pi p}(1)p_o - \\
0 & 0 & 0 & -L_{p}(1) & 0 & 0 & 0 & -L_{pE}(1) & 0 & 0 & 0 & 0 & 0 & -1 & c_2 & & -\lambda_{p}(1)c_o - L_{p}(1)p_o - \\
0 & 0 & 0 & -L_{Ep}(1) & 0 & 0 & 0 & -L_{E}(1) & 0 & 0 & 0 & 0 & 0 & 0 & c_3 & & i - \lambda_{E}(1)c_o - L_{Ep}(1)p_o - \\
-\lambda_{\pi}(2) & 0 & 0 & +L_{p\pi}(2) & -L_{\pi p}(2) & 0 & 0 & +L_{\pi E}(2) & -L_{\pi E}(2) & 0 & 0 & 0 & -1 & 0 & c_4 & & 0 \\
-\lambda_{p}(2) & 0 & 0 & +L_{p}(2) & -L_{p}(2) & 0 & 0 & +L_{pE}(2) & -L_{pE}(2) & 0 & 0 & 0 & 0 & -1 & p_1 & & 0 \\
-\lambda_{E}(2) & 0 & 0 & +L_{Ep}(2) & -L_{Ep}(2) & 0 & 0 & +L_{E}(2) & -L_{E}(2) & 0 & 0 & 0 & 0 & 0 & p_2 & & i \\
+\lambda_{\pi}(3) & -\lambda_{\pi}(3) & 0 & 0 & +L_{\pi p}(3) & -L_{\pi p}(3) & 0 & 0 & +L_{\pi E}(3) & -L_{\pi E}(3) & 0 & 0 & -1 & 0 & p_3 & & 0 \\
+\lambda_{p}(3) & -\lambda_{p}(3) & 0 & 0 & +L_{p}(3) & -L_{p}(3) & 0 & 0 & +L_{pE}(3) & -L_{pE}(3) & 0 & 0 & 0 & -1 & p_4 & = & 0 \\
+\lambda_{E}(3) & -\lambda_{E}(3) & 0 & 0 & +L_{Ep}(3) & -L_{Ep}(3) & 0 & 0 & +L_{E}(3) & -L_{E}(3) & 0 & 0 & 0 & 0 & E_1 & & i \\
0 & +\lambda_{\pi}(4) & -\lambda_{\pi}(4) & 0 & 0 & +L_{\pi p}(4) & -L_{\pi p}(4) & 0 & 0 & +L_{\pi E}(4) & -L_{\pi E}(4) & 0 & -1 & 0 & E_2 & & 0 \\
0 & +\lambda_{p}(4) & -\lambda_{p}(4) & 0 & 0 & +L_{p}(4) & -L_{p}(4) & 0 & 0 & +L_{pE}(4) & -L_{pE}(4) & 0 & 0 & -1 & E_3 & & 0 \\
0 & +\lambda_{E}(4) & -\lambda_{E}(4) & 0 & 0 & +L_{Ep}(4) & -L_{Ep}(4) & 0 & 0 & +L_{E}(4) & -L_{E}(4) & 0 & 0 & 0 & E_4 & & i \\
0 & 0 & +\lambda_{\pi}(5) & 0 & 0 & 0 & +L_{\pi p}(5) & 0 & 0 & 0 & +L_{\pi E}(5) & -L_{\pi E}(5) & -1 & 0 & E_5 & & \lambda_{\pi}(5)c_5 + L_{\pi p}(5)p_5 \\
0 & 0 & +\lambda_{p}(5) & 0 & 0 & 0 & +L_{p}(5) & 0 & 0 & 0 & +L_{pE}(5) & -L_{pE}(5) & 0 & -1 & \phi_1 & & \lambda_{p}(5)c_5 + L_{p}(5)p_5 \\
0 & 0 & +\lambda_{E}(5) & 0 & 0 & 0 & +L_{Ep}(5) & 0 & 0 & 0 & +L_{E}(5) & -L_{E}(5) & 0 & 0 & \phi_v & & i + \lambda_{E}(5)c_5 + L_{Ep}(5)p_5
\end{array}
$$

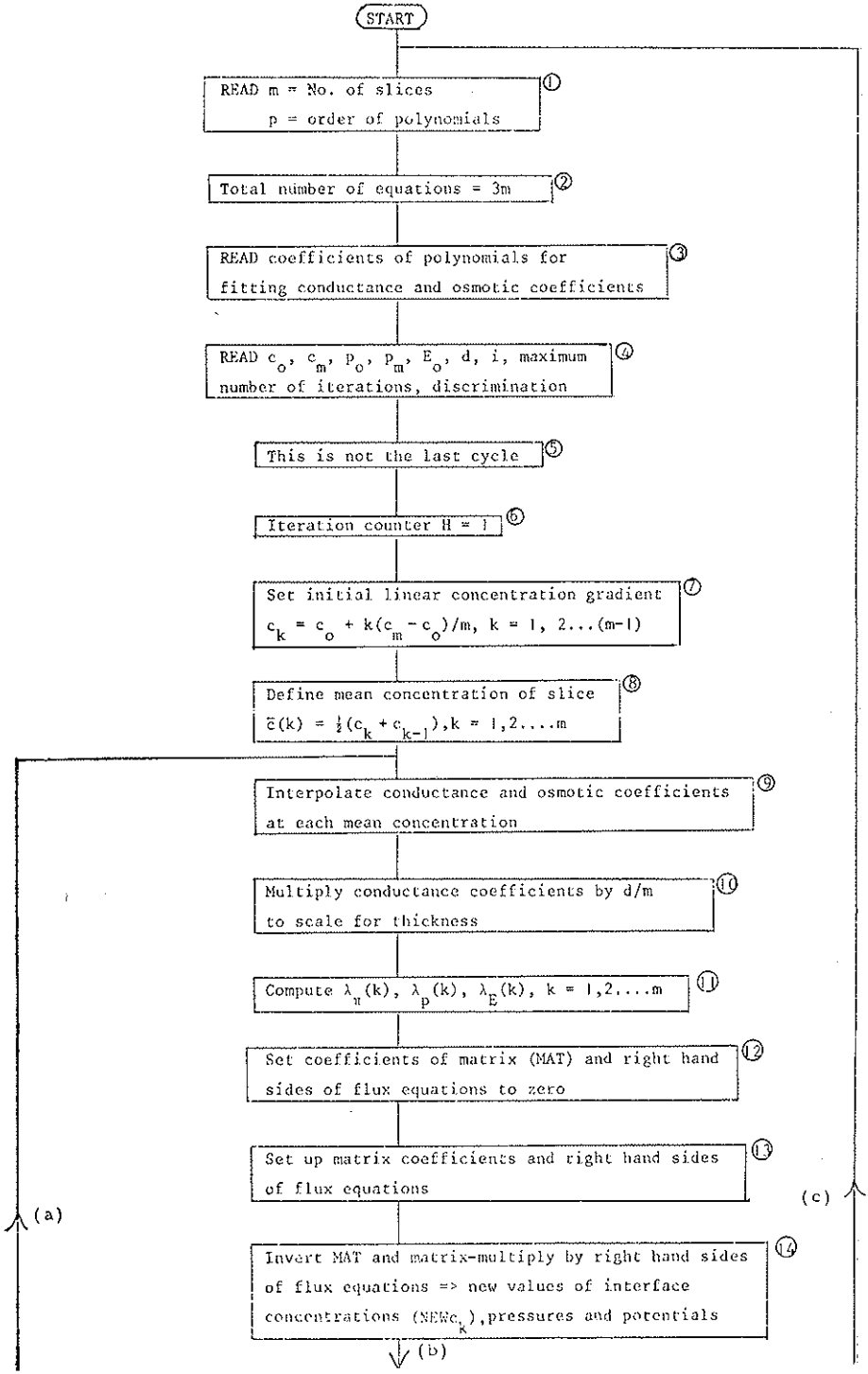

FIG. 1 — Flow diagram.

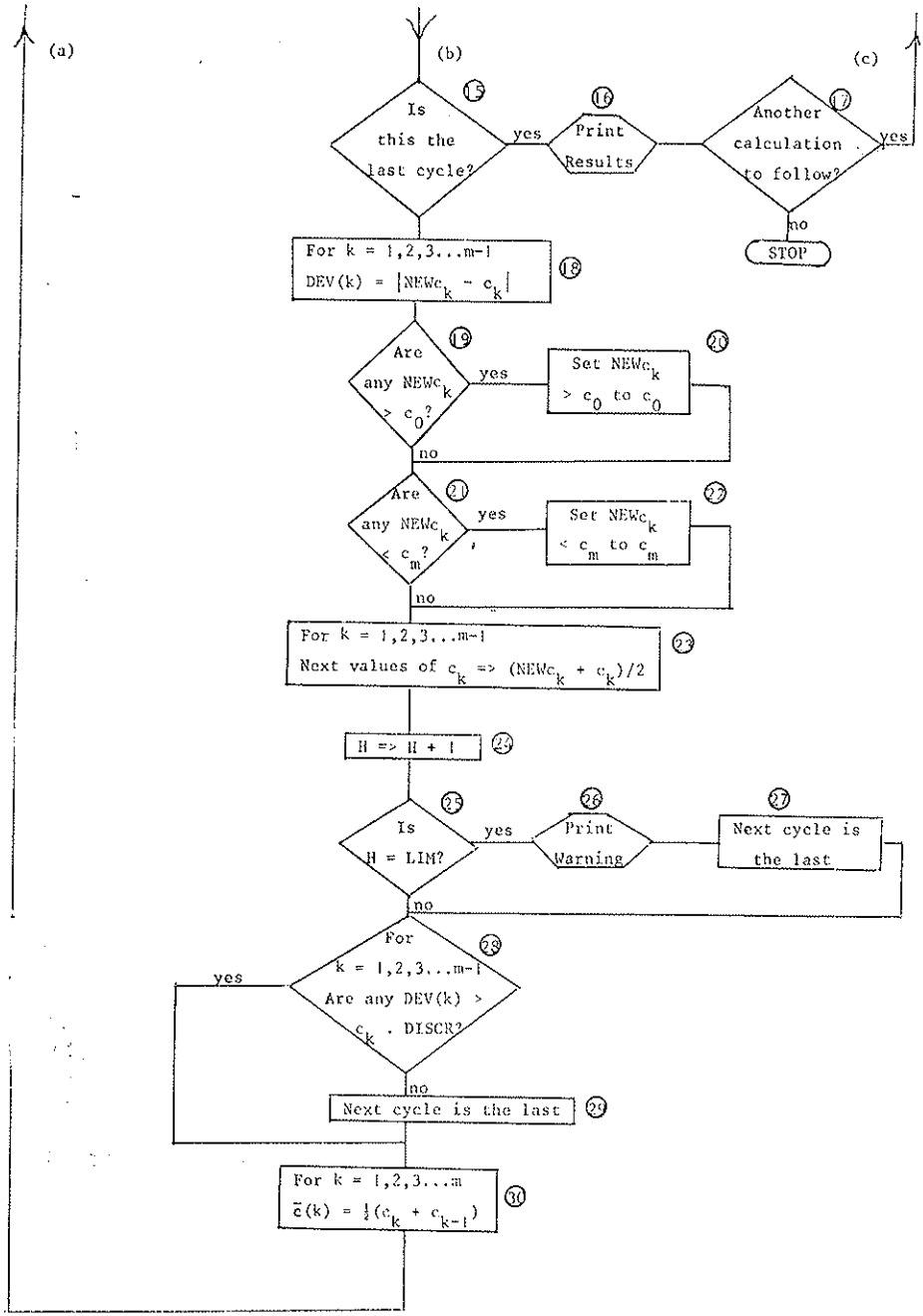

FIG. 1 — Flow diagram (contd).

In setting up the 3m × 3m matrix (Blocks 12 and 13) all elements are first set to zero and then the coefficients are assigned to the required locations on the basis of a set of rules determined by the relative symmetry of most of the flux equations.

Matrix inversion and calculation of new interfacial values of the intensity factors are carried out in Block 14.

Blocks 19-22 are concerned with suppressing apparent negative values of concentration and inhibiting oscillations. For the next cycle of the iteration the interfacial concentrations are chosen to be the means of those used in the previous cycle and the newly calculated values. This procedure speeds up convergence to the desired degree of discrimination.

When the preset discrimination or the preset maximum number of cycles has been reached the results are printed in consequence of Blocks 15 and 16 on the next cycle.

Results of simple tests

In order to examine the accuracy of the polynomials used in the interpolation of the conductance coefficients and of the matrix inversion subroutine two types of simple test calculation were carried out. In the first, the pressure difference Δp and concentration difference Δc_s were set at zero. A value of c_s was chosen and a series of calculations made for different current densities i. This is the configuration of a membrane conductance experiment at constant composition. The calculation involves only the coefficients $\mathcal{L}_E$, $\mathcal{L}_{\pi E}$ and $\mathcal{L}_{pE}$.

The following expectations were exactly confirmed on using our data for the conductance coefficients of Zeo-Karb 315 (*) in contact with NaBr solutions: $(i/\Delta E)_{c_s}$ = constant

(*) The structure and properties of Zeo-Karb 315 membranes have been described previously [11, 12].

(the ohmic conductance) at each value of c_s; similarly $(\phi_j/i)_{c_s}$ is independent of i (constant cation transport number); $(\phi_v/i)_{c_s}$ is independent of i (constant electro-osmotic permeability).

No concentration differences or pressure differences appeared between slices and the potential gradient was always exactly linear. Table 2 compares the computed potentials, cation and volume fluxes for one current at several concentrations with those interpolated directly from experimental data [13, 14]. It confirms that the polynomials for $\mathcal{L}_{\pi E}$ and $\mathcal{L}_E$, $\mathcal{L}_{pE}$ and the matrix inversion procedure are satisfactory in the case of a membrane divided into 20 hypothetical slices.

The next set of test calculations dealt with the case of fluxes and potentials under a concentration gradient ($\Delta c_s \neq 0$) and open circuit ($i = 0$) while keeping $\Delta p = 0$. In Table 3 the experimental values of the fluxes [15, 16] and potentials [17] are compared with those calculated from the previously published conductance coefficients [3] at eight concentration intervals. The calculated values are in the column headed $[\mathcal{L}_\pi(1)]$.

It can be seen that the agreement between the computed and observed potentials is excellent and the agreement is also very satisfactory in the case of the volume fluxes. As an internal check on the mechanics of the computation procedure, it may be noted that the values of ΔE, ϕ_v and ϕ_j all obey the additivity rule for homogeneous membranes [2] i. e.

$$\phi_j(c_1 \rightarrow c_2) + \phi_j(c_2 \rightarrow c_3) = \phi_j(c_1 \rightarrow c_3) \qquad (17)$$

The agreement of the observed and calculated cation fluxes ϕ_j which, in the case of a uni-univalent electrolyte and open circuit is also the salt flux ϕ_s, is not satisfactory. The computed values are too large between dilute solutions and too small between concentrated ones. The discrepancy is usually in the region of a factor of two.

The calculated value of the salt flux is very sensitive to the

TABLE 2 — *Observed and computed potentials and fluxes caused by an electric current of 1 A m^{-2} in a Zeo-Karb 315 membrane 1mm thick in NaBr.*

c_s	ΔE		ϕ_1		ϕ_v	
	obs.	*comp.*	*obs.*	*comp.*	*obs.*	*comp.*
(mol l^{-1})	(m V)		(μmol m^{-2} s^{-1})		(nm s^{-1})	
0.01	1.118	1.117	10.34	10.34	11.13	11.19
0.05	1.031	1.023	10.18	10.17	9.46	9.23
0.10	0.894	0.902	9.90	9.90	7.59	7.68
0.20	0.715	0.712	9.26	9.37	6.11	6.10
0.50	0.423	0.421	8.18	8.18	3.66	3.66
0.995	0.265	0.265	7.38	7.38	2.12	2.12

value of the coefficient $\mathcal{L}_\pi$, the "straight" coefficient coupled to the osmotic gradient ($\partial\pi/\upsilon_1 c_s$). It was pointed out previously [3] that, in the method used to determine the conductance coefficients, $\mathcal{L}_\pi$ was the least reliable because it was extracted only indirectly from the experimental data and the errors of many types of data were accumulated in it.

The observed salt fluxes were obtained by measuring in separate experiments the tracer flux of 82Br up and down tbe concentration gradient and deriving the net flux by taking the difference between the two one-way fluxes [16]. The data have not yet been published in full although our procedure has been described [18, 19]. By differentiating the experimental salt flux curves the coefficient $(\partial\phi_1/\partial c_s)_{\substack{p=0\\i=0}}$ may be obtained.

From this and other data a more directly derived value of $\mathcal{L}_\pi$ may be calculated by using the equation [16]

$$\mathcal{L}_\pi = \frac{t_1^2\ k}{z_i^2\ F} + c_s\left\{c_s\left(\frac{\partial \phi_v}{\partial p}\right)^{i=0} + \left(\frac{\partial c_s}{\partial p}\right)^{i=0}\left[\phi_v - \left(\frac{\partial \phi_1}{\partial c_s}\right)_{p=0}^{i=0}\right] + \left(\frac{\partial \phi_1}{\partial c_s}\right)_{p=0}^{i=0}\left(\frac{\partial c_s}{\partial \pi}\right)\right\} \qquad (18)$$

Here t_1 and z_1 are the transport number and valency of the cations, k tbe membrane electrical conductance and F tbe Faraday. The other parameters have already been defined and subscript $i=0$ alone refers to a pressure driven (hyperfiltration) experiment under open circuit.

Equation (18) has been used with the salt diffusion fluxes and electrical and hyperfiltration data already published [3] to calculate a new set of values of $\mathcal{L}_\pi$ which will be denoted by $\mathcal{L}_\pi$ (2).

The computation of ϕ_1, ϕ_v and ΔE has been repeated using these new values $\mathcal{L}_\pi(2)$ together with the other conductance coefficients as before and the results are also in Table 3 in the columns headed [$\mathcal{L}_\pi(2)$]. It is clear that the agreements of experiment and calculation are still good for ΔE and ϕ_v and now are good in the case of ϕ_1 also. Evidently the behaviour of the membrane Zeo-Karb 315 both under electric current and under concentration gradient can be satisfactorily represented by the coefficients $\mathcal{L}_E$, $\mathcal{L}_{\pi E}$, $\mathcal{L}_{pE}$, $\mathcal{L}_p$ and $\mathcal{L}_{p\pi}$ previously publisbed together with the new values $\mathcal{L}_\pi(2)$ which are given in Table 4. All further computations have been carried out by using this set of coefficients.

Membrane profiles

Results discussed so far have not revealed any new information about membranes. They have shown that a wide

TABLE 3 — *Observed and computed potential and fluxes caused by a concentration gradient of NaBr across Zeo-Karb 315 membrane 1mm thick.*

c_o	c_m	ΔE (m V)			ϕ_i (μmol m^{-2} s^{-1})			ϕ_v (nm s^{-1})		
(mol l^{-1})		$[\mathcal{L}_{\pi}(1)]$	obs.	$[\mathcal{L}_{\pi}(2)]$	$[\mathcal{L}_{\pi}(1)]$	obs.	$[\mathcal{L}_{\pi}(2)]$	$[\mathcal{L}_{\pi}(1)]$	obs.	$[\mathcal{L}_{\pi}(2)]$
0.02	0.01	− 33.6	− 32.8	− 33.7	5.45	1.01	1.48	− 18.7	− 16.5	− 19.0
0.05	0.01	− 76.5	− 73.8	− 76.6	10.15	7.48	7.24	− 53.7	− 57.7	− 56.6
0.05	0.02	− 42.9	− 41.5	− 43.0	4.58	6.47	5.86	− 36.3	− 39.5	− 36.6
0.20	0.10	− 27.8	− 26.9	− 27.8	61.88	40.10	42.76	− 44.6	− 41.0	− 44.6
0.50	0.02	−125.7	−128.0	−126.6	142.9	230.4	197.9	−163.4	−155.4	−161.4
0.50	0.05	− 88.5	− 87.5	− 88.7	144.9	214.0	198.7	−128.5	−118.0	−126.4
0.50	0.10	− 58.2	− 58.0	− 58.6	130.7	208.8	189.6	− 88.3	− 75.8	− 87.4
0.50	0.20	− 30.5	− 30.4	− 30.7	69.9	168.7	151.5	− 43.3	− 34.7	− 43.9

TABLE 4 — *Solute permeability coefficient* $\mathcal{L}_\pi(2)$ *of Zeo-Karb 315 in contact with NaBr solutions at 25°C determined from net tracer fluxes (cf. ref. 3).*

c_s (mol dm^{-3})	$10^9\ \mathcal{L}_\pi(2)$ (mol^2 m^{-3} N^{-1} s^{-1})
0.995	58.00
0.495	28.80
0.492	28.71
0.290	19.54
0.287	19.47
0.187	15.94
0.184	15.77
0.085	12.41
0.082	12.28
0.036	11.01
0.032	10.95
0.020	10.76
0.015	10.68
0.010	10.53

range of flux and potential measurements across a charged membrane can be correlated through the formalism of linear non-equilibrium thermodynamics and that procedures have been developed which enable fluxes etc. to be predicted reliably in experimental situations in which the global behaviour of the membrane is non-linear.

The data from the computer include also the values of E, p and c_s pertaining to the interface between each pair of membrane slices. From tbese data it is possible to build up a picture of the profiles of potential, pressure and concentration in the membrane under conditions of steady flow. Such profiles are not readily determinable by experiment although some earlier workers tried to ascertain them by using composite membranes made up of a small number of slices which could be quickly separated for analysis once steady flow had been established [20, 21].

In our calculations the membrane was treated as made up of twenty equal slices. As examples, the profiles for two concentration pairs: 0.50M/0.20M and 0.50M/0.02M, i.e. a small and a moderately large concentration ratio, will be discussed in detail. The profiles are plotted in Figures 2, 3 and 4. In these the abscissa scale gives the interface number 0 to 20 i.e. k refers to the interface between slices k and (k + 1). When plotted in this way the profiles are independent of the actual thickness of the membrane because the ordinate scales represent intensive quantities. As mentioned above, the fluxes are inversely proportional to the membrane thickness as long as local linearity is oheyed.

The plots of concentration (Figure 2) are surprisingly straight; the only noticeable feature is the slight downward curve close to the dilute side in the 0.50M/0.02M case. Such simple behaviour was not to be expected in a charged membrane in which the solute mobility, as measured by $\mathscr{L}_\pi(c_s)$ (Table 4), is a clear function of concentration.

The plots of potential E (Figure3) are curved and indicate an increase in the apparent potential gradient towards the dilute side of the membrane i.e. where concentrations of counterions Na^+ and co-ions Br^- have their largest ratio.

Most interesting are the plots of pressure p (Figure 4). In each case they indicate a maximum in the pressure about one quarter of the way across the membrane from the dilute

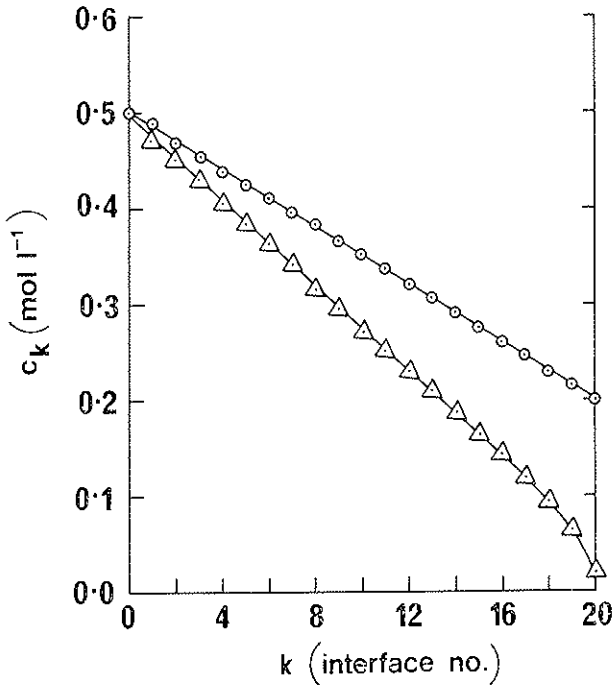

FIG. 2 — Profile of interface concentrations c_k of NaBr.
⊙ 0.50M/0.20M; △ 0.50M/0.02M.

side. The qualitative explanation of this behaviour is straightforward when bearing in mind the almost linear concentration plots of Figure 2. The volume flow, which in the case of NaBr solutions always occurs from dilute to concentrated, across a given concentration interval Δc_s decreases as the mean concentration over the interval increases. However in steady flow ϕ_v is independent of distance and the unrestrained osmotic flux across each region of the concentration profile has to be compensated by a pressure driven volume flux to ensure constancy of ϕ_v. Thus, on the dilute side the osmotic flux is reduced by an oppositely directed pressure gradient and on the

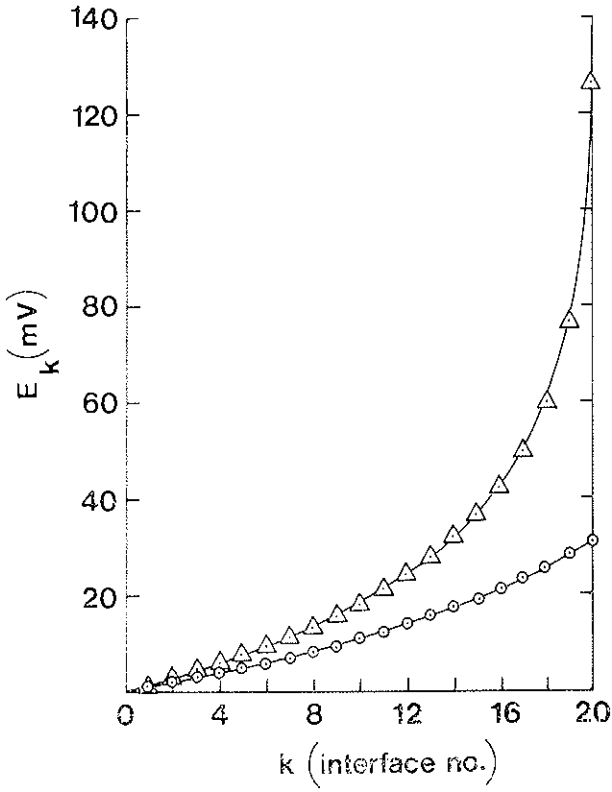

FIG. 3 — Profile of interface potentials E_k relative to Br^- electrode in the more concentrated solution.

⊙ 0.50M/0.20M; △ 0.50M/0.02M.

concentrated side the flux is assisted by the reverse pressure gradient. Quantitatively the curves are complicated by the concentration dependence of the hydraulic permeability coefficient $\mathscr{L}_p$. The effect is quite important in the 0.5M/0.02M case, the maximum pressure being 2.7 atm above ambient but in the 0.50M/0.20M case the maximum is at only about 0.15 atm.

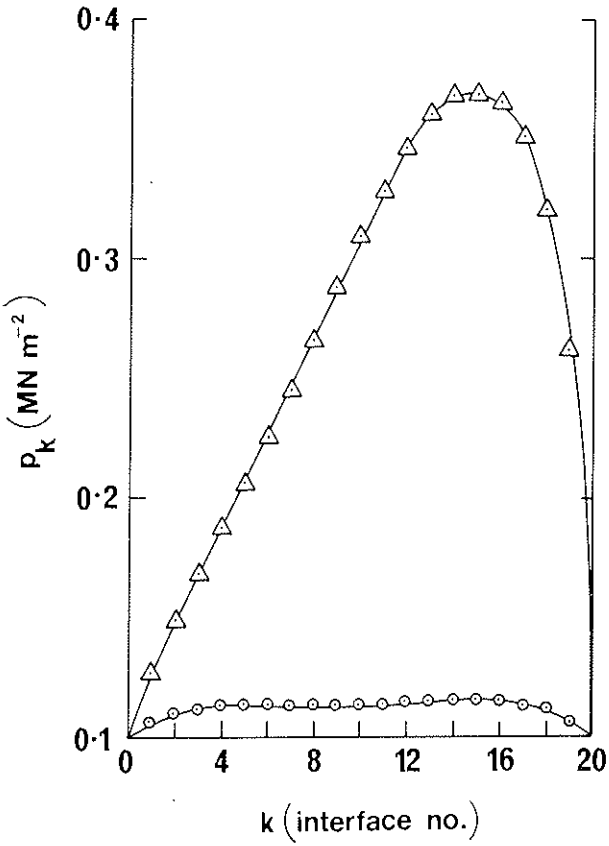

FIG. 4 — Profile of interface pressures relative to external solution at 0.1 MN m^{-2}.

⊙ 0.50M/0.20M; △ 0.50M/0.02M.

Profiles inside the membrane phase

The quantities whose profiles were discussed above refer to the states of solutions which would be in equilibrium with the membrane at the interfaces between the hypothetical slices.

More directly interesting are the conditions within the membrane phase itself because it is the parameters specifying these internal conditions which appear in the now almost classical model treatments of membrane transport, such as that obtained by combining the Nernst-Planck flux equation with the fixed charge model of TEORELL and MEYER and SIEVERS [22].

These internal profiles can also be obtained from our data provided one makes some relatively innocuous approximations about activity and osmotic coefficients when estimating potentials and pressures, i.e. the derived internal profiles do not have absolute thermodynamic rigour.

The internal concentration profiles can be obtained unambiguously by measuring the co-ion concentration in the membrane in equilibrium at each external concentration of interest. The extensive data on the sorption of NaBr by Zeo-Karb 315 [11, 23] can be represented by an equation [24], developed from GLUECKAUF'S treatment of electrolyte sorption [25],

$$\bar{m}\,(\bar{m} + M) = (\alpha\, m)^{2\beta} \qquad (19)$$

where α is a ratio of activity coefficients, characteristic of the electrolyte and close to unity, while β is characteristic of the resin structure. $\bar{m}$, m and M are the molalities of the external solution sorbed co-ions and fixed charges respectively.

Data on the sorption of NaBr are plotted according to equation (19) in Figure 5. From this plot it is found that $\alpha = 1.164$ and $\beta = 0.8055$. Over the concentration range 0.02-0.50 molar the change in membrane swelling is negligible so that M is constant and one may use

$$\bar{c} = c_w\, \bar{m}/55.51$$

and

$$X = c_w\, M/55.51$$

where c_w is the molarity of water in the membrane, 41.25 in the present case. $\bar{c}$ and X are the molar concentrations of

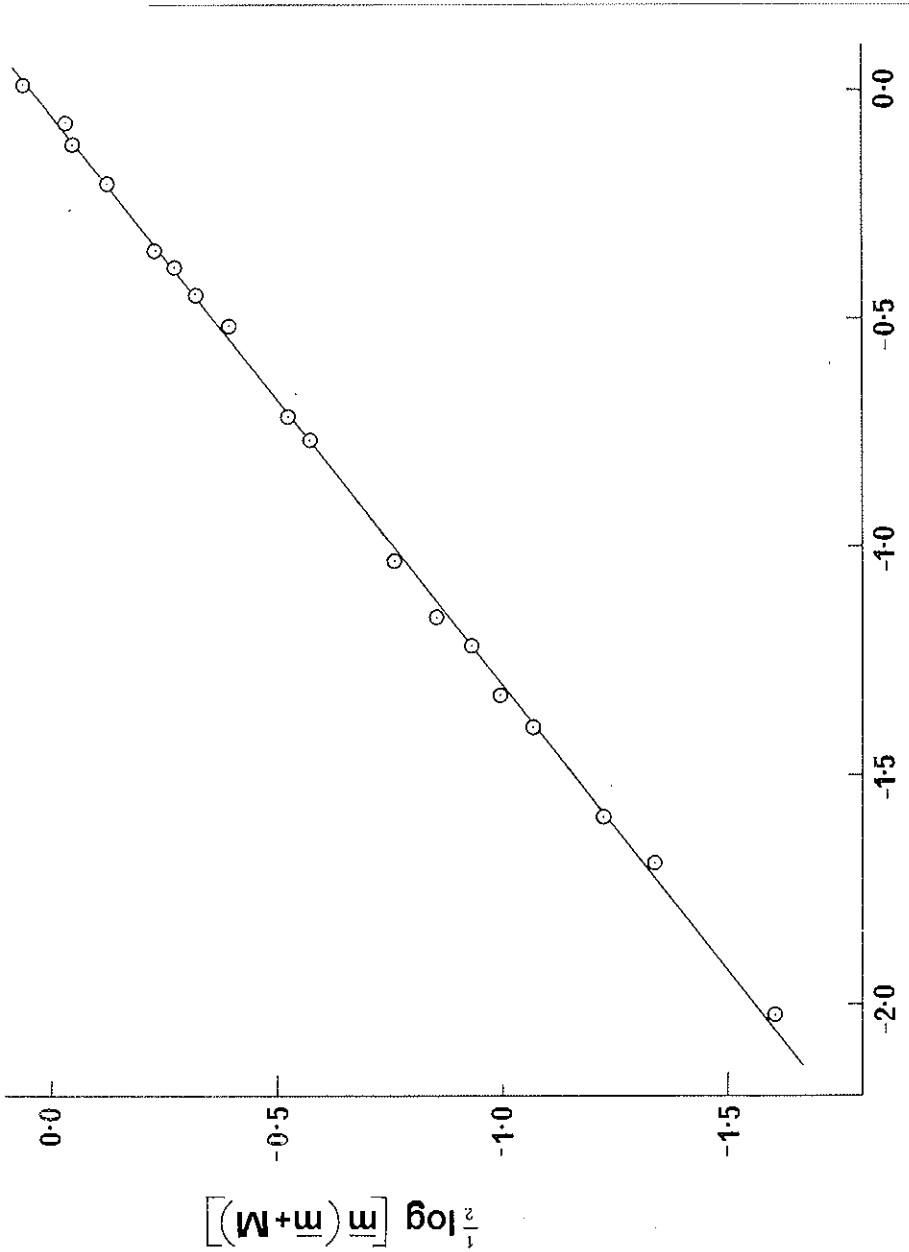

FIG. 5 — log - log plot of internal and external Br concentrations according to equation (19). NaBr in Zeo-Karb 315.

co-ions and fixed charges. For NaBr at 0.50 molar $m \simeq c$ to within 1%. Thus one may write

$$\bar{c}_k = \tfrac{1}{2}\left[-0.550 + \{0.3025 + 4\,(0.805\ c_k)^{1.611}\}^{\frac{1}{2}}\right] \qquad (20)$$

Figure 6 gives the profiles of $\bar{c}_k$ derived from the c_k profiles in Figure 2. It is seen that the internal concentration gradients are very nearly constant in both examples.

The potential E refers to a measurement made with an electrode reversible to Br^- ions. Defining E_0 as the electrode potential in the solution on the concentrated side and E_k that in the solution at interface k, and letting ψ_0 and ψ_k be the corresponding potentials of the liquid phases and $\bar{\psi}_0$ and $\bar{\psi}_k$ the potentials in the membrane at interfaces 0 and k, the following relations hold

$$E_i - \psi_i = E^\circ_{Br} - \frac{RT}{F} \ln c_i y_i \quad (i = 0 \text{ or } k) \qquad (21)$$

$$\psi_i - \bar{\psi}_i = \frac{RT}{F} \ln (\alpha' c_i/\bar{c}_i) \quad (i = 0 \text{ or } k) \qquad (22)$$

Equation (22) concerns the Donnan potential and has been discussed before [24]; α' represents $c_w\alpha/55.51$. E°_{Br} is the standard potential of the bromide electrode and y_i the molar activity coefficient of Br^- in the solution. Setting E_0 as the zero level of potential it is easily found that

$$\bar{\psi}_k - \bar{\psi}_0 = E_k + \frac{RT}{F} \ln \frac{\bar{c}_k\, y_k}{c_0\, y_0} \qquad (23)$$

$(\bar{\psi}_k - \bar{\psi}_0)$ gives the potential difference between the membrane interior at the distance of slice $(k+1)$ relative to that at slice 1 and in evaluating this y_k/y_0 has been approximated by $\gamma_{\pm k}/\gamma_{\pm 0}$, where $\gamma_{\pm k}$ is the mean molal activity coefficient of NaBr at $m_k \simeq c_k$ etc.

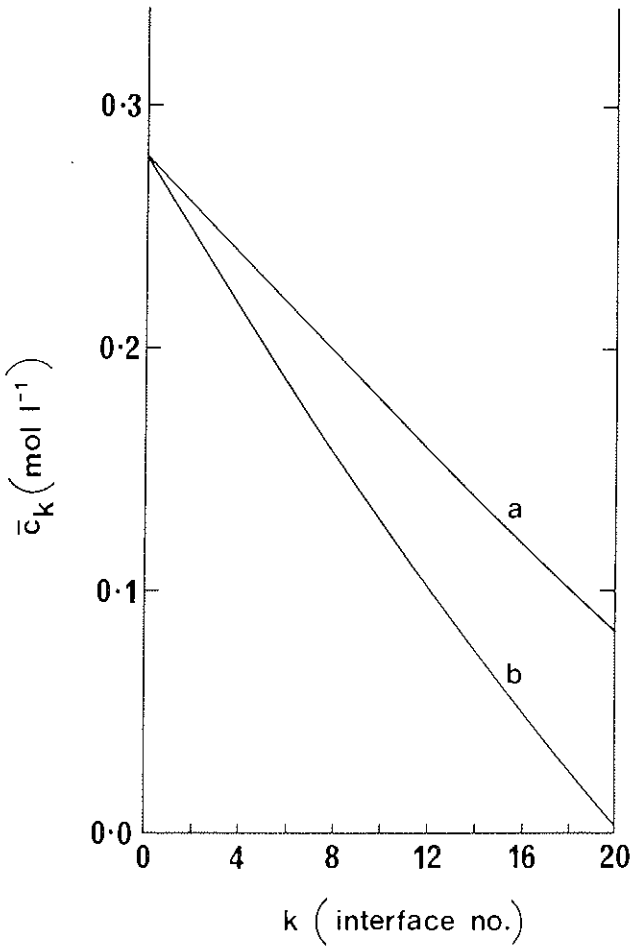

FIG. 6 — Profile of concentration of Br^- ions c_k inside Zeo-Karb 315 membrane in contact with NaBr solutions.
(a) 0.50M/0.20M; (b) 0.50M/0.02M.

The internal potential profiles are plotted in Figure 7. It is seen that the potential plays only a small role in modifying the ion fluxes in the 0.50M/0.20M case where the co-ion concentration is everywhere relatively large. Although the gradient becomes clearly helpful to the co-ions towards the more dilute side it slightly opposes them on the concentrated side. In the 0.50M/0.02M case the same general trends are seen but the potentials are an order of magnitude larger. Over the last two or three slices the co-ions, which are there very dilute, derive considerable help from the potential gradient, which conversely checks the counterion flow. Comparing the $\bar{c}$ and $(\bar{\gamma}_k - \bar{\gamma}_0)$ profiles shows that the "electrical gradient" contribution to the co-ion flux at the dilute side is about one quarter of the "concentration gradient" contribution.

Finally, the internal profile of pressure can be considered. In relating the pressure inside the membrane to that in a contacting solution one must take into account the osmotic pressure difference arising from the difference in ion concentrations on either side of the solution/membrane interface. Thus one has

$$\bar{p}_i = p_i + \bar{\pi}_i - \pi_i \quad (i = 0 \text{ or } k) \tag{24}$$

where $\bar{\pi}_i$ and π_i are the osmotic pressures of the media inside and outside the membrane respectively. For π_i we have used

$$\pi_i = 2\,\theta_i\, c_i\, RT \quad (i = 0 \text{ or } k)$$

where θ_i is the practical osmotic coefficient of NaBr at c_i [10]. To estimate $\bar{\pi}_i$ we have taken the effective osmotic concentration $\bar{c}_{os}$ in the membrane to be given by $55.51\,(2\bar{c}_k + X)/c_w$ and have used as the osmotic coefficient $\bar{\theta}_k$ that of sodium p-toluene sulphonate (the fixed charges in Zeo-Karb 315 are sulphonate groups) at concentration $\bar{c}_{os}$. Thus

$$\bar{\pi}_i = 55.51\,\bar{\theta}_i\, RT\,(2c_i + X)/\bar{c}_w \quad (i = 0 \text{ or } k) \tag{25}$$

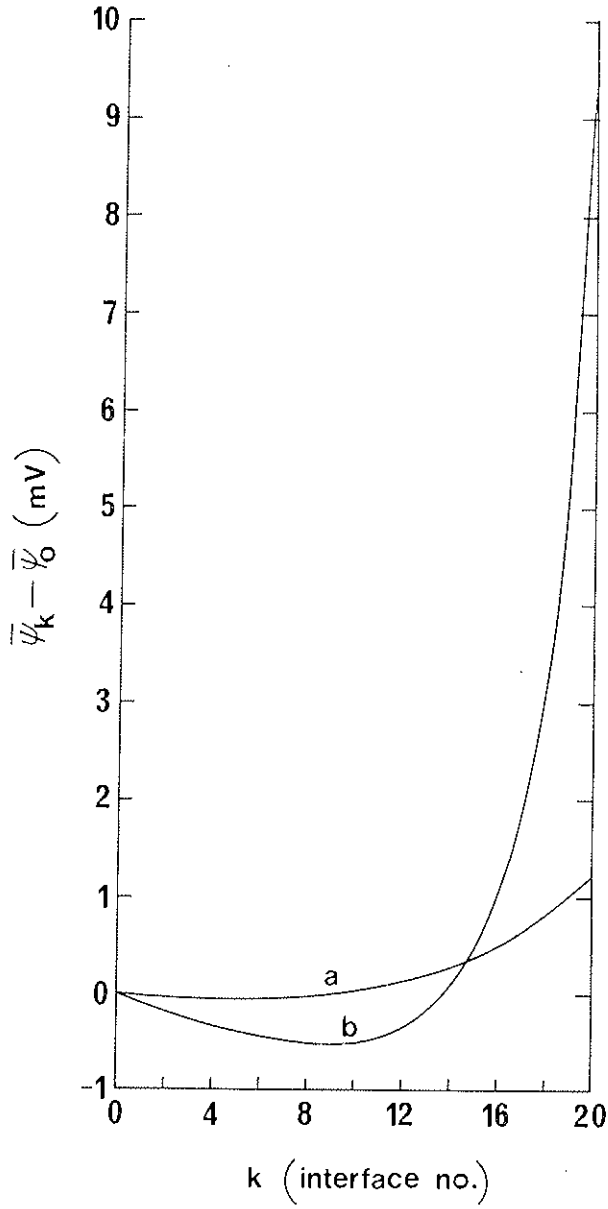

FIG. 7 — Profile of potential inside Zeo-Karb 315 membrane $\bar{\psi}_k$ relative to that just inside more concentrated face $\bar{\psi}_0$.
(a) 0.50M/0.20M; (b) 0.50M/0.02M.

Figure 8 shows the plots of the internal pressure $\bar{p}_k$ versus interface number calculated from p_k in Figure 4. The maxima in Figure 4 are no longer evident. In the 0.50M/0.20M case the pressure plot is almost linear and for 0.50M/0.02M it is linear through most of the membrane but there is a small maximum close to the dilute side. There is a pressure gradient inside the membrane due to the higher relative swelling pressure when the membrane is in contact with a more dilute solution i.e. a phase of higher water activity. It is this pressure gradient which can be regarded as " driving " the osmotic flow of solvent across the membrane.

The summarizing conclusion which emerges from this evaluation of the concentration, potential and pressure profiles in the membrane is that, despite its fixed-charge character and the variation of the conductance coefficients with concentration, overall the conditions are so adapted that in steady flow the membrane does not behave very differently from a simple diffusion barrier with constant diffusion coefficient. This unspectacular finding, if confirmed for some other membranes, could give confidence and comfort to biologists who, because of the immense complexity of the systems they work with, are often forced to use very simplified expressions in the interpretations of their data.

Electrically driven fluxes

One of the most important uses of ion exchange membranes is in electrodialytic desalination. In this process an electric current drives counterions from a dilute into a more concentrated solution. The complete process makes use of anion and cation permeable membranes and the concentration gradient varies along the flow path of the process streams. A detailed electrochemical analysis of the process in terms of membrane characteristics would in any case be a formidable problem. In practice, the details of the membrane per-

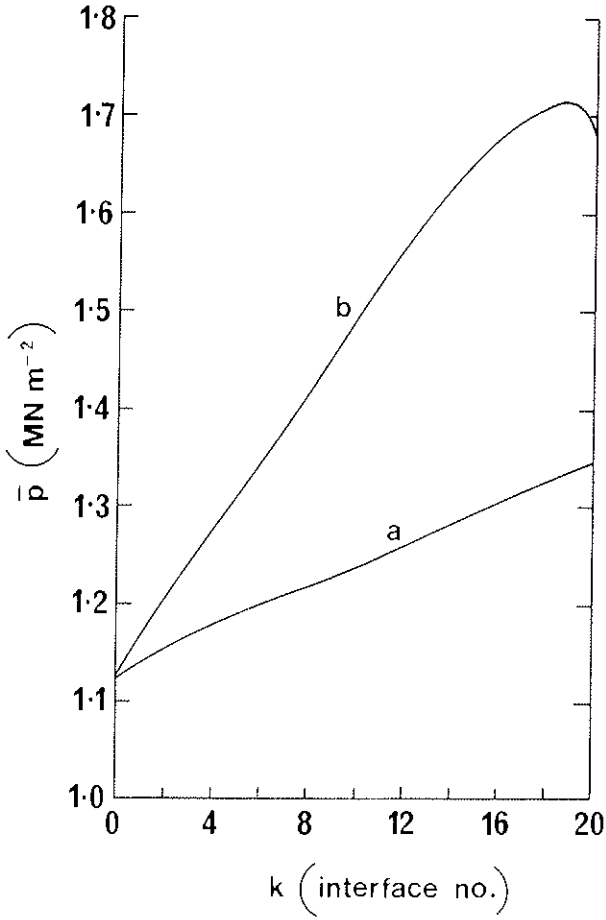

FIG. 8 — Profile of pressure inside Zeo-Karb 315 membrane $\bar{p}_k$ relative to that just outside both faces at 0.1 MN m^{-2}.
(a) 0.50M/0.20M; (b) 0.50M/0.02M.

formance are often overshadowed in importance by the problems of polarization in the liquid phases. For this reason relatively little precise work has been attempted on the fluxes and potentials which arise when a current is passed through a membrane separating two solutions at different concentrations. The subject has however been discussed several times [26, 27] and substantial deviations from ohmic behaviour are expected in some circumstances.

We are reporting here on some computations that have been carried out to examine how Zeo-Karb 315 would behave under concentration differences and current densities in the ranges met with in practical electrodialysis. Such information may be of help in the development later of a more thorough theoretical treatment of the desalination process. Typical results are presented graphically and discussed in the following paragraphs.

In normal electrodialysis the counterion current is directed from dilute to concentrated i.e. i is negative in a cation exchange membrane. Hence the graphs here have been plotted against $-i$; normal electrodialytic flows appear in the lower right hand quadrant in the figures. By fixing the concentration interval, i.e. the force $\Delta\pi$, (and keeping Δp at zero) and exploring different currents, i.e. fluxes, the membrane thickness is no longer a simple scaling factor; it is an important variable. Three thicknesses have been considered: 0.5mm which is typical of commercial electrodialysis membranes, 1.2mm which was the thickness of the membranes used in our experiments and 10mm to observe the effect of an unusually large thickness.

Figure 9 shows current versus voltage plots for three examples. The departures from linearity are small but definite in the 0.20M/0.01M cases at both thicknesses shown, the resistance being lower when the current was from dilute to concentrated. In the 0.50M/0.01M case the resistance was lower due to the higher mean ionic concentrations in the membrane and the deviation from ohmic behaviour scarcely evident.

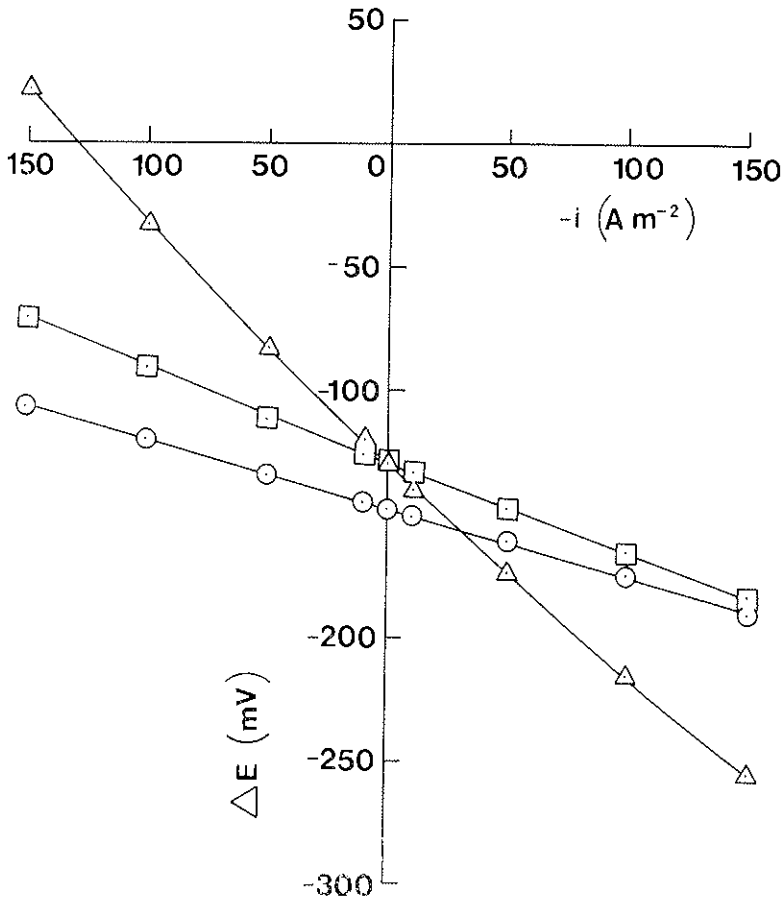

FIG. 9 — Potential difference $\Delta E = (E_0 - E_m)$ versus $-i$ plots for two concentration intervals and thicknesses d.

□ 0.20M/0.01M, d = 0.5mm;

△ 0.20M/0.01M, d = 1.2mm;

⊙ 0.50M/0.01M, d = 0.5mm.

Figure 10 shows the counter-ion fluxes for the same three cases. For practical purposes the plots are straight and almost parallel showing that the transport number of the cations is independent of i and almost of c_0, it is dictated by the concentration of the more dilute bathing solution, 0.01M in every case.

The volume fluxes (Figure 11) have, like the potentials, noticeably curved plots. Whereas ΔE was more sensitive to changes in thickness d than to concentration c_0, ϕ_v is more sensitive to changes in concentration.

In Figure 12 ϕ_1, ϕ_v and ΔE for 0.50M/0.01M and 1.2mm thickness are shown together. This case has been examined in more detail. Figure 12 is entirely consistent with Figures 9, 10 and 11.

The internal concentration profiles for the most positive and the most negative i are compared with the open circuit profile in Figure 13. It is seen that the electric current does modify the profile. When i is negative the internal concentrations of Br^- are increased because the co-ion current is directed from concentrated to dilute. Thus there is a tendency to carry more co-ions into the membrane at the concentrated side than out of it at the dilute side. The concentration gradients have to adjust to a new stationary condition to maintain flux independent of distance. The converse holds with a positive current and the profile bends in the opposite direction. Over the range of currents explored, and much larger currents are not used in electrodialysis because they create excessive polarization, the mean concentration in the membrane is only slightly perturbed. This explains why there are only small departures from linearity in the fluxes and potential differences shown in Figure 12.

Figure 14 shows the $\overline{\psi}_k$ profiles for the same case and Figure 15 the $\overline{p}_k$ profiles. Of course $\overline{\psi}_k$ must adjust directly with i and the plots are remarkable only for the small maxima under negative and zero current which appear close to the

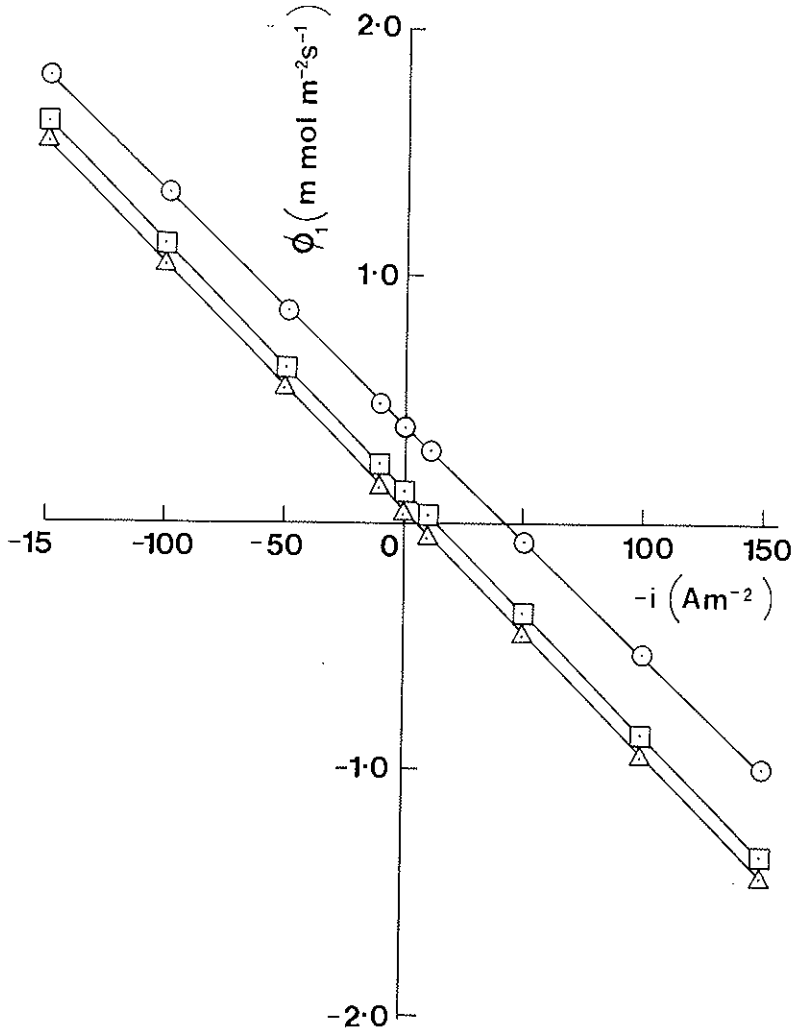

FIG. 10 — Cation flux ϕ_i versus $-i$ plots for two concentration intervals and thicknesses d.

⬚ 0.20M/0.01M, d = 0.5mm;
△ 0.20M/0.01M, d = 1.2mm;
⊙ 0.50M/0.01M, d = 0.5mm.

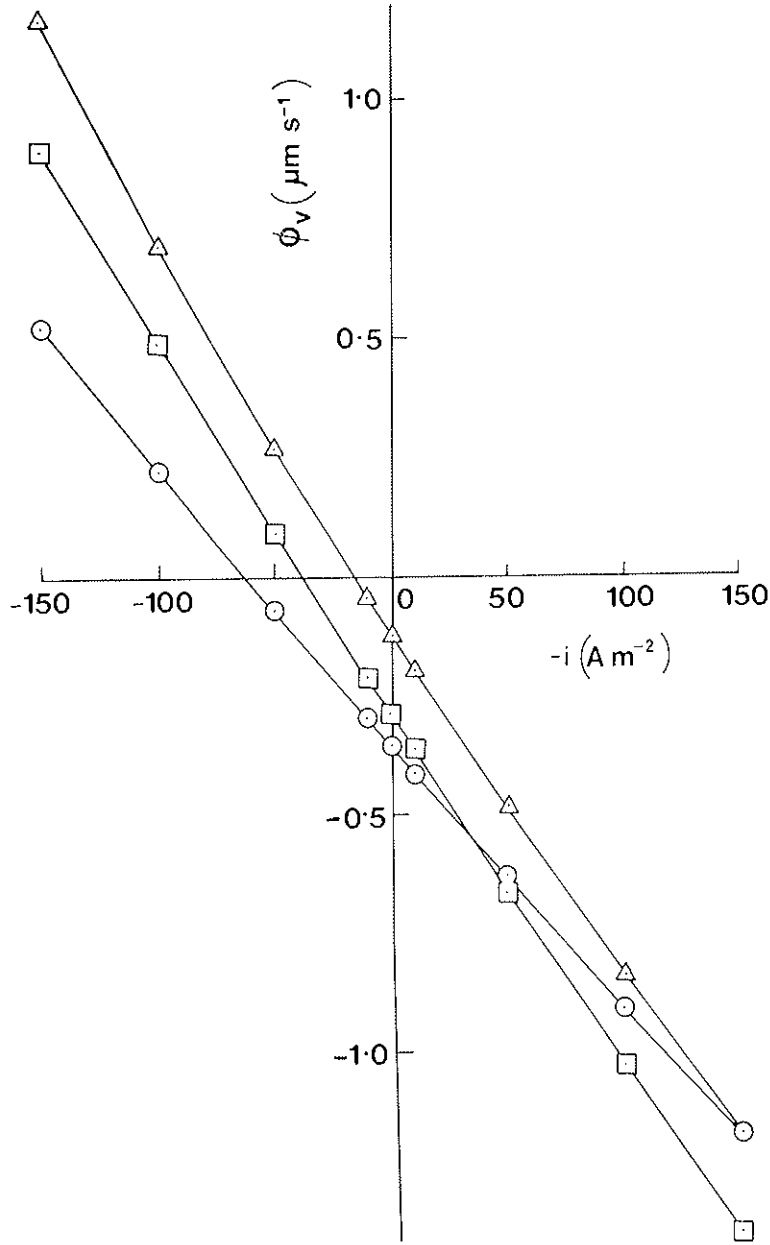

FIG. 11 — Volume flux ϕ_v versus $-i$ plots for two concentration intervals and thicknesses d.

□ 0.20M/0.01M, d = 0.5mm;
△ 0.20M/0.01M, d = 1.2mm;
⊙ 0.50M/0.01M, d = 0.5mm.

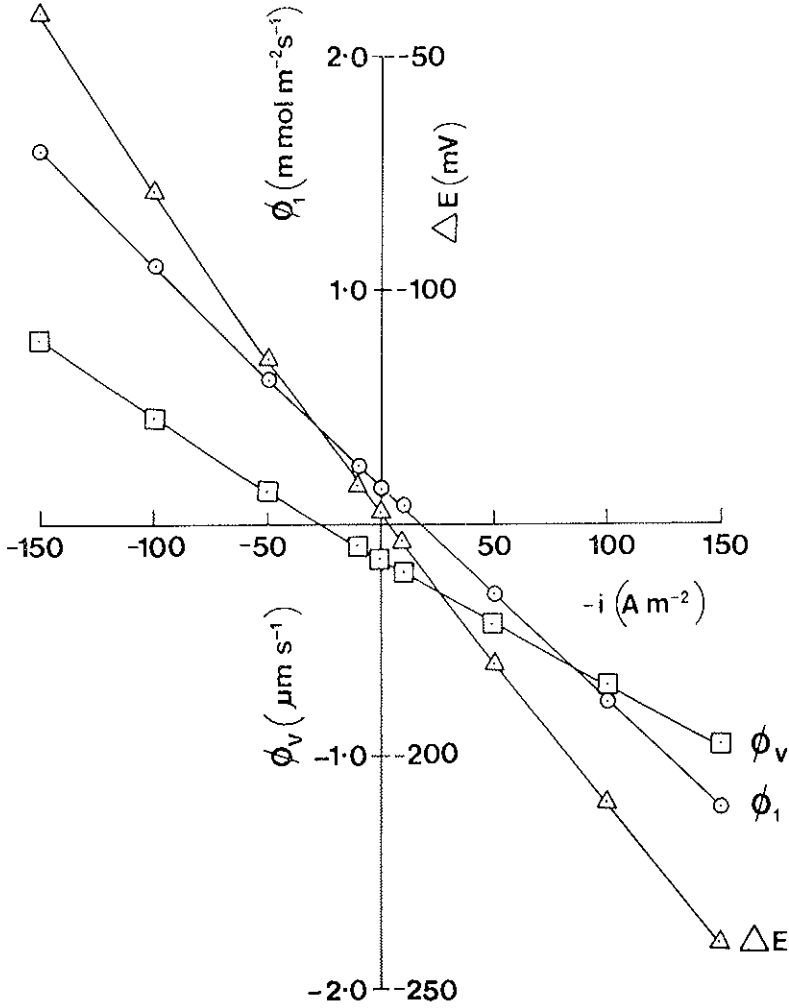

FIG. 12 — ϕ_1, ϕ_v and ΔE versus $-i$ for 0.50M/0.01M NaBr and membrane thickness 1.2mm.

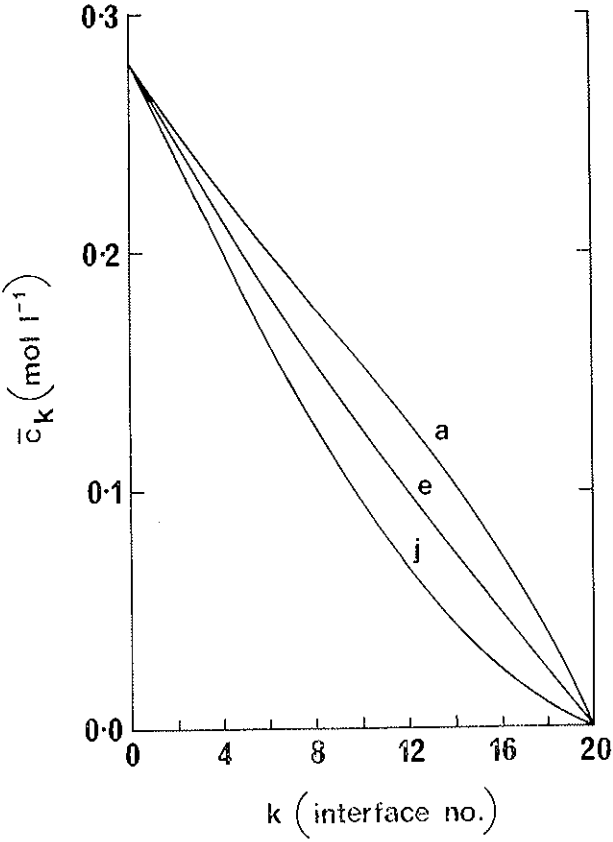

FIG. 13 — Profile of internal Br^- concentration $\bar{c}_k$ for 0.50M/0.01M NaBr and membrane thickness 1.2mm.

(a) $-i = 150$ A m^{-2};

(e) $-i = 0$;

(j) $-i = -150$ A m^{-2}.

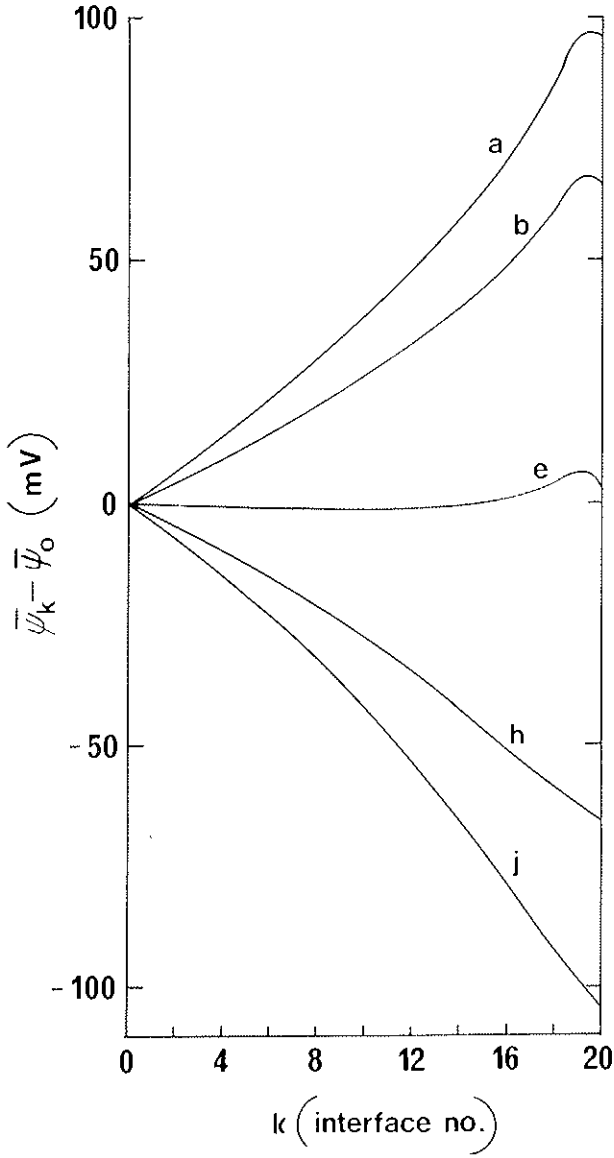

FIG. 14 — Profile of internal potential $\overline{\phi_k}$ for 0.50M/0.01M NaBr and membrane thickness 1.2mm.

(a) $-i = 150$ A m^{-2};
(b) $-i = 100$ A m^{-2};
(e) $-i = 0$;
(h) $-i = -100$ A m^{-2};
(j) $-i = -150$ A m^{-2}.

dilute face. In view of the uncertainty regarding the exact significance in thermodynamic terms of the potentials $\bar{\Psi}_k$, it is not realistic to try to interpret maxima of height 1-3 mV which are not apparent also in E_k.

The maxima in p_k under negative currents and the minima under positive currents are distinct and significant. They result from the concentration dependence of the electro-osmotic permeability of the membrane. The volume transported per coulomb decreases as the concentration increases and the pressure gradients develop in such a way as to maintain ϕ_v constant throughout the membrane. In Figure 15 the extrema are no more than 3 atm above or below the nearer face value and the pressures, allowing for the swelling pressure, are always well above zero.

Figure 16-19 inclusive are a set analogous to Figures 12-15 but deal with the case 0.20M/0.02M and thickness 10mm. In this case the rectifying action of the membrane is seen more clearly in the ΔE versus $-i$ plot and ϕ_v is also curved. ϕ_j remains straight and independent of the strength and direction of the current. This finding, characteristic of all our results, is surprising and should simplify the estimation of electrodialytic performance.

The concentration profiles (Figure 17) now show a great sensitivity to i and witb the largest positive value of i, i.e. from concentrated to dilute, $\bar{c}_k$ was constant at $\bar{c}_m$ over most of the membrane and difficulty was met in completing the iteration procedure to a satisfactory conclusion. Notice also that the concentration interval across the extreme slices was too large to permit the use with complete confidence of mean conductance coefficients to describe the bebaviour of these slices.

The $\bar{\Psi}_k$ plot (Figure 18) is unremarkable. The $\bar{p}_k$ plot (Figure 19) is more interesting. Substantial maxima and minima are apparent. The maxima rise about 8 atm in the case of the largest $-i$. At large positive currents $\bar{p}_k$ apparently becomes 5-10 atm negative. It is tempting to wonder whether,

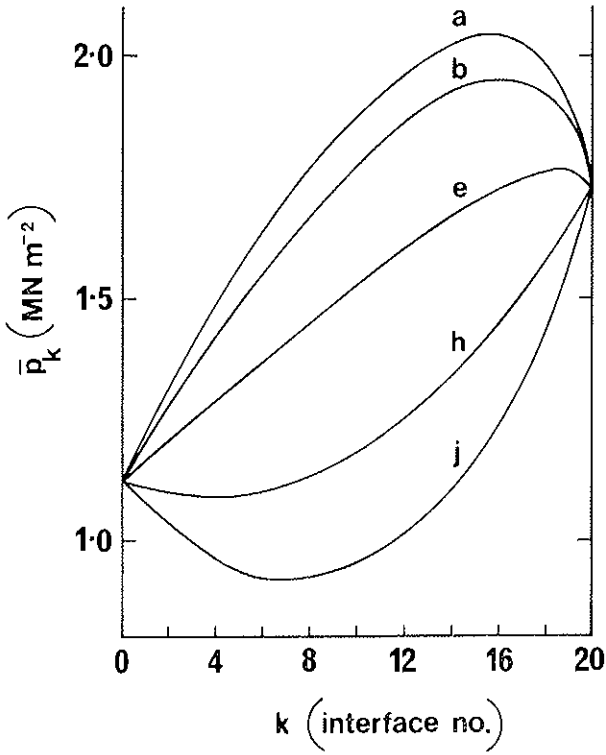

FIG. 15 — Profile of internal pressure $\overline{p_k}$ for 0.50M/0.01M NaBr and membrane thickness 1.2mm.

(a) $-i = 150$ A m^{-2};
(b) $-i = 100$ A m^{-2};
(e) $-i = 0$;
(h) $-i = -100$ A m^{-2};
(j) $-i = -150$ A m^{-2}.

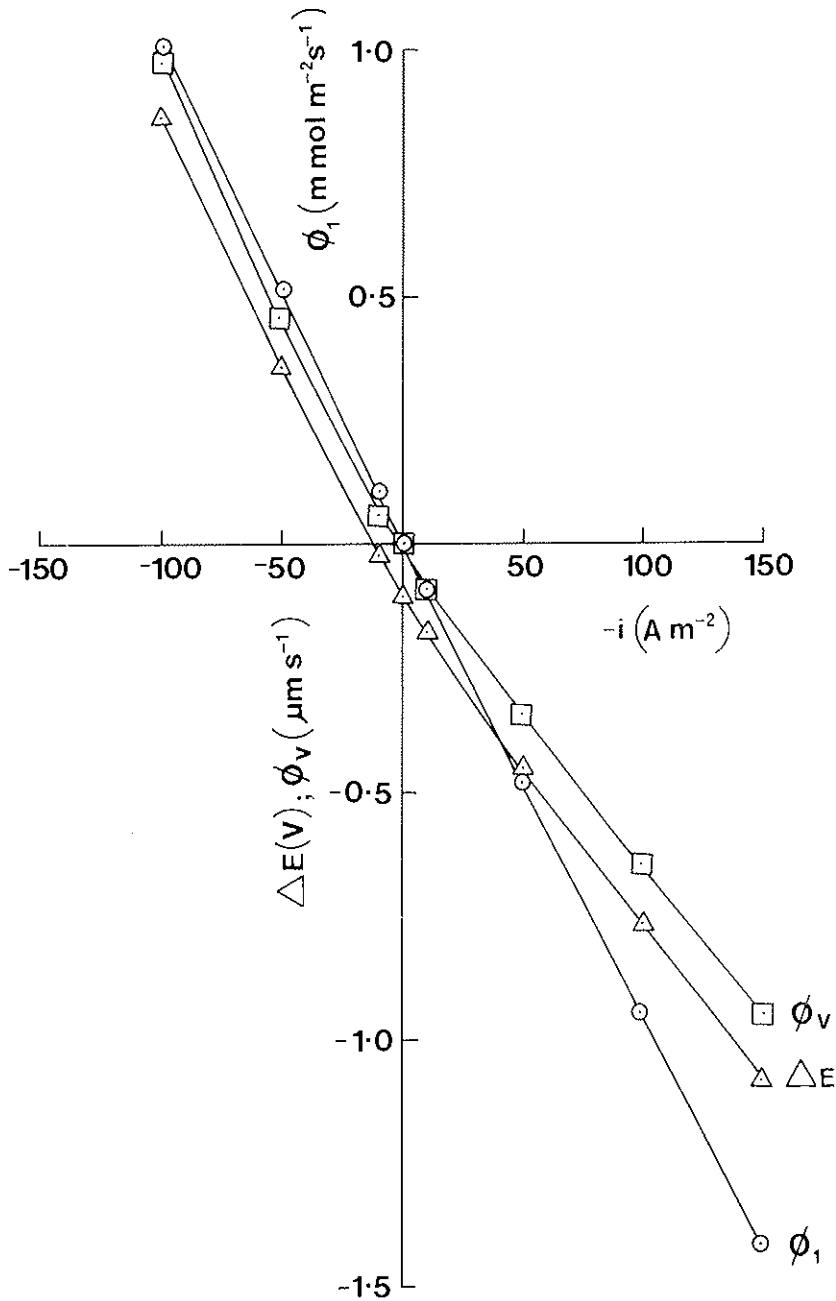

FIG. 16 — ϕ_1, ϕ_v and ΔE versus $-i$ for 0.20M/0.02M NaBr and membrane thickness 10mm.

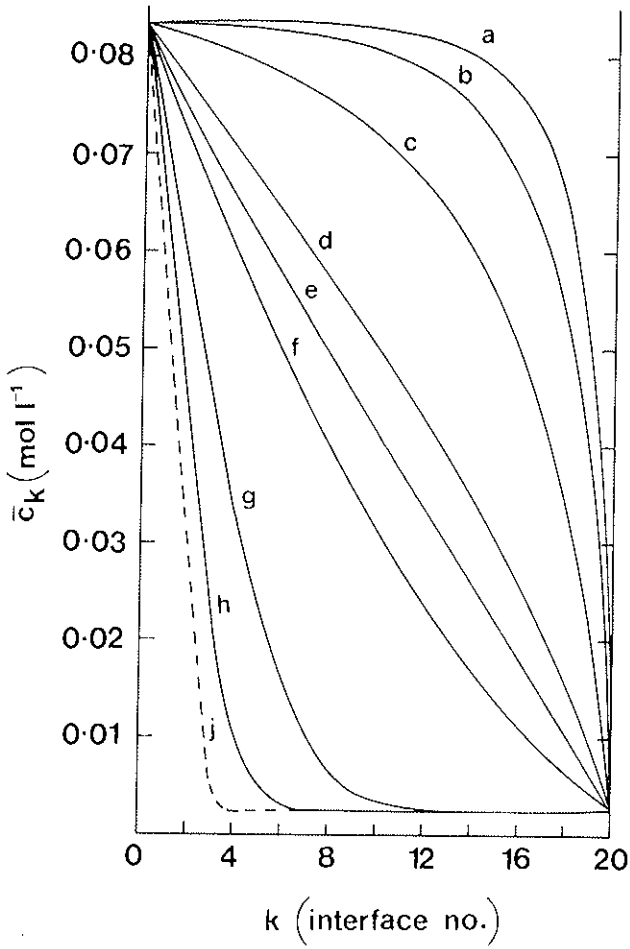

FIG. 17 — Profile of internal Br^- concentration $\overline{c_k}$ for 0.20M/0.02M NaBr and thickness 10mm.

(a) $-i = 150$ A m^{-2};
(b) $-i = 100$ A m^{-2};
(c) $-i = 50$ A m^{-2};
(d) $-i = 10$ A m^{-2};
(e) $-i = 0$;
(f) $-i = -10$ A m^{-2};
(g) $-i = -50$ A m^{-2};
(h) $-i = -100$ A m^{-2};
(j) $-i = -150$ A m^{-2}. (not fully converged)

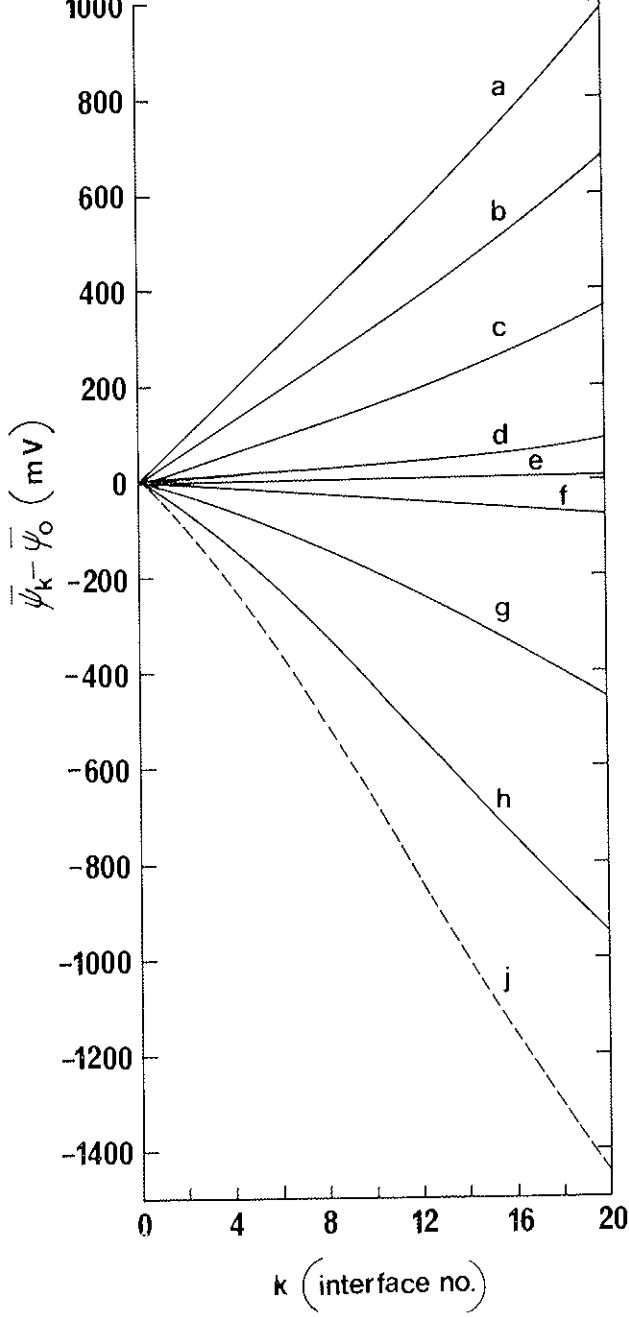

FIG. 18 — Profile of internal potential $\bar{\psi}_k$ for 0.20M/0.02M NaBr and membrane thickness 10mm.

(a) $-i = 150$ A m^{-2};
(b) $-i = 100$ A m^{-2};
(c) $-i = 50$ A m^{-2};
(d) $-i = 10$ A m^{-2};
(e) $-i = 0$;
(f) $-i = -10$ A m^{-2};
(g) $-i = -50$ A m^{-2};
(h) $-i = -100$ A m^{-2};
(j) $-i = -150$ A m^{-2}. (not fully converged)

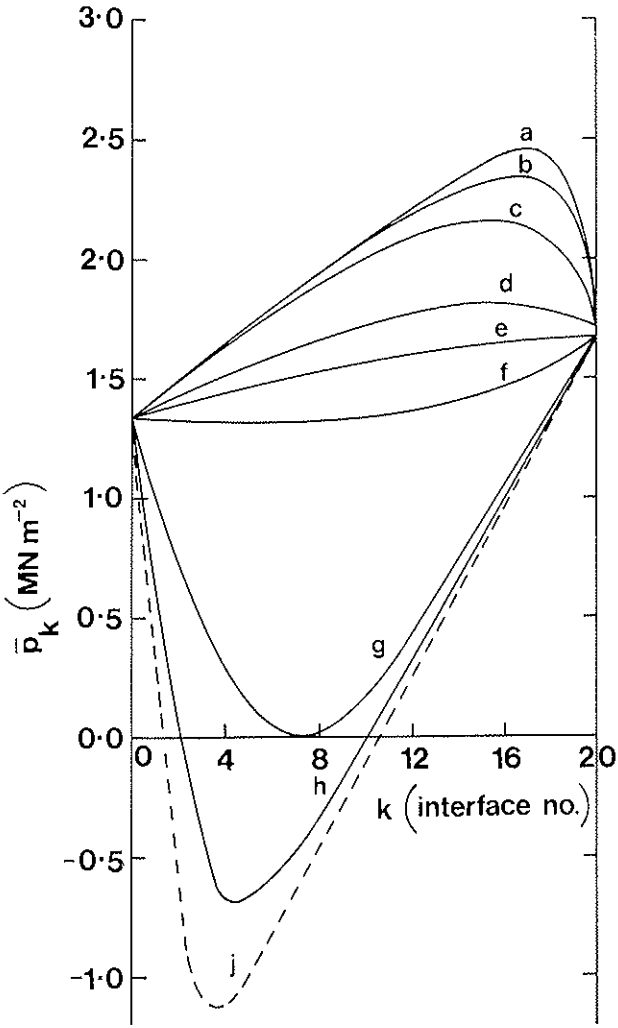

FIG. 19 — Profile of internal pressure $\overline{p_k}$ for 0.20M/0.02M NaBr and membrane thickness 10mm.

(a) $-i = 150$ A m^{-2};
(b) $-i = 100$ A m^{-2};
(c) $-i = 50$ A m^{-2};
(d) $-i = 10$ A m^{-2};
(e) $-i = 0$;
(f) $-i = -10$ A m^{-2};
(g) $-i = -50$ A m^{-2};
(h) $-i = -100$ A m^{-2};
(j) $-i = -150$ A m^{-2}. (not fully converged)

in view of the dependence of membrane properties on pressure, any part of the polarization phenomena observed in electrodialysis may be due to processes within the membrane phase in addition to those widely recognized to occur in the interfacial solution layers. Such effects would not be seen in our plots of ϕ_1 and ΔE because they were computed with coefficients which were assumed independent of pressure. In the normal commercial ion exchange membranes L_p is smaller than in Zeo-Karb 315 and the pressures reached may be much larger. In addition to their effect on fluxes and power consumption such pressure maxima within membranes could lead to accelerated deterioration.

The case of negative pressures at positive currents are thought provoking in another sense. It would be interesting to examine whether there is a decrease in membrane thickness at positive i and also whether sufficiently large negative values of $\overline{p_k}$ could lead to cavity formation and increased resistance due to the inability of the water in the membrane to withstand tension. This is probably unlikely because the swelling water is not truly a bulk phase but is in irregulat capillary spaces between the polymer chains of the membrane matrix.

In conclusion, it may be hoped that a procedure has been developed here which will permit the exploration of membrane phenomena, and particularly of conditions inside the membrane, over a wide range of conditions of practical interest. The procedure is capable of adaptation and development in many ways and it is intended to pursue the work further. Of much greater importance and significance would be the collection of sets of conductance coefficients on a wide range of membranes so that the calculations could be carried out on materials of greater relevance than the long-since-obsolete phenol-sulphonic acid exchanger Zeo-Karb 315 on which we have carried out all of our studies.

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DISCUSSION

Chairman: Prof. P. MEARES

LIQUORI

Is the symmetry in Figure 17 accidental or does it mean something?

MEARES

The Figure is not strictly symmetrical but the near-symmetry is not accidental. The line e, representing the concentration profile at zero current, may be regarded as a base line. A positive current will drive co-ions in electrically at the dilute side less rapidly than they would be driven out electrically at the concentrated side and vice-versa for a negative current. In order to maintain steady flows through the membrane the concentration profiles distort until the combined electrical and diffusion fluxes across all planes are again equal. For small positive (line f) and small negative (line d) currents the potential differences are very nearly equal and opposite, and if the response of the system (although globally non-linear) is not far from linear, one would expect a symmetrical distortion of the concentration profile because an equal, but oppositely directed, diffusion flux would be required to be added to the electrical flux in each case to maintain steady flow. It is more surprising that the near-symmetry in the response of the profiles persists also at much higher positive and negative currents, e.g. lines h and b. In the case of

positive currents, as we saw earlier, the membrane resistance increases with current. It reduces in the case of negative currents. Hence the distortion of the profile is somewhat greater under positive current, (line h) than under an equal negative current (line b). In our membrane the dependence of resistance on current is small and the loss of symmetry in the plots is not large. In other membranes, the range of positive and negative current over which the distortion of the profiles maintains this pseudo-symmetric behaviour may be much smaller. It is particularly interesting that the way in which the system responds internally to an externally imposed change is such as would maintain a more nearly linear response than might have been expected before the calculations were carried out.

TEORELL

Professor MEARES' concentration profiles remind me of some calculations done by PLETTIG in the « thirties » — she was a pupil of NERNST. She calculated that a salt system which contained three ion species could show a concentration *maximum* within the membrane, about five times the concentration of the « outside » solution. By a special « multi-membrane » device (J. Biol. Chem. *113*, 735 (1936)) I could confirm experimentally that this was the case (Zeitschr. Elektrochemie *55*, 468 (1951); Progr. Biophysics, *3*, 356 (1953)). I should perhaps emphasize that the calculations done by PLETTIG and by myself were based on classical PLANCK kinetics. It is interesting to note that even « irreversible thermodynamics » sometimes has to look into the details of the « black box » and find out what is happening there.

MEARES

What we have discovered is that the calculations based on simple principles worked extraordinarily well. You find linear profiles where you might have expected a lot of curvature and general things which have always been anticipated can be confirmed by calculation.

TEORELL

May I just add something here. As you remember about 1954 SCHLOEGL performed similar calculations to yours. The interesting thing for a biologist was that he predicted non-linear pressure gradients within a membrane just as you did. Usually a biologist takes the outside concentrations and calculates everything from these. This may not be permissible, at least not in all cases. Do you agree?

MEARES

In Figure 19 there are some minima at negative pressure. Whether cavities can be generated in the membrane as a result of these parts has yet to be discovered.

TEORELL

This might have biological application. A physiologist by name GUYTON, has claimed there is negative pressure in the interstitial fluid, and A. MAURO of the Rockefeller University has treated some negative pressures which may difficult to understand. Probably they are correct as the irreversible thermo-dynamics predicts it.

SOLLNER

We have an essentially very low-brow problem, the efficiency of electrodialysis. Why don't you use membranes that are one-tenth as thick as the membranes you use now and have the same resistance per square centimeter? Such membranes would be much denser, have a much higher ionic selectivity and smaller electroosmotic water movement. I am sure you would obtain better results if you would make and use membranes with that combination of properties most desirable in your work.

MEARES

I am trying to find out what goes on inside membranes. If thinner membranes are considered the calculations become even less interesting and the lines become straighter. To find out about the changes in profiles we chose to consider a very thick membrane.

Electrodialysis is done with membranes usually about 0.5 mm thick and this is as much for mechanical reasons as for electrochemical ones.

SOLLNER

Well my collodion base membranes are very weak but GREGOR's « Dynel » membranes are very strong. « Dynel » membranes are only 30 to 60 microns thick, much less than half a millimetre. You could make the specific resistance of these membranes ten times higher and rejection would be better.

MEARES

Professor SPIEGLER would know better than I whether there is a problem of taking the electrical heat away from such membranes.

SOLLNER

I think the use of commercial membranes, what the manufacturers sell you, might be a mistake. One should prepare membranes which are most suitable for your purpose; the manufacturers probably will not do this. Once such membranes are developed some manufacturers will gladly take over.

SPIEGLER

I don't know if they fully succeded but I am quite sure that they tried to do it.

STAVERMAN

I should like to ask one question about activity coefficients. Activity is always a nuisance but if you are calculating the differential concentrations, these will be very sensitive to the change of activity. Now I understood that you used two tools, first you determined the real Donnan equilibrium which was different from the theoretical Donnan equilibrium and then you used activities as they appear on the outside fluid — you used the same activities inside. Now, in my opinion, the fact that Donnan equilibrium were different from the theoretical Donnan equilibrium indicates that the activities are different from those in outside as is proposed in your derivation. In my opinion, by using the experimental Donnan equation already the activities in the membrane are fixed, or didn't I understand it?

MEARES

The best answer to give you now is that your question is answered in full in the paper.

INORGANIC ION EXCHANGE MEMBRANES

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INTRODUCTION

It is well known that common ion exchange membranes having an organic matrix are today largely employed in several technological processes; however, their use could even be enlarged if some limitations due to their instability towards temperature, ionizing radiations or oxidizing agents could be overcome. Owing to these limitations, organic ion exchange membranes cannot be suitably used in the fuel cells, in the nuclear technology, in the electrodialysis at high temperature and in several processes where strong oxidizing solutions are employed.

The above limitations are essentially due to the organic nature of the active polymer used and therefore the simpler manner to avoid them is to replace the organic matrix with an inorganic one.

The first inorganic membranes, possessing very high resistance towards acids, temperature, and ionizing radiations and having, at the same time, a high concentration of fixed charges, were obtained thirteen years ago, independently by DRAVNIEKS and BREGMAN [1] and the author [2], who used amorphous zirconium phosphate as an active inorganic material. Successively inorganic membranes have been intensively studied in some laboratories and considerable im-

provements were made especially in the procedures of their preparation [3-20].

As active inorganic materials were employed, however, only some already well known synthetic inorganic exchangers and no efforts were usually made to obtain new materials having specific characteristics for membrane preparation, nor researches were performed to improve some of their properties, such as electrical conductance. Now, the common inorganic ion exchangers usually have higher electrical resistance than the organic exchangers; consequently inorganic membranes too show higher electrical resistance than the commercially available organic ion exchange membranes. Thus, after some partial success in the electrodialytic desalination of brackish waters [21-23], the interest for inorganic membranes, except for precipitate membranes to be used as ion-selective electrodes [24-26], is appreaciably decreased.

In this laboratory a different approach has been used. Taking into account that the electrochemical and osmotic properties of a given inorganic membrane must closely be related to the crystalline structure and to the ion exchange characteristic of the active inorganic material used for its preparation, most of the efforts have been devoted to the synthesis of new inorganic ion exchangers; some of the most promising obtained materials have been selected and then systematic researches have been carried out in order to improve the required characteristics, especially the electrical conductivity. The preparation of the inorganic membranes and the study of their properties was finally accomplished only when several basic information on the used active inorganic materials were well established.

Experimental

Chemicals: All reagents were Merck "pro analysi" products. Kynar (polyvinylidene fluoride) was supplied by Pennsalt Chemical Corporation, Philadelphia, U.S.A.

Preparation of zirconium phosphate having a different degree of crystallinity and its salt forms

Well crystallized zirconium phosphate was prepared according to ALBERTI and TORRACCA's method [27] while amorphous zirconium phosphate was obtained according to GAL and GAL [28].

Semicrystalline materials have been obtained by refluxing the amorphous product in phosphoric acid for different time. The degree of crystallinity of the exchanger increases with refluxing time and with increasing phosphoric acid concentration. These materials will subsequently be indicated by reporting in parentheses the molar concentration of the used phosphoric acid and the refluxing time in hrs.

The materials have generally been stored over P_4O_{10} and for conductivity determinations the fractions between 100 and 200 U.S. Mesh employed.

Monosodium form of zirconium phosphate was prepared as earlier described [29]. Full alkali metal forms were obtained by titrating the H^+ form with 0.1 N (MeCl + MeOH) solution (Me = Li, Na, K, Cs). Mg^{++}, Ca^{++} and Ba^{++} forms were obtained by equilibrating $ZrHNa(PO_4)_2 \cdot 5H_2O$ with $MgCl_2$, $CaCl_2$ or $BaCl_2$ solutions respectively as described in [30]. Further details can be found by referring [31] and references therein.

Synthesis of fibrous cerium (IV) phosphate and its salt forms

Fibrous cerium phosphate was prepared according to ALBERTI and COSTANTINO method [32] and its salts forms by titrating the H^+ form with the suitable hydroxide in presence of added salt.

Preparation of membranes with microcrystals of layered exchangers

The membranes, having Kynar as binder, were obtained

using a method similar to that described by BREGMAN and BRAMAN [8]. Four grams of exchanger were suspended in 20 ml of N, N'-dimetyl acetamide and grinded for two hrs. Then two grams of Kynar were added and the slurry so obtained was mantained at 85-90°C under stirring. This mixture was then spread on a glass plate and the solvent was removed in an oven at 85-90°C. In order to avoid dehydration of the exchanger, the solvent remotion was accomplished in saturated vapour water atmosphere. The plate was then placed in water to detach the membrane from the casting plate. In order to obtain a decrease in porosity, the membrane was held under a pressure of $5 \cdot 10^2$ Kg/cm^2 for 10 hrs. The membranes 0.1-0.3 mm thick were equilibrated in suitable solutions before use.

Preparation of membranes with fibrous exchangers

1 gram of fibrous exchanger was suspended under stirring in one liter of water and then filtered over a « Millipore » plastic filter of 4 cm diameter. The porous membrane so obtained, detached from the filter, was dried in an oven at 30-40°C and after held under a pressure of $2 \cdot 10^2$ Kg/cm^2 for 15 min. Membranes 0.3-0.5 mm thick were obtained.

Apparatus

a) Isoconductance determination of microcrystals of exchanger.

Resistance measurements were carried out with L.K.B. conductivity bridge (type 3216 B) at 1000 c/s using an oscillograph as null-point detector.

The conductivity of a bed of microcrystals of exchanger was measured at 25.0 $\pm$ 0.1°C in a cell similar to that described by PARRISH [33] with platinum electrodes fused in the walls of the cell and a fritted glass plate supporting the ion exchanger. Solutions of suitable concentration were circulated, by means

of peristaltic pumps, and their conductivity measured before and after passing through the exchanger bed. All measurements were taken when the conductivity of the solution became constant. The isoconductance point of the exchanger was found graphically according to SAUER et al. [34].

b) E.M.F. Measurements.

Concentration potentials were measured at 25.0 ± 0.1°C in suitable cells where HCl or NaCl solutions were circulating through the two compartments by means of peristaltic pumps.

Ag/AgCl electrodes, prepared according to BROWN method [35] were used and potential readings made by means of a Leeds and Northrup type K-3 potentiometer supplied with a Null detector mod. 9828; alternatively measurements were performed with a Hewlett-Packard 3490A digital multimeter.

RESULTS AND DISCUSSION

1) *Choice of the active inorganic materials for membrane preparation.*

The choice of an active inorganic material for membrane preparation obviously depends on the particular use to which the membrane is designed.

In Table I are schematically reported the required characteristics that an active inorganic material should have in order to give good membranes for some specific uses.

In our laboratory a particular attention has been devoted to the synthesis and characterization of inorganic exchangers of the class of the insoluble acid salts of tetravalent metals as they are usually very stable towards temperature, ionizing radiations, oxidizing agents, acid solutions and have very high concentration of fixed charges.

In this paper we are exclusively concerned with inorganic

TABLE I — *Characteristics that an active inorganic material should have in order to obtain inorganic membranes for particular uses.*

Membrane use	Required characteristics
Fuel cells, electrodialysis or other processes occurring at high temperature.	High stability towards temperature and low electrical resistance.
Processes occurring in presence of ionizing radiations.	High resistance towards ionizing radiations.
Processes occurring in presence of strong oxidizing agents.	High resistance towards oxidizing agents.
Electrodialysis	Low electrical resistance; low permeability to water.
Hyperfiltration	Low permeability to salt; high permeability to water.
Ion selective electrodes	High selectivity for a given ion or ion sieve properties.
Theoretical studies	Crystalline structure, position of fixed charges and counterions well known.

membranes prepared with the exchangers of this class and therefore some brief considerations on the general characteristics of these exchangers are necessary in order to understand more closely the membrane properties. Particular characteristics of some exchangers selected for membrane preparation will be examined later in connection with membrane requirements.

Initially obtained as amorphous materials [36], most of the insoluble acid salts of tetravalent metals have recently been synthetized as crystalline or semicrystalline materials [31, 37].

Crystalline materials are considerably more stable than the amorphous ones towards hydrolysis of their acids groups [38] and have a well defined chemical composition and structure. At the present, the crystalline exchangers of

the class of the insoluble acid salts of tetravalent metals may conveniently be divided in the following three subgroups:

1) Exchangers having a layered crystalline structure;
2) Exchangers having a fibrous structure;
3) Exchangers having an as yet unknown structure.

Some physico-chemical properties of these exchangers are reported in Table II.

On the basis of these characteristics it can be pointed out that the listed layered exchangers are all promising as active materials for preparation of membranes that are required to possess a good resistance to temperature or ionizing radiations; however zirconium phosphate seems to be the most promising material and has therefore been selected for further investigations. Fibrous exchangers are also very interesting in order to prepare inorganic membranes without binder [32] and some results obtained with cerium (IV) phosphate will be later examined.

Finally thorium arsenate, titanium phosphate and cerium phosphate seem very promising materials for preparation of ion selective electrodes, owing to their high selectivities for certain ions or their ion-sieve properties.

2) *Inorganic membranes prepared with exchangers having a layered structure.*

2.1. *Structural data.*

Before discussing the properties of these membranes, it will be useful to examine the crystalline structure of zirconium phosphate, as worked out by CLEARFIELD and SMITH [39]; the other layered exchangers have essentially the same structure [37].

Each layer consists of zirconium atoms lying in a plane and bridged through phosphate groups located alternatively

TABLE II — *Characteristics of some important exchangers of the class of insoluble acid salts of tetravalent metals.*

Exchanger*	Formula or composition	Ion Exchange capacity (meq/g)	Density (g/cm³)	Concentration of fixed charges (meq/cm³)	Resistance to				
					Temperature °C		Ionizing radiations (rad)	Hydrolisis of acid groups	Acids
					H^+ form;	Na^+ form			
Amorphous zirconium phosphate	$Zr(HPO_4)_{2-x}(OH)_{2x}2\text{-}4H_2O$	4-6			200	—	$>10^9$	low	very good
Layered zirconium phosphate	$Zr(HPO_4)_2 \cdot H_2O$	6.64	2.72	18.1	500	700	$>10^9$ ***	very good	»
» *titanium phosphate*	$Ti(HPO_4)_2 \cdot H_2O$	7.76	2.61	20.3	450	700		low	»
» *tin (IV) phosphate*	$Sn(HPO_4)_2 \cdot H_2O$	6.08	3.12	18.9	360			medium	»
» *zirconium arsenate*	$Zr(HAsO_4)_2 \cdot H_2O$	5.14	3.39	17.4	260			good	»
» *titanium arsenate*	$Ti(HAsO_4)_2 \cdot H_2O$	5.78	3.34	19.3	350			very low	»
» *tin (IV) arsenate*	$Sn(HAsO_4)_2 \cdot H_2O$	4.80	3.75	18.0	340			medium	»
Amorphous cerium phosphate	$Ce(HPO_4)_{2-x}(OH)_{2x} \cdot nH_2O$	2-4						low	low
Fibrous *cerium phosphate*	$Ce(HPO_4)_2 \cdot 3H_2O$	5.20	3.20	16.6	250		$<10^9$ ***	good	good
» *thorium phosphate*	$Th(HPO_4)_2 \cdot 3H_2O$	4.18			250			good	good
Crystalline *cerium phosphate***	$Ce(HPO_4)_2 \cdot 1.33H_2O$	5.78	3.18	18.4	300			medium	good
» *cerium arsenate***	$Ce(HAsO_4)_2 \cdot 2H_2O$	4.38	3.73	16.3	300		$>10^9$ ***	good	good
» *thorium arsenate***	$Th(HAsO_4)_2 \cdot H_2O$	3.78			320			good	good

* Exchangers written in italic been synthetized for the first time in our laboratory.

** Crystalline structure yet unknown.

*** L. SZIRTES and L. ZSINKA - J. Radioanal. Chem. 27, 271 (1974).

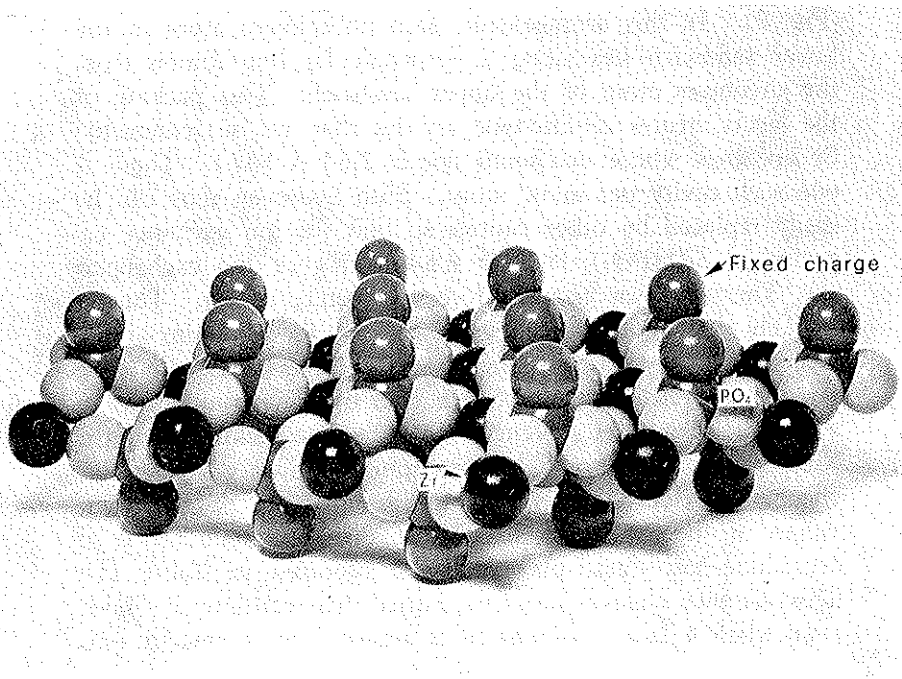

FIG. 1 — Idealized structure of a layer of zirconium phosphate. Oxygens carrying the fixed negative charge are neutralized by counterions not shown in the figure.

above and below this plane; three oxygen atoms of each phosphate group are bonded to three zirconium atoms of the plane, so that an octahedral coordination of oxygen atoms around each zirconium atom is obtained. The fourth oxygen atom bears the fixed negative charge, neutralized by H^+ counterion. Each sandwich can thus be considered as a giant molecule of which a schematic view is given in Fig. 1.

The crystal structure of zirconium phosphate is built up by bonding together these sandwiches, the forces holding the layers being long hydrogen bonds or VAN DER WAALS forces

(Fig. 2). In this arrangement, each phosphorus atom in the lower sandwich lies along a perpendicular line drawn from the zirconium atom of the upper sandwich. This packing of the layers creates zeolitic-type cavities that are interconnected by openings whose maximum size is 2.64 Å [37]. There is one such cavity per metal atom. Both hydrogen ions can be easily replaced by other cations so that the ion exchange capacity of $Zr(HPO_4)_2 \cdot H_2O$ is 6.64 meq/g of the exchanger.

The intralayer bonding forces are quite strong. Thus it is likely that, during exchange of cations, the structure of the layers remains practically unchanged, but the layers can move relative to each other to accommodate the new counterions or a greater amount of solvent. Thus the first maximum of the X-ray powder pattern of a given ionic form of a layered exchanger provides directly its interlayer distance [37]. Some values of the interlayer distance of different ionic forms of crystalline zirconium phosphate are reported in Table III. This distance changes stepwise rather than continuosly; however when a larger counterion is replaced by a smaller one,

TABLE III — *Interlayer distance of some hydrated and anhydrous ionic forms of crystalline zirconium phosphate.*

Ionic form	Interlayer distance (Å)	Ionic form	Interlayer distance (Å)
$Zr(HPO_4)_2 \cdot H_2O$	7.6	$Zr(CsPO_4)_2 \cdot 6H_2O$	14.2
$Zr(HPO_4)_2$	7.6	$Zr(CsPO_4)_2$	9.2
$ZrHNa(PO_4)_2 \cdot 5H_2O$	11.8	$ZrHMg_{0.5}(PO_4)_2 \cdot 3H_20$	9.9
$ZrHNa(PO_4)_2$	7.3	$ZrHMg_{0.5}(PO_4)_2$	7.6
$Zr(NaPO_4)_2 \cdot 3H_2O$	9.9	$ZrH_{0.74}Ca_{0.63}(PO_4)_2 \cdot 3.6H_2O$	10.1
$Zr(NaPO_4)_2$	8.4	$ZrH_{0.74}Ca_{0.63}(PO_4)_2$	7.9
$Zr(KPO_4)_2 \cdot 3H_2O$	10.7	$ZrBa(PO_4)_2 \cdot 2.5H_2O$	9.5
$Zr(KPO_4)_2$	9.0	$ZrBa(PO_4)_2$	7.5

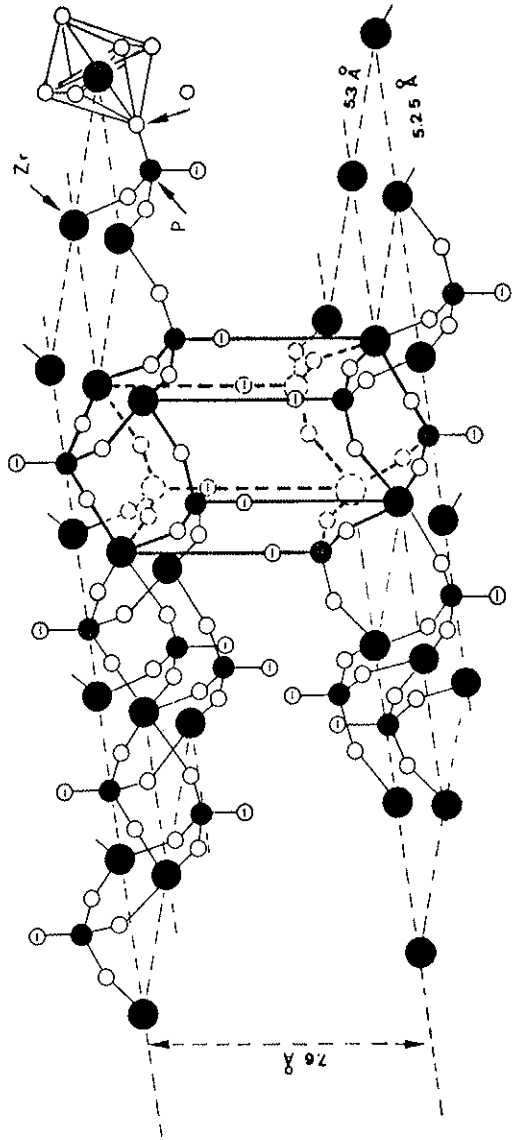

FIG. 2 — Idealized crystal structure of zirconium phosphate. Solid lines show one of the zeolitic cavities created by the arrangement of the layers.

the interlayer distance does not necessarily decrease and formation of large hydrated phases are usually obtained within a large range of counterion composition inside the exchanger [31].

In order to obtain a better understanding of the problems concerning inorganic membranes prepared with layered exchangers, let us now consider an ideal membrane formed by a single crystal of zirconium phosphate. In Fig. 3, three adjacent sandwiches of a crystal having an interlayer distance of 10 Å are schematically shown. The fixed negative charges of oxygens are balanced by an equivalent amount of cations as counterions.

The distance between two adjacent fixed charges in a same plane is 5.3 Å, while that between two charges of adjacent sandwiches depends on the actual interlayer distance of the exchanger. It can be seen from Fig. 3 that, for interlayer spacing lower than 10 Å, this latter distance becomes lower than that between adjacent charges in a same plane, while the opposite occurs for interlayer spacing larger than 10 Å. Thus, when the interlayer spacing of the exchanger is about 10 Å, or lower than this value, the counterions move under the influence of fixed charges of the two adjacent planes; however, when the interlayer spacing is $\gg$ 10 Å, the distance between fixed charges of two adjacent layers becomes too large comparatively to that in a same plane and it can therefore be considered that counterions move under the influence of the fixed charges of a single plane. The planar surface of fixed charges for cm^3 of exchanger at different interlayer distance, is reported in Table IV, while in Table V are reported the distance between two fixed charges and the fixed charge density in a same plane for some layered exchangers.

Finally it must be pointed out that the largest free window connecting the opposite fixed charge planes of a same sandwich is 2.38 Å [40]. Thus it should be very difficult for counterions to cross a sandwich and therefore, a crystal of layered exchanger should exhibit an anisotropic conductance. However, this effect should be very high only for a perfect crystal.

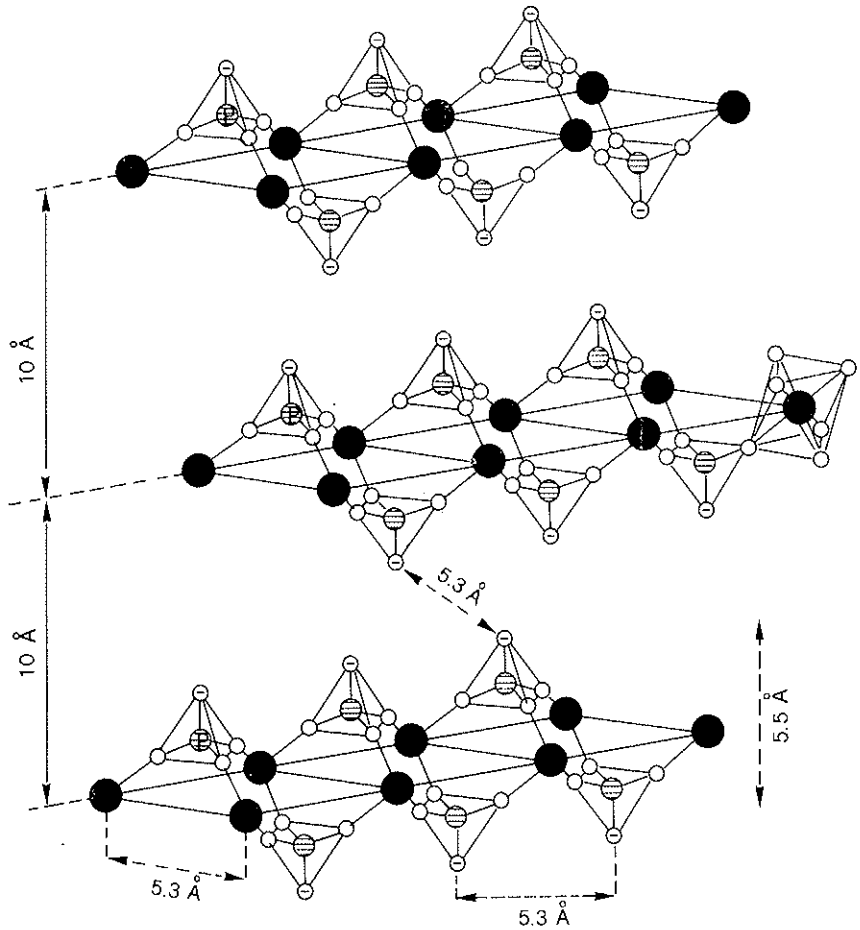

FIG. 3 — Schematic picture of three adjacent layers of zirconium phosphate having an interlayer distance of 10 Å.

TABLE IV — *Internal planar surface of fixed charges and approximate percentage of available internal space of crystalline zirconium phosphate as function of its interlayer distance.*

Interlayer distance d (Å)	Internal planar surface of fixed charges per cm^3 of exchanger (m^2)	Approximate percentage of internal space available for counterions and solvents
7.6	$2.6 \cdot 10^3$	60
9.0	$2.2 \cdot 10^3$	65
10.4	$1.9 \cdot 10^3$	70
12.0	$1.7 \cdot 10^3$	75
16.0	$1.2 \cdot 10^3$	80
19.0	$1.0 \cdot 10^3$	85

TABLE V — *Distance between two fixed charges and their planar density for some layered inorganic ion exchangers.*

Exchanger	Distance between two fixed charges in a same plane (Å)	Planar density of fixed charges (fixed charges/cm^2)
$Zr(HPO_4)_2 \cdot H_2O$	5.3*	$4.2 \cdot 10^{14}$
$Zr(HAsO_4)_2 \cdot H_2O$	5.4*	$4.0 \cdot 10^{14}$
$Ti(HPO_4)_2 \cdot H_2O$	5.0*	$4.6 \cdot 10^{14}$
$Ti(HAsO_4)_2 \cdot H_2O$	5.1**	$4.5 \cdot 10^{14}$
$Sn(HPO_4)_2 \cdot H_2O$	5.1**	$4.2 \cdot 10^{14}$
$Sn(HAsO_4)_2 \cdot H_2O$	5.2**	$4.1 \cdot 10^{14}$

* According to CLEARFIELD et al.

** Calculated from the density and interlayer distance of the exchanger, assuming the same crystalline structure as zirconium phosphate.

In semicrystalline or amorphous materials the holes, or distortion in the structure, may considerably increase the diffusion of ions between opposite planes of the same sandwich.

2.2. *Electrical conductivity.*

The concentration of fixed charges of the crystalline zirconium phosphate, owing to its high ion exchange capacity and density, is appreciably higher than that of commercial organic exchangers; therefore, by assuming a same mobility for counterions as in the organic exchangers, zirconium phosphate should also have a relatively higher electrical conductance.

In Table VI is shown the molarity of fixed charges of

TABLE VI — *Molarity of fixed charges and calculated specific conductance of crystalline zirconium phosphate as function of its interlayer distance.*

Interlayer distance * d (Å)	Molarity of fixed charges (meq/cm³)	Calculated specific conductance (Ω^{-1} cm^{-1}) of H^+-form** assuming: $\alpha = 1$	$\alpha = 10^{-3}$	Calculated specific conductance (Ω^{-1} cm^{-1}) of Na^+-form
7.6	18.1	6.3	$6.3 \cdot 10^{-3}$	$9.0 \cdot 10^{-1}$
9.0	15.3	5.3	$5.3 \cdot 10^{-3}$	$7.6 \cdot 10^{-1}$
10.4	13.2	4.6	$4.6 \cdot 10^{-3}$	$6.6 \cdot 10^{-1}$
12.0	11.4	4.0	$4.0 \cdot 10^{-3}$	$5.7 \cdot 10^{-1}$
14.0	9.8	3.4	$3.4 \cdot 10^{-3}$	$4.9 \cdot 10^{-1}$
16.0	8.6	3.0	$3.0 \cdot 10^{-3}$	$4.3 \cdot 10^{-1}$
19.0	7.2	5.2	$2.5 \cdot 10^{-3}$	$3.6 \cdot 10^{-1}$

* The values of interlayer distance written in italic correspond to known ionic forms of zirconium phosphate.

** Calculation was made by assuming for H^+ and Na^+ the same mobility as in aqueous solution.

zirconium phosphate as a function of its interlayer distance and the specific conductances for H^+ and Na^+ forms, calculated by assuming for these ions the same mobility as in aqueous solution.

Let us now examine some preliminary experimental results on the conductance of zirconium phosphate. Unfortunately, until now, it has not been possible to obtain single crystals of zirconium phosphate large enough for conductivity or transport studies. Crystals having a size of 0.1 mm have already been prepared by CLEARFIELD for structure investigation [41]; we are now attempting to prepare larger crystals.

Suitable large crystals being not available at present, conductivity must be determined by using plugs of microcrystals according to the method described by SAUER and SPIEGLER [34]. In this method the specific conductance of a plug of microcrystals is plotted against the specific conductance of the interstitial solution. By gradually increasing the concentration of the interstitial solution a point is reached in which the pure solution and the plug plus interstitial solution have the same specific conductance (isoconductance point). This point corresponds to the specific conductance of the crystalline material and is easily found by the intersection of the plot with a line drawn at 45° to the abscissa (Fig. 4).

The experimental value of the specific conductance of a well crystallized sample of $Zr(HPO_4)_2 \cdot H_2O$ was $5.6 \cdot 10^{-6}\ \Omega^{-1}\ cm^{-1}$. Taking into account the calculated values reported in Table VI, this small value of the specific conductance indicates a very low average mobility of the H^+ ion on crystalline structure even if a degree of dissociation of 10^{-3} was taken for the functional $\geqslant$P-OH groups.

In order to increase the conductance of zirconium phosphate we are now following three different lines:

a) we are attempting to decrease the activation energy of conduction by creating some defects or by introducing some impurities in the crystalline structure;

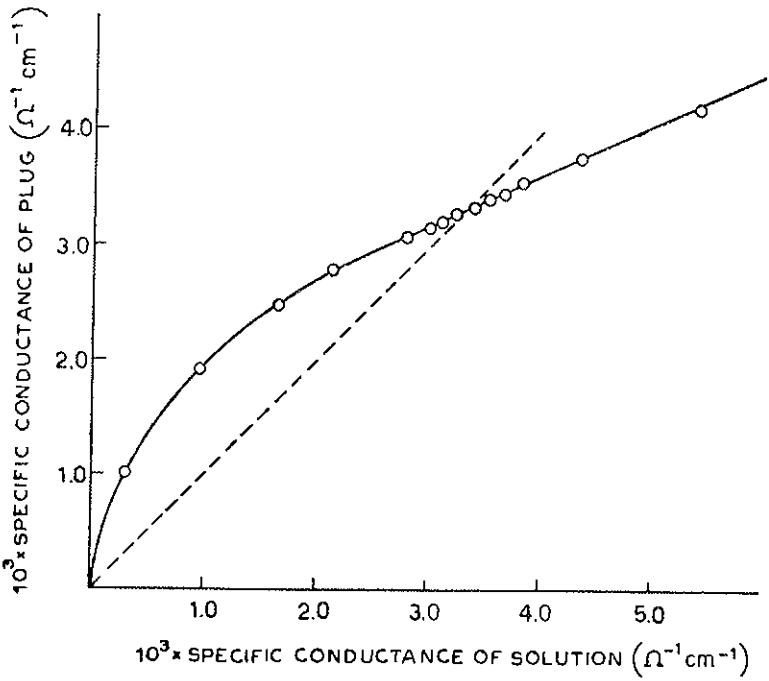

FIG. 4 — Specific conductance of a zirconium phosphate (Bio-Rad) plug.

b) we are replacing the hydrogen ions with other counterions, such as alkali metal ions, so as to increase the degree of dissociation of the phosphate groups;

c) we are trying to decrease the steric impediments for the movement of counterions within the exchanger by enlarging the interlayer distance.

Let us now examine separately the experimental results until now obtained along these lines.

a) *Effect of defects of the crystalline structure on the electrical conductance.*

A first investigation has been carried out by preparing $Zr(HPO_4)_2 \cdot H_2O$ having a different degree of crystallinity. Well crystallized $Zr(HPO_4)_2 \cdot H_2O$ was obtained according to the ALBERTI and TORRACCA method [27], while materials having a variable degree of crystallinity have been prepared by refluxing the amorphous product in phosphoric acid of various concentrations for different time.

The isoconductance values of the investigated materials, listed according to their decrease in degree of crystallinity, are reported in Table VII.

It can be seen that conductance considerably increases with the increasing disorder of the structure of the exchanger. Different causes can be responsable for such an increase, e.g.,

TABLE VII — *Specific conductance of zirconium phosphates in H^+ form, obtained by different methods of preparation and ordered according their degree of crystallinity.*

Preparation method	Specific conductance $\Omega^{-1}\,cm^{-1}$
Direct precipitation at 20°C	$8.4 \cdot 10^{-3}$
Refluxing method (2.5 – 48)*	$1.9 \cdot 10^{-3}$
» » (7 – 48)	$6.6 \cdot 10^{-4}$
» » (10 – 48)	$1.2 \cdot 10^{-4}$
» » (10 – 100)	$9.4 \cdot 10^{-5}$
» » (12 – 500)	$3.7 \cdot 10^{-5}$
HF method (fast precipitation)	$3.0 \cdot 10^{-5}$
HF method (slow precipitation)	$< 5.0 \cdot 10^{-6}$

* Numbers in parentheses are the molarity of phosphoric acid and the time of refluxing respectively.

the formation of some groups $>P<^{OH}_{OH}$ which are much more dissociable than the $\geqslant$P-OH ones, an increase of the surface of the exchanger, a lower activation energy due to a different distribution of fixed charges in the same plane, weaker hydrogen bonds, and higher amount of water between the layers.

In order to have some information on the variation of the conductance with the degree of disorder in the crystal structure of the exchanger, the specific conductances of two materials having a different degree of crystallinity, were measured as a function of the temperature (Fig. 5), and the activation energies for the hydrogen ion conduction were evaluated. It was found that, although the conductances of amorphous and crystalline materials were noticeably different at the same temperature, their activation energies for hydrogen conduction were of the same order of magnitude. (2.7 and 3.2 Kcal/mole, respectively). A tentative explanation of the above results could be given by assuming that in zirconium phosphate, like some ionic solids, transport takes place essentially by motion of interstitial defects. These defects are due to the fact that, at any temperature, there exists a certain number of counterions removed from the normal site to an interstitial site, the fraction N of such ions being

$$N = \sqrt{P}\ e^{-\frac{\Delta G_f}{R T}}$$

where P is the number of interstitial sites available to each normal counterion and ΔG_f is the free energy of formation of the interstitial defect. The most important factor in the energy of formation of the interstitial ion is very likely the electrostatic energy necessary to tear away the counterion from the fixed charge. In turn, this energy is dependent on the anionic field strength of the fixed charges and on the hydrated ionic radius of the counterion. Thus, as the fixed anionic charges of both amorphous and crystalline zirconium phosphate are the same, i.e. the $\geqslant$P-OH functional groups, the energy of formation of

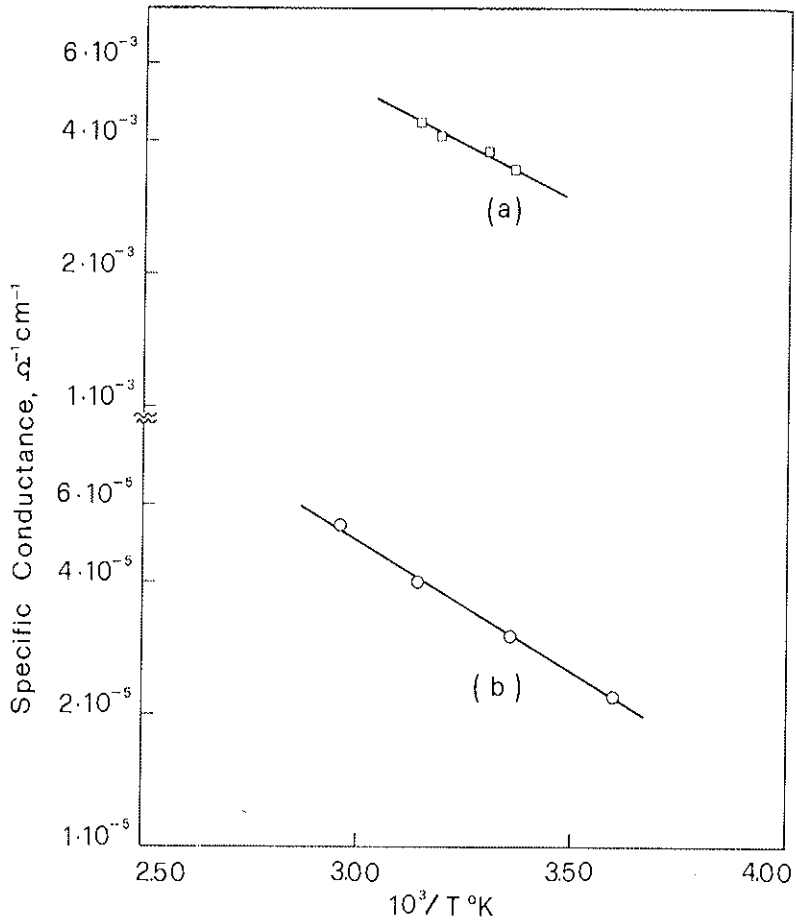

FIG. 5 — The specific conductance, in logarithmic scale, of amorphous (a) and crystalline (b) zirconium phosphate, as function of the reciprocal of the absolute temperature.

interstitial ions too should be the same, provided the hydration of counterion in the two materials is also the same. The slightly lower activation energy for hydrogen ion conduction in the amorphous material could be due to its higher water content relatively to the crystalline material (Table II) and weaker hydrogen bonds.

Several authors [42-44] have reported that in sodium silicates glasses, where ionic transport is most likely to occur by the motion of interstitial defects [45], the conductance depends exponentially upon the applied electrical field. We are therefore performing similar experiments for zirconium phosphate.

Finally, in order to have a deeper insight into the main factors influencing the conductance, we are also investigating zirconium phosphate materials having controlled defects or containing some impurities. For such a purpose we have already prepared some samples of crystalline zirconium phosphate in which a few percentage of phosphate groups has been substituted with arsenate groups. We have also replaced a few percentage of zirconium atoms with Ti or Sn(IV) atoms. Also attempts are in course in order to reduce Sn(IV) to Sn (II) e.g. by introducing Cr^{2+} as counterion in the exchanger. It is hoped that for each Sn(IV) reduced to Sn(II) there is the formation of a hole in the plane of the metal atoms and at the same time, the formation of six $>P<^{OH}_{OH}$ groups. Furthermore we are examining the possibility to substitute a few percentage of zirconium with other elements having an oxidation number three or five.

Some of the obtained materials are now under investigation, and results will be reported upon subsequently.

b) *Effect of the nature of the counterions on the electrical conductance.*

One or both the hydrogen ions of $Zr(HPO_4)_2 \cdot H_2O$ can be easily replaced for Li^+, Na^+, K^+, Ca^{++} and Sr^{++}, while

the Na^+ of $ZrHNa(PO_4)_2 \cdot 5H_2O$ can also be easily replaced for Rb^+, Cs^+, Ag^+, Mg^{++}, Ba^{++} or even divalent transition metal ions. Thus, in recent years, several ionic forms of zirconium phosphate have been prepared and investigated [31]. We are now studying the electrical conductance of the zirconium phosphate as a function of the size and electrical charge of counterions.

The specific conductances of some salt forms of zirconium phosphate are reported in Table VIII.

It can be noted that the electrical conductance strongly decreases with the increase of electrical charge of the counterions. This decrease could be related to a stronger interaction with the anionic fixed charges of the exchanger. The dependence of the specific conductance on the ionic size of counterions seems instead to be less important as if the smaller activation energy for conduction of larger cations were more than balanced by a greater steric hindrance. The conductance seems to decrease with increasing radius of the alkali metal ion; however, it must be noted that the ion exchange processes required to obtain the various ionic forms of zirconium phosphate could appreciably modify either the surface area or the degree of

TABLE VIII — *Specific conductance of different ionic forms of crystalline zirconium phosphate.*

Ionic form	Specific conductance $\Omega^{-1}\,cm^{-1}$
$Zr(HPO_4)_2 \cdot H_2O$	$3 \cdot 10^{-5}$
$Zr(LiPO_4)_2 \cdot 4H_2O$	$2.7 \cdot 10^{-4}$
$Zr(NaPO_4)_2 \cdot 3H_2O$	$1.2 \cdot 10^{-4}$
$Zr(KPO_4)_2 \cdot 3H_2O$	$1.7 \cdot 10^{-4}$
$ZrMg_{0.7}H_{0.6}(PO_4)_2 \cdot 3H_2O$	$1.3 \cdot 10^{-5}$
$ZrBa(PO_4)_2 \cdot 2.5H_2O$	$4 \cdot 10^{-5}$

crystallinity of this exchanger. Thus definitive conclusions cannot be derived until a better knowledge of the dependence of conductance on the external surface or on structural disorder of the exchanger will not be acquired.

In order to have a better understanding of the effect of replacement of the hydrogen with other counterions, samples of zirconium phosphate having a different degree of crystallinity have been converted into monosodium form and their specific conductances were measured.

Also in this case it was found that the conductance was strongly dependent on the degree of crystallinity of the material. A comparison among conductances of hydrogen and monosodium form of zirconium phosphate, at various degree of crystallinity, is shown in Table IX. It can be seen that the replacement of one H^+ with Na^+ appreciably increases the conductance of the well crystallized zirconium phosphate, however it does not appreciably modify the conductance of semicrystalline materials while even decreases that of the amorphous material.

Regarding this latter material, since its H^+ ion can be gradually exchanged by Na^+, it is possible to follow the variation of the conductance as a function of the degree of conver-

TABLE IX — *Comparison between specific conductance of zirconium phosphate in dihydrogen and monosodium form at various degree of crystallinity.*

Material	Specific conductance of the dihydrogen form Ω^{-1} cm^{-1}	Specific conductance of the monosodium form Ω^{-1} cm^{-1}
Amorphous	$8.4 \cdot 10^{-3}$	$5.5 \cdot 10^{-3}$
Semicrystalline (10−48)	$1.2 \cdot 10^{-4}$	$5.0 \cdot 10^{-4}$
Crystalline (HF method)	$3.0 \cdot 10^{-5}$	$1.4 \cdot 10^{-4}$

sion of the exchanger. In a previous paper [46], it was found that there is an initial increase and then a decrease of the conductance with the increasing degree of conversion, the maximum in conductivity being obtained at about 10 % of Na^+-conversion (Fig. 6).

Steric hindrance may be responsable of the conductance decrease at high Na conversion [46].

c) *Effect of the distance among the fixed charges on the electrical conductance.*

We have already seen that the distance between the fixed charges of two adjacent planes of a layered exchanger depends on its interlayer distance. Now we have found that there exists the possibility of obtaining same ionic forms of crys-

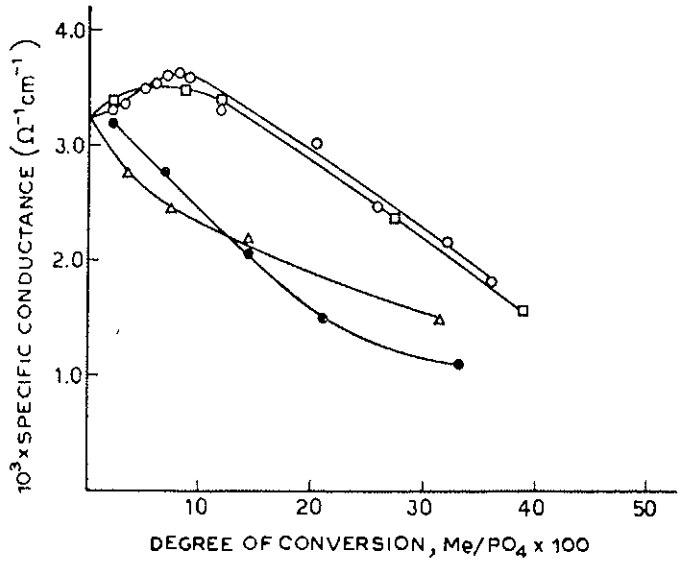

FIG. 6 — Specific contuctance of zirconium phosphate (Bio-Rad) at different degrees of conversion in salt form. △ Li^+; ○ Na^+; □ K^+; ● Cs^+.

talline zirconium phosphate, such as the dihydrogen form, at various interlayer distance [31]. For example, when $Zr(HPO_4)_2 \cdot H_2O$ is converted into the monosodium form and then regenerated into the dihydrogen form, a material having a larger interlayer distance than the original product is obtained. The process can be schematically written as

$$\underset{(7.56\ \text{Å})}{Zr(HPO_4)_2 \cdot H_2O} \xrightarrow{Na^+} \underset{(11.8\ \text{Å})}{ZrHNa(PO_4)_2 \cdot 5H_2O} \xrightarrow{H^+} \underset{(10.4\ \text{Å})}{Zr(HPO_4)_2 \cdot 8H_2O}$$

where the numbers in parentheses are the interlayer distances.

Even larger interlayer distances have been obtained by not complete regeneration of the monobarium form of zirconium phosphate [47].

We are now determining the electrical conductance of these materials. The interpretation of preliminary results is however difficult since the ion exchange processes required to enlarge the interlayer distance are very likely also lowering the degree of crystallinity; furthermore it must be considered that breaking of crystals during exchange could also increase the external surface of the exchanger. In this connection, it must be pointed out that the mobility of counterions on the surface is expected to be higher than their internal mobility, owing to surface disordered structure, presence of a few percentage of $>P<^{OH}_{OH}$ groups, absence of steric hindrance and contact with free water. Therefore surface increase can play an important role for low conducting materials such as well crystallized zirconium phosphate. In order to have more reliable comparison among conductivities of exchangers we are now determining their specific surface areas.

Concerning the comparison between $Zr(HPO_4)_2 \cdot H_2O$ and $Zr(HPO_4)_2 \cdot 8H_2O$, this latter material was dehydrated again to monohydrate form over P_4O_{10} and the conductance again

determined. The following sequence for the specific conductance of these materials was obtained:

$$(10\text{-}100)\ Zr(HPO_4)_2 \cdot H_2O \rightarrow Zr(HPO_4)_2 \cdot 8H_2O \rightarrow Zr(HPO_4)_2 \cdot H_2O$$
$$9.4 \cdot 10^{-5}\ \Omega^{-1}\ cm^{-1}) \quad (1.6 \cdot 10^{-3}\ \Omega^{-1}\ cm^{-1}) \quad (1.0 \cdot 10^{-3}\ \Omega^{-1}\ cm^{-1})$$

It can be noted that the ion exchange processes required in order to obtain the octahydrate form and the successive dehydration appreciably increase the conductance of $Zr(HPO_4)_2 \cdot H_2O$.

However specific conductance of the octahydrate form is higher than that of the monohydrate zirconium phosphate and therefore the mobility of counterions increases by increasing the interlayer distance from 7.6 to 10.4 Å. A decrease of steric hindrance, a weaker hydrogen bonds and a higher amount of interlayer water can be responsible for such an increase.

It is worthnoting that, when the interlayer water is removed, the interlayer distance again decreases to its original value. It would be very interesting to prepare layered exchangers having a few stable bridge linking two phosphate groups of adjacent layers, so that the interlayer distance of the exchanger were independent from the size of the counterions and water content. By using different molecules linking the phosphate groups, different interlayer distance could be obtained. Even free water, and consequently a higher electrical conductance, could be expected for exchangers having a very large interlayer distance. Such partially bridged exchangers could also find interesting applications as molecular sieves or as catalysts and we are therefore investigating various possibilities of obtaining them.

2.3. *Concentration potentials of inorganic membranes prepared with zirconium phosphate having different degree of crystallinity.*

As an example showing the dependence of the membrane properties on the active material used for its preparation, let

us examine the potential of concentration of membranes prepared with zirconium phosphate having different degree of crystallinity. The variations of the concentration potentials as a function of $-\log a'_{HCl}$, for three different zirconium phosphate materials, are shown in Fig. 7.

It can be noted that the ideal behaviour of the membrane is lost at lower and lower concentrations as the degree of crystallinity of the exchanger is increased. It is also worthnoting that the experimental points fit fairly well the theoretical curves calculated according to the T.M.S. theory [48, 49], when the values of U = 0.60 and 0.66 are used for crystalline and semicrystalline zirconium phosphate respectively. A better agreement is obtained if the weak character of the functional groups P – OH is also taken into account.

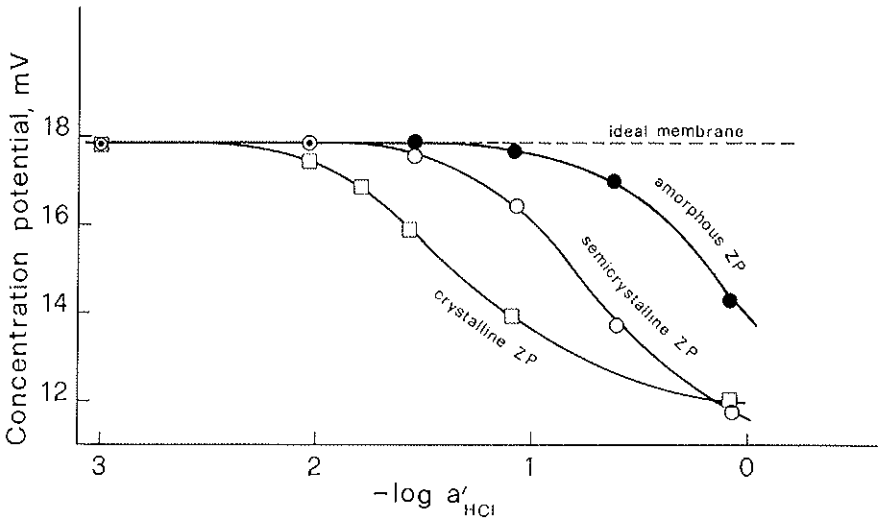

FIG. 7 — Concentration potentials as function of - log a'_{HCl}, for membranes prepared with zirconium phosphate having different degree of crystallinity ($\frac{a'_{HCl}}{a''_{HCl}} = 2$).

The effective density of the fixed charge for these membranes has been evaluated according to the graphic method of MEYER and BERNFELD [49] and the obtained values are reported in Table X. These values are considerably lower than those expected from the fixed charges concentration of the exchangers. This indicates that a large part of the internal fixed charges remains inactive. The agreement between T.M.S. curves and experimental values obtained from heterogeneous membranes can thus be explained assuming that the active fixed charges in these membranes are essentially those of the external surface of the grains. Owing to the small size of well grinded zirconium phosphate (> 200 mesh) the free intergranular spaces are also very small, so that Donnan salt exclusion could take place on it. Thus the examinated membranes can be considered as porous quasi homogeneous membranes with charged pores. It is clear that considerable improvements have to be expected for membranes prepared with exchangers in which the most part of the internal fixed charges are also active. This goal could be obtained by exchangers having controlled defects, large interlayer distance and free water between the layers.

TABLE X — *Effective fixed charge density evaluated according to the method of Meyer and Bernfeld for some inorganic membranes prepared with zirconium phosphate at various degree of crystallinity.*

Zirconium phosphate used for membrane preparation	Effective fixed charges density (meq/cm³)
Amorphous	$5.0 \cdot 10^{-1}$
Semicrystalline (10 – 48)	$1.1 \cdot 10^{-1}$
Crystalline (HF fast precipitation)	$2.5 \cdot 10^{-2}$
Crystalline (HF slow precipitation)	$5.7 \cdot 10^{-3}$

3) *Inorganic membranes prepared with fibrous exchangers*

Some years ago we have success in preparing cerium (IV) phosphate, a new inorganic ion-exchanger having a fibrous structure [32]. Successively we have also obtained fibrous thorium phosphate [50] and it is very likely that other fibrous exchangers may be obtained in the next future.

An electron micrograph of a sample of cerium (IV) phosphate, showing its fibrous nature is shown in Fig. 8.

Fibrous inorganic ion-exchangers are very interesting because they can be used to prepare inorganic ion exchange papers, or thin-layers, that have heen already employed as chromatographic or electrophoretic supports [51]. These inorganic ion exchange sheets can be also utilized, as described in the experimental part, to prepare inorganic membranes without binder.

The specific conductance of cerium (IV) phosphate in different ionic forms is reported in Table XI.

Like zirconium phosphate, the conductance of fibrous cerium phosphate also seems to be too low relatively to its high concentration of fixed charges. From the titration curve of cerium (IV) phosphate with 0.1 N (NaCl + NaOH), previously reported [32], it is possible to evaluate an average apparent pK value of about 3, and in consequence, a degree

TABLE XI — *Specific electrical conductance of cerium phosphate materials.*

Material	Specific conductance $\Omega^{-1}\,cm^{-1}$
Amorphous cerium phosphate	$1.7 \cdot 10^{-3}$
Fibrous cerium phosphate in H^+-form	$5.6 \cdot 10^{-4}$
Fibrous cerium phosphate in Li^+-form	$3.6 \cdot 10^{-3}$
Fibrous cerium phosphate (H-Na-form)	$1.2 \cdot 10^{-3}$

FIG. 8 — Electron micrograph of a sample of fibrous cerium (IV) phosphate (Magnification 10,000 X).

of dissociation about $8 \cdot 10^{-3}$ for acid phosphate groups bonded to Ce(IV).

Taking into account that the density of fixed charges of fibrous cerium(IV) phosphate is 16.6 meq/cm^3, a conductance of $4.6 \cdot 10^{-2}\ \Omega^{-1}\ cm^{-1}$ can be calculated, if the same mobility as in water is assumed for the proton. Thus mobility of the proton in cerium phosphate is noticeably lower thant in aqueous solution.

By gradually replacing the protons with sodium ions, it was found that conductance increases between 0-20% of conversion and then remains about constant (Fig. 9). It is likely that the initial increase is due to a lower interaction of Na^+ with $\geqslant P\text{-}O^-$ groups; however, for high Na^+ conversion steric hindrance may balance this effect.

Fig. 10 shows the variation of the concentration potential for a cerium (IV) phosphate membrane in hydrogen form as function of $-\log a'_{HCl}$ of the more concentrated solution.

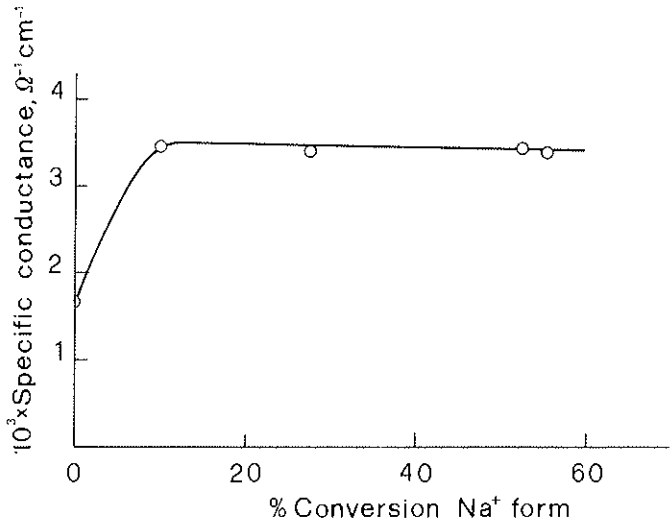

FIG. 9 — Specific conductance of fibrous cerium phosphate as a function of its conversion in Na^+ - form.

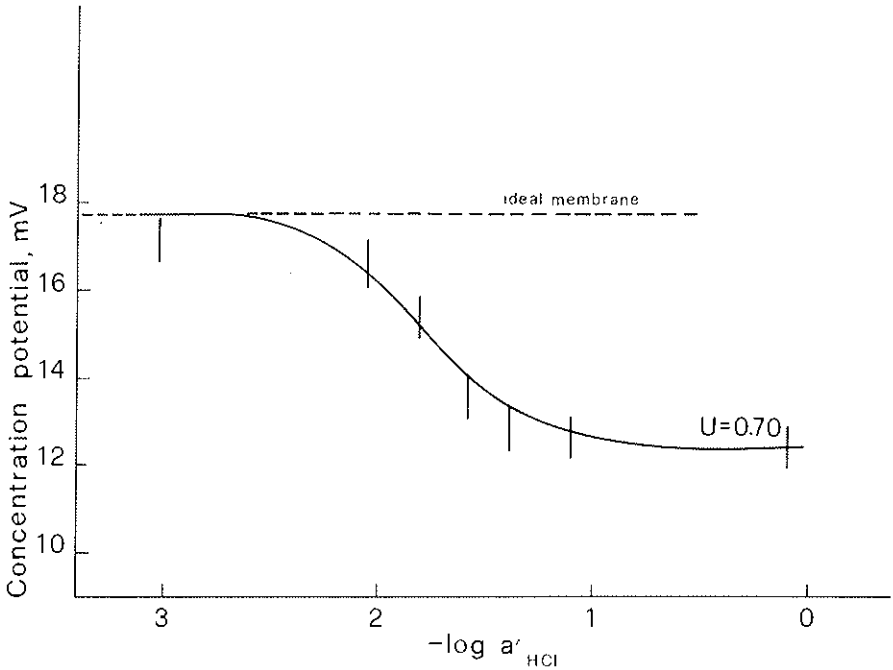

FIG. 10 — Concentration potentials of fibrous cerium phosphate membrane in H^+-form, as function of $-\log a'_{HCl}$ ($\frac{a'_{HCl}}{a''_{HCl}} = 2$). The experimental points fit fairly well the curve calculated according T. M. S. theory for U=0.7 and $K = 5 \cdot 10^{-3}$. Calculated concentration of fixed charges: $2.7 \cdot 10^{-2}$ meq/cm³.

The ratio $\frac{a'_{HCl}}{a''_{HCl}}$ was 2. The full curve has been calculated according T.M.S. theory for U = 0.7 and $K = 5 \cdot 10^{-3}$. The experimental points fit fairly well this curve. By graphic method [49] a concentration of fixed charges of $2.7 \cdot 10^{-2}$ meq/cm³, can be evaluated. Considerations similar to those already discussed for zirconium phosphate can be made also in the case of cerium phosphate. It is likely that the mobility of internal counterions is considerably lower than the external

ones so that the membrane can be considered as a porous membrane with charged pores, the pores being the free spaces between the fibers of the exchanger.

Also in this case, the electrochemical properties of the membrane could be considerably improved if the mobility of internal counterions were increased.

Investigations on the effects of the degree of crystallinity on conductance of cerium phosphate are now in progress.

4) *Inorganic ion exchange membranes for particular uses.*

4.1. *Inorganic membranes to be used at high temperature.*

Layered exchangers are all very stable toward temperature. Concerning crystalline zirconium phosphate, it was found that condensation of $\geqslant P-OH$ group to pyrophosphate occurs in the range 450-550°C. Still higher resistance to temperature can be obtained by replacing H^+ for other counterions so avoiding the condensation of the phosphate groups.

For example, ion exchange experiments in molten salts at temperature higher than 600-700°C can be easily carried out with $Zr(NaPO_4)_2$ [52].

The stability of heterogeneous inorganic membranes obviously also depends on the stability of the binder used for their preparation. Some binders until now investigated for preparation of inorganic membranes to be employed at high temperature are glass fibers, silicates, Kynar and Teflon.

4.2. *Inorganic membranes for hyperfiltration of brackish waters.*

As shown in Table I, the characteristics required for hyperfiltration are a high salt reflexion coefficient associated with a high permeability to the water.

In this connection membranes prepared with fibrous exchangers could be interesting owing to their porous structure

formed by the interlacing of the fibres. Fibrous exchangers could prove also to be suitable active materials for dynamically formed membranes of the type studied by KRAUS et al. [53-55]. From a theoretical point of view, hyperfiltration experiments on single crystals of layered exchangers could also be interesting since the reflexion coefficient and permeability to the water could be studied as function of the distance of fixed charges of the two adjacent planes.

4.3. *Inorganic membranes to be used as ion-selective electrodes.*

Some of the new crystalline inorganic exchangers exhibit very interesting ion-sieve properties (Table XII). For example, thorium arsenate in hydrogen form, owing to its very compact crystalline structure, can exchange anhydrous Li^+ ion, but not Na^+ or larger cations [56]. A single crystal of this exchanger could thus prove to be a good ion selective electrode for Li^+ ion.

TABLE XII — *Ion sieve properties and particular ion selectivity of some inorganic ion exchangers of the class of insoluble acid salts of tetravalent metals.*

Exchanger	Alkali metal ion exchanged by H^+ form	High ion selectivity for:
Layered zirconium arsenate	Li; Na; K; Cs	
Crystalline cerium arsenate	Li; Na; K	
Layered titanium phosphate	Li; Na	
Crystalline thorium arsenate	Li	
Amorphous zirconium phosphate		Cs^+
Layered zirconium phosphate in monosodium form		Ca^{++} and other divalent cations
Fibrous cerium phosphate		Pb^{++}, Hg^{++}

Unfortunately crystals large enough for such a study have not been obtained at the present.

The selectivity of some inorganic exchangers towards certain cations (see Table XII) could be utilized in order to obtain ion-selective electrodes. Owing to the very high selectivity of fibrous cerium phosphate towards Pb^{++} and Hg^{++} [57], investigations on very compact membranes of this exchangers are in progress.

4.4. *Inorganic membranes to be used for solid state electrochemistry.*

Interest in solid state electrochemistry has grown in recent years. In order to avoid excessive Joule-heat losses in systems operating with high current densities (electrolyzers, batteries) or excessive cell impedence in systems operating at very low current levels (gauges, electrochemical memories, coulometers) solid electrolytes having specific conductance values around $10^{-2}\,\Omega^{-1}\,cm^{-1}$ are required. Only a few solid electrolytes are presently known to have such a favourable conductance and most of them only at high temperature [58]. It has been seen that some inorganic ion exchange material, exhibit conductance values around $10^{-2}\,\Omega^{-1}\,cm^{-1}$ and therefore they could also find practical application in solid state electrochemistry.

At present, we are examining the possibility to use cerium phosphate or zirconium phosphate in Li^{+} forms as solid electrolytes for lithium solid state cells.

4.5. *Inorganic layered exchangers as dynamic membranes.*

We have already seen that steric hindrance to diffusion of counterions essentially depends on the interlayer distance of the layered exchanger and, in turn, the interlayer distance depends on the size and hydration of the counterions (Table III).

Concerning the hydrogen form of zirconium phosphate, we have seen before that the largest opening connecting two adjacent cavities is 2.64 Å and therefore counterions having a size larger than this values cannot diffuse inside the layered structure. For this reason, when $Zr(HPO_4)_2 \cdot H_2O$ is equilibrated with solution containing cations of large size, ion exchange does not occur. However, it was found recently in our laboratory [59] that, when a small amount of Na^+ ion is added, this ion acts as an ion exchange catalyst and the large cation is quickly taken up. The catalytic mechanism is as follows. Primarily Na^+ is exchanged by $Zr(HPO_4)_2 \cdot H_2O$:

$$\underset{(7.6\ \text{Å})}{Zr(HPO_4)_2 \cdot H_2O} + Na^+ + 5H_2O \rightarrow \underset{(11.8\ \text{Å})}{ZrHNa(PO_4)_2 \cdot 5H_2O} + H_3O^+ \qquad (1)$$

The layers are spread apart so that the large cation is now able to enter inside the enlarged structure. Since the exchanger prefers the large cation, Na^+ is displaced:

$$ZrHNa(PO_4)_2 \cdot 5H_2O + Me^+ + zH_2O \rightarrow ZrHMe(PO_4)_2 \cdot xH_2O + Na^+ + yH_2O \qquad (2)$$

$(5+z = x+y)$.

and it can give again new $ZrHNa(PO_4)_2 \cdot 5H_2O$ phase according the process (1), and so on.

This interesting phenomenon could be employed in order to obtain dynamic membranes. Let us suppose, for instance, a single crystal of zirconium phosphate separating two solutions containing a large cation at different concentration. Owing to the narrow interlayer distance of this exchanger, diffusion of the large cation does not occur. However, by adding a small

amount of Na^+, the interlayer distance is increased and diffusion of the large cation can now occur. It can be noted that this diffusion could be stopped if $Zr(HPO_4)_2 \cdot H_2O$ is again formed, e.g. by acidifying the solution.

4.6. *Inorganic membranes for theoretical studies.*

Membranes prepared with single crystal of layered exchangers are very attractive as a model for theoretical studies. In fact, owing to their well established crystalline structure, membranes prepared with these exchangers may represent a unique possibility to relate their electrochemical and osmotic properties to the kown reticular arrangement of fixed charges and counterions, as well as to steric factors due to resistance to the diffusion of counterions in their reticular structure. In particular, the layered structure of these exchangers could give the opportunity of studying the conductance and transport properties in a single plane of fixed charges as a function of their distance. On the other hand the possibility of obtaining a same ionic form at different interlayer distance of the exchanger could give the opportunity of investigating the electrical mobility of a given counterion as a function of the distance between fixed charges of adjacent layers.

We are now trying to obtain such a large crystal for theoretical studies.

Conclusion

Our studies have led to a deeper and more quantitative understanding of some of the basic problems involved; however, inorganic ion-exchange membranes still are in the first stage of their development. Intensive investigations have to be pursued especially those on the synthesis of new suitable active materials and in the direction to improve the electrical mobility of counterions. Preparation of inorganic membranes

having an electrical conductance comparable to that of organic membranes but a greater stability towards temperature, radiations and aggressive chemical environments, seems now to be an actual possibility, so that practical applications in some processes where the organic membranes, owing to tbeir degradability, cannot be employed, are hoped in a short time.

Potential employments as ion selective electrodes, as solid electrolytes for solid state electrochemistry, as membranes for hyperfiltration of brackish water, as well as their use as a model for theoretical studies could renew attention for inorganic ion excbange membranes so giving new stimulus for further investigations.

We are now harvesting the first fruits of our systematic researches, this indicating that our way was right and we are therefore encouraged to go further on it.

ACKNOWLEDGEMENTS

The author thanks Drs. U. COSTANTINO, M. G. BERNASCONI, R. BERTRAMI, J. S. GILL and M. L. LUCIANI for useful discussions and Mr. R. GIULIETTI for the assistance in the experimental work.

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DISCUSSION

Chairman: Prof. P. MEARES

SOLLNER

I should like to congratulate Prof. ALBERTI on two scores. First, he has done a beautiful piece of work and, secondly, he is a courageous man. Prof. ALBERTI has taken up a problem that has been lying dormant for about 30 years. Some of you will recall that MARSHALL in the late thirties and early forties prepared inorganic ion exchange membranes, consisting of compressed clays, green earth, etc., which he used successfully as membrane electrodes, some of them rather specific for certain ions. It was a beautiful piece of work, but MARSHALL'S membranes were very delicate and reacted very slowly; it took hours before final stable potentials were obtained. Also, these membranes disintegrated in course of time due to swelling. I think I inflicted a mortal blow to the use of these membranes as membrane electrodes by introducing the oxydized collodion membrane and the other collodion base membrane as membrane electrodes in 1943. Since then, as far as I know, nobody but Prof. ALBERTI has the courage to go back to the inorganics. Congratulations, Prof. ALBERTI.

ALBERTI

Thank you very much for your congratulations.

SLAYMAN

I noticed in the micrograph which you showed of the fibrous membranes that apparently there were fairly large water-filled interstices, through the membrane. I would be surprised, given such an arrangement, if the macroscopic transport properties of the membrane would reflect the fixed charges rather than simply the water-filled interstices. Am I correct about the spaces?

ALBERTI

The micrograph in question was obtained by a sample of cerium phosphate previously dispersed in water to show the fibrous nature of the exchanger. Membranes are instead obtained by pressing a sheet of cerium phosphate and therefore the interfibre distance depends on the applied pressure. By treating opportunely these membranes some bonds among interlacing fibres can be formed so giving some reticulation.

SLAYMAN

Does that change the basic picture of what looks like large spaces through the membrane?

ALBERTI

Large interfibre spaces are present in not-pressed cerium phosphate membranes. Now Donnan salt exclusion is small in large spaces and, therefore, the electrochemical properties of these membranes are not good. However, their electrochemical properties can be improved by reducing the interfibre distance by means of pressure.

It is likely that the active fixed charges are essentially those in the fibre surface, owing to the high activation energy for conduction of counterions in crystalline exchangers.

MEARES

Would you agree that the reason why the potential falls at a fairly low concentration below from the value for the ideal membrane is because the potential is degraded by conduction in the water-filled spaces between the bundles of fibres that we can see in the micrographs? I think this is partly what Dr. SLAYMAN is concerned about.

PATERSON

I would like to associate myself with Prof. SOLLNER'S congratulations and with Prof. SLAYMAN'S criticisms because I, too, began my career on zirconium phosphate and we didn't succeed in crystallizing it the way you have. In that work, however, I also studied diffusion in particles of zirconium phosphate aggregated in the way, (I assume), that your membrane is created and the diffusion properties through that aggregated particle were certainly greatly increased towards the value in free aqueous solution and I believe that, although under equilibrium conditions your selectivities are excellent, in membrane performance the interstitial pores will dominate the properties of your membrane and it would, to a large degree, lose its excellent selectivity.

MONNIER

I am sure that from your cerium phosphate membranes physiologists could build perhaps an improved model of Prof. TEORELL'S oscillator which shows so many features of living membranes. Prof. TEORELL has used sintered glass, and this membrane of cerium or ziconium phosphate, with its exchange properties and its well defined mobilities for various ions would be, perhaps, an invaluable material to visualize the remarkable property of Prof. TEORELL'S oscillator.

ALBERTI

I should be very glad if these membranes could be useful for such experiments.

MEARES

Have you considered filling the cerium pbosphate paper with lipids and then studying its properties? That also could have some interest perhaps in relation to biological systems.

ALBERTI

No. I haven't considered these possibilites. Thank you for your suggestion.

CHEMICAL ENGINEERING PROBLEMS REGARDING REVERSE OSMOSIS PROCESS OPERATION

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ABSTRACT

Processes involving transport phenomena in membranes, when considered globally, appear to include a variety of elementary phenomena wbich can be analyzed with methods typical of chemical engineering. The majority of these are phenomena which, although taking place in a continuous phase rather than in the membrane itself, are strongly influenced by the presence of the membrane.

Typical examples are:

1) Concentration polarization, and related mass transfer phenomena, with emphasis on the convection towards the interface typical of membrane phenomena.

2) Countercurrent processes with reflux, and optimization.

3) Mass transfer with chemical reaction at an interface, as taking place when enzymes are supported on the membrane.

4) Active transport in the membrane, and its analysis on the basis of the theory of mass transfer with chemical reaction.

Of these, only the fourth one takes place in the membrane itself. Research work, done at our Institution on some aspects of the phenomena considered, is briefly reviewed.

Introduction

Processes involving transport phenomena in membranes, when considered globally, appear to include a variety of elementary phenomena which can be analyzed with methods typicap of chemical engineering. The majority of these are phenomena which, although taking place in a continuous phase (the bulk of the solution) rather than in the membrane itself, are strongly influenced by the presence of the membrane.

In this work a detailed analysis of the most significant of these phenomena, the concentration polarization which occurs in an hyperfiltration process, is presented. It is our aim to emphasize the necessity of developping accurate analytical models for the description and for the control of this process, also when it is considered in its most simple form, the unstirred batch.

The unstirred batch cell has been generally considered of no practical importance; however the system is of some interest to determine membrane properties such as the intrinsic membrane rejection [1, 2], and for some applications when any stirring or recirculation can damage the products. Moreover the study of the flow regimes which characterize this hyperfiltration system is important in connection with the possibility of developping an analytical model apt to describe with a high degree of accuracy the fluodynamic field and particularly the salt concentration profiles generated in the cell and their evolution in time.

In order to solve the problem some simplifying hypotheses on the physical properties of the system such as constant, density, diffusivity and viscosity of the solution, and constant rejection of the membrane are introduced [3].

The line we will follow in our analysis consists in obtaining first of all a model which enables to solve exactly the problem in the case where the physical assumption indeed hold true; therefore if agreement with the experimental data

is not observed, one is sure that some of the assumptions must be relaxed.

Some interesting approximate analytical solutions have been presented recently by different authors [4, 5]. However also when the agreement between the experimental and theoretical results is satisfactory, it is possible to conclude that at least for intermediate range of time, no one of the previous available theories agree with experiments within 10% [6].

In contrast with the case of an exact solution, this disagreement can be due either to the approximation of the analytical solution or to the inadequacy of the physical model. Furthemore, we have also obtained some new approximate solution accurate enough for all the values of the parameters characterizing the process.

Basic equations and boundary equations

In the hypothesis of constancy of the transport properties of the solution and of the rejection coefficient of the membrane, the unknown functions in an unstirred batch system (see fig. 1) are tbe velocity, $v(x, t)$, the pressure $p(x, t)$, and the concentration $c(x, t)$. The equation governing an incompressible isothermal, non-homogeneous, one dimensional flow are the continuity, diffusion and the momentum equations.

The continuity equation shows that the transverse velocity v is a function only of time t. The diffusion equation gives the concentration c as function of t and of the coordinate x normal to the membrane; the third one, i.e. the momentum equation, gives the pressure p as function of x and t.

The boundary conditions are:

$$c(x, o) = c(\infty, t) = c_o \tag{1}$$

$$R^o v(o, t) \cdot c(o, t) = D c_x(o, t) \tag{2}$$

$$\Delta\pi - \Delta P = K v(o, t) \tag{3}$$

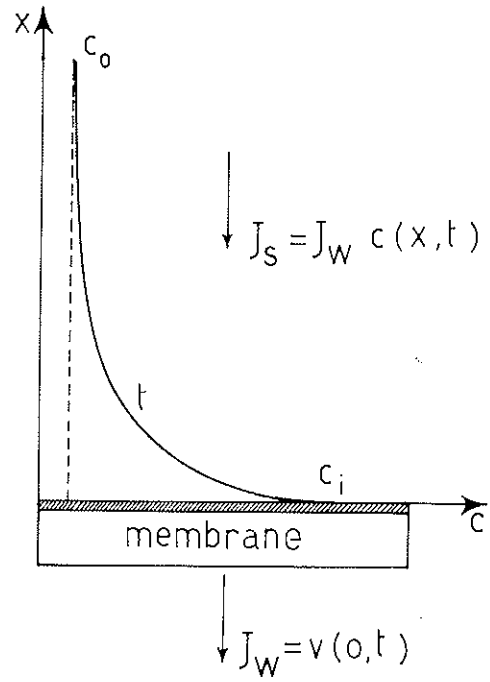

FIG. 1 — Schematic model of an hyperfiltration unstirred batch system.

where D is the binary diffusion coefficient, $\Delta\pi$ is the difference between the osmotic pressure of the interfacial salt solution on both sides of the membrane, ΔP is the hydrostatic pressure difference across the membrane and K is the so called membrane constant.

Assuming ideal solutions, the osmotic pressure difference $\Delta\pi$ can be approximate as follow:

$$\Delta\pi = R^o\pi_o c(o, t) \ / \ c_o \qquad (4)$$

where c_o and π_o are respectively the initial concentration and the corresponding osmotic pressure.

Introducing the following dimensionless variables

$$\tau = \left(\frac{\Delta P}{K}\right)^2 \cdot \frac{t}{D}; \quad \gamma = x\,\frac{\Delta P}{KD}; \quad r = \frac{c(x,t)}{c_o} - 1;$$

$$v = \delta\, r_o + \delta - 1 \tag{5}$$

and the dimensionless parameters R^o and δ defined as follow:

$$R^o = 1 - \frac{c_e}{c(o,t)}; \qquad \delta = \frac{R^o \pi_o}{\Delta P} \tag{6}$$

the diffusion equation and relative boundary conditions become

$$\left.\begin{aligned} & r_\tau + v\, r_\gamma = r_{\gamma\gamma} \\ & r(\gamma, o) = r(\infty, \tau) = o \\ & r_\gamma(o, \tau) = v(r_o + 1) \end{aligned}\right\} \tag{7}$$

where $r_o = r(o, \tau)$.

Different analytical procedures can be used to solve the non-linear equations 7. In some recent papers [7, 8] an integral method has been proposed, which simplifies the analytical procedure withouth any lack in accuracy. The following integral equation has been obtained:

$$r(\gamma,\tau) = \frac{1}{2\sqrt{\pi}} \int_o^\tau \left\{ \frac{\gamma\, r_o}{2(\tau - s)} - V\left[R^o + r_o\left(R^o - \frac{1}{2}\right)\right] - \right.$$

$$\left. - r_o \frac{\delta}{2}\left[\frac{H}{\tau - s} - r_o\right]\right\} (\tau - s)^{-1/2}\; e^{-[1-\delta+(\gamma-\delta_H)/(\tau-s)]^2\,(\tau-s)/4}\; d\,s \tag{8}$$

As already noted by LIU and WILLIAMS [9], equation 8 for high values of time τ shows two different asymptotic behaviours which experimentally are characterized in a first case by a finite value of the transverse velocity (rejection loss regime) and in the second case by a limiting value of the transverse velocity equal to zero (diffusion wave regime).

Equations describing these two different asymptotic behaviours have been obtained and are respectively:

for a rejection loss regime ($v_\infty \neq o$) (9)

$$r_o(\infty) = - \frac{2v_\infty}{1v_\infty\ 1}\left[R^o + r_o(\infty)\left(R^o - \frac{1}{2}\right)\right]\lim_{\tau\to\infty} erf\left[1v_\infty\ 1\ \frac{\tau^{1/2}}{2}\right]$$

which occurs if and only if $R^o - 1 + \delta < o$;

for a diffusion wave regime

$$r_o = r_o(\infty)\{1 - exp\,[(R^o - 1 + \delta)^2\,\tau]\ erfc\,[(R^o - 1 + \delta)\,\tau^{1/2}]\} \qquad (10)$$

which occurs when $R^o - 1 + \delta > o$.

A simple asymptotic solution has been also derived which describes the transition region when $R^o - 1 + \delta = o$; it is

$$r_o = r'_o(\infty)\ \delta\ (\tau/\pi)^{1/2}\ /\ [1 + r_o(\infty)\ (\tau/\pi)^{1/2}] \qquad (11)$$

Approximate solutions were also obtained for the two regimes, which agree in a large interval of time with the numerical solutions.

In fig. 2 a comparison between numerical and approximate solutions, in the case of a rejection loss regime is reported. In fig. 3 and 4 the approximate solutions in term of dimensionless flux as a function of τ are compared to experimental data.

The agreement is satisfactory.

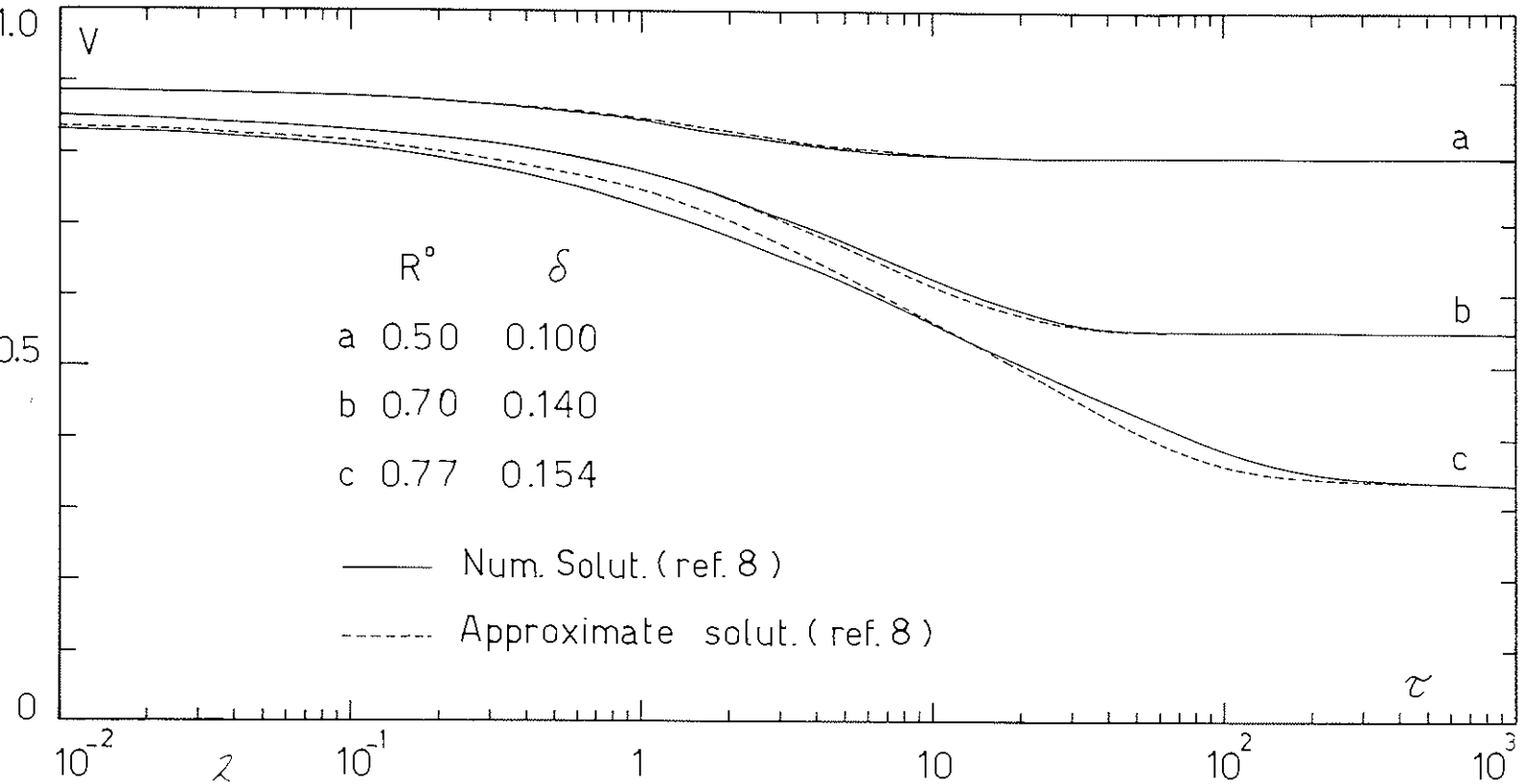

FIG. 2 — Dimensionless flux V as a function of dimensionless time τ in a rejection loss regime. Comparison between numerical and approximate analytical solution.

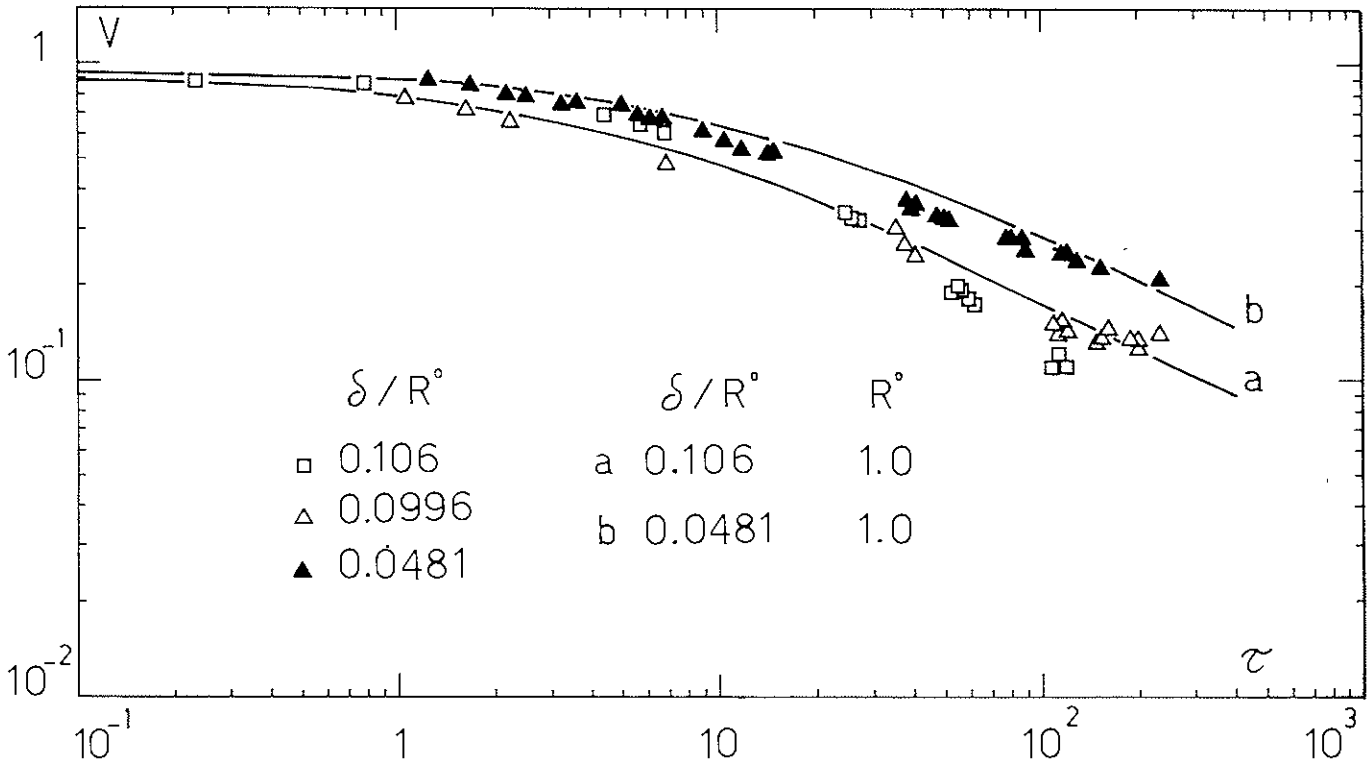

FIG. 3 — Dimensionless flux V as a function of dimensionless time for three different experimental runs in a diffusion wave regime. ———— Numerical solution.

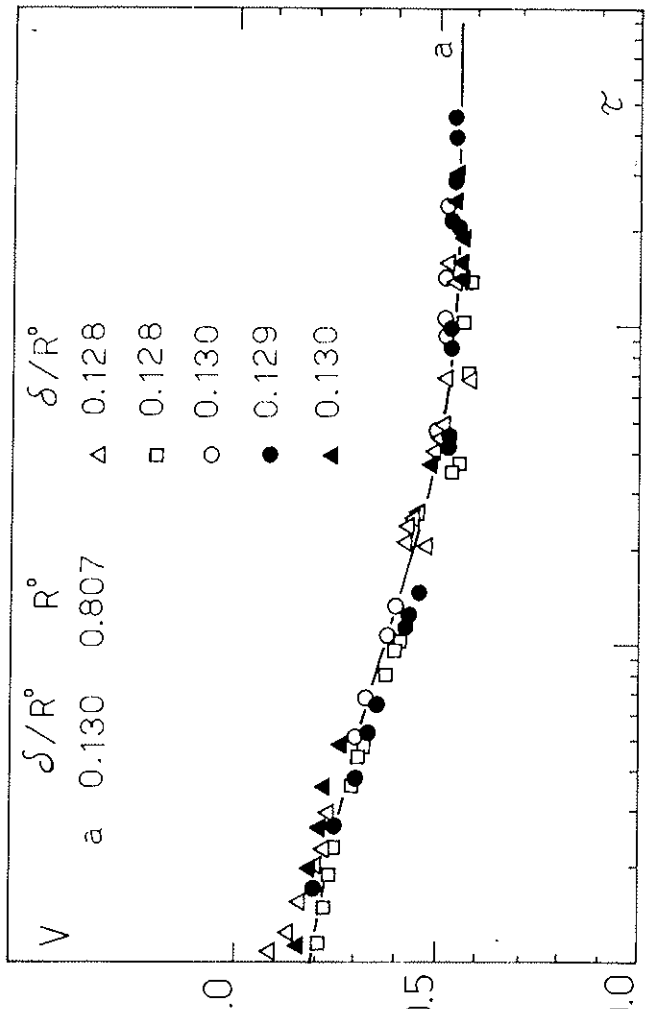

FIG. 4 — Dimensionless flux V as a function of dimensionless time for five different experimental runs in a rejection loss regime. ——— Numerical solution.

However if the dimensionless excess of concentration r is plotted as a function of time, it is possible to observe a quite good agreement between the experimental results and the proposed approximate equations only for high values of the dimensionless time τ. At low values of τ discrepancies are significant. This result indicates that one of the physical assumption present in the model is not correct.

An analysis has been therefore developed postulating that the rejection coefficient is no more a step function ($\tau = o$, $R^o = o$; $\tau > o$, $R^o = \text{cost.} \neq o$), but progressively increases with time approaching its asymptotic value.

However the rejection coefficient dependence from the physical quantities, as pressure bulk concentration and membrane permeability, is not known.

We have therefore studied a semitheoretical solution of the problem, making use of the experimental results [10, 11].

The main features of the model were the same than in the previous case, and particularly: *a*) zero thickness membrane; *b*) two dimensionless geometry and semi-infinite axial dimension upstream and down-stream of the membrane; *c*) constancy of the applied pressure; *d*) constancy of the transport properties of the solution (diffusion coefficient), viscosity, density; *e*) negligible salt accumulation and pressure drop in the porous plate supporting the membrane; *f*) same membrane permeability for water and solutions; *g*) validity of the Van't Hoff equation to evaluate the osmotic pressure.

The differential mass balance equations upstream and downstream of the membrane were considered. The simultaneous solution of these equations was dependent on a function $f(\tau)$ which was defined as the difference between the dimensionless salt concentration on the two faces of the membrane. A force balance across the membrane on the basis of assumption *f*) and *g*) gives

$$f(\tau) = r_o - r'_o = \frac{v_\omega - v_\tau}{r\, v_\omega} \qquad (12)$$

For the solution of the problem a previous evaluation of $f(\tau)$ was required. Therefore from the experimental data of flow rate at different times, by a least square method, it was searched for the best polynomial fitting of the interface salt concentration difference. The system of differential equations with the pertinent boundary conditions was solved numerically by a finite difference procedure. In fig. 5, plots of the dimensionless interface salt concentration upstream of the membrane versus time calculated from models based on different physical assumptions are reported and compared with experimental results in a typical rejection loss regime. It is quite evident that at least in the range of low applied pressure the best agreement between theory and experiments is obtained with the model which assumes a variation of the flow rate as a consequence of concentration polarization. However a more significant result is furnished by the time dependency of the effluent salt concentration. Figure 6, is a plot of computed dimensionless salt concentration versus dimensionless time. The curves refer to different models.

Due to the assumption of a constant rejection coefficient curves 2, 3 and 4 are characterized by a positive derivative in the entire τ range, wbilst only curve 1 is characterized by an initial negative curvature according to an increase of R^o with time. A significant qualitative agreement exists between this curve and the experimental results reported in fig. 7. Since it is impossible to make a direct measure of r'_o this value could be determined from the salt concentration measured at a definite distance from the membrane interface, upon definition of the transfer function which relate r'_o to r^o. Neverthless the quite good qualitative and the satisfactory quantitative agreement seem to prove that the variable rejection model well describes concentration polarization phenomena in hyperfiltration batch system. In figure 8, a typical curve showing rejection coefficient R^o variation with time, calculated on the basis of the model is presented. The results indicate that in the unstirred batch system rejection variation occurs in a time interval

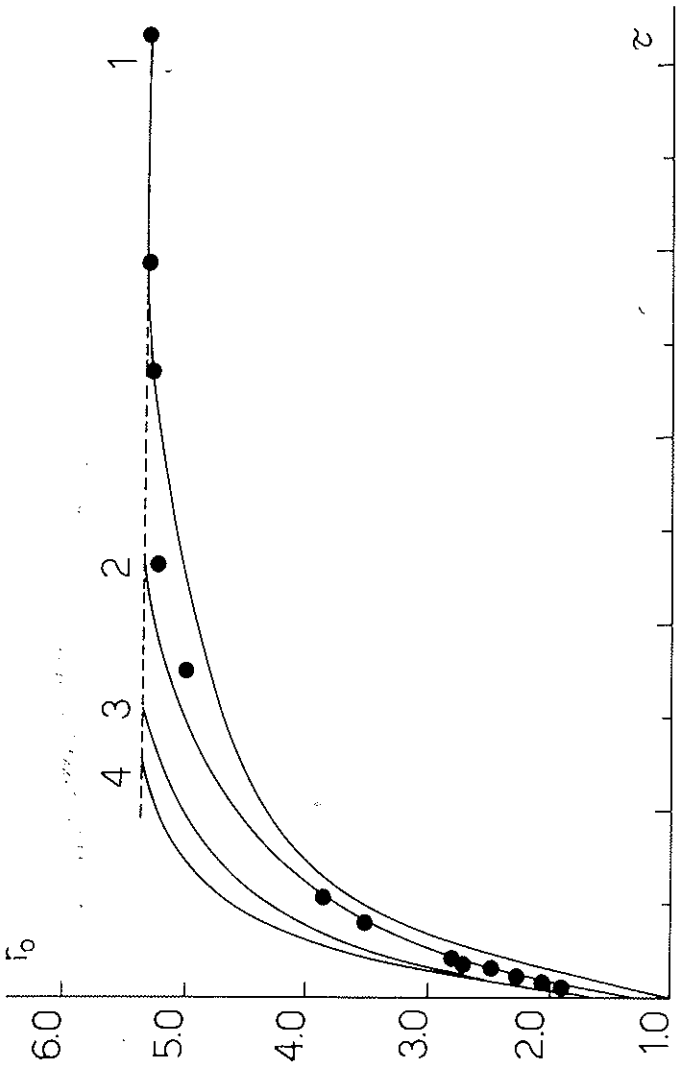

FIG. 5 — Plot of the interface NaCl concentration upstream of the membrane vs. time; $\Delta P = 4$atm; $\tau_o = 0.534$, $R^o = 0.814$. Key: Curve 1, variable rejection model; Curve 2, constant rejection model [3]; Curve 3, ASTARITA *et* al. model [2]; Curve 4, RARIDON et al. model [4].

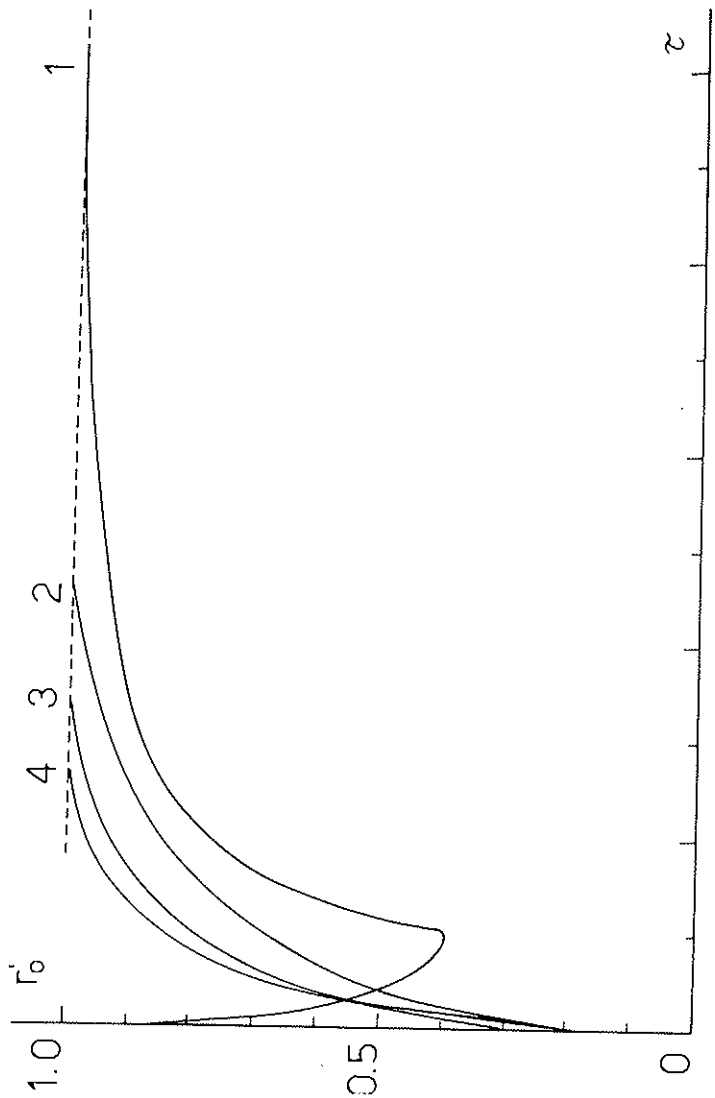

FIG. 6 — Plot of the interface NaCl concentration downstream of the membrane *vs.* time. Curve 1, variable rejection model; Curve 2, constant rejection model [3]; Curve 3, ASTARITA *et al.* model [2]; Curve 4, RARIDON *et al.* model [4].

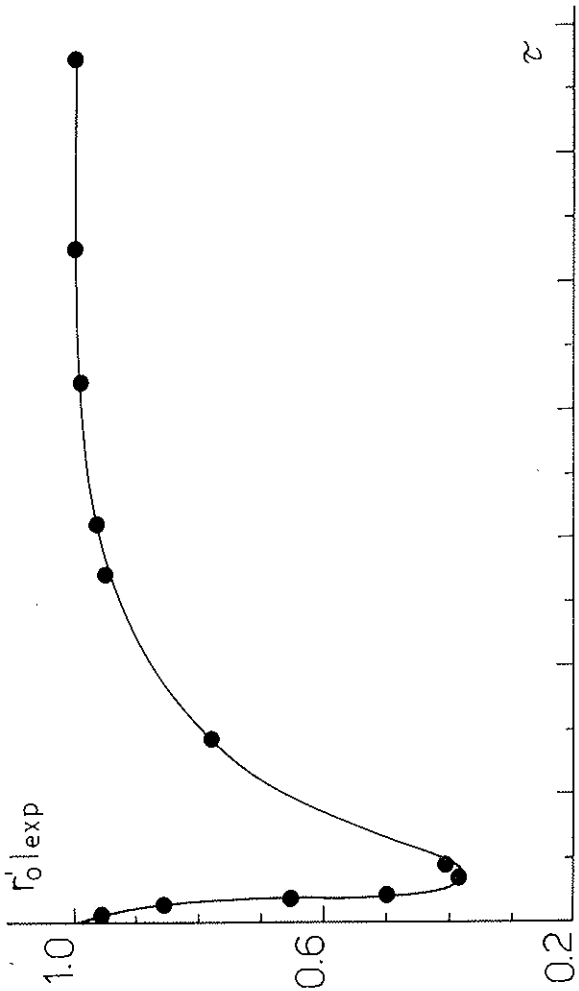

FIG. 7 — Plot of effluent NaCl dimensionless concentration vs. time, $\Delta P = 4$ atm, $\pi^\circ = 0.534$, $R^\circ = 0.814$.

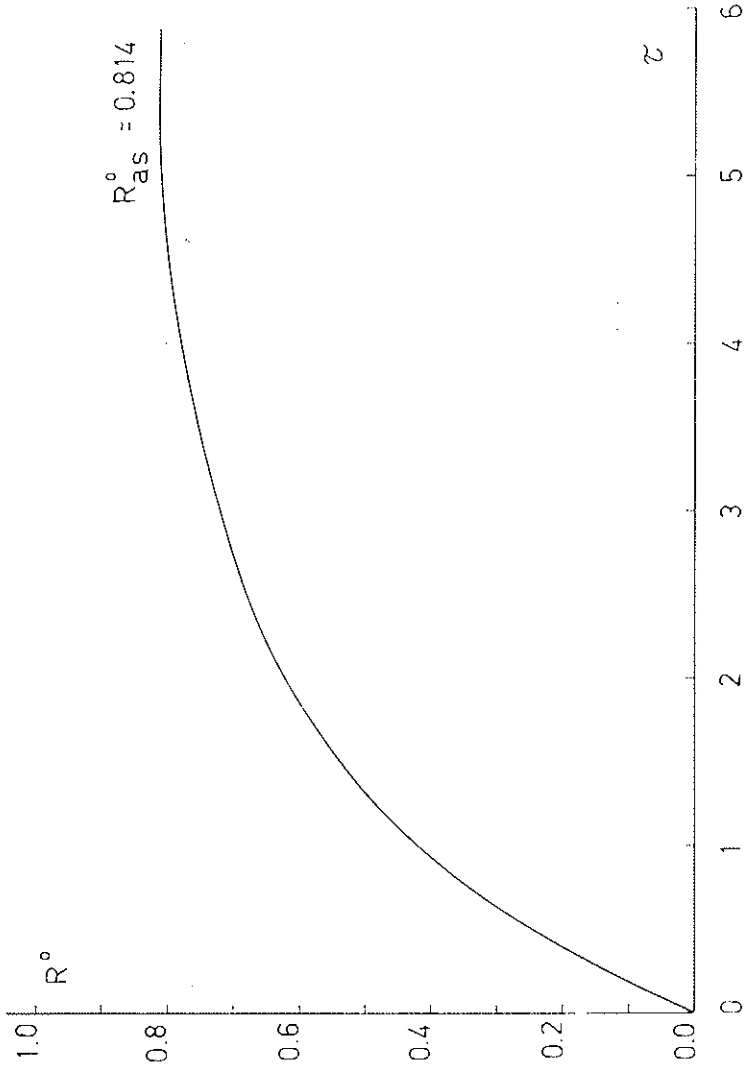

FIG. 8 — Plot of calculated R° vs. time.

of the order of twenty minutes in a typical run before reaching the asymptotic value. This time interval decreases if we consider stirred batch system or recirculating systems, due to the fact that concentration polarization is less significant in these processes. This consideration, however, is not valid when working with industrial plants or in general with multicomponent solutions containing impurities. In fact in these situations significant flux decline and often subsequent rejection increase are observed also in recirculating systems, in time intervals of the order of hours. These phenomena which can be present indipendently from any compaction of the membrane can be interpreted on the basis of dynamic membrane formation [12]. From recent results, it appears that dynamic membrane formation may be a fairly general phenomenon, requiring only *a*) a suitable matching of sizes of the species in solution and the pores in the substrate, and *b*) an attachment mechanism [12]. Experimental results indicate that very low $AlCl_3 \cdot 6H_2O$ concentration (of the order of 3.10^{-4} M) in standard electrolytic solutions produce significant flux decline and subsequent rejection increase if low annealed cellulose acetate membranes are used in the process [13]. In figure are reported for example experimental results obtained in hyperfiltration of an $LiCl + AlCl_3 \cdot 6H_2O$ solution on cellulose acetate membranes annealed at different temperatures.

Shown in figure 9, is the volume flux Q thorough three membranes as a function of time. The membranes are designated according to their annealing temperature, i.e. the CA60 membrane was annealed at 60°C.

Prior to the zero time shown in the figure, the volume flux was measured for several hours with 10^{-4} M LiCl feed and found to be stable at the initial values shown in the figure. At time $t = 0$, the feed was changed to a mixture of 10^{-4} M LiCl and 3.10^{-4} M $AlCl_3 \cdot 6H_2O$ and there was initially a very sharp decline in the rejection of Li^+ exibted by the CA60 and CA75 membranes. Over a longer time period, there was a decline in volume flux for these same membranes, as shown

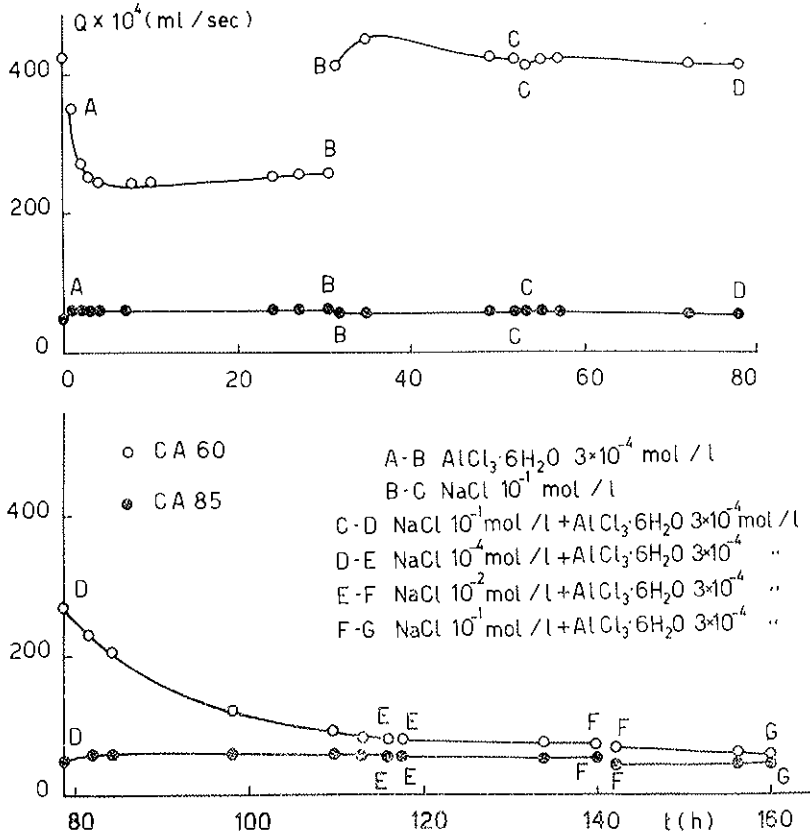

FIG. 9 — Volume flux, Q, vs. time for three asymmetric cellulose acetate membranes.

in the figure, with an accompanying increase in the rejection of Li^+.

Small concentrations of soluble iron are present in virtually all hyperfiltration systems and the strong similarity between the acqueous chemistry of iron and aluminum suggests that effects similar to those reported in [13] may be important even with highly annealed membranes over long time periods.

On the other side every industrial hyperfiltration or ultrafiltration plant works continuously for time intervals of the order of 24 or 48 hours between subsequent shut off and shut on due to the necessity of controlling fouling with cleaning the membranes.

Therefore if rejection variation is significant in time interval of hours it has to be taken into account in the optimization of the process.

The importance of a detailed study of concentration polarization phenomena which occurs in these processes is also connected to the possibility of utilizing UF or HP cells as enzymatic reactors, with the enzymes " immohilized " at the membrane-solution interface.

Processes, where a chemical reaction catalysed by the immobilized enzymes is accompanied to the mass transport through selective membranes, seems today of great interest for their industrial potentiality. Besides systems formed immobilizing enzymes on membranes reproduce biological situation and can be used as models for studying in laboratory some basic biological processes.

The ultrafiltration of protein solutions has been recently investigated for the concentration polarization phenomena and their influence on the overall process. The results obtained have been generally interpreted on the basis of the so called gel-polarization model which assumes the formation of an adherent gel layer of concentrated protein on the pressurized face of the ultrafiltration membrane, a formation occuring under any fluodynamic regime [14] (i.e. unstirred batch system, stirred batch system continuous recirculating ultrafiltra-

tion system). The results of experimental investigations seem to indicate that the dynamic formation of an enzyme gel-layer on an UF membrane may be suggested as an additional mode of preparing immobilized enzymes without special chemical or physical procedures [15, 16]. Experiments have been carried out in an unstirred ultrafiltration cell at a costant temperature of 30 ± 0.1°C and at different applied pressure, on dynamically formed enzyme membranes using acid phosphatase as enzyme.

Sodium p-nitrophenyl phosfate in 0,05 M sodium citrate/ citric acid buffer, pH 5-6, was used as a substrate.

Interesting results have been obtained particularly in terms of Michaelis constant K'_m. In fact a value of $1{,}35.10^{-4}$ M, about seven fold lower than that obtained at 30°C in separate experiments with the enzyme acting in free solution, was calculated from the experimental results.

In this kind of process the concentration polarization phenomena plays two different roles; the first one consists in controlling the concentration profiles of the enzymatic protein on the pressurized face of the membrane to the point that an enzymatic gel layer of constant thickness is built up at the membrane-solution interface; the second one consists in the built up of substrate and products concentration profiles on the dynamically formed enzymatic membrane.

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DISCUSSION

Chairman: Prof. P. MEARES

MONNIER

As a physiologist I have been interested in food problems I would like to ask what are the prospects of the reversed osmosis as a source of fresh water. Is there now some major improvement in that field?

DRIOLI

If you speak with people working in reverse osmosis they will give you a very optimistic prognosis and what they say is that in the next 10 years reverse osmosis industrial applications will improve, both in food industry and waste water treatment. Professor SPIEGLER will tell you about sea water desalination. The process I showed, where mass transfer and a chemical reaction are combined at the membrane solutions interface, is a kind of process which is now deeply studied. It seems to be a field of immense industrial interest.

MONNIER

Then you fully deserve all our congratulations.

POST

I got the impression you were making the alkaline phosphatase sit still and and it was not free to move about in solution. Could you explain that point?

DRIOLI

There are two different ways of looking at the problem. One way is this one: the concentration polarization phenomena, occurring in protein solutions ultrafiltration, can be described with the so called « gel-model ». It is assumed that at the interface membrane/ solution a gel layer is formed when the concentration of the protein equals the gelification point. If now the substrate is ultrafiltered through this dynamically formed gelled-enzyme composite membrane, we have at the same time a chemical reaction catalized by the enzyme and selective mass transport. The enzyme is working in a very good situation: high concentration, physical or chemical bonding, etc. Back diffusion is generally not significant and can be avoided in different ways. So what I am doing is trying to use this kind of approach to « immobilize » the enzymes on the membrane. This approach can also be applied to studies on biological membranes.

The second idea is just to use the concentration polarization even though you don't need to arrive at the gelification point. You can in fact control the concentration profiles in this enzymatic reactor where the concentration of enzymes is very high at the membrane-solution interface and very low in the bulk of the reactor. The second kind of approach is more useful, for example, if you have to work with e.g. cellulose.

VIII

ARTIFICIAL MEMBRANES: THERMODYNAMICS AND TRANSPORT. I

FUNCTION AND STRUCTURE OF MEMBRANES

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1. Introduction

The studies of membranes and of drugs have in common that a wide gap exists between the study of *function* and the study of *structure*. Of both subjects the point can be made that knowledge of the structure does not imply any knowledge at all about the function and reversely complete knowledge of the function does not imply any knowledge of the structure.

The discipline of the investigators of structure and function respectively is also different in general. Structural investigations are generally performed by organic chemists and biologists, functional investigations by physicists, physico-chemists and physiologists.

This controversion is conducive to misunderstanding as both groups tend to ignore or underrate the intricacies of the field of the other group and to propose inadmissible simplifications in that field. It should be realised that for quite some time we must expect that both kinds of study are relevant in their own right and that the ultimate connection between these studies requires a detailed knowledge about molecular dynamics which is not yet available.

On closer inspection the studies of function and of structure of membranes are not so far apart as suggested above. A des-

cription of the functioning of a membrane without any reference to the structure treats the membrane as a "black box" characterised hy a number of coefficients. From the thermodynamics of irreversible processes, relations can be derived hetween the coefficients reducing the number of "thermodynamical coefficients" to $\frac{1}{2}$ N (N + 1) for a system of a membrane and N-components. These relations not only imply the so-called Onsager reciprocal relations but also the requirement of thermodynamics that — in a linear treatment — the coefficient relating a flow to a force must have the same value for forces of different origin, mechanical, electrical or chemical, provided the forces are expressed in the same units.

In view of the fact that in this black box treatment nothing is known or supposed about the processes in and the structure of the membrane, a surprising number of relations between measurahle quantities can be derived [1] from it. Several of these relations have heen checked experimentally and these experiments generally confirm the validity of the thermodynamical relations [2]. This is not absolutely necessary since the membrane is subjected to different strains in different experiments and therefore the "state" of the membrane, implying the value of the coefficients could be different. The observed validity of the thermodynamical relations confirms the usefulness of this treatment as a starting point. At the same time from the derivation of these relations it follows clearly that the validity of these relations cannot prove any particular model of the membrane or of the permeation process. Any model not yielding the thermodynamical relations should be viewed with suspicion. Finally, we remark that the black box treatment [1] produces a relation between mechanical and electrical transport numbers which to my knowledge has never been checked.

The advantage and the merit of the hlack box treatment of being independent of any model turns into a disadvantage if one hopes to understand the physical nature of the permeation process. Also, the thermodynamic coefficients are constants

only for a given system of a given composition. About the concentration dependence of the coefficients thermodynamics can give no information. In order to keep the number of necessary experiments within limits and also in order to arrive at a physical picture of the permeation process, the first additional information needed is the value of the concentrations inside the membrane. This involves additional measurement of the partition coefficients of all components. If the outside concentration is — in an equilibrium experiment — C_i and the concentration inside the membrane is $c_i\ (1 - \phi_m)$, with $1 - \phi_m$ the volume fraction of liquid in the membrane, then the partition coefficient of component i is defined as

$$K_i = \frac{c_i\ (1 - \phi_m)}{C_i} \qquad (1)$$

The partition coefficient can depend strongly on the concentration. This dependence can be determined in a sufficient number of equilibrium experiments, for instance with radioisotopes.

In order to describe not only a system of given composition but all systems of a given membrane and given components at different concentration, the measurement must be expressed in coefficients which may be expected to be independent or only slightly dependent on the concentration. For this purpose the friction constants introduced by SPIEGLER [3] following LAITY [4] and KLEMM [5] may serve.

They can be defined in various ways. In this paper we will use the following notation

$$-X_i = \Sigma_k\, r_{ik}\, c_k\, (u_k - u_i) = \Sigma_k\, r_{ik} \left(J_k - \frac{c_k}{c_i} J_i\right) \qquad (2)$$

where X_i is the force acting on molecules or ions of component i, u_i is the average velocity of these molecules and

$$J_k = c_k\, u_k \qquad (3)$$

is the flow in molecules per unit area per second.

We will sometimes use the symbols

$$\left.\begin{aligned} f_{ik} &= c_k\, r_{ik} \\ f_{ki} &= c_i\, r_{ik} \end{aligned}\right\} \qquad (4)$$

and

with the understanding that the reciprocal relation

$$r_{ik} = r_{ki} \qquad (5)$$

does not hold for the f_{ik}.

We see from the middle expression in (2) that the r_{ik} do not depend on the coordinate system used.

2. Constancy of friction constants

In order to describe the functioning of a membrane not only with respect to a certain mixture of given composition but with respect to a given set of components at varying concentration and under different boundary conditions, it is essential to have at one's disposal coefficients wich may be expected to be constants.

The friction coefficients defined above may be expected to comply with this requirement and are actually often found to do so.

Since this is a critical point concerning the foundations of a description of membrane functioning, we will discuss in some detail 2 variations which should not affect the friction coefficients in a first approximation: variation of boundary conditions, variation of concentration.

2.1. *Variation of Boundary Conditions*

The simplest example of variation of boundary conditions is given by experiments to check the reciprocal relations as discussed above. This check has confirmed the validity of the relations in many cases.

Another example is given by the comparison between stationary and non-stationary experiments. Also here constancy of the friction coefficients is generally assumed. Finally, in the technology of water desalination by reverse osmosis one is interested in the membrane behaviour at very high flow rates where the macroscopic membrane behaviour is not linear anymore.

I do not know of explicit experiments to check the constancy of the friction coefficients under such conditions. But the fact that even at high flow rates the average displacement of the molecules per second is small compared to $\sqrt{\frac{kT}{m}}$, the average velocity of the molecules, is an argument to trust that constant friction coefficients can be used to describe the process. Without this assumption the description of the functioning of the membrane is virtually impossible. On the other hand the effect of the pressure together with the fouling of the membrane on the state of the membrane has to be checked experimentally.

2.2. *Variation of concentration*

Since the r_{ik} describe the average friction force exerted by one molecule i on one molecule k and reversely, this force can remain a constant at different concentrations only in case there is no strong thermodynamic interaction, that is in ideal solution.

Most solutions of experimental interest are not ideal at all. However, in a limited range of concentrations the activity coefficients usually change not very much and in the same range the friction coefficient may be expected to remain approximately constant.

2.3. *Variation of the environment*

Some of these variations have been investigated carefully by SMIT [6]. He used Vycor glass membranes and solutions

of various sugars, i.c. raffinose, sucrose and mannitol in water. He confirmed the reciprocal relations for these systems and he found that the friction coefficients r_{ik} are independent of the concentration. He calculated the partition coefficient K, from the concentration dependence of the macroscopic permeability coefficients. He found for mannitol, sucrose and raffinose in the membranes K-values very near to unity whereas for pentaerythritol K = 1.10.

He also found that the value of the friction coefficient between solute and solvent in free solution as deduced from measurements of free diffusion, r^f_{10}, is proportional to r_{10} in the membrane for mannitol, sucrose and raffinose. This is a strong indication that the friction between these solutes is of the same nature and magnitude in the different environment of the membrane and in free solution. In fact it is easily seen that in that case

$$r^f_{10} = r_{10} (1 - \phi_m) \theta^2 \qquad (6)$$

where $1 - \phi_m$ is the "pore volume" of the membrane and θ^2 the tortuosity factor. On the other hand for the pentaerythritol where K = 1.10 the ratio between r^f_{10} and r_{10} is about twenty percent higher. This is understandable from the fact that the higher partition coefficient is an indication of a thermodynamical or chemical interaction between membrane and solute which must be accompanied by a hydrodynamical interaction.

These results have led Smit to a definition of pore model membranes based upon a quantitative relation. The pore model is said to apply for membranes and solutes characterised by a partition coefficient of unity and a constant ratio between the friction coefficients in the membrane and in free solution for different solutes. Thus, a structural model is defined in terms of the results of permeation measurements.

3. Effect of the partition coefficient

Although differences in friction coefficients can cause differences in permeation selectivity of membranes, one expects that pronounced differences in selectivity will originate from differences in the partition coefficient, reflecting differences in thermodynamical or chemical interaction.

Partition coefficients can depend strongly on the concentration, in contrast to friction coefficients and this will affect the membrane behaviour strongly. An interesting example of this effect has been observed in my laboratory by Talen [7]. He used the same glass membranes as Smit and investigated among others solutions of poly(ethylene glycol) in water. He met with many experimental difficulties, in particular strong absorption of solute in the membrane, indicating that the partition coefficient is much larger than unity. The experimental difficulties make his quantitative results unreliable but qualitatively they can probably be trusted. For low concentrations ($C_1 < 3$ g/l) he finds a negative reflection coefficient in ultrafiltration experiments, indicating that this is a case of *anomalous osmosis*. This was in complete accordance with negative osmotic pressures found by Elias [8] for very similar or identical systems. At first sight it appears surprising that these negative values sharply decrease at increasing concentrations and are converted to values up to $+0.8$ at concentrations of 10 g/l and higher. Thus, a substance permeating faster than water and entraining large amounts of water at low concentrations, is strongly reflected at higher concentrations.

The phenomenon can be understood from the concentration dependence of the partition coefficient in a model with constant friction coefficients as is shown by Staverman, Kruissink and Pals [9]. In fact, if we assume a Langmuir isotherm or a similar relation for the amount of absorbed solute as a function of concentration, we see that the partition coefficient defined by (1) starts with a high value and declines sharply at increasing concentration of solute so as to approach a nearly

Anomalous Osmosis

$$\sigma = 1 - K \frac{r_{10}(1 - \varnothing) + f_{om} v_1}{r_{10}(1 - \varnothing) + f_{1m} v_o}$$

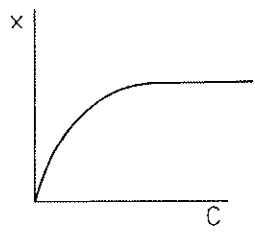

FIG. 1*a* — Absorption Isotherm

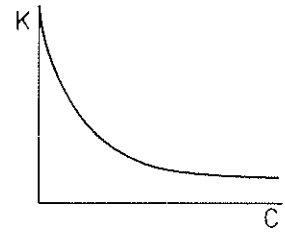

FIG. 1*b* — $K \approx \frac{X + (1 - \varnothing_m) C}{C}$

constant value (Fig. 1). Expressing the reflection coefficient in the friction coefficient we have

$$\sigma = 1 - K \frac{r_{1o}(1 - \varnothing_m) + f_{om} v_1}{r_{1o}(1 - \varnothing_m) + f_{1m} v_o} \qquad (7)$$

from which it is seen that σ may become negative for very large K and approach unity for small K provided the fraction in (7) is small.

Physically we can visualise the process by assuming that at low solute concentrations the pore wall is covered by a procentually high fraction of the solute. These solute molecules diffuse in the direction of low solute concentration entraining the water molecules in the pore, thus giving rise to anomalous osmosis. At higher concentration the proportion of absorbed molecules to free solute molecules in the pores and to free molecules in the outer solutions will become much smaller and owing to the friction of the large molecules with the membranes a positive reflection coefficient near unity may result.

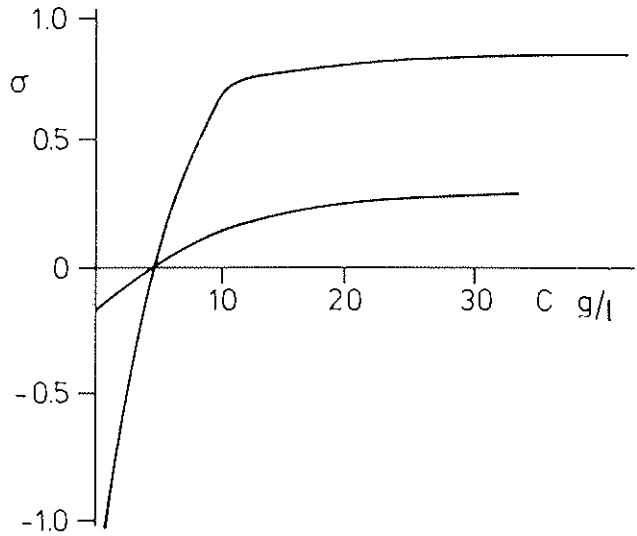

FIG. 2 — Poly-ethylene glycol in water; Vycor glass membrane; σ from ultrafiltration (J.L. TALEN, THESIS, LEIDEN, 1963).

If we consider the PEG molecules as "carriers" of the water molecules at low concentrations the remarkable observation is made that such a carrier only operates at very low concentrations as is found both experimentally and theoretically.

Thus, the important role that the partition coefficient and in particular the concentration dependence of the partition coefficient can play has been elucidated.

CONCENTRATION PROFILES

The next step in the description of the relation between structure and function of the membrane consists of investigating, theoretically and experimentally, the concentration profile inside the membrane. In stationary cases with flows and forces

in the linear region linear concentration profiles are found. Assuming that the friction coefficients remain constant with changing concentrations and also for large flows, the cases of non-stationary and of non-linear flows can be described with the same coefficients as the linear and stationary cases.

This is of interest in the first place because any stationary experiment passes through a non-stationary state from which additional information about the characteristic constants can be derived.

In 4.1 we discuss the non-stationary phase of osmotic measurements with zero volume flow [10], in 4.2 we consider non-linear phenomena in reverse osmosis experiments [11].

4.1. *Non-stationary diffusion,* $J_v = 0$

In a two-component system with a membrane we have three friction coefficients r_{10}, f_{om} and f_{1m}. The concentration of solute is assumed to be $c_1 \ll c_o$.

The fundamental equations are

$$X_o = \frac{r_{10}\, c_1 + f_{om}}{c_o} J_o - r_{10}\, J_1 \tag{8}$$

$$X_1 = - r_{10}\, J_o + \frac{r_{10}\, c_1 + f_{1m}}{c_1} J_1 \tag{9}$$

Assuming a pressure gradient ∇p and concentration gradient ∇c, the forces become

$$X_o = - v_o \nabla p - \nabla \mu_o \tag{10}$$

$$X_1 = - v_1 \nabla p - \nabla \mu_1 \tag{11}$$

with

$$\nabla \mu_o = - v_o \nabla \pi = - \frac{c_o}{c_1} \nabla \mu_1 = - \frac{RTc_1}{c_o} \nabla \ln c_1 = - \frac{RT}{c_o} \nabla c_1 \quad (12)$$

where we have assumed constant activity constants and $\nabla \mu_m = 0$.

Substituting (10), (11) and (12) into (8) and (9) we obtain

$$- \nabla p - \nabla \pi = \frac{r_{10} c_1 + f_{om}}{c_o v_o} J_o - \frac{r_{10}}{v_o} J_1 \quad (13)$$

$$- \nabla p - \frac{c_o v_o}{c_1 v_1} \nabla \pi = - \frac{r_{10}}{v_1} J_o + \frac{r_{10} c_o + f_{1m}}{c_1 v_1} J_1 \quad (14)$$

Subtracting (13) and 14) using

$$c_o v_o + c_1 v_1 = 1 - \phi_m \quad (15)$$

we find

$$(1 - \phi_m) RT \nabla c = \{(1 - \phi_m) r_{10} + v_1 f_{om}\} c_1 J_o - \{(1 - \phi_m) r_{10} + v_o f_{1m}\} c_o J_1 \quad (16)$$

In the case

$$J_v = v_o J_o + v_1 J_1 = 0 \quad (17)$$

J_o and J_1 are of the same order of magnitude, and since $c_1 \ll c_o$ the first term of the r.h.s. of (16) can be omitted. Writing further

$$c_o = \frac{1 - \phi_m - c_1 v_1}{v_o} = \frac{1 - \phi_m}{v_o} \quad (18)$$

(16) can be written

$$(1 - \phi_m)\, RT\, \nabla c = - \{(1 - \phi_m)\, r_{10} + v_o f_{1m}\} \frac{1 - \phi_m}{v_o} J_1 \quad (19)$$

and comparing with Fick's law

$$J_1 = - D_{II}\, \nabla c \quad (20)$$

we see that the diffusion coefficient in the membrane D_{II} is expressed in the friction coefficients by

$$D_{II} = \frac{RT\, v_o}{(1 - \phi_m)\, r_{10} + v_o f_{1m}} \quad (21)$$

We call the outer phases I and III and the membrane phase II and consider 6 different sets of boundary conditions, cases 1 *a*, 1 *b*, 1 *c* and 2 *a*, 2 *b* and 2 *c*. In cases 1 stirring is applied in I and III, in cases 2 no stirring is applied.

For the concentrations at $t = 0$ we have: in cases *a* $c^{II} = 0$; in cases *b* $c^{II} = K(1 - \phi_m)\, c^I$ and in cases *c* $c^{II} = K(1 - \phi_m)\, c^I \left(1 - \frac{x}{d}\right)$, where d is the membrane thickness, while in all cases $c^{III} = 0$ at $t = 0$ (see fig. 3).

Thus, cases *a* start with an empty membrane, cases *b* with a membrane in equilibrium with c^I and cases *c* with a linear concentration gradient, in quasi equilibrium with both c^I and c^{III}.

In the stirred cases the general solutions of the differential equations read for the outer concentrations

$$c^I(t) - c^{III}(t) = \sum_{n=1}^{\infty} A_n\, e^{-\frac{t}{\tau_n}} \quad (22)$$

in which the A_n are different for the three cases *a, b, c,* but the τ_n are not.

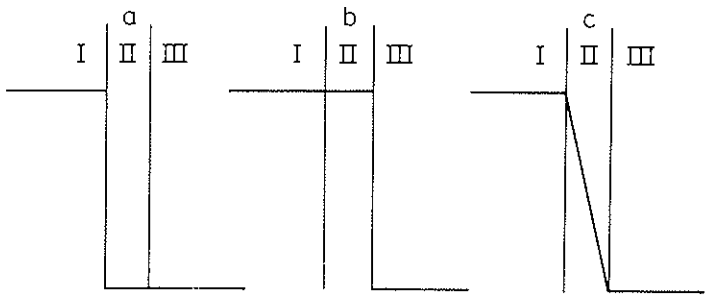

FIG. 3 — Non-stationary diffusion, $J_v = 0$ — 6 cases; boundary conditions at $t = 0$ — 1. stirred; 2. non-stirred.

The τ_n can be written

$$\tau_n = \frac{D_{II}\alpha_n^2}{d^2} \tag{23}$$

where the α_n are the solutions of the equation

$$\operatorname{tg} \alpha_n = \frac{-\alpha_n (V_I + V_{III}) K (1 - \phi_m) V_{II}}{K^2 (1 - \phi_m)^2 V_{II}^2 - V_I V_{III} \alpha_n^2} \tag{24}$$

where V_I, V_{II} and V_{III} are the volumnina of the phases.

The equations can be solved numerically by a computer. Generally $V_{II} \ll V_I$ and V_{III} and is τ, the largest relaxation time, characteristic for the measured concentration difference $c^I(t) - c^{III}(t)$. This quantity τ_1, to be calculated by putting $\operatorname{tg} \alpha = \alpha$, yields $\alpha_1^2 = K(1 - \phi_m) V_{II} \frac{I + III}{V_I V_{III}}$ and τ_1 from (23).

In this case $c^I(t) - c^{III}(t) = c^I(o)\, e^{-t/\tau_1}$ after a short starting period.

KRUISSINK [12] and also ŠPAČEK and KUBIN [13] cal-

culated a solution which is identical with ours under certain restrictions for case 1 *a*.

As K D_{II} appears together in the value of τ_1 they cannot be calculated separately from the observed relaxation times.

In the unstirred cases besides D_{II} also $D_I = D_{III}$ play a part.

It appears that a quantity r defined by

$$r = (1 - \phi_m)\, K \sqrt{\frac{D_{II}}{D_I}} \tag{25}$$

is characteristic. In the unstirred cases the concentration in the outer cells is not uniform, but in an automatic osmometer $c^I(o) - c^{III}(d)$ can be measured. It appears that extrapolation towards $t \to 0$ yields different values for

$$\lim_{t \to o} [c^I(0, t) - c^{III}(d, t)]$$

In cases *a* and *b* we find the value $\frac{c^I(0,0)}{1 + r}$ for this limit, in case *c* the value of the limit is $c^I(0,0)$. The physical reason for the difference is that in cases *a* and *b* a very fast exchange of solute takes place on the non-equilibrated membrane surface. This is important in case one wants to determine the apparent osmotic pressure

$$\Pi_{exp} = (\Delta P)_{J_V = o} = \sigma\, \Pi_{theor} \tag{26}$$

where σ is the reflection coefficient. In case *a*

$$\Pi_{exp} = \lim (\Delta P)_{J_V = o} = \sigma\, RT \frac{c^I}{1 + r} \tag{27}$$

and in case *c*

$$\Pi_{exp} = \sigma\, RT\, c^I \tag{28}$$

TABLE 1

c^I	g/100 ml	D_Π cm^2/sec	Π_{exp} cm	r	K
0.4842	case *c*	1.16×10^{-7}	4.2	0.31	0.97
0.6614		1.16	6.1	0.27	0.89
0.8678		1.16	8.1	0.28	0.88
1.0869		1.16	10.9	0.27	0.87
0.4042	case *a*	1.53×10^{-7}	3.4	0.32	0.90
0.6614		1.55	5.1	0.29	0.87
0.8678		1.54	6.7	0.32	0.88
1.0869		1.51	9.2	0.31	0.87

Thus, from non-stationary measurements the characteristic permeability coefficients can be determined.

An example is given by the case of polystyrene (MW 20,000) in toluene measured with ultracella f-membranes. Table 1 shows some results.

The observed values of r and K are in good accordance. Besides, the value of

$$\frac{[\Pi_{exp}]_{case\ c}}{[\Pi_{exp}]_{case\ a}} \quad \text{case } c = 1.24;\ 1.20;\ 1.21;\ 1.19$$

whereas the theoretical value $1 + r = 1.29$.

4.2. *The concentration profile in reverse osmosis;* $\Delta P \gg \Delta\Pi$; $J_v > 0$

Again we consider a system of two components 0 and 1 with $c_1 \ll c_0$, but this time the volume flow in the direction

opposite to the osmotic flow is large due to the presence of a large pressure gradient ∇p.

Macroscopically this case, which is of practical interest for water desalination, has been treated by SPIEGLER and KEDEM [14] and by JAGUR-GRODISKY and KEDEM [15]. We will consider the concentration profile, essentially with their method, in some detail locally.

The equations (8) to (16) can be applied to this case. But in this case the two terms in the r.h.s. of (16) are of equal magnitude.

Defining
$$A = \frac{(1 - \phi_m)\, r_{10} + v_1 f_{om}}{(1 - \phi_m)\, RT} \qquad (29)$$

and
$$B = \frac{(1 - \phi_m)\, r_{10} + v_o f_{1m}}{(1 - \phi_m)\, RT}$$

(16) can be written, writing c or c_1 indiscriminately

$$\frac{dc}{dx} = \nabla c = A\, J_o\, c_1 - B\, J_1\, c_o \qquad (30)$$

Following GUGGENHEIM [16] we express concentrations in r, defined by

$$r = \frac{c_1}{c_o} \qquad (31)$$

and write for the gradient $\frac{dr}{dx} = \frac{1 - \phi_m}{c_o v_o} \cdot \frac{\nabla c}{c_o}$

Dividing (30) by c_o we find

$$\frac{dr}{dx} = \frac{1 - \phi_m}{c_o v_o} A J_o r - \frac{1 - \phi_m}{c_o v_o} B J_1 = zr - \frac{1 - \phi_m}{c_o v_o} B J_1 \qquad (32)$$

where $$z = \frac{1 - \phi_m}{c_o v_o} A J_o \qquad (33)$$

On differentiation with respect to x (32) yields

$$\frac{d^2r}{dx^2} = z\frac{dr}{dx} \qquad (34)$$

giving $$\frac{dr}{dx} = \left(\frac{dr}{dx}\right)_{x=o} e^{zx} \qquad (35)$$

and $$r = r' + \frac{1}{z}\left(\frac{dr}{dx}\right)_o (e^{zx} - 1) \qquad (36)$$

and especially $$r'' = r' + \frac{1}{z}\left(\frac{dr}{dx}\right)_o (e^{zd} - 1) \qquad (37)$$

where r' and r'' are the values of r at the membrane surface of inflow and outflow respectively. Eq. (36) represents the concentration profile in the membrane. Fig. 4. It contains the characteristic quantity z which has the dimension of a reciprocal length, z is proportional to the flow rate J_o and independent of the membrane thickness. The physical nature of z will be discussed below. The composition of the outflowing liquid is determined by the ratio $\frac{J_1}{J_o}$ and in case the diffusion and stirring in phase III is very fast compared to the diffusion in the membrane we may put

$$\frac{c^{III}}{c_o^{III}} = \frac{1}{K}\frac{c''}{c_o''} = \frac{1}{K} r'' = \frac{J_1}{J_o} \qquad (38)$$

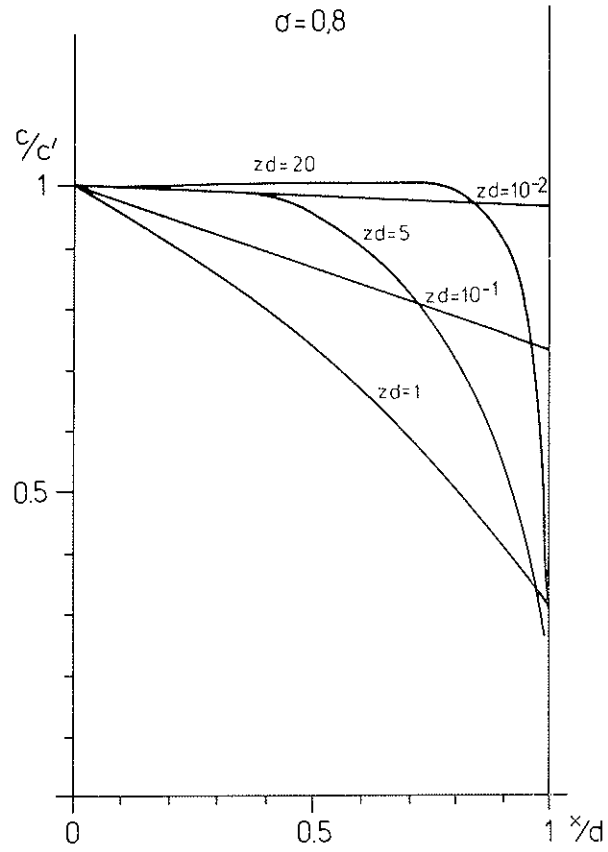

FIG. 4 — Concentration profile in reverse osmosis for different values of zd.

(38) and (33) in (32) give for x = o

$$\left(\frac{dr}{dx}\right)_o = z\,(r' - \frac{B}{AK}\,r'') \tag{39}$$

or with (37) and some rearrangement

$$\frac{r''}{r'} = \frac{KA}{(KA-B)\,e^{-zd} + B} \tag{40}$$

$$\text{The retention is } R = \frac{c^I - c^{III}}{c^I} \tag{41}$$

Taking $r' = K\frac{c^I}{c_o^I}$, $r'' = K\,\frac{c^{III}}{c_o^{III}}$ and $c_o^I \approx c_o^{III}$, this gives

$$R = 1 - \frac{r''}{r'} = 1 - \frac{KA}{(KA-B)\,e^{-zd} + B}$$

or

$$R = \frac{R_\infty\,(1 - e^{-zd})}{1 - e^{zd}\,R_\infty} \tag{42}$$

with

$$R_\infty = (1 - \frac{KA}{B}) \tag{43}$$

Eq. (42) is identical with (29) of SPIEGLER and KEDEM [14]

$$R = \frac{(1-F)\,\sigma}{1 - \sigma\,F}$$

if we identify $\sigma = R$ and $F = e^{-zd}$.

Our derivation is somewhat more general than that of SPIEGLER and KEDEM as we have deduced it from consideration of the local concentration inside the membrane.

The concentration profile in a membrane witb given values of $1 - \phi_m$ and A is for a given value of $\frac{J_o}{c_o}$ determined by a characteristic length

$$z^{-1} = \frac{c_o V_o}{(1 - \phi_m)\ A J_o} \quad (33).$$

If the membrane thickness exceeds z^{-1}, $dz \gg 1$, then the maximum retention is reached and increase of thickness or increase of flow rate does not increase the retention any more. In this situation the concentration is nearly constant at the entry side of the membrane and changes sharply near the surface of outflow in a layer of thickness z^{-1}.

This can be understood from consideration of the diffusion process. In fact, z^{-1} is the distance which is covered by the diffusion and by the convection $u_o = \frac{J_o}{c_o}$ in the same time. A concentration gradient does not originate from a difference in flow rate of the two components, but from a change in the ratio of the flow rates with x. The concentration gradient at x gives rise to a diffusion flow, which smears it out over a distance z^{-1} but not far beyond it.

The ratio of the flow rates can change for two reasons; either by a change of $\frac{A}{B}$ or by a change of K. If $\frac{A}{B}$ increases with x, retardation of solvent increases with respect to solute. If K increases with x, the solute is subjected to a force in the direction of positive x. Both effects, increase of $\frac{A}{B}$ and of

K, tend to accelerate the solute and thus to give rise to a negative value of $\frac{dc}{dx}$ over a distance of the order of z^{-1} upstream from the spot where the change occurs.

In a homogeneous membrane the only value of x where K and $\frac{A}{B}$ change is at $x = d$. Our treatment can also be applied to a bilayer or multilayer membrane with changes of K and $\frac{A}{B}$ for various values of x.

In this treatment J_o and J_1 can be considered as the independent variables, while every layer is characterised by a certain value of $\frac{A}{B}$, determining the minimum value of $\frac{u_o}{u_1} = \frac{J_o c_1}{J_1 c_o}$ and of z, depending on A and on $u_o = \frac{J_o}{c_o}$, while change of K expressed in $\frac{d \ln K}{dx}$ or $\frac{d u_1}{dx}$ has the effect of a local extra force on the solute.

5. Conclusions

The functioning of a given membrane with respect to a liquid mixture of given composition can be completely described by a number of macroscopic coefficients, which can be determined without any assumption or information concerning the concentrations inside the membrane.

The macroscopic coefficients can be determined experimentally from experiments on stationary as well as non-stationary

states. Between these coefficients relations can be derived from thermodynamics which are observed experimentally to hold in the linear region of small flow.

In order to describe the membrane behaviour with respect to change of concentration and to large flows the macroscopic coefficients are not sufficient. Additionally, partition coefficients must be determined and the thermodynamic coefficients have to be transformed into friction coefficients, which may or may not be independent of the concentration.

Strong concentration dependence of the membrane behaviour can generally be ascribed to the concentration dependence of the partition coefficients. However, in cases where the partition coefficients depend strongly on the concentration one must expect that also the friction coefficients will depend on the concentration.

It is proposed to restrict the use of the pore model to systems in which the partition coefficients are very near to unity and the friction coefficients of a number of solutes with a given solvent have the same ratio inside the membrane and in free solution respectively.

The clue to understanding membrane behaviour in non-stationary cases and in cases of large non-linear flows is probably given by calculating concentration profiles within the membrane starting from the assumption that the friction coefficients are constant.

A number of examples of such calculations is given for homogeneous membranes. Inhomogeneities, in particular, a multilayer structure of the membrane, will profoundly affect the concentration profile. If the macroscopic coefficients of the separate layers are known, the concentration profile can be calculated in principle and from it the macroscopic membrane bebaviour.

Thus, the process of understanding membrane functioning proceeds from a macroscopic description in terms of a black

box towards a microscopic description in terms of inhomogeneous parts with partition and friction coefficients.

My thanks are due to Dr. SMIT and Drs. HENKENS for contributions to this manuscript and for very stimulating discussions.

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DISCUSSION

Chairman: A. J. STAVERMAN

SLAYMAN

Your plot of concentration profiles had one trace which rose above the abscissa, and it wasn't clear physically why this should happen.

STAVERMAN

Could I have the slide back? There is one nearly straight curve with a small slope and another curve rises above that one but it does not have a maximum. You see these different curves sometimes crossing and sometimes not.

MEARES

If there is no lower limit on the negative values the reflection coefficient can take, then it would appear that the solvent, which is undergoing anomalous osmosis, can actually go faster than the solute which is generating this flow. This may not be mechanistically true, but it is true in sofar as the thermodynamics is concerned. I noticed Dr. PATERSON said that he had a limit on his electro-osmotic velocity because the water could not go faster than the ions which were pushing it. There are some scattered observations in the literature which suggest that the water may go faster than the solute.

STAVERMAN

The solute molecules drag the water in the direction of the dilute solution. But there are many more molecules of water than of solute. The flow is concentration times velocity. I expect that the velocity of the water molecules will be not so large as that of the solute molecules. Of course we talk about reflection coefficients. The whole idea of reflection coefficients is already linked to the idea of a dilute solution, the components are not symetrical. Any other questions?

KEDEM

You mentioned the problem of various driving forces giving the same coefficient. An old puzzle of that kind has been whether the osmotic pressure and hydrostatic pressure, which are always united in DARCY's law, really give the same flows. Has anybody come up with an answer to that, as far as you know?

STAVERMAN

If I understand your question correctly, this is exactly the point that I stressed in the beginning of my lecture: the macroscopic relations said to be derived from irreversible thermodynamics will be valid only in case all coefficients do not change if the experimental conditions are changed (as they necessarily are in any check). This does not only apply to $L_{ik}=L_{ki}$, but also to attributing the same coefficient L_{ik} to forces of a different nature such as $v_k \Delta P$, $z_k \Delta\overline{\Phi}$ and $\Delta\mu_k$. Considering this, one is perhaps surprised that these macroscopic relations are confirmed at all, rather than being surprised when they are not. But if the « state of the membrane » is not changed by the experiment, a force of a given magnitude should give a flow of given magnitude irrespective of the nature of the force.

You may say, that this reduces these relations to a *definition* of the concept of a « constant state of the membrane », but in my opinion this is a useful concept.

SOLOMON

In the very first table that you put on the board, it wasn't clear how the friction between the solute and the membrane was included because it seemed that you mentioned only the solute solvent and solvent-solute friction and you didn't specifically include friction with the membrane.

STAVERMAN

Dr. SMIT determined all the coefficients so we had the membrane completely characterised. From this he calculated the three friction coefficients v_{10}, f_{om} and f_{im}. Of these v_{10} can be compared to something else, the v_{10} in free solution.

POLARIZATION AT MEMBRANE/SOLUTION INTERFACES

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INTRODUCTION

Transport processes through synthetic membranes have been developed in the past two decades for practical separation processes, and membrane technology is emerging as a full-fledged separations method. While much of this progress, based on the work of pioneering efforts in the earlier part of this century [1, 2] was motivated by the desire for the desalination of brackish water and primarily supported by the Office of Saline Water, U.S. Department of the Interior (*), as well as other governmental, academic and industrial institutions, its applicability is much wider. Consider, for instance, the development of artificial kidneys and of blood oxygenators.

In the course of the study of the scientific and engineering aspects of these processes it was found that often mass-transfer bottlenecks develop in the immediate vicinity of membrane-solution interfaces. These effects are usually called « *polarization phenomena* » (although *electrical* polarity is not always

(*) Now part of the Office of Water Research and Technology.

evident). This name is used to indicate substantial resistances to the transfer of dissolved matter (and perhaps in some cases of solvent molecules) from the solution to the membrane, or vice versa, causing a slow-down of the desired overall transport process. For the engineer this means the design of plants with large membrane areas, which, in turn, causes high investment costs. Hence there exists an incentive to study these interfacial phenomena in the hope of finding a way to reduce the mass transfer resistance.

One wonders to which extent these phenomena are important in *life processes*. Living membranes are usually several orders of magnitude thinner than synthetic membranes used for industrial or bioengineering applications. At first sight, one might therefore think that polarization in the solution layers adjacent to biological membranes contributes an even higher percentage of the resistance to the overall transport process from the extracellular solution into the cell. Yet the molecular structure of many biological membranes is much denser than that of synthetic membranes used in practical applications. In fact, the permeabilities (as opposed to the *specific* permeabilities, which are the permeabilities of membranes reduced to *unit thickness*) of some biological membranes are often not radically different from those of their much thicker synthetic counterparts [3]. Therefore the experience gained in technical membranology is of some relevance for the study of transport in biological membranes, and for medical applications also.

The purpose of the present paper is to present a summary and status report on the study of interfacial polarization in electrodialysis at the Sea Water Conversion Laboratory, University of California - Berkeley, which is one (but by no means the only) institution which performs studies in this field. The processes studied in the membrane research group of this Laboratory are *electrodialysis* (in which ions and water are transported across membranes under the influence of *electrical*

forces), and *hyperfiltration* (*reverse osmosis*) in which the driving force is *pressure*. The experimental results described here were obtained under realistic conditions of solution flow (i. e. actual plant hydrodynamics), except experiments with laser interferometry, which were performed in a small, bench-scale cell.

Electrodialysis

The *principle* of electrodialysis, as described in ref. [4] is illustrated in Fig. 1. Electrodialysis units consists of a number of narrow compartments through which saline water is pumped. These compartments are separated by alternating kinds of special membranes, which are permeable to cations or anions respectively. The terminal compartments are bounded by electrodes for passing direct current through the whole stack. Fig. 1(a) shows schematically the stack before passage of the current.

When the electrodes are connected to a direct-current source, ion travel begins and hence an electric current passes through the unit, as shown schematically in Fig. 1(b), which focuses attention on a group of compartments well within the stack. In the center compartment positive ions travel from right to left, negative ones in the opposite direction, and both kinds leave the compartment through the membranes. If each membrane were permeable to both kinds of ions, no concentration change would result because for each positive ion leaving the center compartment into the left compartment, another positive ion would enter from the right. Similarly, the amount of negative ions in the center compartment would also not change. The anion-permeable membrane on the right, however, does not admit cations from the right compartment to replenish the center compartment, and the cation-permeable membrane on the left similarly acts as a barrier for the negative ions in the left compartment. As a result, the salt concentration de-

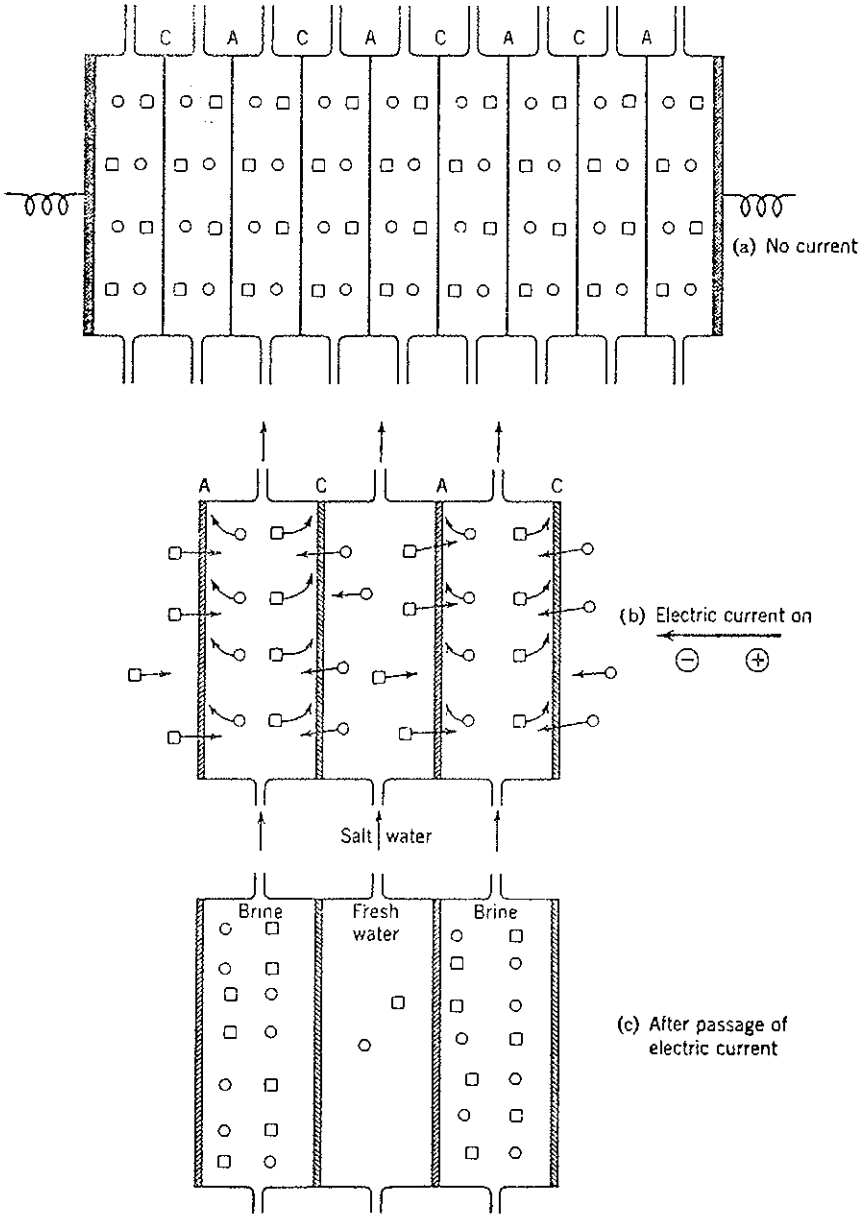

FIG. 1 — Principle of electrodialysis. (4) ○ Positive ion (e.g., sodium), □ Negative ion (e.g., chloride). "C" and "A" cation and anion permeable membrane, respectively. Ion migration under action of electric current causes salt depletion in alternate compartments and salt enrichment in adjacent ones.

creases in the center compartment and increases in the neighboring compartments as shown in Fig. 1(c).

In practical electrodialysis installations which contain from ten to hundreds of compartments between one pair of electrodes, the passage of electric current thus creates fresh water (« diluate ») and brine (« concentrate ») in neighbouring cells. In other words, half of the cells carry partly desalted water and half carry brine. The solutions in the electrode compartments are contaminated with the products of the electrode reactions which occur as a result of the passage of the current.

The principle of polarization in electrodialysis is illustrated in Fig. 2. Since the transport number of the anion in the solution is lower than in the anion-exchange membrane, the amount of negative ions transported by the electrical current from the solution to the face of this membrane is not sufficient to make up for the negative ions removed through the membrane. The balance is made up by diffusion of ions from the solution to the membrane surface. At the other membrane surface, more negative ions arrive by electromigration than are carried away by this mechanism. In the steady state, the concentration remains constant, because the excess of ions is removed into the solution by diffusion.

In an electrodialysis cell, the solutions flow past the membranes and a continuous velocity gradient extends from the membrane face to the center of the flow channel, which is filled with a turbulence-promoting spacer material, causing a complex flow pattern. As a first approximation, and for illustration only, the « Nernst diffusion layer » model is used in Figure 2 to describe the situation: it is assumed that there is a completely stationary layer close to the surface, and beyond this layer there is complete mixing. Within the layer, all transport processes occur by diffusion and electromigration only.

If this assumption is made, the solution concentration profiles in the steady state are straight lines, as shown in Figu-

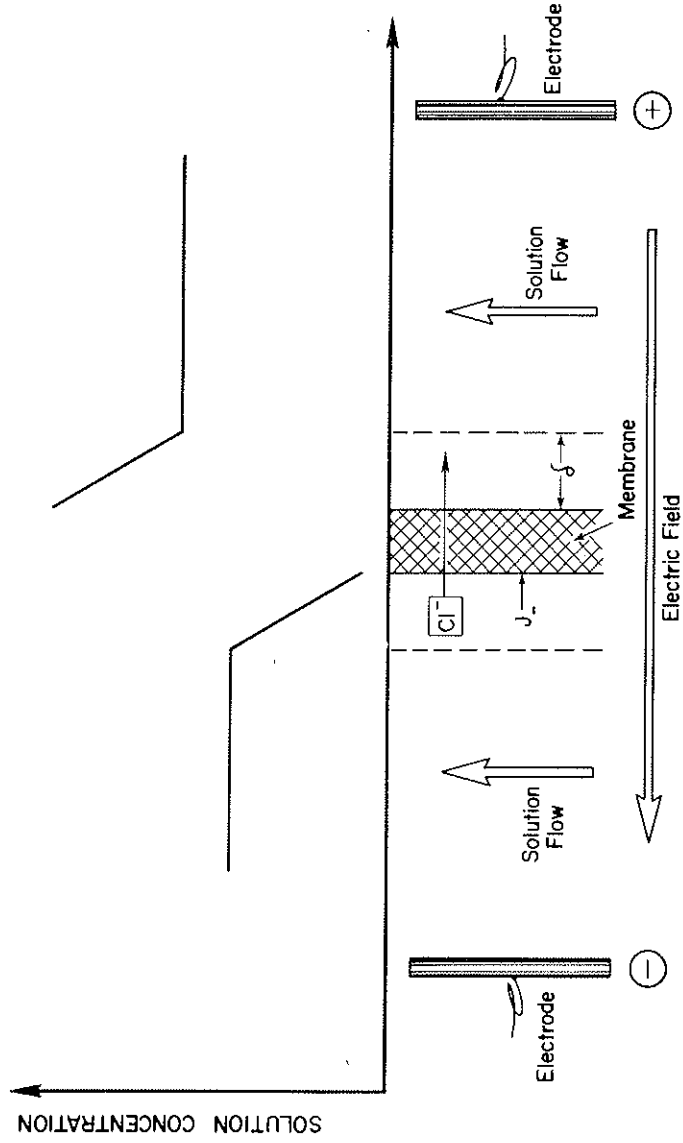

FIG. 2 — Schematic of field and ion flow at anion-exchange membrane (7).

re 2, but irrespective of the assumptions about the structure of the diffusion layer, the electrolyte concentration near the membrane surfaces is low in the diluate compartments and high in the brine compartments. The lower the salt concentration in the diffusion layers within the diluate compartments, the bigher the voltage necessary to maintain constant current, because the concentration changes cause a membrane potential opposing the applied voltage. Also, the effect of the electrolyte depletion in the diluate compartments on the resistance outweighs the opposite effect of the increased concentration in the brine compartments.

At high current densities, an additional effect is important. In this case, there are not enough ions of the electrolyte in the vicinity of the membrane, and hence some hydrogen and hydroxyl ions, derived from water, pass through the cation and anion-exchange membranes, respectively, to maintain constant current. This phenomenon has been referred to as « water splitting » but since this term also refers to the production of hydrogen and oxygen gas, we now prefer the term « acid-base generation » (*).

If acid-base generation could be excluded, current-voltage curves for membrane-solution interfaces should exhibit limiting-current plateaus, similar to polarographic plateaus [5, 6]. In other words, limiting salt transport can occur, but the existence of a limiting salt ion-current plateau is not necessarily reflected in an electric-current plateau because of acid-base generation, which introduces highly conductive H^+ and OH^- ions. It should be noted that the determination of the salt ion-current plateaus is of decisive importance for the evaluation of different operating conditions and designs of electrodialysis units, because these plateaus express quantitatively the maximum desalting rates obtainable with a given design under the operating conditions chosen in the measurement.

(*) The phenomenon has been long known as the "Bethe-Toropoff effect" [J. Phys. Chem. *88*, 686 (1914); ibid. *89*, 597 (1915)].

Electrochemical studies of polarization in electrodialysis.

When allowance is made for acid-base generation by correcting the measured currents for the variation of Coulombic efficiency with current density, plateaus of the « corrected current » were indeed obtained in a system containing an anionexchange membrane (111BZL 183, Ionics, Inc., Watertown, Mass., U.S.A.), bracketed by KCl solutions of average concentration 0.01M [7]. These limiting currents appeared at different locations of the long, tortuous flow path of the solutions parallel to the membrane. Further evidence for the existence of limiting anion flows came from experiments [8] in which diluate samples, taken at different points along the tortuous path of a dilute solution of potassium chloride flowing past the same anion-exchange membrane were analyzed (Fig. 3).

The situation for cation-exchange membranes remains to be clarified. Although limiting currents have been observed by other authors with small laboratory samples of membranes in the absence of forced flow of the solutions [9, 10, 11], no limiting ion-flows or currents have been observed so far in the flow apparatus in our Laboratory, described in reference [7].

Optical studies of polarization in electrodialysis [12].

The interferogram of a polarized diffusion layer near an electrode or membrane surface (or of any solution segment exhibiting a concentration gradient and thus a refractive index gradient) records the deviation of the refractive index at various locations from its value at some reference point where the concentration remains constant. Unless the concentration gradient is quite small, however, secondary diffraction effects (deflection of light beams towards or away from the membrane surface) distort the interferogram considerably; quantitative evaluation of the concentration profiles is then not pos-

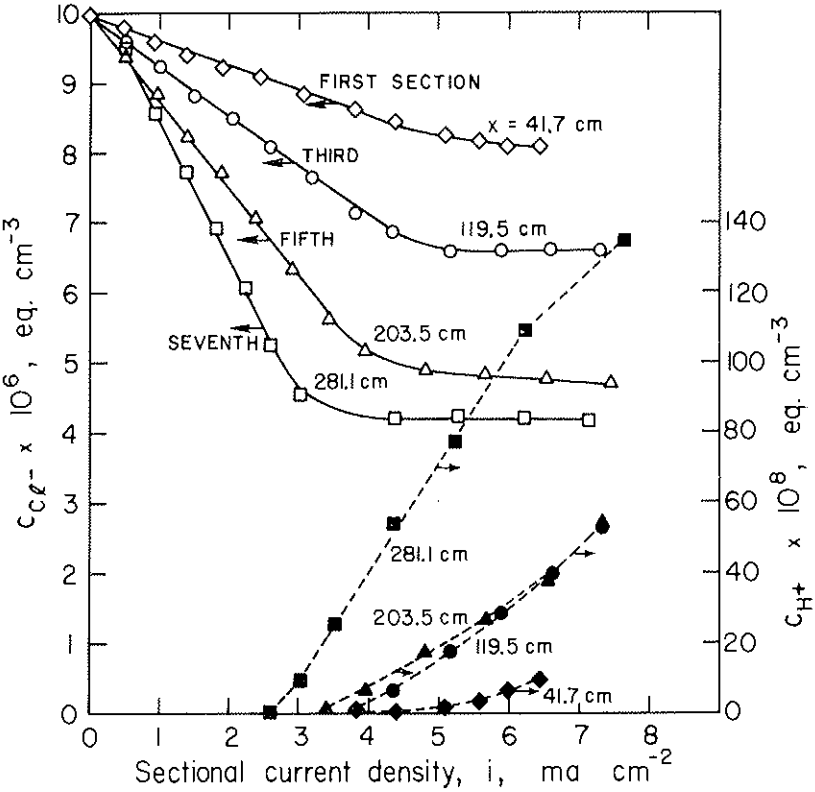

FIG. 3 — Limiting salt transport and "acid-base generation" for anion-selective membrane (8). On increasing current density, salt-transport reaches plateau. Acid-base generation occurs just before plateau. Membrane: 111 BZL 183 (Ionics, Inc.). Average solution flow rate: 11 cm/sec^{-1}. "Ionics" Mark II spacer between solution and neutral separator. x = distance from solution inlet to sampling point. Temperature 25°C. Solution inlet concentration: 0.01M KCl. System as in ref. (7); with neutral separators composed of sheets of cellulose dialysing tubing (Fisher Scientific Co., Pittsburgh, Pa., U.S.A.) attached to an inert porous sheet ("Hostalen G", high-density porous polyethylene, Porvair, Ltd., Estuary Rd., King's Lynn, Norfolk, England).

sible unless fairly complex correction procedures are applied. Only where the refractive index (or concentration) exhibit a minimum (or maximum) do these distortions disappear. Under certain conditions both the location of these points and the local deviation of the refractive index from the index at the reference point are exactly represented on the observed interferograms. A detailed analysis of the interpretation of such extrema in interferograms has been presented by BEACH [13].

If the direction of the current is suddenly reversed, however, concentration extrema appear whose position is unequivocally determined, because at the extremum there is no gradient of the refractive index and hence no deflection of the beam towards or away from the membrane. The appearance of concentration extrema in the concentration profile near membrane-solution interfaces following sudden current reversal had been predicted long before the optical studies to be reported were performed [14].

In our method, current is first passed through the arrangement: solution/membrane/solution for a time of several seconds (« prepolarization period »), and then suddenly reversed. This causes a concentration minimum in the diluate (easily visible as a minimum of the interference fringes), which gradually moves from the membrane-solution interface into the body of the solution. The migration of the minimum is followed by motion photography. The trajectory of the minimum, determined in this manner, is then compared, by computer methods, to trajectories of the minima predicted for different interfacial salt concentrations at the moment of current reversal. For this prediction, an equation developed by ROSEBRUGH and MILLER [15] in a straightforward manner from the classical Nernst-Planck theory of salt-ion migration was used. Agreement between observed and computed trajectories is seen as proof of the classical model of migration of K^+ and Cl^-, on which the theory is based; disagreement (observed at high prepolarization times or high current den-

sities, hence low interfacial salt concentration) is taken as proof of additional modes of current transfer, e.g. by H^+ and OH^- (« acid-base generation ») which the theory does not take into account.

Consider the passage of current of constant density, i(a cm^{-2}), in a system consisting of a perfectly semipermeable membrane bounded by two KCl solutions (Fig. 2). After passage of current for t_1(s) (« prepolarization time ») the current is suddenly reversed. A detailed mathematical treatment of this situation for metal-solution interfaces dates back to 1910 [15]. The concentration distribution in the left boundary layer (Fig. 2) at time t(s) after current reversal is given by equation [29] of that reference. This equation, slightly rearranged, and using our notation is

$$\frac{\Delta c}{\bar{i}} = \frac{\Delta\tau\delta}{D\mathcal{F}}\left[(1-\xi) - \frac{8}{\pi^2}\cdot\sum_m \frac{1}{(2m-1)^2}\left\{2 - \exp\left[-(2m-1)^2\left(\frac{\pi}{2\delta}\right)^2 Dt_1\right]\right\}\cdot\right.$$

$$\left.\cdot\left\{\exp\left[-(2m-1)^2\left(\frac{\pi}{2\delta}\right)^2 Dt\right]\right\}\cdot\left\{\cos(2m-1)\frac{\pi}{2}\xi\right\}\right]$$

where δ(cm) is the thickness of the diffusion layer, $\xi(>0)$ the « reduced » distance from the membrane surface (absolute value of distance, x, divided by δ), and Δc(eq cm^{-3}) is the difference between the local and bulk concentration respectively at a reduced distance ξ from the membrane surface and at time t. D(cm^2s^{-1}) is the diffusion coefficient of the electrolyte (KCl) in free solution, and $\mathcal{F}$(C eq^{-1}) is Fadaday's constant. $\Delta\tau$ is the difference between the transport numbers of the counterion in membrane and solution respectively. *m* represents the series of whole numbers. A similar equation applies when the salt concentration in the left diffusion layer (Fig. 2) is *enriched*, which occurs when the direction of the current is inverted ($i > 0$).

By differentiation of eq. (1) the trajectory of the concentration minimum can be found, and the interfacial concentration at the time of current reversal computed therefrom [12]. Fig. 4 shows interferograms obtained in an unstirred solution with a small O'BRIEN-type interferometer [16, 17] using a Ne-He laser as a light source.

By comparing experimental with computed trajectories, it was found that for 0.01 M KCl solution and current density of 2.3 milliamp cm^{-2} (25°C), acid-base generation became significant when the interfacial concentration dropped to the order of $10^{-3} - 10^{-4}$. We do not know whether this result holds also for other electrolytes, membranes, and flow conditions, but considering that the concentration of H^+ and OH^- in free water is of the order of 10^{-7}N (albeit the mobility of H^+ is almost one order of magnitude larger than the mobility of K^+), it is of interest that side-effects already set in at a moderate degree of electrolyte depletion at the membrane/solution interface.

It is also of interest that these optical studies thus lead to conclusions quite similar to those obtained by R. PASSINO's group in an independent electrochemical study [18].

SUMMARY

Studies are reported on phenomena at membrane/solution interfaces during the industrial process of electrodialysis which separates particles present in aqueous solutions by means of differential migration fluxes in synthetic membranes. Most experiments were performed under realistic hydrodynamic conditions, such as are found in industrial plants.

The experimental results agree well at relatively low current densities with those computed from the Nernst-Planck migration equations, assuming a fixed diffusion layer. Discrepancies appear in the polarization region (high current den-

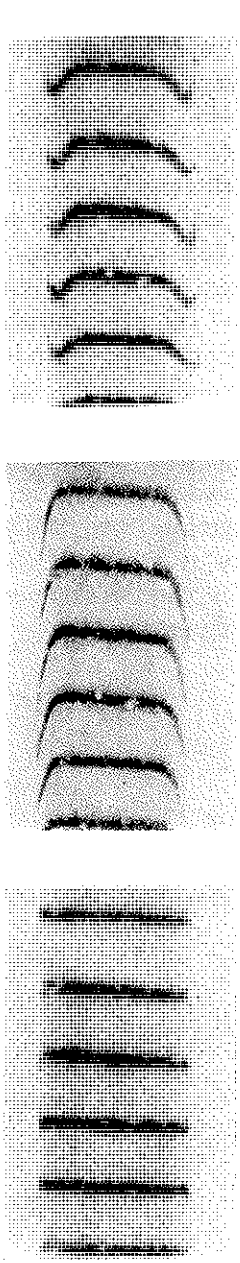

FIG. 4 — Interferograms of the solution in the wedge interferometer during electrodialysis at different states of concentration near the interfaces (12). Cation-exchange membrane 61AZL183 (Ionics, Inc., Watertown, Mass.) borders solution, above; Ag/AgCl electrode below. Distance between electrode and membrane 0.435 cm. Stagnant aqueous KCl solution of 0.01 M concentration. Electrical current density 2.3 mA cm^{-2}. Temperature 25°C. Light source: 1 m Watt Ne-He laser. A) before starting current; uniform concentration. B) after 18 sec. prepolarization (2 sec. before current reversal); salt depletion at the interfaces extends into the bulk. C) 11 sec. after current reversal: salt enrichment at the interfaces extends into the previously depleted layers creating concentration minima.

sities) where acid-base generation masks the flow of the salt ions. For an anion-exchange membrane (Ionics BZL 183, Watertown, Mass.) limiting ion flows, albeit not limiting electric-current flows (i.e. plateau currents) were found all along the flow path (parallel to the membrane) of dilute potassium chloride solutions. For a cation-exchange membrane, (Ionics 61 AZL 103) no limiting ion flows were found, and there is less acid-base generation. Results obtained by laser-interferometric observation of the interface confirm the findings of R. PASSINO's group that appreciable acid-base generation sets in before the solution at the interface is depleted of electrolyte [18].

Transport-induced phenomena at membrane/solution interfaces represent major bottlenecks in the development of membrane technology. Therefore their study, and, hopefully, the invention of devices for reduction of the interfacial resistance, are key elements in the development of separations by means of synthetic membranes.

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DISCUSSION

Chairman: A. J. STAVERMAN

STAVERMAN

May I start with a question myself? I understand that you notice what you call the water splitting reaction from the change of pH and I undestand the water is split at sufficiently low concentrations of salt. Is that change of pH not compensated on the other side of the cell where you have production of OH^- ions? I would expect that you wouldn't notice a change of pH.

SPIEGLER

You're quite right, on the one side of the membrane acid is formed, on the other side of the membrane after a time delay there appear hydroxyl ions. A diagram would look exactly like Dr. MAC ROBBIE's diagram that we saw, where water split into hydrogen ions and into hydroxyl ions. One can measure the pH in the solutions on both sides of the membrane.

STAVERMAN

Are there any other questions?

MEARES

May I make a comment and also ask a question about your interferometry technique? The comment is that although one frequently talks about the membrane solution/interface as though it were something which was clearly defined in space, when we consider what the membrane solution interface is really like, there must be at least in a polymeric-type membrane, many chain segments trailing out of the membrane probably for a few hundred Å. These would be similar to any other organic particles with charged groups in their effect on the surrounding water structure. They must have an effect on the water structure and, bearing in mind also the very large electric field strength in the double layer, I can believe that the water ionization in that region may give rise to ion concentration one or two orders of magnitude different from that in pure water under zero potential gradient.

My question is about the use of the wedge interferometer. Most of your slides refer to stagnant solution, while an important question in electrodialysis is how much pumping power it is worth expending on circulating the solutions. Can you use this technique in the flowing system? Also, in the stagnant system, it is really conceptually correct to speak of a diffusion layer as though it were something which had a discreet thickness together with an outer boundary which you could locate.

SPIEGLER

We've done some experiments with flowing solutions and we've found that at relatively low Reynolds numbers it is still possible to use this instrument. In the turbulent range the diffusion layer is too thin, however. I don't know if it is conceptually correct, but the proof of the pudding lies in the eating. In the interferogram a distinct diffusion layer is visible. The concept is a gross approximation, however. Even with much more extensive interferometric instrumentation, researchers have succeeded in seeing diffusion layers

only at very low Reynolds numbers. In turbulent flow, I don't think it is feasible with our instrument.

TEORELL

I wish to ask whether you have observed instability or periodical phenomena in your systems. The reason for my question is that some years ago I published some measurements on a rather thin bed of ion exchange powder surrounded by identical salt solutions and flow current across it, just like you. At higher current densities we could measure a « tilting » of the concentration profile within the membrane. The tilting could vary periodically with time and the membrane potential showed time variations similar to action potentials (Arkiv Kemi (Roy. Ac. Sci. Stockholm), *18*, 401, 1961). Perhaps these observations could have a biological counterpart.

SPIEGLER

I was very interested to hear about your work on these oscillations in the early '60s. In 1959 Dr. C. FORGACS of the Negev Research Institute in Beersheba showed me records taken during the operation of his industrial unit, and there was a very clear small voltage oscillation at constant current. The frequency of this oscillation was in the order of seconds. In the interferometric experiments we have usually not seen oscillations but that may be because the instrumentation wasn't adapted to such observations. In industrial electrodialysis units there happen the oscillations which you have been studying for many years.

MONNIER

Electro depletion of an aqueous compartment adjacent to a membrane was very familiar many years ago to biochemists who used this

simple system to deionize protein solutions. But now it's not so familiar to neuro-physiologists in spite of the fact that a cell membrane, in many cases, is in the ideal situation to show polarization through electro depletion. For instance, the nerve fibres, and even the giant fibres are always surrounded by cylindrical cells — the famous SCHWANN cells — which are notable for their many narrow channels which connect the nerve fibre surface to the outside medium. But these channels are very tortuous. They form a maze of small uncharged canals. And when you excite the fibre with an electric current, the canals situated in the stimulated area are depleted of the free ions which they normally contain through diffusion with — in the outside medium. Thus ionic depletion of the outside surface of a stimulated fibre is certainly real — it cannot be neglected. Its exact functional importance has yet to be ascertained, of course. But we are grateful to you for showing that ionic depletion can be a very large prenomenon.

EQUILIBRIA AT MEMBRANE/SOLUTION INTERFACES

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Abstract

The characteristic behaviour of the intensive state parameters is analysed for different membrane systems. The correlation of the local and overall transport equations and the classification of membranes requires the examination of potentials for discontinuities at the membrane-solution boundaries. Membrane function cannot be fully defined by monitoring the flows at a plane within the membrane without taking into account the contribution of surface equilibria to the steady state processes. This is due to the fact that the surfaces may comprise sources and sinks of heat and chemical species.

Introduction

As a concrete example of linear force-flow relations most of us think first of Ohm's law. For systems more complicated than a single resistor, the same tradition remains helpful. The overall functioning of a network obeys Kirchhoff's law: Kirchhoff's current law requiring local conservation, and Kirchhoff's voltage law requiring uniqueness of potential at each point. Including a broader range of transport processes, each element

is fully defined by external measurements of "through" and "across" variables. Kirchhoff's laws are thus the boundary conditions each subsystem imposes on its neighbours [1].

The stationary state performance of a membrane separating two reservoirs is phenomenologically defined by measurements of state parameters and their change with time, in the reservoirs. Flows and forces in the membrane are defined through conservation laws and continuity; that is, the boundary conditions imposed by the reservoirs on the membrane.

If we want to go beyond this black box approach, we have to write local flow equations which can incorporate information on membrane structure, and on properties of the permanents. Their integration leads back to discontinuous equations and thus provides an interpretation of the overall coefficients in terms of characteristic physical parameters. According to the classification suggested in DE GROOT and MAZUR'S book [2] such a procedure is possible for membranes whose thickness as well as openings are of macroscopic dimensions, e.g. an assembly of inert capillaries. From our point of view, that would mean that we analyse in detail only quite unselective systems. This may lead to interesting results in some special situations [3, 4, 5] but any theoretical approach must include selective membranes in order to be generally useful. In fact, it is possible to formulate and integrate local flow equations for membranes which constitute a separate phase, without continuous macroscopic aqueous channels — many model calculations have been carried out in this direction. However, the definition of flows through a separate phase, and the relation between local and overall forces must be based on a careful examination of conservation laws and continuity of potentials at interfaces between membrane and solution.

Conservation laws at interfaces

Focusing on phenomena at the membrane boundaries, we shall assume the simplest possible external conditions: large,

well-stirred reservoirs, so that compositions are practically constant, no gradients are maintained in the solutions, and the membrane is in a stationary state.

The thermodynamic bookkeeping in each of the compartments separated by the membrane is translated into flows through the appropriate conservation laws following the number of mols of component i in compartments ′ and ″ (n'_i and n''_i respectively) and their change with time ($\dot{n}'_i$, $\dot{n}''_i$). If there is no exchange of matter with surroundings, and no chemical reaction, the increase in one compartment is balanced by a decrease in the other and is equal to the overall flow AJ_i through the membrane of area A. J_i monitored at any place x in the membrane is equal to $-\dot{n}_i/A$. However, not in every case is the relation between flows in the continuous membrane phase and the changes in the compartments as simple as this.

To start with a familiar example, consider the "exchange flow" of a non-electrolyte,

$$J_D = \frac{C_{s\ (average)}}{J_s} - J_v \qquad (1)$$

conjugate to the osmotic pressure difference, $\Delta\Pi_s$. The local exchange flow measured in the membrane would be

$$\bar{J}_D = \frac{\bar{J}_s}{C_s} - \bar{J}_v \qquad (2)$$

where bars denote parameters inside the membrane. From the conservation of matter, and incompressibility:

$$\bar{J}_s = J_s = -\dot{n}'_s/A \qquad (3)$$

and

$$\bar{J}_v = J_v = \frac{-\Sigma \dot{n}_i \bar{V}_i}{A} \qquad (4)$$

($\bar{V}_i$ = molar volume).

The exchange flow near a surface, just inside the membrane, is not equal to that defined for the discontinuous system. Hence, the coupling between exchange flow and volume flow contains the discontinuity of C_s, at surface equilibrium.

$$\sigma \equiv -(J_D/J_V)_{\Delta\Pi=0} \tag{5}$$

$$1-\sigma = (1+J_D/J_V)_{\Delta\Pi=0} = (1+\bar{J}_D/J_V)_{\Delta\Pi=0K} \tag{6}$$

$$K = \bar{C}_s/C_s \tag{7}$$

The velocity of the solute dragged along with the solvent depends on the properties of the membrane itself, but the overall reflection coefficient is determined also by the distribution coefficient of the solute between membrane and aqueous solution.

One might say that this discontinuity depends on the particular flows chosen. The next example demonstrates clearly the intrinsic contribution of surface equilibria.

If species i participates in a reaction taking place at the interfaces, the number of mols of i crossing the membrane per unit time, $\bar{A}J_i$, will not be equal to $-\dot{n}'_i$. Nevertheless, in the discontinuous black-box equations the definition $J_i \equiv \dot{n}_i/A$ remains valid. According to our assumption the reaction takes place on the membrane and not in the bulk of the solutions, and from measurements performed in the reservoirs only, one cannot discern whether a given species is produced and consumed at the surfaces, or passes across. As a concrete example, consider a redox reaction catalysed by an enzyme located on the membrane surfaces. Choose a reaction according to the following pattern:

$$HQ\,(\text{aqueous}) \rightleftharpoons \overset{+}{H}\,(aq) + Q\,(aq) + \varepsilon\,(\text{membrane})$$

Q and HQ are impermeable, the electron is transferred to a membrane component, and *via* a redox chain across the mem-

brane. The mass balance, including the degree of advancement, ζ, of the reaction, is:

$$AJ^{+}_{H} \equiv -\mathring{n}'_{H} = \bar{A}J_{H} - \frac{d\zeta}{dt} \qquad (8)$$

$$AJ_{e} \equiv -\mathring{n}'_{HQ} = \bar{A}J_{e} = \frac{d\zeta}{dt} \qquad (9)$$

and hence

$$\frac{J_{H}}{J_{e}} = \frac{\bar{J}_{H}}{J_{e}} - 1 \qquad (10)$$

At zero gradient for the proton and in the absence of coupling *within* the membrane, an overall 1:1 negative coupling will be observed. If the electron acceptor is protonated and diffusible in the membrane, and thus one proton is transferred per electron *across*, i.e. $\bar{J}^{+}_{H}/\bar{J} = 1$, then

$$J_{H}/J_{e} = \bar{J}_{H}/\bar{J}_{e} - 1 = 0 \qquad (11)$$

The stoichiometry of proton per electron is determined by the character of the surface reaction as much as by the actual carrier mechanism in the membrane.

Finally, consider a non-isothermal system, with flow of heat and matter across the membrane. The appropriate conservation law is the first law of thermodynamics for open systems, taking into account the flow of energy linked to the flow of matter.

The total change in inner energy of each compartment is given by exchange of heat with the surroundings, and the energy flow, J_{ψ}, through the membrane. The flow conjugate to the temperature gradient ($X_T = \frac{1}{T}\frac{dT}{dx}$) is the heat flow, J_q [6].

$$J_{q} = J_{\psi} - \Sigma h_{i}J_{i} \qquad (12)$$

where h_i is the partial molar enthalpy of component i. Let the membrane be permeable to water only as e.g. dense cellulose acetate separating salt solutions. Since it constitutes a separate non-aqueous phase [7], the partial molar enthalpy of water in the membrane, $\bar{h}_w$, will in general be different from that in the aqueous solution. In this case $\bar{J}_q$ in the membrane will be different from the reduced heat flow between the compartments: ΔH, the heat of dissolution per mol of water, is absorbed or liberated at the surfaces. In formal terms:

$$J_q = J_\psi - J_w h_w \tag{13}$$

$$\bar{J}_q = \bar{J}_\psi - \bar{J}_w \bar{h}_w \tag{14}$$

From conservation laws: $\bar{J}_\psi = J_\psi$, $\bar{J}_w = J_w$, therefore, $J_q = \bar{J}_q - (h_w - \bar{h}_w) J_w$. The heat transferred per mol at $\Delta T = 0$, the so-called total or overall heat of transfer, is:

$$Q^* = \left(\frac{J_q}{J_w}\right)_{\Delta T = 0} = \left(\frac{\bar{J}_q}{J_w}\right)_{\Delta T = 0} + \Delta H = Q_m + \Delta H \tag{15}$$

$$\Delta H = \bar{h}_w - h_w$$

Q_m is the heat of transport in the membrane phase. Even in the absence of thermodiffusional coupling in the membrane itself (i.e. $Q_m = 0$), the overall heat of transport does not vanish, if $\Delta H \neq 0$ [8].

Potentials at interfaces

If all surface processes are fast relative to the transport across the membrane, equilibrium is always maintained between membrane and contiguous solutions. This means that the temperature and the electrochemical potentials for all species must be continuous at the interfaces [9]. If one writes con-

sistently all local equations in terms of these gradients, boundary conditions are straightforward:

$$\int_0^{\Delta x} d\tilde{\mu}_i = \tilde{\mu}_i - \tilde{\mu}''_i \equiv \Delta\tilde{\mu}_i \qquad (16)$$

$$\int_0^{\Delta x} d\bar{T} = T' - T'' \equiv \Delta T \qquad (17)$$

(Δx – thickness of the membrane). Integration of local phenomenological equations and model calculations are indeed greatly simplified if $\tilde{\mu}_i$ is used [10, 11, 12]. However, in many cases other intensive parameters are interesting per se, and often more readily measurable or interpretable.

Electric potential, pressure etc. may and do "jump" near the membrane surface. In reality, there is of course no discontinuity but a very steep gradient; however, as long as we are not interested in the detailed structure of the interface, we may adequately describe the situation by two values for these potentials, inside and outside the membrane, near $x=0$ and $x=\Delta x$.

We shall discuss phase boundary potentials for three systems: a assembly of inert capillaries, a fixed charge membrane, e.g. a collodion-based permselective membrane [13], and a widely-used solvent membrane, dense cellulose acetate. Table 1 summarizes their behaviour.

A membrane with wide aqueous pores may be regarded as a mechanically stabilized region in an aqueous solution, in which composition, etc. may change continuously.

Realization of the discontinuity of electric potential and composition at the surface of a charged gel was a breakthrough for membrane theory. TEORELL [14] and MEYER-SIEVERS [15] replaced the continuous Helmholtz approach by the fixed-charge model in which one regards the membrane

TABLE 1 — *Intensive parameters at membrane surfaces.*

	Assembly of capillaries, wide pores, inert matrix	Fixed charge membrane, narrow pores. Permeants: water, salt	Dense cellulose acetate. Permeant: water
Continuous	All potentials and their gradients	$\tilde{\mu}_i$, T, μ_w, $\sim\mu^c$ (salt) h_w	
Discontinuous		ψ, p, μ^c (ions), activity of water	p, $\mu^c{}_w$ $\mu^o{}_w$ (p, T), h_w

μ^c = the composition dependent part of the chemical potential.
ψ = electric potential.
p = pressure.

as a separate phase, and assumes Donnan equilibria at the phase boundaries. With these assumptions the quantitative treatment of a selective membrane became possible. As indicated in the Table, the composition-dependent part of μ_s, i.e. the total activity of the salt, is equal inside and outside (apart from a small pressure dependent contribution), while the activities of the single ions are different. For the water in the membrane two approaches are possible and, consistently followed, lead to the same result: either we consider the membrane as a single solution, or, following the traditional colloid terminology, we see the gel as two interpenetrating continuous phases. The polymer matrix, due to its elasticity, creates a pressure in the aqueous interstices, and water establishes an osmotic equilibrium at the surfaces. This is the point of view indicated in the Table.

The discontinuity of composition at the surfaces of a solvent membrane, i.e. partition coefficients different from unity, were assumed already by Overton. The significance for the

rate of solute permeation is self-evident, but there are other, less obvious, consequences. Pressure in the membrane may not be meaningful in this highly structured polymer phase, but at any rate there is no continuity of pressure and water activity. An externally applied pressure difference is an effective driving force for water, though there is no hydrodynamic flow in the membrane: hyperfiltration may take place by a diffusion mechanism.

When the external compartments are maintained at different temperatures, T itself is continuous, but its gradient creates a discontinuity in composition. The influence of temperature on μ^{o}_{w} is different in the two phases:

$$\int_{T'}^{T''} \frac{\partial \mu_w}{\partial T}\, dT = \int_{T'}^{T''} \bar{h}_w dT \neq \int_{T'}^{T''} h_w dT = \int_{T'}^{T} \frac{\partial \mu_w}{\partial T}\, dT \quad (18)$$

The *overall* difference in μ_w must be the same, hence the contributions of composition are necessarily different: an externally applied temperature gradient creates an internal concentration gradient.

New complications arise with very thin membranes. In a black lipid membrane the thickness of the lipid core may be less than that of the electric double layers, which we regarded as a discontinuity. We shall not go into these problems here. The basic analysis of continuity remains useful, though a more detailed examination of the transition region may be necessary.

Surface equilibria and coupling

The Onsager symmetry relation holds for the local as well as the overall equations and thus discontinuities of forces and flows must be interrelated. For equal local and overall flows a simple relation between potentials is guaranteed. Take for

example, the well-known relation between electric potential and composition [16]

$$-F\Delta\phi = \sum \frac{\tau_i}{Z_i} \Delta\mu_i^c \qquad (19)$$

Since the transport numbers are ratios between J_i and the electric current I, flows which are equal locally and overall, this equation must hold both for the outside and inside potentials. It is indeed readily seen from surface equilibria that also

$$-F\bar{\Delta}\phi = \sum \frac{\tau_i}{Z_i} \bar{\Delta\mu}_i^c \qquad (20)$$

The overall reflection coefficient, on the other hand, cannot be determined by a measurement in the membrane alone, as was shown above.

The redox potential, or chemical potential of the electron, must be discontinuous as a consequence of a surface redox reaction [13]. It was not included in Table 1, simply because there is not enough agreement in the literature even on notations and definitions, for such a brief summarizing statement. Without any further analysis we may say now that the surface redox reaction chosen above must enable the maintenance of stationary gradients. Electron flow across the membrane will be zero in the presence of non-vanishing driving force for the electron. Onsager symmetry for the system requires:

$$\frac{\tilde{\Delta\mu_e}}{\tilde{\Delta\mu_H}} = \left(\frac{\Delta\mu_e - F\Delta\phi}{\Delta\mu_H + F\Delta\phi}\right)_{J_\epsilon = 0} = -\left(\frac{J_H^+}{J_e}\right)_{\tilde{\Delta\mu_H} = 0} = 1 \qquad (21)$$

The meaning of this equation is that as a consequence of the surface reaction, proton and electron can drive each other uphill.

Thermo-osmotic water flow, against a considerable osmotic gradient, was observed in cellulose acetate [8].

However, the most striking example of coupling between the flow of heat and matter is the spontaneous transport of water between a hot and a cold reservoir. It should be realized that the direction of water flow is not determined by the parameters of the bulk phases alone but depends on the mode of transfer. If the reservoirs are separated by a vapour gap, as is the usual case, water will be transported through it to the cold reservoir. The flow of heat associated with the flow of water (in this case $Q_m = 0$, $Q^* \equiv \Delta H$, in eq. 15) will be in the same direction. On the other hand, if water were to pass between the reservoirs through ice instead of vapour, liberating heat at the entrance into the "membrane" and consuming it at the exit, the flow of heat associated with the flow of water would still be from the hot to the cold reservoir but the flow of water would this time be in the opposite direction. The same effect can be achieved, of course, by any membrane for which $h_w - \bar{h}_w > 0$, provided the ΔH term is dominant in eq. 15.

Surface equilibria are an integral part of the transfer *process,* and may contribute mechanisms of coupling which cannot be observed by monitoring flows inside the membrane only, and certainly not predicted from the state of the reservoirs alone.

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MEMBRANE/SOLUTION POLARIZATION IN DYNAMIC CONDITIONS

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1. *Introduction*

The concentration polarization arising between two electrolytic solutions separated by a charged membrane is one of the most important phenomena in the electrodialysis process. A charged membrane is selective towards ions and an electric current flow induces variations of electrolyte concentrations at the membrane/solution interfaces. Zero polarization condition exists only for $i \to o$, but from a practical point of view, polarization becomes significant only when the interfacial concentration tends to zero on the dilute side, with increase of electric resistance and power consumption. At the same time, on the concentrate side there is a concentration increase which causes membrane fouling when scaling ions are present.

Polarization phenomena have been extensively studied [1-5] by means of Nernst-Planck equation [6, 7], or by electric analog models [8, 9]. Recently mass transfer equations taking into account the hydrodynamic conditions of the system [10-13] were also used.

Other approaches start from optical interferometry [14-16] or from overpotential measurements [17-20]; both methods allow more accurate determination of interfacial concentrations to be made under different experimental conditions.

In this work results obtained with the current interruption technique on an electrodialysis cell under prevailing laminar flow conditions are reported.

2. *Theoretical*

The following assumptions are made:

1) transport of solute ions takes place according to Nernst-Planck equation;

2) ion diffusion coefficients and transport numbers in solution and in membranes are constant;

3) electroosmotic water transport is negligible;

4) interactions between ions, water and membrane fixed charges are negligible;

5) the ohmic resistance of the membrane is independent of solution concentration and current density;

6) membrane structural polarization is negligible;

7) the laminar velocity profile is fully developed within the tested cell section;

8) dynamic and kinematic viscosities are independent of concentrations.

The method of calculating interfacial concentration, starting from the Nernst-Planck equation is based on the analysis of the differences of electrochemical potential in the system formed by two solutions separated by a charged membrane, measured by reversible electrodes, during electric current flow and at current interruption. In the first case:

$$\Delta V = -(R_d + R_c + Rm) \cdot i + \Delta V'_b + \Delta V''_b + E_m + E'_d + E''_d \quad (1)$$

As previously reported [21], it is possible to isolate the potential between the two boundary layers:

$$E(i) = \eta_t - R_m \cdot i = \Delta V'_b + \Delta V''_b + \eta_c \qquad (2)$$

where

$$\eta_c = E_m + E'_d + E''_d \qquad (3)$$

When current is interrupted, ohmic potentials drop to zero ($\eta_m = 0$, assumption 6) and the residual potential is described by eq. (3).

The following additional assumptions are made:

9) activity coefficients are constant;

10) the bulk solution concentration is the same on both sides of the membrane;

11) the imposed hydrodynamic conditions are the same in both cell compartments and hence [21]:

$$c''_m - c_o = c_o - c'_m = \Delta c \qquad (4)$$

Developing equations (2) and (3) with respect to c'_m, it is possible to obtain two equations for the interfacial concentration, the first while the current is flowing in the system and the second at the instant of current interruption.

The two equations are:

$$c'_m = \frac{2\ c_o}{1 + \exp\ [E(i)/K]} \quad \text{when } i > O \qquad (5)$$

and

$$c'_{m,\,o} = \frac{2\ c_o}{1 + \exp\ [\eta_c/e_o]} \quad \text{when } i = O \qquad (6)$$

In the first case c'_m can be directly calculated while in the second it is generally evaluated by extrapolating the overpotential decay to zero time. This extrapolation is necessary because in the first 40 μs after current interruption inductive and capacitive tensions caused by the electric measuring equipment overlap concentration overpotential.

The choice of the extrapolating function is very important because the overpotential decay is very rapid in the first few seconds after current interruption.

In previous works [20, 21] extrapolation was carried out on overpotential measurements but results were not satisfactory, especially at high current densities.

In this paper a simple diffusion model was studied in order to find a physically consistent extrapolation expression for interfacial concentrations.

First the influence of flow conditions was investigated. Velocity distribution in laminar flow within a rectangular duct like the cell compartments tested is controlled by the following differential equation:

$$\frac{1}{\mu}\frac{dP}{dz} = \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} \qquad (7)$$

which can be solved [22] by a series solution:

$$u_{x,y} = \left[\frac{4a^2}{\pi^3 \mu}\frac{dP}{dz}\sum_{n=1,3,5}\frac{(-1)^{\frac{n-1}{2}}}{n^3}\left(1 - \frac{\cosh\left[n\pi(b/2 - x)/a\right]}{\cosh(n\pi b/2a)}\right)\cos\frac{n\pi y}{a}\right] \qquad (8)$$

Coordinate origin on the middle the membrane at the inlet channel section.

Fig. 1 shows mean velocity distribution obtained from eq. (8).

It can be observed that mean velocities within 500 μ and 50 μ layers adjacent to the membrane surface are less than 18% and 2% of the nominal mean velocity, respectively.

Consequently at least in the first seconds after current interruption the influence of the velocity field on the relaxation of the concentration boundary layer ($\delta \leq 500\ \mu$) can be assumed to be negligible.

The general diffusion equation in a bidimensional system is:

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial z^2}\right) - \frac{u\,\partial c}{\partial z} - v\,\frac{\partial c}{\partial x} \qquad (9)$$

Assuming:

$$v = O$$

$$u \cong O \qquad \text{for } x \leqslant \delta$$

$$\frac{\partial^2 c}{\partial z^2} \cong O$$

(9) is reduced to

$$\frac{\partial c}{\partial t} = D\,\frac{\partial^2 c}{\partial x^2} \qquad (10)$$

After current interruption the membrane is considered to be ion impermeable as $D_m/D < 0.1$ [23] and hence:

$$\left(\frac{\partial c}{\partial x}\right)_{x=o} \cong O$$

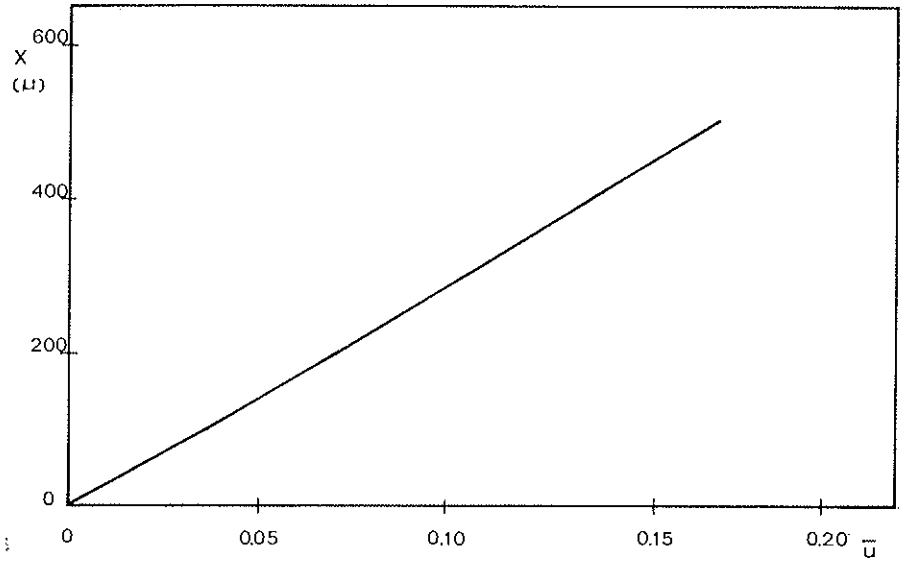


FIG. 1 — Mean adimensional velocity within the concentration boundary layer.

Concentration is assumed to be constant at $x \geqslant 3\,\delta$ (*) hence:

$$(c)_{x>3\delta} = c_o$$

Equation (10) has been solved numerically (finite differences method) for linear and parabolic boundary layer concentration profiles (Fig. 2).

Concentration and time have been entered in tbe computer program in the following adimensional form:

$$\overline{c}_{x,t} = \frac{c'_{x,t} - c'_{m,o}}{c'_o - c'_{m,o}} \qquad (11)$$

$$\overline{T} = t\,D/\delta^2 \qquad (12)$$

(*) In fact concentration should remain constant only for $x \to \infty$ but calculations have shown that when the boundary condition $c = c_o$ is set for $x = 3\delta$ the error is negligible.

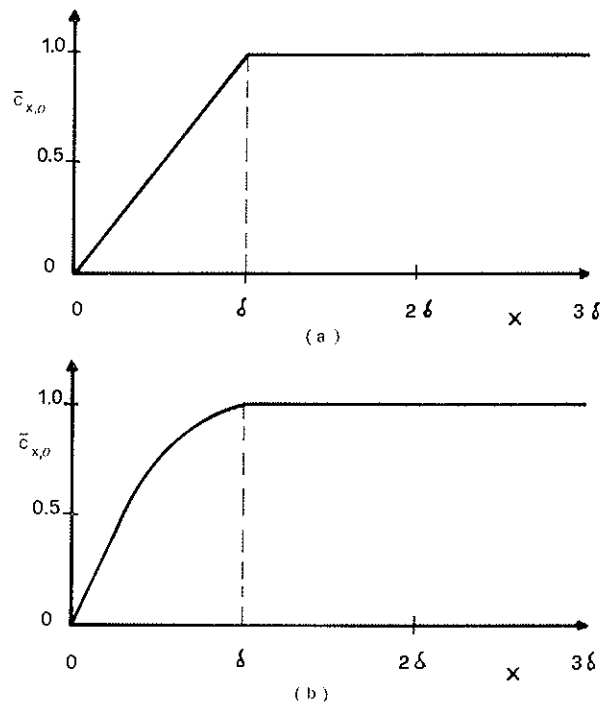


FIG. 2 — Linear and parabolic boundary layer concentration profiles.

It can be demonstrated that the $(\overline{c_{x,t}}, \overline{T})$ solution depends only on the shape of the concentration profile and is independent of initial conditions. Obviously, with parabolic concentration profiles relaxation times are shorter. From now on, only linear profiles will be considered.

Relaxation of $\overline{c_{m,t}}$ (membrane interface) has been plotted on Fig. 3.

For $\overline{T} \leq 2 \cdot 10^{-1}$ concentration relaxation at membrane interface can be interpolated with good accuracy by the function

$$\overline{c_{m,t}} = \alpha \, \overline{T}^{0,5} \qquad (13)$$

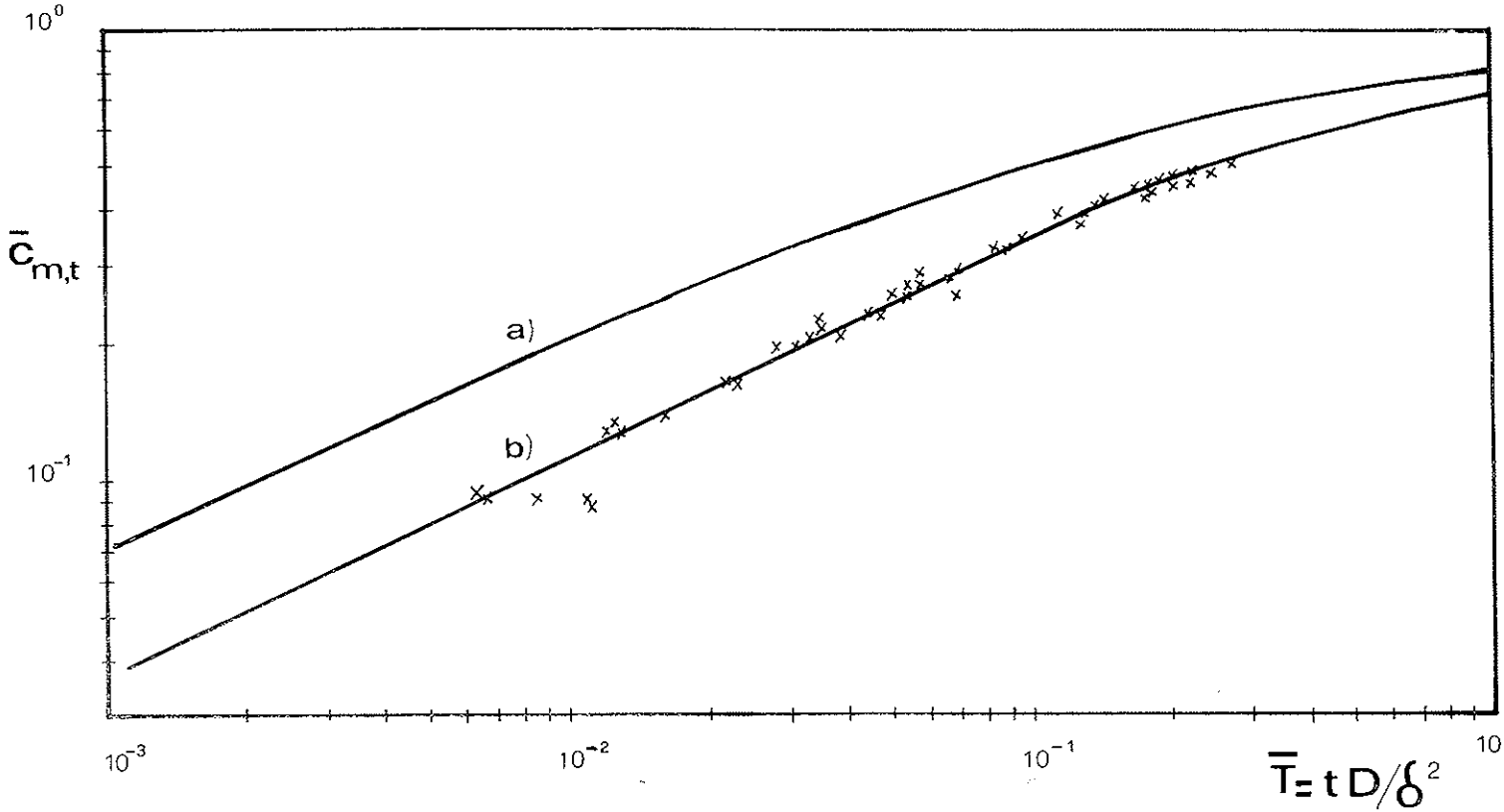


FIG. 3 — Relaxation of interfacial concentration. Continuous line: theoretical boundary layer profile: *a*) parabolic; *b*) linear.

or, in a dimensional form

$$c'_{m,t} - c'_{m,o} = \alpha' t^{0,5} \qquad (13\ bis)$$

which corresponds to the approximate solution found in [20].

Equation (13 *bis*) has been used to extrapolate to zero time interfacial concentration data obtained from overpotential measurements. Extrapolated $c'_{m,o} < 10^{-7}$ N were discarded because in these polarization conditions assumptions 2, 3, 4 do not hold.

Determination of concentration boundary layer along the membrane length.

In previous works [6, 18] the thickness δ of the concentration boundary layer for a developing laminar flow was derived from hydrodynamic considerations assuming constant concentration at membrane interface and geometrical similitude between velocity and concentration boundary layer profiles.

For fully developed laminar flow and developing and developed concentration boundary layer the thickness δ was derived adopting an integral method [10] based on the resolution of eq. (9) and on the assumption of a parabolic profile for the concentration boundary layer.

A different approach which enables the determination of δ from electric experimental measurements only was followed: from Nernst equation

$$\delta = B \cdot \frac{\Delta c}{i} \qquad (14)$$

while Ohm's law gives a relation

$$f(\delta, i) = O \qquad (18)$$

δ or i were then derived from (14) and (18).

(18) has been derived as follows: total resistance measured by a couple of reversible electrodes on both sides of the

membrane is made up by resistances of bulk solutions, of dilute and concentrate boundary layer and of membrane.

Counterelectromotive force caused by concentration polarization has also to be taken into account.

If electric currents in the z direction are negligible, from Ohm's law:

$$\Delta V - \eta_c = (R_d + R_c + R_m + R'_b + R''_b)\ i \qquad (15)$$

$$R'_b = \int_0^{\delta} \rho \, dx \qquad (16)$$

$$R'_b = \frac{\delta}{\Lambda\ \Delta c} \ln \frac{c_o}{c_o - \Delta c} \qquad (17)$$

and

$$R''_b = \frac{\delta}{\Lambda\ \Delta c} \ln \frac{c_o + \Delta c}{c_o} \qquad (17\ bis)$$

hence

$$\Delta V - \eta_c = \left(R_d + R_c + R_m + \frac{\delta}{\Lambda\ \Delta c} \ln \frac{c_o + \Delta c}{c_o - \Delta c}\right) i \qquad (18)$$

and from (14) and (18)

$$i = \frac{\Delta V - \eta_c - \dfrac{B}{\Lambda} \ln \dfrac{c_o + \Delta c}{c_o - \Delta c}}{R_d + R_c + R_m} \qquad (19)$$

substituting (19) in (14)

$$\varepsilon = \frac{B \cdot \Delta c\ (R_d + R_c + R_m)}{\Delta V - \eta_c - \dfrac{B}{\Lambda} \ln \dfrac{c_o + \Delta c}{c_o - \Delta c}} \qquad (20)$$

3. *Experimental apparatus and procedure*

Table 1 summarizes the main geometrical and operational characteristics of the electrodialysis system used in this study. Fig. 4 shows a flow diagram of the experimental setup.

The cell, similar to that used by RAYAN and coll. [24], was modified by adding two perspex frames, the interior of which defined the flow compartments. In order to eliminate interferences, terminal "working" electrodes were screened by neutral membranes.

In all experiments, the working fluid in both dialyzate and brine cells was the same solution of potassium chloride in distilled water. Initial concentrations of 0.05 and 0.01 N at 25± 0.1 °C were used. KCl was chosen in order to eleminate liquid junction potentials, as both ions have the same mobility. KCl solutions concentrated tenfold were used in the neutral and electrode compartments. This higher concentration associated with higher flow velocity, ensured that the ohmic potential drop across the fluid in the neutral and electrode cells was very low compared with that across the rest of the system. Diffusion through neutral membranes was experimentally found to be negligible. The concentration changes were detected by conductivity cells and recorded.

Potential measurements along the flow path were carried out by six couples of calomel electrodes of the capillary type having a negligible asymmetry potential. The distance between each electrode couple was 48mm.

The electric lay-out is shown in Fig. 5. In order to avoid the reverse flow of electric current due to the anode and cathode potentials immediately after interruption of polarizing current, a diode in series with the constant current generator was used. Potentials were measured by an electronic D.C. multichannel voltmeter and recorded by a printer with a scanning speed of 7 channels/s.

The concentration overpotential relaxations were also recorded by a camera connected to a dual beam oscilloscope.

TABLE 1 — *Characteristics of the experimental system.*

STACK

Number of compartments: 1 dialyzate, 1 brine
Width of the channel: a = 13mm
Length of active membrane: ι = 298mm
Depth of the channel: b = 23mm
Number of neutral compartments: 4
Membrane tested: Anion ionics CZL-183
Neutral membranes: Asahi Chem. Co., Japan
Active membrane surface area: S = 38.70 cm²

FLOW CONDITIONS

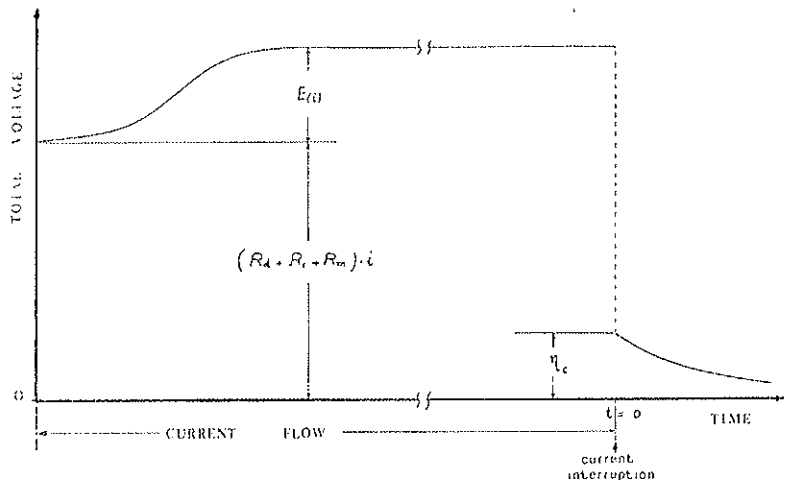
Nominal mean velocity: u = 1 ÷ 6.5 cm/s
Reynolds number: N_{Re} = 190 ÷ 1200

ELECTRIC CONDITIONS

Total voltage applied: = 0 ÷ 30 V
Mean current density: i = 0 ÷ 11.6 mA/cm²

ELECTRODES

Working electrodes: Pt Plated 13×300mm
Distance between probe couples: 48mm
Probe electrodes: Calomel capillary type, 6 couples
Distance of the first probe from cell inlet: 24mm



QUALITATIVE TOTAL VOLTAGE CURVE VERSUS TIME

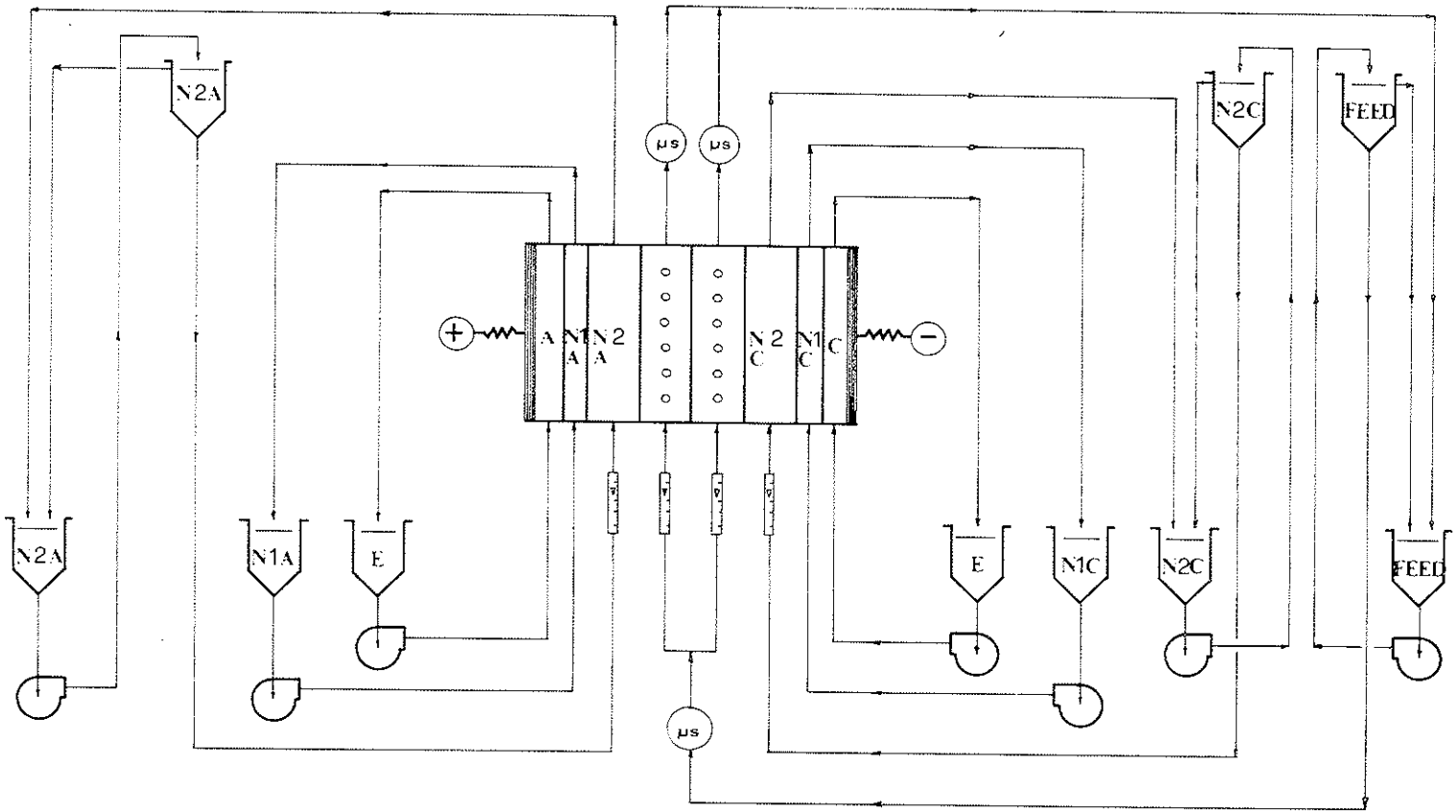


FIG. 4 — Flow diagram of experimental setup: N1, N2 = neutral compartments; A = anode; C = cathode.

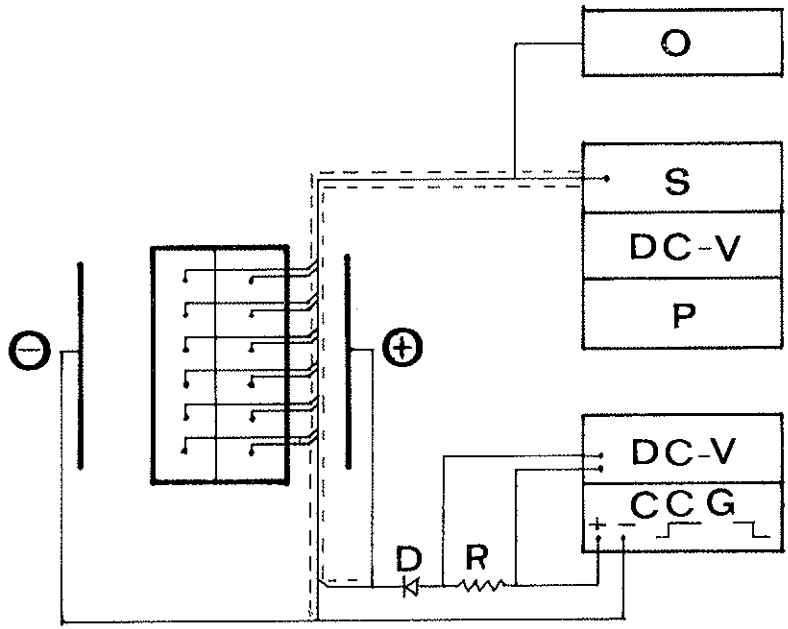


FIG. 5 — Electric lay-out: O = oscilloscope; S = scanner; DC-V = voltmeter; P = printer; CC G = constant-current generator; D = diode; R = calibrated resistance.

The steps in a typical run were as follows. The feed solutions were stirred and recirculated through the system at the desired flow rate. Potential differences between the solutions in the two compartments separated by the membrane were continuously recorded, both during the passage of the constant current until steady state conditions were reached, and after current interruption. The resistance of the brine and dialyzate cell, plus membrane, was obtained from potential measurements recorded at the instant of application of the constant current.

The time to reach steady state polarization increased with current density up to 9 minutes.

All the components of the experimental setup were made from inert, non metallic materials in order to avoid corrosion and contaminations with other ions.

4. *Results and discussion*

Firstly the effects of the electric field on the membrane structure were investigated.

It is known [25] that when a direct current flows through a charged membrane its resistance can vary with time even after steady flow conditions are reached.

This effect, yet to be fully understood, has been associated [26, 27] with the formation of persisting oriented electrical dipoles, i. e. "electrets", generated hy the application of the electric field.

In the experimental conditions previously described, no "electret" potentials were detected after a two minute concentration overpotential relaxation and asymmetry potentials only between reversible electrodes were monitored.

It is likely that the electric fields applied to the membrane studied were too weak to induce "electret" potentials. Overpotential experimental data have been used in equation (6) in order to obtain interfacial concentration $c'_{m,t}$ at different times after current interruption. Interfacial concentration at zero time was calculated, by least squares extrapolation with (13 *bis*) from $c'_{m,t}$ data collected during the first 3 seconds after interruption.

Interfacial concentration $c'_{m,o}$, potential and overpotential data were used in (20) in order to calculate concentration boundary layer thickness δ.

Fig. 6 gives some examples of calculated δ and $c'_{m,o}$ along the membrane. Fig 7 shows correlation between δ, the membrane length and mean nominal velocity.

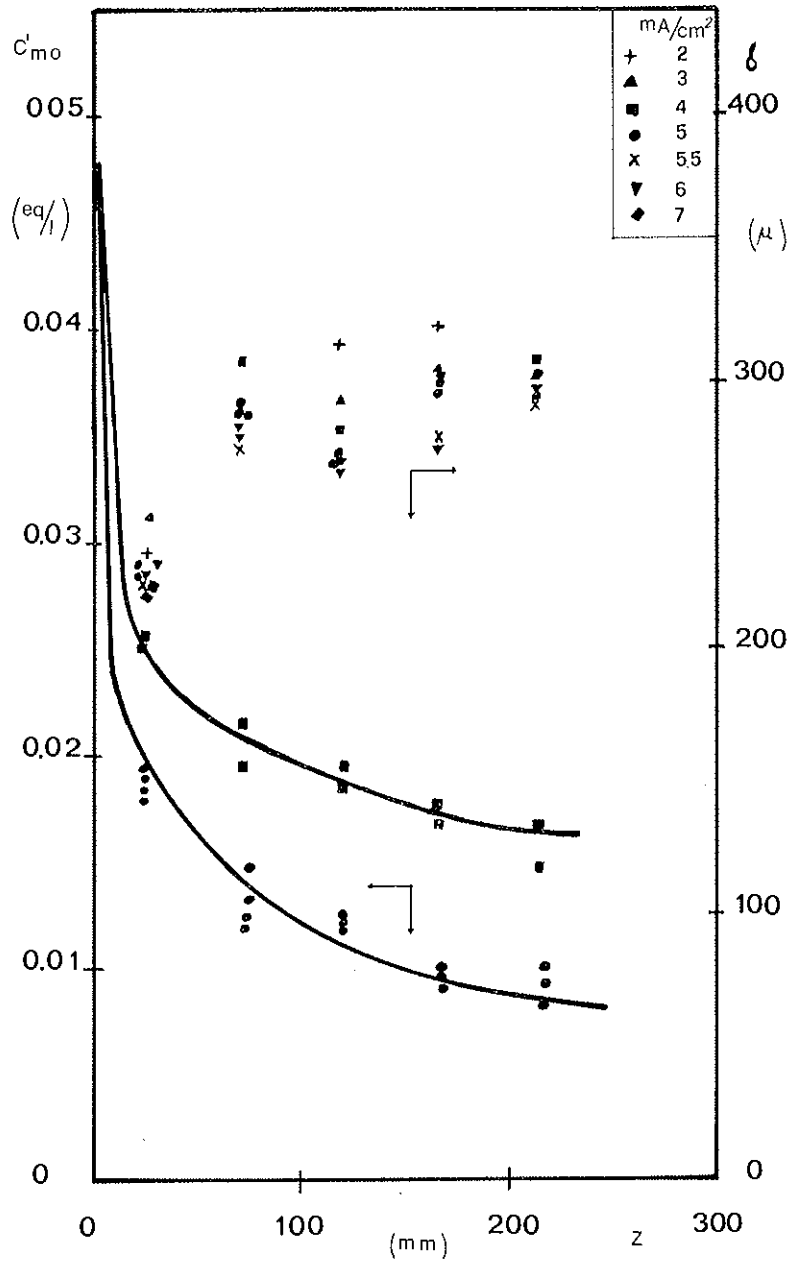


FIG. 6 — Interfacial concentrations (continuous lines) and boundary layer thickness versus membrane length: Co = 0.05 N; u = 3.7 cm/s.

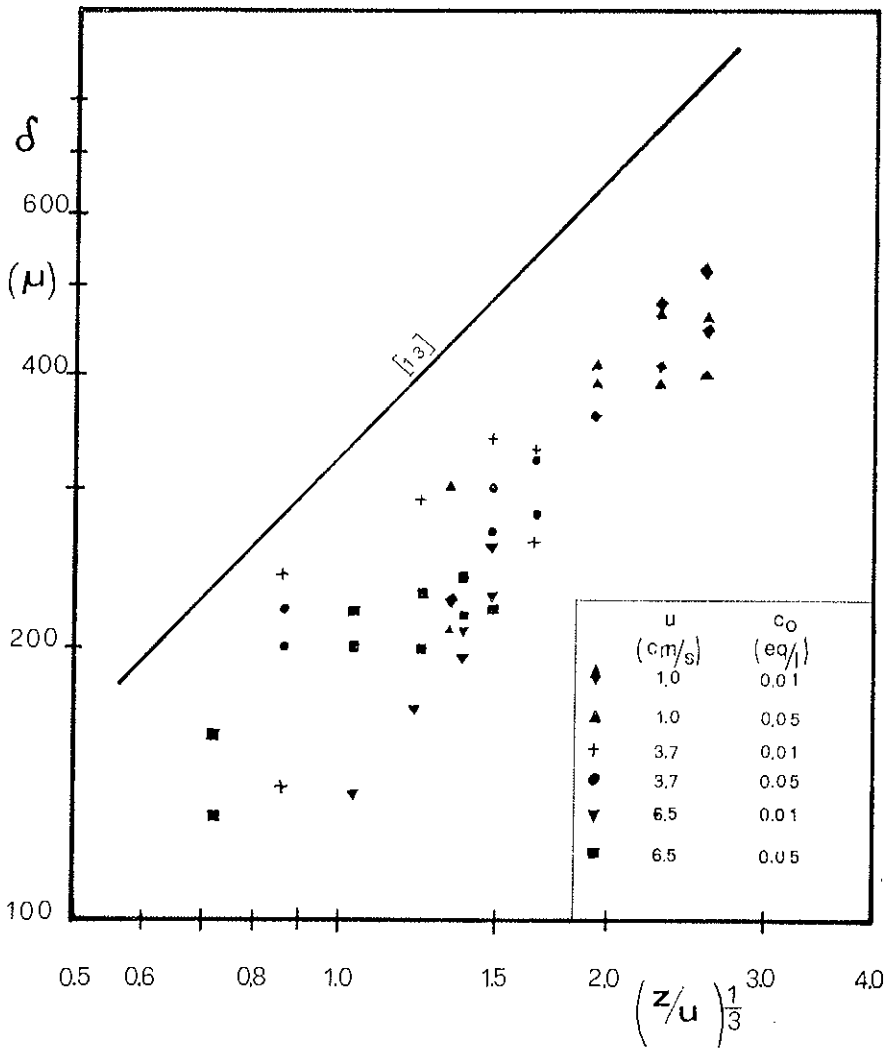


FIG. 7 — Correlation between boundary layer thickness, linear velocity and lenght of the membrane.

The correlation is of the type

$$\delta = \beta \, (z/u)^{1/3} \qquad (21)$$

which is similar to the equation reported in [13], derived from hydrodynamic considerations. The latter is plotted on the same diagram.

Equation (21) is an empirical function based on data obtained only from experimental electric parameters.

Local current densities were determined by (19) and it was found that current density variation with membrane length was very low (less than 10% variation); hence no correction was applied to nominal current densities for different electrode probes.

Adimensional concentration and time were obtained using $c'_{m,o}$, $c'_{m,t}$, δ, t in (11) and (12).

Predicted interfacial concentration relaxation (10) and data obtained from experimental measurements compared on Fig. 3 show good agreement up to t = 11 s.

Fig. 8 gives some examples of interfacial concentrations calculated during current flow (5) and at current interruption (6) versus current density.

For $c_o = 10^{-2}$ N, interfacial concentrations obtained with the two methods diverge when $c'_{m,o}$ is of the order of 10^{-4} N which is defined as the "critical" concentration and is practically independent of flow rates.

It is worth noting that SPIEGLER and coworkers obtained similar results [16] with a completely different experimental technique, based on interferometric measurements.

For each critical interfacial concentration it is possible to obtain a corresponding critical current density (i*) by means of (19) and (14).

The limiting current density defined by Nernst equation

$$i_{lim} = \frac{F D c_o}{(t_i - t_i) \delta} \qquad (22)$$

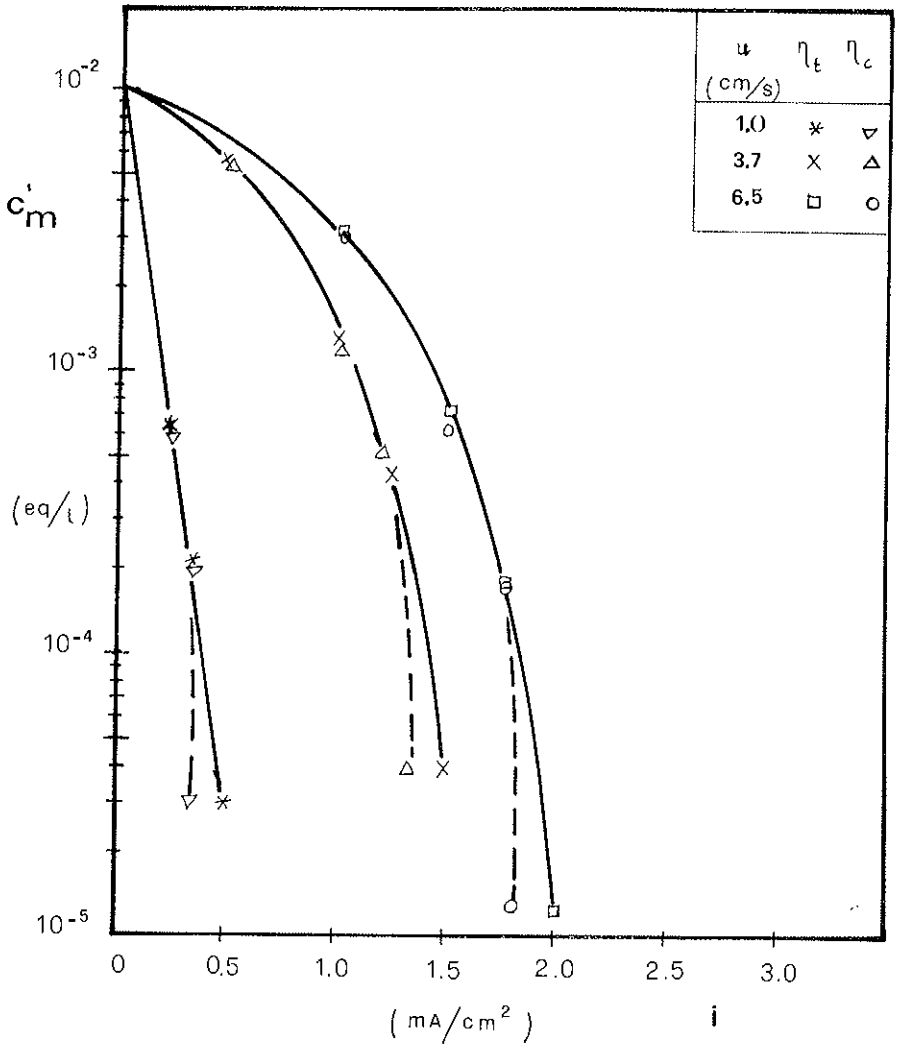


FIG. 8 — Interfacial concentrations calculated during the current flow (η_t) and at current interruption (η_c) vs current density. C_o = 0.01 N.

is practically coincident with the critical one because $c_o \simeq \Delta c$. When $c'_{m,o} < c_{critical}$ for a given current density, concentrations calculated at current interruption are lower than those determined during current flow. It follows that measured total resistances are lower than the calculated ones after current interruption.

The difference between interfacial concentration values obtained with the two methods can probably be ascribed to a decrease of Cl^- transport number in membrane with current density.

Indeed, considering (5) and (6), in order to have the same interfacial concentration

$$\frac{E(i)}{K} = \frac{\eta_c}{e_o} \tag{23}$$

and substituting K and e_o

$$\frac{E(i)}{\dfrac{FD}{(\bar{t}_i - t_i)\Lambda} + \dfrac{2RT}{F}(\bar{t}_i - t_i)} = \frac{\eta_c}{\dfrac{2RT}{F}(\bar{t}_i - t_i)} \tag{24}$$

If $(\bar{t}_i - t_i)$ decreases K increases slightly while e_o decreases. Consequently from (5) and (6), $(c_{m,o})$ during current flow keeps almost constant while $(c_{m,o})$ at current interruption is substantially lowered.

The experimental setup does not allow discrimination between the ionic species competing with Cl^- ions in current transport, but it seems likely that for the relatively high Cl^- interfacial concentration given, the competing ion is K^+ at least for current densities close to the critical one.

An analytical correlation derived in ref. [27] between an adimensional $\bar{i}_{lim}$ and an adimensional flow parameter $\bar{Z}$ defined as [27]

$$\bar{i}_{lim} = \frac{i_{lim}\ b}{4\ FD\ c_o} \tag{25}$$

$$\bar{Z} = \frac{4 \cdot z \cdot D}{b^2\ u} \tag{26}$$

is represented by a continuous line in Fig. 9; i_{lim} was derived from current voltage curves.

Experimental critical current density data obtained as reported above were used in equation (25) and plotted on the same Fig. 9.

The correlation seems to be good in respect of nominal mean velocity but it is less satisfactory when dependence on channel length is considered.

5. *Conclusions*

The current interruption method has proved to be a useful tool in the study of polarization phenomena and, with the use of a simple diffusion model, the divergence point between interfacial concentration data obtained during current flow and after current interruption shifted one order of magnitude lower than previous results [20].

It is also possible to determine diffusion boundary layer thickness and current density distribution without making use of any hydrodynamic model.

Good agreement was found on comparing boundary layer thicknesses determined by this and other methods.

A critical interfacial concentration was defined for which other phenomena must be taken into account. The corresponding critical current density is considered to be highest operational current density for industrial plants fed with pretreated saline water i. e. without scaling ions. In these conditions, electric resistance is not too high and allows an increase of

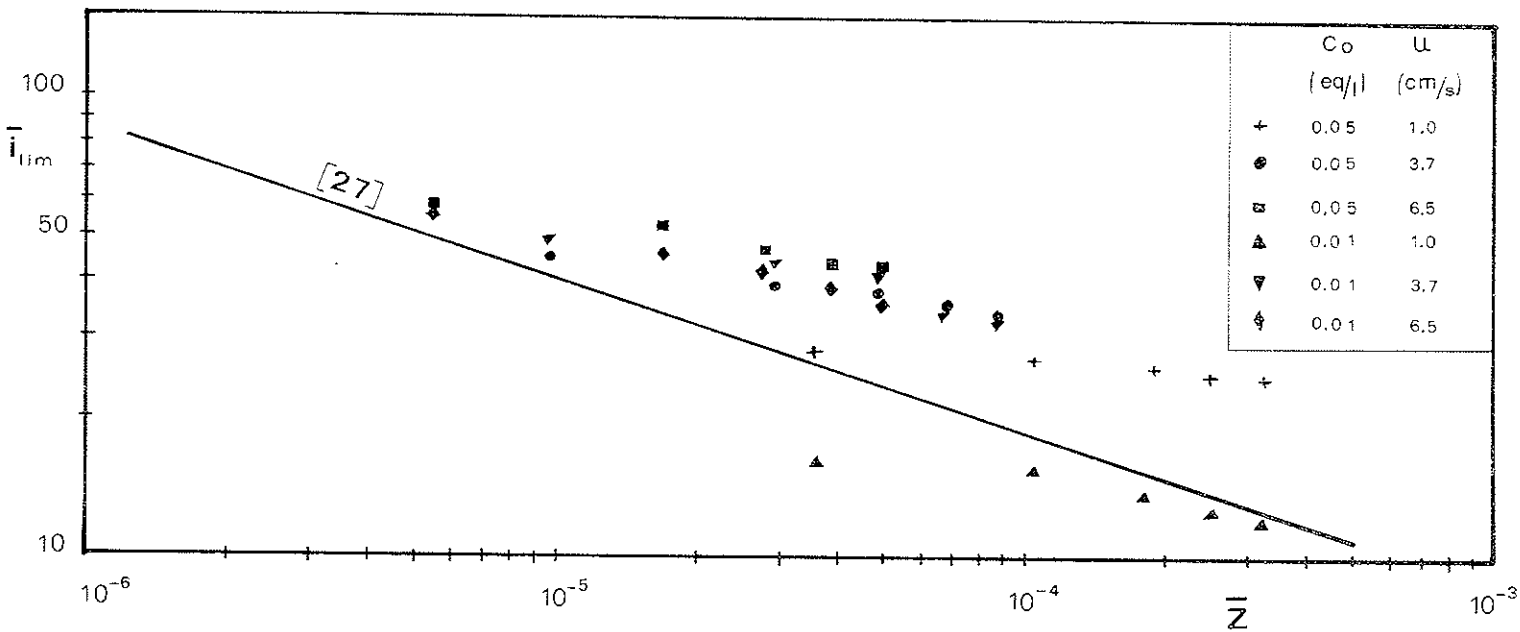


FIG. 9 — Adimensional limiting current density as a function of the flow speed parameter.

product flow rate per unit membrane area without high power penalties, as was demonstrated on a long run industrial scale test [28].

Abstract

Different attempts to describe polarization phenomena have used electrical analog models or mass transfer and hydrodynamic equations, when the system is in a steady state condition.

In this paper polarization phenomena are investigated in steady and transient conditions, using pertinent transport equations under concentration and electrical driving forces.

Concentration at the membrane/solution interface can be determined by means of Nernst-Planck equations in steady state conditions and after current interruption.

In the first case it can be directly calculated, while in the second it is generally evaluated extrapolating the overpotential decay to time zero.

This extrapolation is required because the electric tension decay caused by circuit capacity and inductance during the initial 40 μsec, after current interruption, overlaps the concentration relaxation overpotential, which is a much slower phenomenon that can be easily recorded.

In this paper a simplified diffusion model is used to calculate, for a given bulk concentration, the relaxation equation of interfacial concentration. This relation is used to extrapolate concentration data derived from overpotential measurements to the instant of current interruption.

Experiments were carried out on an electrodialysis vertical upflow cell, working under prevailing laminar flow conditions ($N_{Re} \leqslant 1100$). The membrane tested was an anion Ionics CZL 183 placed between two rectangular cross-section compartments, without turbulence promoters. KCl solutions at initial concentrations 0.05 and 0.01 N were used.

Potential differences were continuously measured by six coup-

les of reversible electrodes at different heights in both compartments.

Constant current flow was applied until steady state conditions were reached, then the current was interrupted and the overpotential relaxation monitored.

Experimental data show that structural polarization of the tested membrane (electret potential) is negligible for current densities up to 10 mA/cm^2, lasting less than 9 minutes.

Interfacial concentrations obtained by the two methods, as indicated above, are compared. A good agreement is found for current densities up to 3 mA/cm^2. For higher values of current densities the two values diverge. This indicates that Nernst-Planck equations do not take into account phenomena which, under these conditions, become significant.

The type of divergence is such that measured total resistances are lower than those predicted through Nernst-Planck equations. Electrodialysis plants can therefore operate safely at higher current densities than usually accepted.

NOMENCLATURE

a	mm	Cell width
b	mm	Cell depth
B	A cm²/eq	$B = \frac{FD}{\bar{t}_i - t_i}$
c	eq/l	Electrolyte concentration
c_m	eq/l	Interfacial concentration
$c_{m,t}$	eq/l	Interfacial concentration at time t
c_o	eq/l	Bulk concentration
$c_{x,t}$	eq/l	Electrolyte concentration at coordinate x and time t
$\bar{c}_{x,t}$	—	Adimensional concentration at coordinate x and time t defined by eq. (11)
D	cm²/s	Diffusion coefficient of the electrolyte
E_d	V	Diffusion potential
$E_{(i)}$	V	Potential difference between the two boundary layers
E_m	V	Membrane potential
e_o	V	$e_o = \frac{2RT}{F}(\bar{t}_i - t_i)$
F	coul/eq	Faraday constant
i	mA/cm²	Nominal current density defined as total current/active membrane area
i*	mA/cm²	Critical current density
$\bar{i}_{lim}$	—	Adim. limiting current density defined by (25)
i_{lim}	mA/cm²	Limiting current density
K	V	$K = \frac{B}{A} + e_o$
l	mm	Membrane lenght
P	g/cm s²	Pressure

R	erg/°K$_{mol}$	Gas constant
R_i	$\Omega \cdot cm^2$	Electric resistance per unit area (defined by subscripts)
S	cm^2	Active membrane surface
t	s	Time
T	°K	Absolute temperature
$\overline{T}$	—	Adim. time defined by eq. (12)
$\overline{t_i}$	—	Counterion i transport number in membrane
t_i	—	Counterion i transport number in solution
u	cm/s	Nominal mean velocity in z direction defined as flow rate/channel section
$u_{x,y}$	cm/s	Linear velocity in z direction at coordinates x, y
u_b	cm/s	Mean linear velocity on a surface at distance x from the membrane $u_b = \frac{a}{2} \int_0^{a/2} u_{x,y}\, dy$
$\bar{u}$	—	$\bar{u} = u_b/u$
v	cm/s	Flow velocity in x direction
x	mm	Coordinate of the compartment depth
y	mm	Coordinate of the compartment width
z	mm	Coordinate of the compartment length
$\overline{Z}$	—	Adimensional flow parameter

GREEK LETTERS

α	—	Constant in equation (13)
α'	eq $s^{-1/2}/l$	Constant in equation (13 *bis*)
β	cm $s^{-1/3}$	Constant in equation (21)
δ	10^{-6} m	Boundary layer thickness
Δc	eq/l	Difference of concentration
ΔV	V	Overall difference of potential
ΔV_i	V	Difference of potential (defined by subscripts and superscripts)
η_{ic}	V	Concentration overpotential

η_m	V	Membrane overpotential
η_t	V	Overall overpotential
ρ	$\Omega \cdot$ cm	Resistivity of solution $\rho = \frac{1}{\Lambda c}$
Λ	$cm^2/eq\ \Omega$	Equivalent conductivity of the electrolyte
μ	g/s cm	Dynamic viscosity

SUPERSCRIPTS AND SUBSCRIPTS

b	Diffusion boundary layer
c	Concentrate
d	Dilute
m	Membrane
|	Referred to dilute side of membrane
||	Referred to concentrate side of membrane

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DISCUSSION

Chairman: A. J. STAVERMAN

PASSINO

This work was done in cooperation with Dr. TIRAVANTI and Dr. ROZZI, who are present.

TEORELL

I can't resist making a comment here to indicate that the « unstirred layers » may have a biological significance. In biological diffusion problems one often forgets to add a possible « unstirred layer » to the thickness of what can be measured under the microscope. Some measurements performed by I. SCHULMAN and myself in 1938 indicated that the thickness of unstirred layer was of the of 30, at mobile oil films and at plane cellophane membranes (Trans. Faraday Soc. 34, 1337, 1938).

KEDEM

This question is also for Prof. SPIEGLER: you have more sensitive methods now to see when the water splitting starts, the point at which something goes wrong, with a very reliable prediction, as one sees from the other lines. Does this point coincide with observable pH changes?

PASSINO

We haven't done any measurements. We intend to but for the time being we haven't done anything.

SPIEGLER

We measure the pH routinely on a continuous basis in a pilot plant but we haven't correlated it to the optical experiments because the hydrodynamics are very different. I believe the field is open for additional research. Dr. PASSINO used two terms here — the critical current density and the critical concentration. Do we really know yet whether the interfacial concentration is the critical parameter or that the current density is critical for given conditions?

PASSINO

I think we should do more work on that. I can't say anything because we didn't reach any conclusion yet on this point, but we think that besides the water splitting we should take into consideration potassium transport in this case, because we did all the calculations on chloride transport through the anion membrane; there might be some competition from potassium, but we don't have any quantitative data to show yet.

STAVERMAN

Is there another question?

MEARES

I should just like to comment on Dr. PASSINO's observation that the apparent boundary layer thickness obtained from purely hydro-

dynamic measurements is not necessarily identical with that obtained from purely electrical parameters. A good time ago we made some measurements in which we were studying this polarization phenomenon simply through steady-state current/voltage curves. We used the film thickness as an adjustable parameter to fit the electrochemical measurements and we kept the hydrodynamic conditions exactly constant. We then exchanged the membrane for a polished silver plate and determined the film thickness by a conventional electrode kinetic method. When we compared this with the value found with the membrane we found that the results were considerably different. Much larger values were obtained with membranes. Had we been calculating the film thickness from purely hydrodynamic parameters we would have always got the same result because the hydrodynamics were kept absolutely constant. Thus it is certainly an observation of ours that the apparent film thickness from electrochemical observation of polarization is a function of the type of surface on which the polarization is taking place as well as of hydrodynamic flow parameters in the system as a whole.

IX

GENERAL ASPECTS OF MEMBRANES PHENOMENA

THE USE OF MODELS IN THE STUDY OF COMPLEX EFFECTS AT MOSAIC MEMBRANES (*)

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I. *Introduction; the Electrocapillary Becquerel Phenomenon; Electrostenolysis*

The author's interest in unusual and complex membrane effects started more than 45 years ago in the laboratory of the late Prof. Herbert Freundlich in Berlin, in his days the foremost authority in colloid science. FREUNDLICH's monumental "Kapillarchemie" [1] was then the outstanding, encyclopedic reference work in this field. In the chapter on "Electrocapillarity", in a special section on "Anomalous Osmosis, Electrostenolysis, Electrocapillary Becquerel Phenomenon", FREUNDLICH reviewed these puzzling electrochemical membrane effects which had been discovered sixty and more years previously. All of them were challenging electrochemical problems; in addition, the two first mentioned phenomena were of some biophysical interest because they had been used as the basis of some speculative explanations of various *in vivo* processes. Anomalous osmosis had been adduced as the mechanism of otherwise un-

(*) Section VI in cooperation with Dr. Gerald M. Shean.

explainable liquid movements across membranes in living systems, and electrostenolysis as a basis of *in vivo* oxidation and reduction processes.

None of the various explanations of the mechanisms of the three previously mentioned effects, some presented by well-known investigators [2], made sense to me. Some were based on far-fetched *ad hoc* assumptions, and none could withstand a rigorous physicochemical analysis.

First I tried to clarify the mechanism of the *Electrocapillary Becquerel Effect,* the spontaneous formation of deposits of Ag, Cu, etc. in the metallic state which occurs at the precipitation membranes formed by the interaction of solutions of Ag, Cu, etc. salts with solutions of alkali sulfides, selenides and tellurides. A search of the literature on electrode electrochemistry and the nature of the electrical conductance of solids, then a rather unknown field, led within two months to the still accepted explanation of the mechanism of the electrocapillary Becquerel effect [3], which is based on classical electrochemistry.

As a matter of course, I turned next to the attempt to elucidate the mechanism of "*Electrostenolysis*", the formation of heavy metal deposits at inert, or supposedly inert, porous diaphragms and membranes when a current is passed through the system, an effect first described in 1819. It had been the subject of considerable, often rather diffuse and inconclusive controversy and was considered by some investigators as an effect *sui generis*. The study of the literature, particularly of the then still fairly limited literature on the electrochemistry of membranes, led to a ready, so-to-speak common sense explanation of the two outwardly very similar but basically much different effects described in the literature under the heading of electrostenolysis [4]. This work has stood the test of time very well (1).

(1) See for instance the recent remark by BRENNER [5] "The subject of the electrolytic processes at membranes has been capably reviewed and discussed by SOLLNER [4] and, thus, there is no need for further treatment here".

Having solved within one year two of the three long controversial electrochemical membrane problems discussed at some length in FREUNDLICH's Kapillarchemie [1], I felt ready to attempt next to elucidate the still essentially unknown mechanism of the third effect in this group, "*Anomalous Osmosis*". Without knowing it, I entered thereby a lifelong career in physicochemical membrane research. The most interesting and intellectually satisfying phases of this work were the elucidation of involved membrane effects, the prediction of still unobserved effects, and the conception and the experimental tests of more or less involved membrane model systems. Some of these models deal with the elucidation of the mode of action of various types of *mosaic membranes*. A condensed synopsis of this work, some of it still in progress, is the subject of this paper.

II. *Anomalous Osmosis*

The term "anomalous osmosis" denotes all those osmotic phenomena arising with solutions of electrolytes, which with respect to magnitude or direction seem to be contrary to the common experience with nonelectrolytes. With the nonelectrolytes the direction of the flow of liquid across porous membranes which separate a solution from pure solvent (or a more dilute solution) is toward the side of the more concentrated solution ([2]), and occurs at a rate roughly proportional to the concentration difference. With electrolyte solutions the rate of movement of liquid across the membrane depends in an involved manner on both the absolute concentration, the con-

([2]) In 1949 LAIDLER and coll. [6] reported a still unique contrary instance. With solutions of urea the flow of liquid is towards the side of the more dilute solution, at least under certain conditions. Though the mechanism of this effect may not be settled definitely, it is clear that its mechanism is entirely different from that of the negative osmosis observed with electrolyte solutions; see also a later paper by GRIM [7].

centration difference of the two solutions, and the nature of the membrane. When an abnormally copious flow of liquid, ordinarily at fairly low concentrations, is directed towards the more concentrated solution one speaks of "anomalous positive osmosis". In the relatively rare instances when, only at fairly high concentrations of certain electrolytes, a flow of liquid occurs toward the side of the pure solvent (or the more dilute solution), one speaks of "negative anomalous osmosis" or, "negative osmosis". Negative osmosis is always much smaller than the anomalous positive osmoses obtained with the same membrane, and occurs at much higher concentrations.

Figure 1 shows some data on anomalous osmosis by JACQUES LOEB [8], the great physiologist, one of the foremost experimental investigators of this effect. The pressure rises in this Figure indicate a water movement to the side of the more concentrated solution as observed after 20 minutes in a conventional osmotic apparatus. The difference between these pressure rises and the corresponding pressure rise observed with a solution of equal concentration of an arbitrarily chosen reference nonelectrolyte was considered as the measure of the water movement due to anomalous osmosis. Analogous pressure drops below the zero line, found in certain instances with some electrolytes, are denoted as negative osmosis.

Space does not permit either a review of the literature on anomalous osmosis or a critical evaluation of the several theories proffered in the various attempts to explain the mechanism of this effect. This has been done elsewhere [9]; none of the attempted explanations could withstand a critical examination [9].

I approached the problem of anomalous osmosis firmly convinced that the mechanism of these much discussed twin effects must be explainable without *ad hoc* assumptions by the unprejudiced use of the available information on the electrochemistry of membranes. MICHAELIS' then relatively new studies on the electrochemistry of collodion membranes, mainly

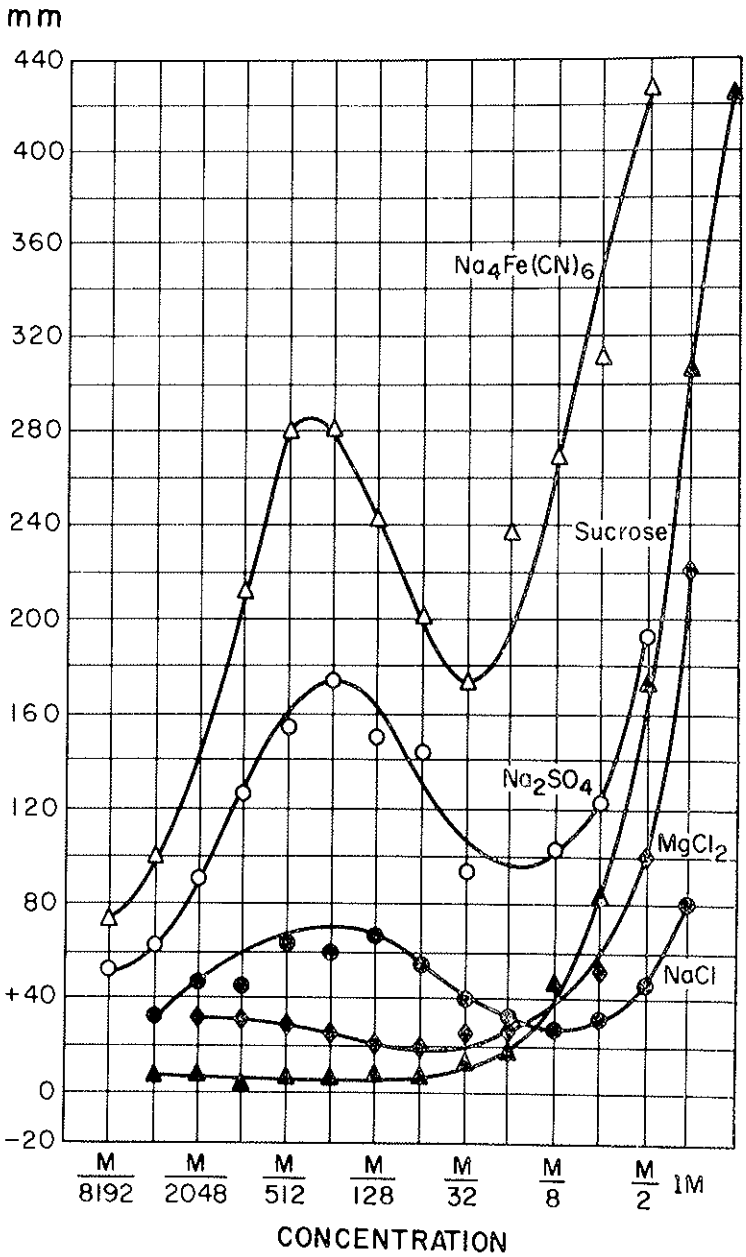

FIG. 1 — Anomalous osmosis arising on the dialysis of the solutions of various electrolytes at different concentrations against water; pressure rises in mm water observed after 20 minutes (after. J. LOEB [8]).

their electromotive actions in concentration cells [10] seemed the most substantial source of information. While repeating some of MICHAELIS' experiments one could not avoid observing that all common membranes, such as collodion membranes and parchment paper, show gross macroscopic heterogeneities, with more dense and less dense patches lying next to each other. Thinking about this and the modes of preparation of membranes in general, it appeared *a priori* overwhelmingly probable that their pore systems are irregular arrays of branching and interconnected channels of uneven width arranged in a more or less random network. Strange as it seemed, this fact had been completely overlooked in the electrochemical membrane literature, though, as I found later, it had already been well documented in the literature on ultrafilters [11]. The idea of *membrane heteroporosity* and the concomitant conception of *membranes as pore-width mosaic structures* opened up new vista.

From MICHAELIS' [10] investigations on the electromotive behavior of collodion membranes in concentration cells it was clear that pores of different width in the same matrix yield different pore potentials. Once the knowledge of this fact and of the concept of membrane heteroporosity interacted, the conclusion was automatic: adjacent pores or group of pores of different width are the sources of pore potentials of different magnitudes; these sources of different E.M.F.s are connected to each other electrically by the two solutions; as a consequence, a closed electrical circuit exists, a current flows. This current according to classic electrokinetics must of necessity cause an electrosmotic transportation of liquid across the one or the other pore or set of pores. This electroosmotic cross membrane movement of water, which is superimposed on ordinary osmosis, is the phenomenon commonly referred to as "*anomalous osmosis*".

This idea is illustrated schematically in Figure 2, redrawn from the original paper on this subject [9]. It illustrates the

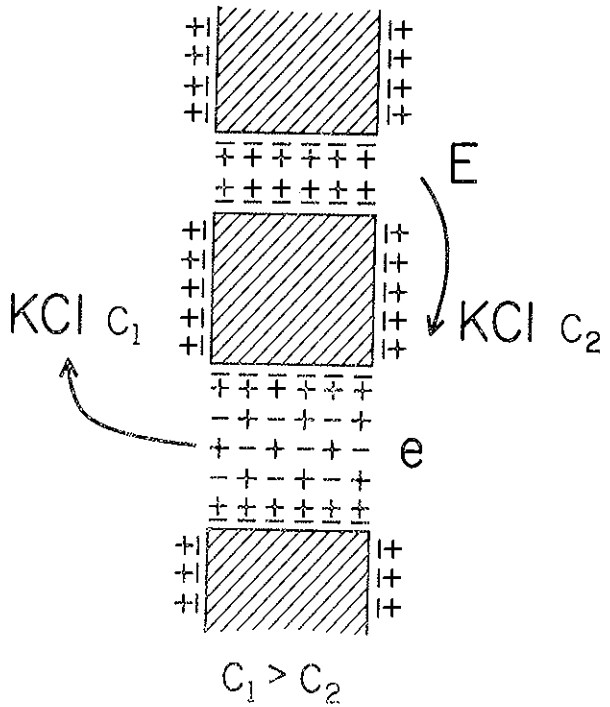

FIG. 2 — Schematic representation of the mechanism of anomalous osmosis. The upper, narrower pore yields a higher pore potential, E, than that arising at the wider pore, e. As a result, a positive current flows clockwise in the direction of the solid arrow and causes an electroosmotic movement of water through the wider pore from the side of the more dilute to the side of the more concentrated solution so that anomalous positive osmosis arises.

results of membrane heteroporosity in a KCl concentration cell, the simplest possible case, since K^+ and Cl^- have virtually the same diffusion velocities. The narrower pore yields the higher pore potential, and a (positive) current flows in a clockwise direction as indicated by the solid arrows. This current causes an electroosmotic flow through the wider pore, from the more dilute to the more concentrated solution. This means that

positive anomalous osmosis occurs [9]. Developing this concept for systems with electrolytes whose cations and anions have different diffusion velocities was a fairly simple task [12]. Everything fell readily into place including a clear, well-defined insight into the experimental conditions which give rise to anomalous negative osmosis.

This conception of the mechanism of anomalous osmosis was soon tested in cooperation with Dr. A. GROLLMAN [13]. For this purpose we constructed macro-model systems whose main functional parts were two membranes of different porosities which interacted with each other in a closed electrical circuit through two solutions of the same electrolyte at different concentrations. The chances of obtaining measurable electro-osmotic water transport in these model were, *a priori,* not favorable: the driving E.M.F.s in these macro-systems were about of the same order of magnitude as those assumed to be operative in the ordinary single-membrane experiments; however the linear distances, and therefore the resistances in the solutions in the two-membrane macro-models were several orders of magnitude larger than in the conventional single membrane cells. The currents in the macro-models were therefore correspondingly smaller, and consequently also the rates of electro-osmotic water transport which could be expected.

A detailed report on the numerous considerations that went into the selection and into the construction of our macro-model systems is outside the scope of this paper. Suffice it to present here a schematic drawing of the rather primitive and inefficient apparatus used in these experiments (Fig. 3) and to state that we consistently obtained measurable, although small, water movements in the predicted direction. This movement of liquid stopped immediately when the flow of the current was interrupted by the interposition of an insulator [13].

This work on the mechanism of anomalous osmosis soon was widely recognized and, particularly gratifying and important to me, it found the approval of the Nobel prize winners

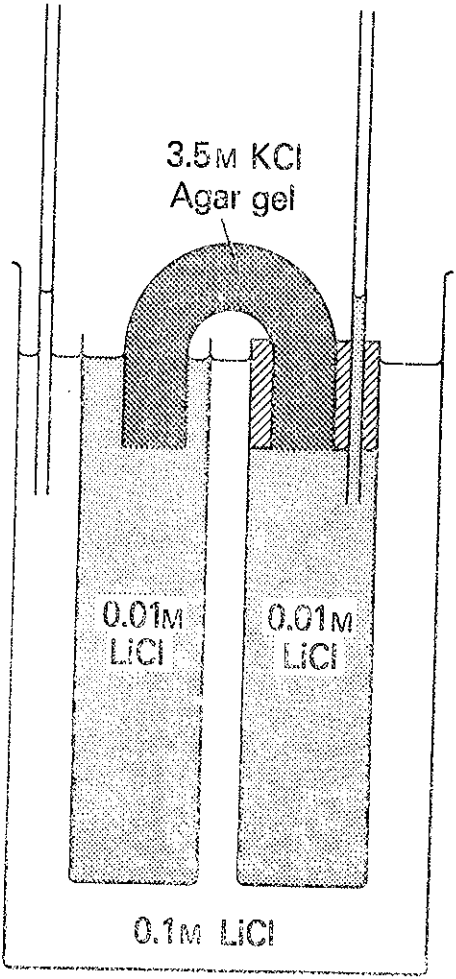

FIG. 3 — First crude two-membrane model to test the pore-width mosaic theory of anomalous osmosis [13].

WALTHER NERNST and FRITZ HABER. It was generally accepted without serious doubts until SCHLÖGL [14], twenty years ago, suggested that the membrane may be considered as a huge polyion which moves with respect to the water in its pores in the electric field created by the diffusing electrolyte. To my knowledge, experiments to prove this concept have not been reported. It is entirely possible that both proposed mechanisms operate simultaneously. This topic has been considered more recently also by KEDEM and KATCHALSKY [15].

III. *The Electrical and Permeability Properties of Mosaic Membranes which are Composed of Exclusively Anion and Exclusively Cation Permeable Parts*

That all heterogeneities in membranes which give rise to locally different electromotive forces, not only heteroporosity, must of necessity produce local electrical circuits had already been pointed out in my first paper on anomalous osmosis [9]. A system very suitable for applying this concepts had recently been discussed in collaboration with HOFFMANN by R. HÖBER [16] whose classical book "Physikalische Chemie der Zelle und der Gewebe" [17] for many years had been the best source of information on biological membrane research and its physico-chemical background. HÖBER and HOFFMANN [16] had elaborated on the electrolyte permeability of *mosaic membranes* which are composed of highly anion selective and highly cation selective parts. Their theoretical reasoning seemed to me basically erroneous and I started therefore to look into this problem.

It was immediately evident that by far the simplest charge mosaic membranes are those which are composed of ideally anion selective and ideally cation selective parts. The main questions were: are such mosaic membranes permeable to electrolytes, and if so, what is the correlation of their electrolyte

permeability to the electrochemical properties of the component parts of which such membranes consist.

These questions are discussed most advantageously by reference to the sequence of line drawings of Figure 4 which are redrawn from the original paper [18].

Figure 4a shows schematically a system in which a mosaic membrane separates a lower compartment of invariable volume from an upper compartment. The striated structure in the figure indicates the membrane. The lower compartment in Figure 4a is filled with 0.1 *N* and the upper one with 0.01 *N* KCl solution. The electronegative, cation-permeable (anion-impermeable) parts of the membrane are indicated by minus signs, and the electropositive, anion-permeable (cation-impermeable) parts by plus signs.

From Figure 4a it is obvious that cations move through the electronegative parts of such membrane and anions through its electropositive parts, neutralizing each other electrically. Such mosaic membranes are therefore permeable to the electrolyte. A continuous movement of the electrolyte occurs across the membrane until equilibrium between the two compartments is established. This is the answer to the first of the previously stated questions.

The second question, the quantitative relationship of the electrolyte permeability of the mosaic membranes and the electrochemical properties of their component parts, is readily answered by the consideration of systems in which the cation-permeable and anion-permeable membrane parts are rearranged spatially separated from each other, as shown in Figure 4b. The U-tube in this figure contains in its left arm an assembly of the electronegative, exclusively cation-permeable parts of the membrane of Figure 4a, in its right arm an assembly of the electropositive, exclusively anion-permeable parts of this membrane. The lower part of the system of Figure 4b is filled with 0.1 *N* KCl solution; the two compartments above the two membranes contain 0.01 *N* KCl. The only processes which can occur

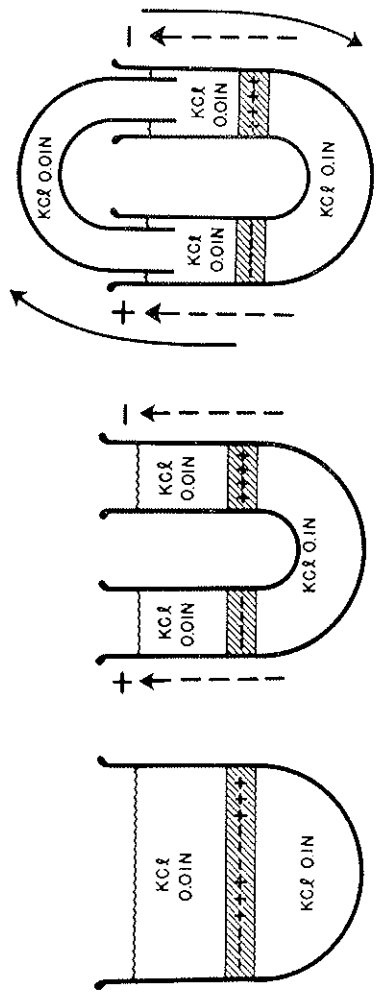

FIG. 4 — Pictorial development of the theory of electrolyte permeability of mosaic membranes that are composed of ideally anion-selective and ideally cation-selective parts. *a)* Mosaic membrane with adjacent cation-selective and anion-selective parts. *b)* Spatial and electrical separation of the cation-selective and anion-selective parts of the membranes. *c)* The spatially separated cation-selective and anion-selective parts joined electrically by a bridge of solution in an all-electrolytic circuit.

in the system of Figure 4b are the build-up of a hydrostatic pressure in the lower compartment and the establishment of the membrane concentration potentials across the two membranes. Thereafter the system is at rest, in a state of equlibrium ([3]).

The magnitude and the signs of the two membrane potentials in the system of Figure 4b are given by NERNST'S equation, written in terms of ion activities. They amount to + 55.1 and − 55.1 mv at 25.0°C; their signs are shown by the plus and the minus signs towards which broken-line arrows point.

The essential feature of the situation represented in Figure 4a, in which the cation and the anion permeable membrane parts do interact with each other, may be re-established by connecting the two upper compartments of Figure 4b by means of a liquid bridge containing the same solution, as shown in Figure 4c. The system of Figure 4c represents an all-electrolytic electrical circuit. A (positive) current flows in a clockwise direction through the system, as is indicated by the solid arrows. The strength of the current, I, is given by Ohm's law, $I = E/\rho$ where E is the sum of the two membrane concentration potentials and ρ the total resistance of the system. That the potential difference between any two points in the system of Figure 4c is defined at any given moment by KIRCHHOFF'S second law is obvious.

The current flowing in the cell of Fig. 4c is transported through the negative membrane in the left arm of the system exclusively by cations which move clockwise, in the direction of the broken-line arrow. Through the positive membrane in the right arm the electricity is transported exclusively by an equivalent quantity of anions which move in a counterclockwise direction, also indicated by a broken-line arrow. Thus, the number of the equivalents of electrolyte which move in a given time in the system of Figure 4c from the concentrated to the

([3]) The establishment of two Donnan membrane potentials is associated with membrane hydrolysis across the two membranes, a very minor side effect which can be disregarded here.

dilute solution must be numerically identical with the number of faradays which flow in the system during the same period.

This theory of mosaic membranes is the first instance in which a complex membrane system was shown to be amenable to quantitative theoretical analysis. However, at that time, in 1931, it could not be verified experimentally; suitable membranes which combine extreme ionic selectivity with a moderate or low resistance were not available and methods suitable for the preparation of such membranes had not been developed.

This situation changed abruptly in the middle thirties when TEORELL [19] and soon thereafter MEYER and SIEVERS [20] in greater detail, proposed the to-day dominant fixed charge theory of ionic membranes and thereby opened up a new era of electrochemical membrane research. MEYER and coll. [20], moreover, also described methods of preparing high charge density membranes and obtained membranes of a formerly unknown degree of electrochemical activity.

Beginning in 1940 the author with the help of a small but excellent group of collaborators developed positive and negative collodion matrix membranes of very high electrochemical activity and any desired degree of porosity, particularly "permselective" membranes which combine extreme degrees of ionic selectivity with low resistance [21-25]. Such "permselective" membranes were soon used by NEIHOF and SOLLNER [26, 27] to test the before outlined theory of mosaic membranes. For this purpose it was necessary to construct model systems which in all essential features are identical with the all-electrolytic ring system of Figure 4c and permit the continuous accurate determination of the strength of the current which flows in the system. The determination of the quantity of electrolyte moved to the more dilute solution is not a major problem. This test of the theory can be achieved with two different experimental arrangements.

The more straightforward, but experimentally more difficult approach [27] is to measure continuously by means of probe

electrodes, the potential difference across an element of the circuit of known, invariable resistance.

From these data the current strength is computed. Figure 5a shows in a schematic manner all the essential features of this arrangement; the locations of the probe electrodes are indicated by Y and Y'. Figure 5b is a schematic drawing of the actual rather elaborate experimental arrangement used. The electrical identity of the systems of Figures 5a and 5b is obvious.

The other approach was to determine the current strength in the system of Figure 4c by cutting the system at a suitable point and attaching to the two open ends of the interrupted circuit two symmetrical electrodes which can reversibly take the current from and return it to the system [26]. The two electrodes may be connected to each other through a coulometer whereby a closed circuit is reestablished, or a microameter of very low resistance may be inserted which is read at close intervals. In systems with halide solutions the electrodes of choice are the appropriate silver-silver halide electrodes as shown in the schematic Figure 6a. The essential features of the electrochemically equivalent experimental model [26] are shown in Figure 6b. In more elaborate models (not shown here), nonspecific electrodes, e.g., $Cu \mid CuSO_4$ electrodes in conjunction with agar plugs as bridges, were used. It might be added that with the rather dense membranes utilized in these model experiments the electroosmotic water transport which did occur was much too small to affect the experimental result to a detectable degree.

Table 1 presents some typical results obtained with models of the outlined nature. The sixth column gives the corrections for the leakage of electrolyte across the membranes during the experimental period which were due to the deviation from ideality of the selectivities of the membranes used. Typical data of the equivalents of electrolyte and of electricity moved in a variety of experiments are given in the last two columns

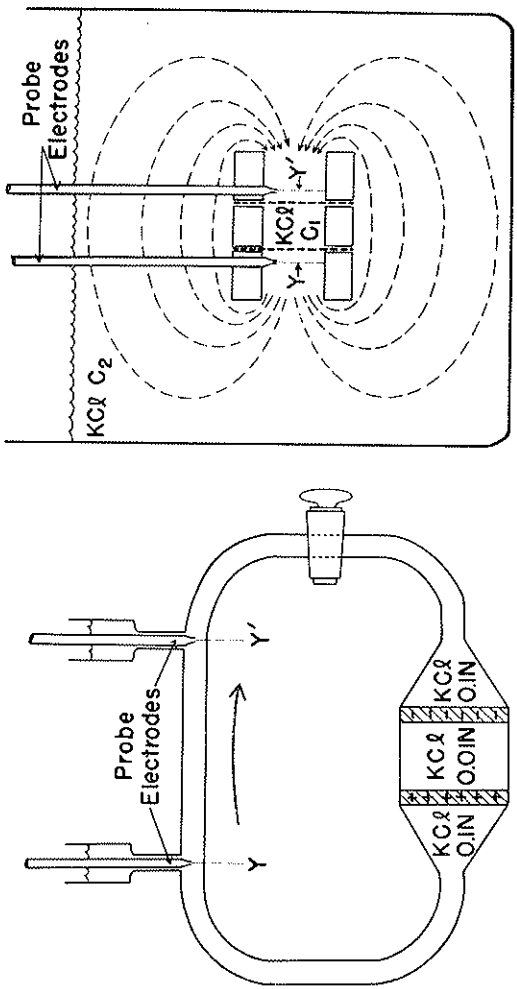

FIG. 5 — All-electrolytic mosaic membrane models. *a*) A schematic model. *b*) The experimental model.

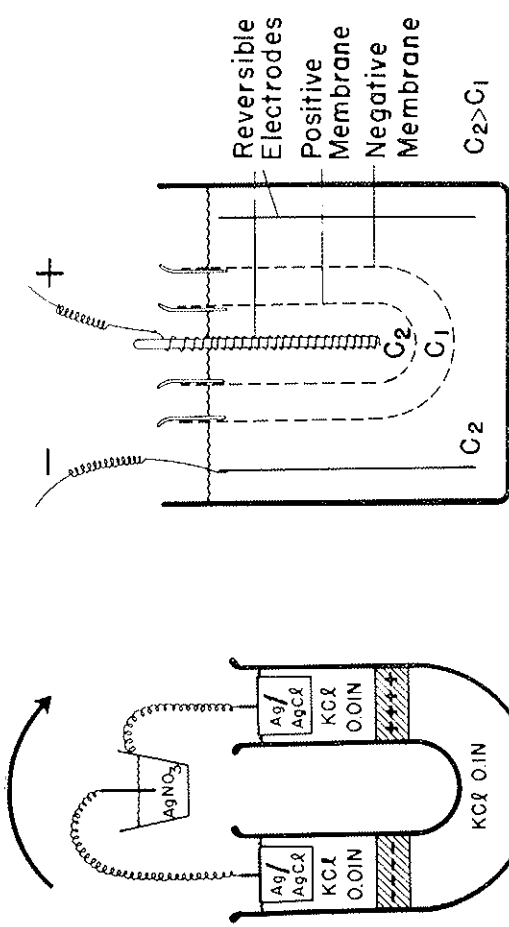

FIG. 6 — Mosaic membrane models with auxiliary electrodes through which the current flows. *a*) A schematic model. *b*) The experimental model (simplified).

of Table 1. In view of the experimental difficulties encountered in these experiments, the agreement between these two sets of data seems more than satisfactory and does not require any further comment.

IV. *A Physicochemical Cell Model which Simultaneously Accumulates Anions and Cations against Concentration Gradients*

The just-outlined successful experimental tests [26, 27] of the theory of charge-mosaic membranes by means of macro-models was a strong incentive to speculate, and theorize on the physicochemical behavior of other conceivable mosaic membrane systems. This was the more attractive since it was now possible to test any new idea without delay with the new type membranes of formerly unattainable degrees of electrochemical activity [21-24].

One obviously attractive problem for such theorizing was the functional electrochemical behavior of charge-mosaic structures composed of ideally cation- and ideally anion-selective membranes in which the two membranes do not interact electrically with each other, although they are interposed between the same two solutions. Considerations along these lines led, as had been hoped, to the idea of a macro-two-membrane system which should achieve the simultaneous accumulation of anions and cations against concentration gradients, a process ubiquitous *in vivo* but never achieved in the laboratory.

The essence of this idea of an accumulation model is based on the predictable functional behavior of a novel, clearly defined macro-model membrane system whose functional parts are an ideally cation-selective and an ideally anion-selective membrane, across which, under proper conditions, *two* Donnan equilibria are established, one with respect to cations, the other with respect to anions [28].

TABLE 1 — *A Comparison of the Quantities of Electrolyte and of Electricity Moved in Mosaic Membrane Model Systems which are Composed of Anion- and Cation-Selective Parts.*

Electrolyte Solutions		Electrodes	Volume of dilute solution	Increase in concentration of dilute solution	Correction for leak	Equivalents of electrolyte moved equiv. X	Quantity of electricity moved F X
Electrolyte used	Concentrations on closing of circuit						
	equiv./liter		ml	equiv./liter	equiv./liter	10^{-6}	10^{-6}
KCl	0.050/0.001554	None	19.00	0.001308	0.000046	24.0	24.6
LiCl	0.050/0.004898	None	19.00	0.000657	0.000009	12.3	12.2
KIO_3	0.050/0.004938	None	19.00	0.000435	0.000019	7.90	8.08
KCl	0.050/0.00521	Ag|AgCl	70.0	0.00116	None	81.2	82.2
KCl	0.100/0.01050	Ag|AgCl	65.0	0.00105	0.00008	63.1	61.8
KCl	0.050/0.001500	Cu|$CuSO_4$-agar	80.0	0.000420	None	33.6	33.5
K_2SO_4	0.050/0.001681	Cu|$CuSO_4$-agar	74.0	0.000417	0.000064	26.1	25.7

We consider first a simple system in which a cation-permeable, anion-impermeable membrane separates an "inside" solution which is kept constant at 1×10^{-3} *N* with respect to H^+ by the continuous addition of nitric acid of high concentration (which represents the continuously developing metabolite in the living cell) and an "outside" solution which is maintained, best by a flow arrangement, at 1×10^{-5} *N* with respect to HNO_3 and 1×10^{-3} *N* with respect to NH_4Cl. In this system H^+ will exchange against NH_4^+ until the Donnan membrane equilibrium with respect to these ions is reached:

$$\frac{[H^+]_{in}}{[H^+]_{out}} = \frac{[NH_4^+]_{in}}{[NH_4^+]_{out}} = \frac{10^{-3}}{10^{-5}} = \frac{100}{1}, \qquad (1)$$

when the concentration of NH_4^+ ions in the inside solution will be 1×10^{-1} *N*. The counter-ions of the NH_4^+ ions are the NO_3^- ions which were added continuously to the system in the form of HNO_3 while the H^+ ions exchanged across the membrane for NH_4^+ ions.

Next we consider an analogous system in which an exclusively anion-permeable membrane is interposed between an "inside" solution, the concentration of which with respect to NO_3^- is kept constant at 1×10^{-3} *N* by the addition of HNO_3, and an "outside" solution which, as in the preceding case, is maintained at 1×10^{-5} *N* with respect to HNO_3, and at 1×10^{-3} *N* with respect to NH_4Cl. After the Donnan equilibrium with respect to the anions is reached we find:

$$\frac{[NO_3^-]_{in}}{[NO_3^-]_{out}} = \frac{[Cl^-]_{in}}{[Cl^-]_{out}} = \frac{10^{-3}}{10^{-5}} = \frac{100}{1}; \qquad (2)$$

the concentration of Cl^- in the inside solution will be 1×10^{-1} *N*, with an equivalent amount of (nondiffusible) H^+ ions being present to maintain electroneutrality.

These two membrane equilibria are shown in Figure 7 in which a cation permeable and an anion permeable membrane

are located next to each other in the same outside solution of constant composition.

If the two "inside" solutions of Figure 7 were now connected by means of an electrolytic bridge, a closed all-electrolytic circuit would be established. The system would now be composed of two concentration cells arranged in series in a closed circuit; it would be closely similar to the above discussed mosaic systems of Figure 4c. However in the present case the system would not discharge to an equilibrium state, but a stationary state would be reached in which the rate of accumulation of electrolyte and the rate of discharge balance. Qualitatively it is obvious that the lower the electrical resistance of the short-circuiting pathways of the current through the solutions, the lower must be the degree of electrolyte accumulation which is obtained in the stationary state.

Finally we consider a system with the same two membranes, shown in Figure 8, in which the two inside solutions are continuously mixed mechanically and thus maintained at the same concentration with respect to all their ionic constituents while at the same time no current can flow in the system. This may be achieved, as is indicated in Figure 8, by means of a pump which moves liquid in a manner which prevents a direct electrical contact of the liquids inside the two membranes while in the return flow the same is achieved by letting the liquid drop back into the left compartment.

In the model of Figure 8 the two membranes are inserted side by side in the same outside solution which is kept continuously 1×10^{-5} N with respect to NHO_3 and 1×10^{-3} N with respect to NH_4Cl. The solution inside the two membranes in kept 1×10^{-3} N with respect to both H^+ and NO_3^- ions. (For the sake of simplicity we assume here that the rates of exchange of the cations and of the anions across the two membranes are the same.)

In the system of Figure 8, H^+ ions will continuously exchange across the cation-permeable membrane for NH_4^+ ions

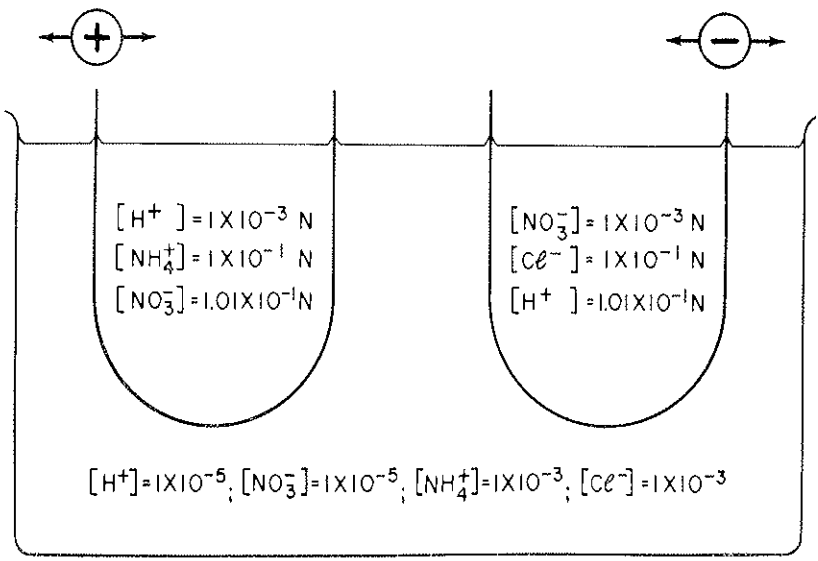

FIG. 7 — The two Gibbs-Donnan membrane equilibria with the same "outside" solution across an exclusively cation-permeable membrane (left side) and across an exclusively anion-permeable membrane (right side).

until the Donnan equilibrium with respect to the cations, defined by Equation 1, is reached; similarly, NO_3^- ions will exchange for Cl^- ions across the anion-permeable membrane until Equation 2 is satisfied. The final equilibrium state of our system is shown in Figure 8. Both the NH_4^+ and the Cl^- ions are accumulated one hundred-fold, up to 10^{-1} *N*, from 10^{-3} *N* in the outside solution.

Using permselective collodion matrix membranes [21-24] in model systems essentially identical with that shown in Figure 8 [28], electrolyte accumulations up to 22 times the outside concentration were observed by Dr. Rex Neihof. In unpublished, later experiments with somewhat improved membranes and using a refined experimental technique up to 35-fold accumulation was obtained. The calculated equilibrium

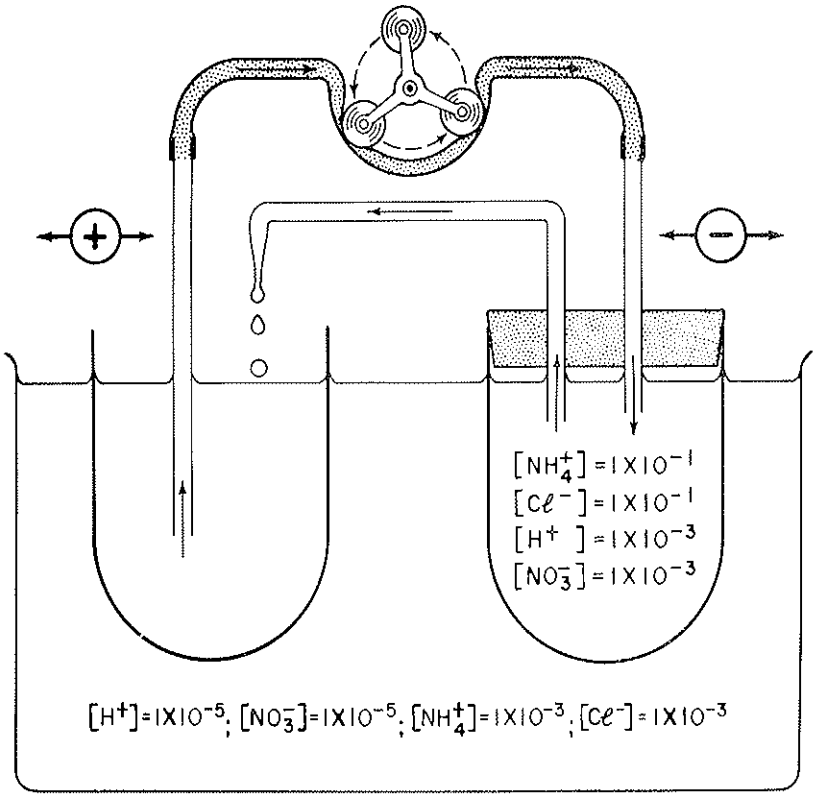

FIG. 8 — The simultaneous accumulation of cations and anions against concentration gradients by the combination of an exclusively cation-permeable permselective membrane and an exclusively anion-permeable permselective membrane in a system in which the solutions in the two "inside" compartments are mixed mechanically but are separated from each other electrically. The concentrations of the ions in the inside solution are the equilibrium concentration calculated for ideal leakage-free systems.

ratio of accumulation has not yet been reached in full, undoubtedly because of the back-leakage of the nominally impermeable counter-ions across the two membranes which increases greatly as the degree of electrolyte accumulation increases. Ultimately a stationary state is reached in the experimental

models, in our experiments at accumulation ratios of up to 35:1 instead of the 100:1 ratio, predicted hy the theory for systems with membranes of ideal ionic selectivity.

It might be added that in an accumulation system with two or more species of accumulating ions of the same charge, while it drifts towards the steady state condition, transitory degrees of accumulation of the one or the other species of ions are feasible which exceed those described by the Donnan equation [29].

V. *Anomalous Osmosis Revisited; Electrosmosis in Charge-Mosaic Membranes*

Although the ahove outlined theory of the mechanism of anomalous osmosis and the verifying model experiments [9, 12, 13] seemed to have settled this matter 45 years ago, at least from the physicochemical point of view, the question of the importance of anomalous osmosis in transport processes *in vivo* was, and still is, wide open and the subject of steadily increasing interests among physiologists. For this reason Dr. M. B. VISSCHER of the Department of Physiology at the University of Minnesota suggested shortly before World War II that the author should renew his interest in the physical chemistry of membranes and invited him to start a research program on the electrochemistry of membranes, particularly on anomalous osmosis.

Right at the beginning of this project it was observed that membranes cast from solutions of commercial collodion preparations consistently yielded much smaller anomalous osmotic effects than those reported 20 odd years earlier by LOEB [8] and seen ten years before by the author [9].

The inability to reproduce fully the earlier observations was very disturbing in itself, and more so because the ability to produce very large anomalous osmotic effects would have been a great help in the planned investigation.

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It was originally for this purpose that we started the development of the previously mentioned methods of preparing membranes of high electrochemical activity, that means high charge density membranes [21-25]. First we prepared negative, preferentially cation permeable collodion membranes of high electrochemical activity having virtually any desired degree of porosity, namely, by the oxidation of membranes cast from the available commercial collodion preparations [21]. These "oxidized collodion membranes" yielded anomalous osmotic pressure rises 4 to 5 times higher than those observed by LOEB; the largest pressure rises occurred at somewhat higher concentrations than in LOEB'S experiments.

Later, we also prepared a new type of excellent, very stable electropositive membranes by the irreversible adsorption of protamine on preformed collodion membranes [22]. These "protamine collodion membranes" were the preferentially anion selective analogues of the oxidized collodion membranes. Protaminized membranes of the proper porosity yielded anomalous osmotic pressure rises of the same magnitudes as the oxidized collodion membranes. The protamine collodion membranes also gave unexpectedly large negative osmoses which were of the same magnitude as LOEB'S positive anomalous osmotic effects. The most pronounced negative osmoses occurred at unexpectedly high concentrations, in the range of 0.5 to 1.5 *M*.

This and related later work [21-24] on the preparation of electrochemically highly active membranes of high porosity were the necessary precursors of the systematic studies on anomalous osmosis reported below. It also led to the preparation of the "permselective" membranes which were employed in the above described experimental test of the theory of mosaic membranes [26, 27]. The use of permselective membranes in various physicochemical studies, including their use as membrane electrodes has been reviewed elsewhere [30].

In the course of our experimental studies on anomalous osmosis with the new types of electrochemical highly active

membranes we began to realize that the conventional method of estimating the magnitude of the anomalous effects by measuring pressure rises after a stated period, and comparing these pressure rises with those produced by an arbitrarily chosen nonelectrolytic reference substance, was deficient on three major scores. First, the conventional systems are poorly defined. One starts with a solution dialyzing against pure water which in the course of the experiment becomes a solution of ever increasing concentration. The dynamic membrane potentials arising under these conditions decrease rapidly particularly in the initial period. This makes such experimental systems a difficult subject for a detailed electrochemical analysis. Second, pressure rises in a given period are not a true measure of the anomalous osmotic water movement that occurs under a given set of conditions since, with the rather highly porous membranes used, back filtration plays an increasingly disturbing role as the absolute pressures increase. With the pressure rises obtained with our activated membranes, greater than 1000 mm of water, back filtration is rather significant. The third shortcoming of the conventional method of determining anomalous osmosis is the reliance on the osmotic efficacy of an arbitrarily chosen nonelectrolyte reference substance. This is obvious from Figure 9 which shows osmotic water pressure rises with nonelectrolytes after twenty minutes obtained with one of our membranes used routinely in experiments on anomalous osmosis [31].

Once these three shortcomings of the conventional way of measuring anomalous osmosis are clearly understood the remedies in the first two instances are obvious and easy.

First, anomalous osmosis should be studied in systems with solutions whose concentrations and concentration ratios remain reasonably constant during the experimental periods. This can be achieved by using solutions with low, e.g., 2:1 concentration ratios. This also would have the additional advantage that such systems are more akin to those *in vivo* than those in which the concentration ratios are very high.

The second of the conventional experimental shortcomings can be minimized by determining transport rates against very small constant pressures. In this case the influence of back filtration is minimized and moreover can be accounted for accurately. This change in technique would also yield results of higher biophysical interest, since liquid movements *in vivo* occur ordinarily against small pressure differences.

The third of the enumerated shortcomings, the coice of a proper reference substance for each electrolyte, was resolved by an, at first sight, improbable looking technique, namely, the use of each electrolyte as its own reference solute.

All existing theories of the mechanism of anomalous osmosis indicated that this end could be accomplished by abolishing the electrokinetic charge of the membrane, so that even with electrolytes only normal osmotic effects could occur. In other words, the ideal model of an ordinarily charged membrane is the self-same membrane in the isoelectric, on the macroscopic level uncharged state. Accordingly, the contribution of anomalous osmosis towards the over-all osmotic effect with a membrane in a charged state would be the difference between the osmotic effects with the same membranes in the charged and in the isoelectric state. The change of the membrane from the charged to the uncharged state, or *vice versa,* should occur without any essential change in the geometrical structure of the membrane.

For the preparation of membranes of this type the material of choice was oxyhemoglobin with an isoelectric point of 6.75. With oxyhemoglobin as the membrane activating material, the osmotic effects with the membrane in the isoelectric state can be determined with solutions which require only the most minute additions of acid or alkali to adjust them to the proper pH; more important, the membrane will be positively or negatively charged in solutions containing acid or alkali of such low concentrations that the osmotic effects due to these additives will be insignificant.

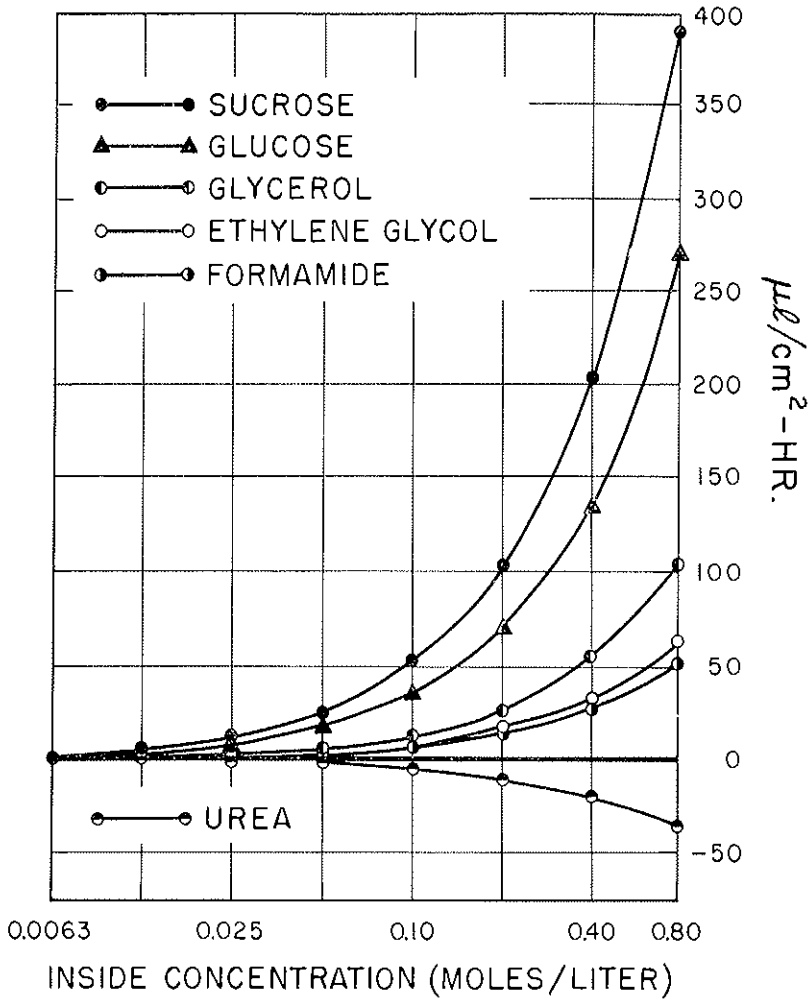

FIG. 9 — The rates of movement of liquid across a collodion membrane of medium porosity separating solutions of several non-electrolytes at different concentrations from distilled water.

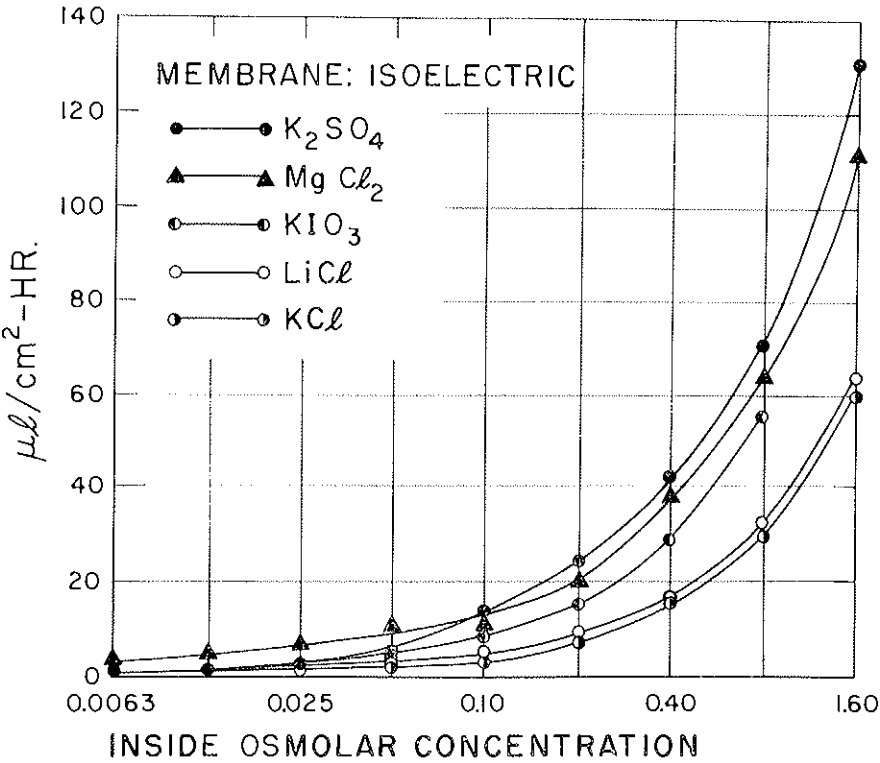

FIG. 10 — Rates of movement of liquid across an oxyhemoglobin membrane of medium porosity in the isoelectric state separating solutions of various electrolytes with concentration ratios of 2:1. (The indicated concentrations refer to the more concentrated solutions.)

Here is not the place to present the details of the experimental work on anomalous osmosis which was based essentially on the outlined considerations [31]. It must suffice to present here the results of this work in a series of self-explanatory figures, Figures 10 to 13. The crosshatched areas in Figure 13 indicate the range of (osmolar) concentrations of particular interest in mammalian physiology.

From the physiological point of view it is of considerable

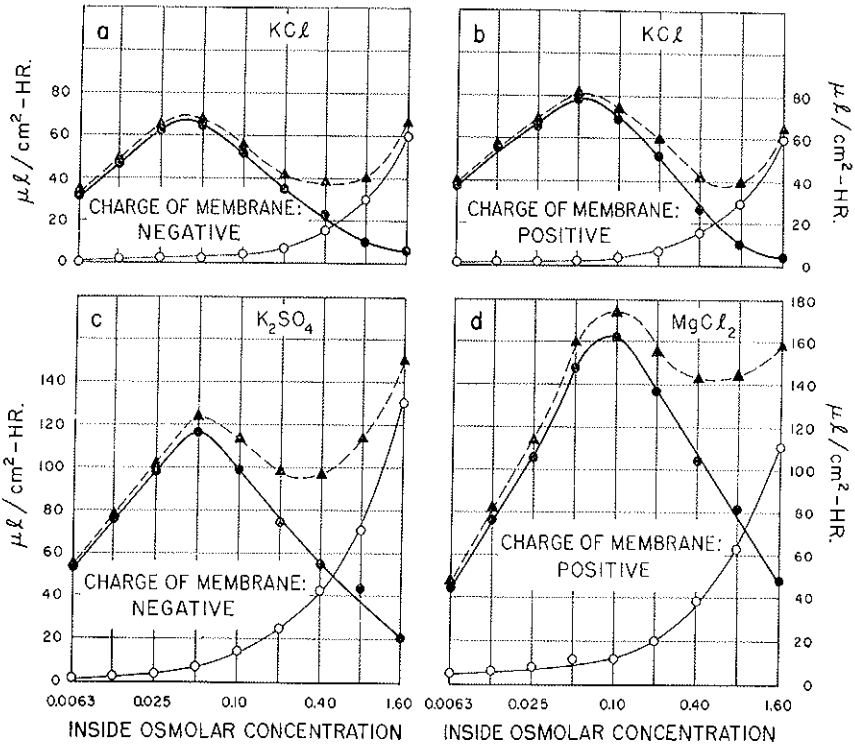

FIG. 11 — Rates of movement of liquid across an oxyhemoglobin membrane of medium porosity in the isoelectric and in a charged state separating solutions of an electrolyte with a concentration ratio of 2:1. Four instances in which anomalous osmosis is positive at all concentrations; —O—, membrane in the isoelectric state; —▲—, membrane in a charged state; —●—, true anomalous osmosis.

significance that fairly large true anomalous osmotic effects occur in the range of concentrations of mammalian body fluids, as is evident from Figure 13. At concentration levels below those occurring in the mammalian organism, anomalous osmosis is an even more effective mechanism, a fact which might be of particular interest in plant physiology.

Of greater specific interest for mammalian physiology, however, than the just discussed single-solute systems with 2:1 concentration ratios are systems containing more than one

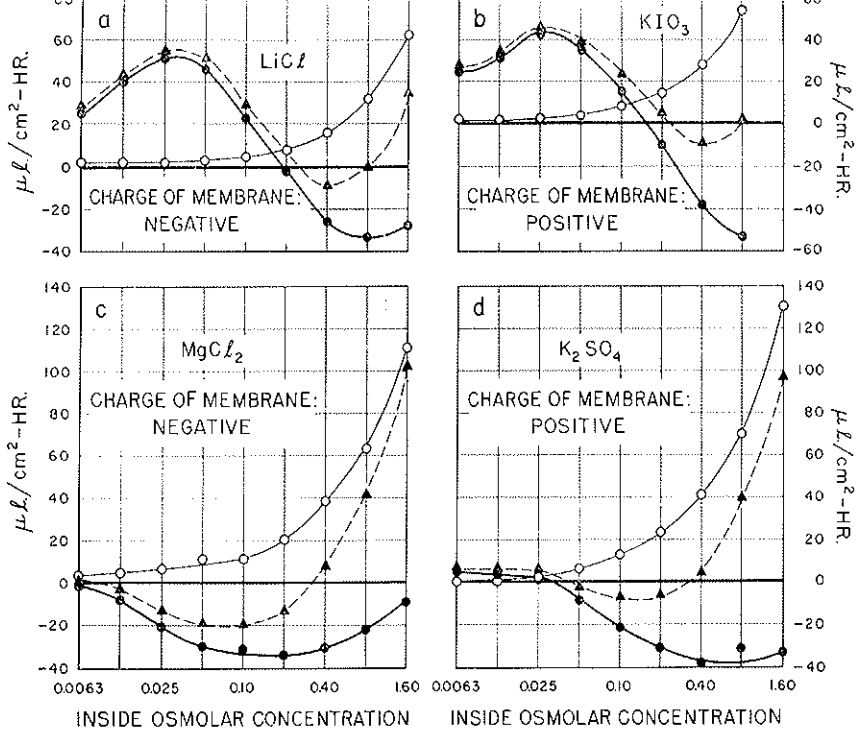

FIG. 12 — Rates of movement of liquid across an oxyhemoglobin membrane of medium porosity in the isoelectric and in a charged state separating solutions of an electrolyte with a concentration ratio of 2:1. Four instances in which anomalous osmosis is negative in the whole or in a part of the tested range of concentrations. —○—, membrane in the isoelectric state; —▲—, membrane in a charged state; —●—, true anomalous osmosis.

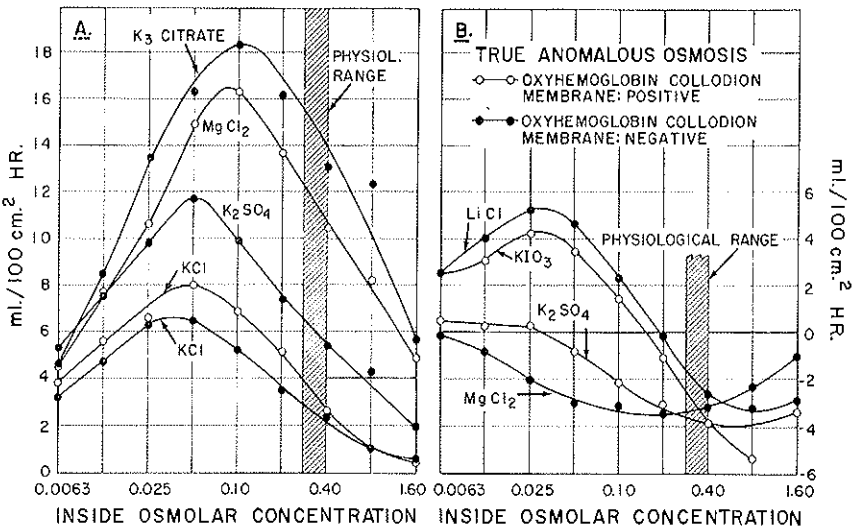

FIG. 13 — True anomalous osmosis across an oxyhemoglobin membrane of medium porosity. Fig. 13a, five instances of electrolytes with which the true anomalous osmosis is positive at all concentrations; Fig. 13b, four instances of electrolytes with which the true anomalous osmosis is negative in the whole or in part of the tested range of concentrations.

solute, particularly systems of this nature in which two solutions of identical over-all concentration (in the physiological range) are separated by the membrane [32]. The data presented in Figures 10 to 13 make it possible to predict semiquantitatively the extent of anomalous osmosis which can be expected in a particular system with two or more solutes.

The rates of true anomalous osmosis in two-or-more-solute systems can be determined by measuring the liquid transport rates with the membrane in the charged and in the isoelectric state, in the same manner as was done in the single-solute systems of Figures 10-13.

Several experiments of this general nature with positive membranes and solutions of equal osmolar concentration are summarized in Table 2 [32]. The results with negative membranes, omitted here, are analogous. In Table 2 a movement of liquid from the outside to the inside solution is indicated by a plus sign; a movement in the opposite direction by a minus sign.

Table 2 shows that true anomalous osmosis occurs at a very considerable rate in systems with more than one solute. With iso-osmotic, 0.40-osmolar solutions of electrolytes, the true anomalous osmotic liquid transport rates found are in the range of 8 to 30 ml/100 cm^2-hr. If one of the solutes is a nonelectrolyte these rates are about twice as high.

The potential physiological significance of the data on transport rates given in Table 2 is apparent from the fact that these rates are, for instance, several times as high as the highest reported rates of intestinal absorption. They are of the same order of magnitude and in some cases considerably higher than the unidirectional rates of fluid movement which have been postulated as the physical basis of intestinal absorption. However, it must be emphasized that the foregoing data do not prove in any way that anomalous osmosis actually is an important mechanism in the translocation of liquid in the mammalian organism; it only furnishes the proof that such a process is a possibility from the strictly physicochemical point of view.

TABLE 2 — *Representative Rates of Transportation of Liquid and by True Anomalous Osmosis Across an Oxyhemoglobin Collodion Membrane in the Electropositive and in the Isoelectric State.*

Inside solution		Outside solution		Rates of transport of liquid Membrane		Δ True anomalous osmosis (ml/100 cm²-hr)
Solute	Concentration (osmolarity)	Solute	Concentration (osmolarity)	Charged (ml/100 cm²-hr)	Isoelectric (ml/100 cm²-hr)	
KCl	0.40	Glucose	0.40	+13.2	−7.0	+20.2
$MgCl_2$	0.20	Glucose	0.20	+70.0	+2.2	+67.8
$MgCl_2$	0.40	Glucose	0.40	+53.1	+1.7	+51.4
K_2SO_4	0.40	Glucose	0.40	−16.2	+0.8	−17.0
$MgCl_2$	0.40	KCl	0.40	+17.3	+3.4	+13.9
$MgCl_2$	0.40	KIO_3	0.40	+31.2	+1.4	+29.8
$MgCl_2$	0.40	K_2SO_4	0.40	+15.4	−1.8	+17.2
$MgCl_2$ + glucose	0.20 0.20	Glucose	0.40	+58.5	+0.2	+58.3
$MgCl_2$ + KCl	0.20 0.20	KCl	0.40	+8.7	0.0	+8.7
$MgCl_2$ + KCl	0.20 0.20	K_2SO_4 + KIO	0.20 0.20	+12.1	−3.7	+15.8

We turn now again to some *macro-model studies on anomalous osmosis.*

The successful test of the theory of mosaic membranes composed of low porosity highly cation and highly anion selective parts by means of macro-model studies reviewed in a preceding section led in a straightforward manner to the problem of the electroosmotic movement of water in charge-mosaic membrane systems. Of particular interest were, of course, systems with widepored membranes with which the electroosmotic water transport per faraday is such larger than with narrowpored membranes (4). The question was not whether such an electroosmotic water transport would arise, but only its magnitude. Its occurrence could be predicted *a priori* without any doubt since electroosmosis is inevitably linked with the electromigration of ions across charged porous membranes. The anticipated effect would represent a new type of anomalous osmosis, anomalous osmosis at charge-mosaic membranes.

In itself an interesting problem in membrane physics, the question of an *electroosmotic water movement across charge-mosaic membranes* had for a prolonged period also been the subject of considerable biophysical speculation concerning the mechanisms of the apparently rather involved mass movement produced by membranes in living systems.

The obvious way to determine the magnitude of the predicted electroosmotic water movement in macro-models was the construction of an appropriately modified apparatus like that

(4) The electroosmotic efficacy of membranes of graded porosities was determined in some preliminary experiments by CARR, MCCLINTOCK and SOLLNER [33]. For instance in a series of polystyrene-sulfonic acid-collodion membranes, the least porous had a water content of 9 per cent by volume, the most porous a water content of 75 per cent. The electroosmotic transport of water through the most dense membranes was 3.2 moles per faraday with KCl, and 6.4 moles of water per faraday with LiCl. As the porosity of the membranes is increased, electroosmosis per faraday increases; with membranes of the porosity of dialyzing membranes, the values with KCl were 50 moles of water per faraday and with LiCl, 90 moles of water per faraday [33].

shown in Figure 6b with a high charge density positive low resistance membrane and a similar negative membrane as the functional component parts.

Space does not permit even a condensed review of the numerous considerations [34] which went into the construction of our charge-mosaic two-membrane macro-model, such as the optimum ranges of membrane porosities, the resistances of the electrolyte solutions to be used, and of the optimal concentrations and concentration ratios of the two solutions, etc. Suffice it to remark that sucrose was added to the more dilute solution at a concentration which approximately balanced the sum of osmotic and anomalous osmotic effects which arise on *open* circuit at the two membranes. The difference between the remaining moderate water movement on open circuit and the rate of liquid movement on *closed* circuit represents the electroosmotic effect due to the interaction of the two membranes on closed circuit. Figure 14 is a self-explanatory line drawing of the apparatus used; its similarity and functional identity with Figure 6b is obvious.

With this apparatus we studied a variety of systems on open and on closed circuit. The protocol of a representative experimental run is given in Table 3. The day to day reproducibility of such experiments was $\pm$ 20 %.

Table 4 summarizes the results of experiments in systems with several electrolytes and polystyrene sulfonic acid collodion matrix membranes of different porosities, but with the same permselective protamine collodion membrane.

To determine approximately the effective resistance of the mosaic system on closed circuit, a decade resistance box was added to the circuit. In a system with Ag | AgI electrodes and a PSSA membrane of 55 % water content, the current, without added resistance, was 13 ma. As the resistance was increased by known increments, the corresponding drop in current was recorded. The resistance of the original system without added resistance was then calculated to be 14Ω, and 9Ω with the milliammeter removed from the circuit. The electro-

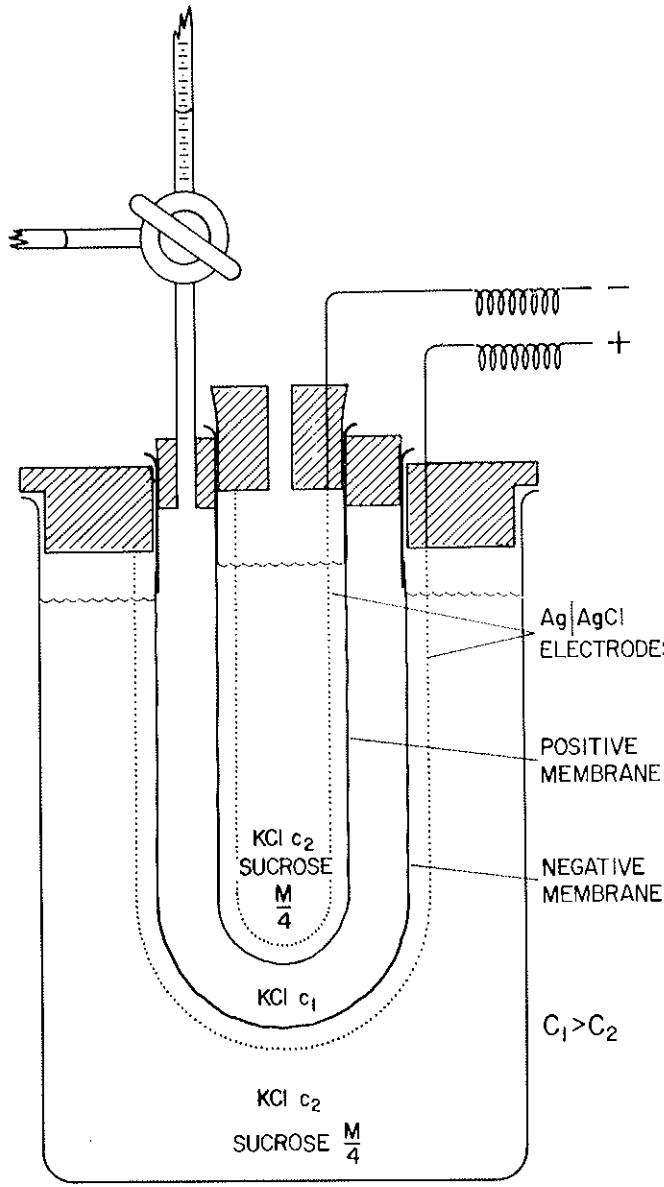

FIG. 14 — Apparatus used for the measurement of the electroosmotic effects arising from the interaction of a selectively anion- and a selectively cation-permeable membrane (schematic).

TABLE 3 — *The Volume Effects on Open and Closed Circuit in the System Ag |AgCl | 0.002 M KCl, 0.25 M Sucrose | wide pored PSSA Membrane | 0.2 M KCl | Permselective Protamine-Collodion Membrane* (*) *| 0.002 M KCl, 0.25 M Sucrose | AgCl | Ag.*

Time interval on open circuit	Volume change	Time interval on closed circuit	Current	Volume change
min.	µl (**)	min.	ma	µl (**)
5	−18			
		5	10.0	−32
5	−19			
		5	9.5	−29
5	−16			
		5	9.0	−28
5	−17			
20	−70	15	9.5 (mean)	−89

$\frac{-70}{20} = -3.5$ µl/min. $\qquad \frac{-89}{15} = -5.9$ µl/min.

Electroosmosis = −5.9 + 3.5 = −2.4 µl/min.
= 0.133 mmole of water/min. − 9.5 ma
= 23 moles of water/faraday

(*) Permselective membrane, resistance = 10 ohms-cm² in 0.1 *M* KCl.

(**) The minus sign indicates movement of liquid from the concentrated solution to the dilute solutions.

osmotic water transport under these conditions, due to the increased current, was now 3.4 µl/min., as compared to 2.5 µl/min. with the milliammeter in the circuit [34].

In extrapolating from the results obtained with the macro-model systems to the effects to be expected with true mosaics, i.e. in microsystems, one basic feature of the macro-systems must be stressed, namely, the distances over which the two membranes interact. While the potentials which arise across the two membranes (with given solutions) are independent of

TABLE 4 — *Electroosmotic Effects in the Mosaic System Ag | AgX | 0.002* M *Electrolyte, 0.25* M *Sucrose | wide pored PSSA Membrane | 0.2* M *Electrolyte | Permselective Protamine-Collodion Membrane* (*) *| 0.002* M *Electrolyte, 0.25* M *Sucrose | AgX | Ag.*

Water content of PSSA Membrane	Electrolyte	Number of experiments	Electroosmosis
vol. per cent			moles of water/faraday
15	KCl	2	16
30	KCl	7	23
45	KCl	6	34
55	KCl	5	42
30	NaI	3	29
45	NaI	3	43
55	NaI	2	47
30	LiCl	2	32
30	LiI	5	33
55	LiI	1	60

(*) Permselective membrane, resistance = 10 ohms-cm² in 0.1 *M* KCl.

the size of the membranes, the intensity of the current is determined by the sum of tbe resistances of all the parts of the circuit or circuits through which the current flows.

In *true micro-mosaic membranes* the electronegative and electropositive parts of the membrane are separated from each other by small, microscopic or even submicroscopic distances. Accordingly, the pathways of the current through the solutions will be of the same order of magnitude as the dimensions of the individual microelements of the membrane, and the sum of all the local microcurrents and the total current density will be large.

In *macro-model systems* of the type shown in Figure 14 the length of the pathway of the current through the solutions is of the order of at least several millimeters. Thus one may conclude that at micro-mosaic membranes the local currents could easily be greater by two orders of magnitude, or more, than those measured in our macro-models [34]. This seems to indicate that under the appropriate conditions anomalous osmotic water transport effects across micro-charge-mosaic membranes *in vivo* might be of physiological significance. Whether or not water transport processes of this nature are de facto of importance *in vivo* seems still to be an open question.

The successful utilization of auxiliary electrodes in the study of sign-of-charge-mosaic membrane model systems [34] indicated that the same approach should also be tried in the case of *pore-width mosaic macro-models* [35]. Experiments of this nature would constitute an extension with an improved technique of the work on pore-width macro-model systems described many years earlier [13].

To measure the magnitude of this effect with the aid of Ag | AgCl electrodes, two membranes of the same charge but of different porosities are used in an arrangement analogous to that shown in Figure 14:

Ag|AgCl	Dilute electrolyte solution	Dense membrane	Con-centrated electrolyte solution	Membrane of high porosity	Dilute electrolyte solution	AgCl|Ag,

the arrow indicating the flow of the positive current when electronegative, preferentially cation permeable membranes were used [35].

In such "pore-width" mosaic model systems, CARR and SOLLNER [35] determined with a variety of solutions, the

rate of volume change of the more concentrated solution, first on open and then on closed circuit, measuring in the latter instance the quantity of electricity which flowed during the experimental periods. The difference between the two rates of water movement must be due to the flow of the current and represents the expected "anomalous" osmosis in these pore-width mosaic macro-models.

The membranes used were electronegative polystyrene sulfonic acid-collodion membranes [23]. The more dense membrane in all experiments was a "permselective" membrane permeable only to nonelectrolytes having molecular weights of less than 100. The more porous membranes were prepared by swelling permselective membranes in 97 per cent ethanol; they were permeable to nonelectrolytes with molecular weights up to about 100,000. The concentration potentials in cells 0.1 *M* / 0.01 *M* KCl or LiCl with the more dense membranes were at 25.0°C + 54.0 to + 55.0 mV. Across the more porous membranes these potentials were about + 10 to + 15 mV with KCl, and differed not much from zero with LiCl.

For use in the model experiments LiCl was the electrolyte of choice for two reasons: first, because Li^+ transports in electroosmosis more water per faraday than any other univalent cation [33]; second, because with LiCl solutions the difference between the E.M.F.s arising across the more dense and across the more porous membrane is higher than with any other uni-univalent electrolyte. The concentrations of the solutions were the same as those in the charge-mosaic models, 0.2 *M* and 0.002 *M* [34]. The more dilute solution, as in the charge-mosaic model, contained also sucrose of 0.25 *M* concentration to minimize the effect of normal osmosis.

In one typical experiment with such a "pore-width" mosaic model, a current of 1.3 milliamp was obtained; the concomitant electroosmotic flow was 34 moles of water per faraday into the compartment of the concentrated solution. In another test the current was 1.9 milliamp accompanied by an electroosmosis

of 42 moles of water per faraday [35]. These results give additional support to the concept that anomalous osmosis through ordinary membranes is due to heteroporosity. In a true micromosaic the differences in pore size may be considerably less than in our model; this would reduce the electroosmotic effect; however, the linear pathways of the current in micro-mosaic systems are likely to be smaller by two or more orders of magnitude, so that the sum of the local currents would be correspondingly higher and therefore also the total concomitant water transport [35]. As in the case of the above discussed charge-mosaic membranes, the potential biophysical significance of electroosmotic water transport through pore-width mosaic membranes is still an open question.

VI. *Some Current Studies on Macro-Mosaic Membrane Model Systems with Liquid Ion Exchanger Membranes* (*)

The mosaic membranes treated in the preceding Sections were without exception porous structures of ion exchanger character, though this latter fact was originally not clearly understood. Likewise, the mosaic model systems described above consisted of porous membranes of ion exchanger nature, differing in pore width or in the sign of their charge.

In this concluding Section we shall briefly outline some current studies on several mosaic membrane macro-model systems in which at least one of the membranes consists of a liquid phase of ion exchanger character, that means models in which at least one of the membranes is a *liquid ion exchanger membrane*.

Since the physical chemistry of this type of membranes, aside from the fact of their use in various commercial membrane electrodes, is not yet widely known, the basic physical chemistry of liquid ion exchangers must here be outlined briefly.

(*) In cooperation with Dr. Gerald M. Shean.

HABER and KLEMENSIEWICZ [36] were the first to describe certain liquid membranes whose electromotive behavior in concentration cells indicated that they were of high ionic selectivity. However, the quantities of ions which permeated across these membranes were in most instances immeasurably low. BEUTNER, a former student of Haber, investigated the electromotive behavior of many liquids and accumulated much useful empirical information [37]. BEUTNER's studies together with OSTERHOUT's [38] work on liquid membranes of moderate ionic selectivity and fairly high ionic transmissivity have furnished much of the background information for the fairly recent development of "liquid ion exchanger membranes" of extreme ionic selectivity which are of interest in this section.

The older literature on liquid membranes in electrolytic systems has been critically reviewed by MICHAELIS in 1922 [39] who presented an amazingly clear and advanced critical analysis of the electrochemistry of liquid membranes. He emphasized the main physical factors which must be the basis of any theory of these membranes, particularly the distribution of the potential-determining ions between membrane and the adjacent solutions. On reading his presentation one has the feeling that MICHAELIS just missed using the term "ion exchanger" which 50 years ago was not yet widely known. More recently, the literature on liquid ion exchanger membranes was reviewed by KAHLWEIT [40] and by SOLLNER [41].

For many years it was obvious to the senior author that progress of the electrochemistry of liquid membranes would depend on the development of membranes which in many ways would be analogous to the "permselective" porous membranes, referred to repeatedly before, which combine extreme ionic selectivity with high transmissivity for the nominally permeable ions. What was required for the preparation of membranes of this nature were strong-acid and strong-base ionogenic compounds which in their various ionic forms are fairly soluble in water immiscible organic liquids, and only sparsely soluble

in water, in other words, materials with high distribution coefficients in favor of the organic phases.

The protracted but essentially futile search for such substances came to an end in 1958 when *liquid anion exchangers* became available commercially and enabled us to start a study of the basic physical chemistry of liquid anion exchanger membranes [42-45]. *Liquid cation exchangers,* being of much less industrial interest, became later also available, but the currently obtainable preparations still do not fulfill our requirements *in toto.*

Other investigators who have made early contributions to the physical chemistry of ion exchanger membranes include BOTRÉ and SCIBONA [47], BONNER and LUNNEY [48], THOMPSON and ROSS [49], and EISENMAN [50].

The currently used *liquid ion exchangers membranes* are solutions in water-insoluble organic solvents of substances consisting of an ionogenic group which is attached to an organic molecule of proper size and configuration to make these compounds, with molecular weight from about 300 to 600, very sparingly soluble in aqueous electrolyte solutions. Typical ion exchange compounds are for example the quaternary ammonium base trioctyl methyl ammonium hydroxide, available commercially in the chloride form, and the strong acid cation exchange compound dinonylnaphthalene sulfonic acid, a mixture of isomers.

The uptake by liquid exchangers of ions from adjacent solutions shows regularities strikingly similar to those observed with solid ion exchangers. Strong acid and strong base liquid exchangers readily exchange ions with neutral solutions; their degree of saturation with counter-ions is practically independent of the pH in a wide range round the neutral point. Weak base and weak acid liquid exchangers do not react significantly with neutral solutions of strong electrolytes. They do so only, when in their salt forms, in contact with slightly acid or slightly basic solutions respectively.

The molecules which carry the ionogenic groups diffuse freely within the liquid exchanger phases, in some instances probably in part in the form of micelles. The dielectric constants of liquid exchangers are low, with hydrocarbon solvents in the range of 2 to 3, with chlorinated solvents about 10 to 12, and about 35 with nitrobenzene and nitro-toluene. The *electric resistivities of the liquid exchangers* are very high, a definite proof that a very high degree of association exists between the functional groups of the liquid exchanger and their counter-ions. In other words, the counter-ions taken up from an aqueous solution by ion exchange, form in the organic phase essentially undissociated salts with the ion exchanger compound. In moving from the aqueous phase into the organic exchanger phase, and vice versa, the exchanging ions undergo a fundamental change in state, from hydrated to essentially unhydrated forms, and the inverse. Strong inorganic electrolytes as such are not dissolved to a detectable degree in most liquid exchangers, this means that there is no invasion of the exchanger phase by "non-exchange" electrolyte, contrary to the situation prevailing with solid exchangers (including porous permselective membranes). The water content of the liquid exchangers is minimal in most instances.

Liquid ion exchange membranes are best studied in cells in which the liquid membrane floats on (or lies below) two solutions which are separated by a glass wall. Our membranes were ordinarily solutions of ion exchange compound in benzene, toluene, o-xylene, o-dichlorobenzene, nitrobenzene, CCl_4, decane, etc. In transport experiments, both aqueous solutions as well as the membrane phase are stirred.

The first to describe the electromotive behavior of such liquid ion exchanger membranes in concentration cells were BOTRÉ and SCIBONA [47]; the slope of their potential versus log concentration curves, however, deviated widely from that predicted by the Nernst equation. With our membranes the *concentration potential* in cells of the type $2c_1$ KCl | liquid

membrane | c_1 KCl may reach the thermodynamically possible maximum values within a few tenths of a millivolt in a concentration range of several hundredths to several tenths normal [41-43].

Bi-ionic potentials in cells of the type A^+L^- c_1 | liquid anion exchange membrane | A^+M^- c_1, and their cationic analogs, show that an ion which is higher in the Hofmeister series than the other, impresses its charge on the other solution, as it does with porous permselective membranes. The numerical values of the ionic specificities which may be computed from these data [41-46], are of the same order as those observed with the porous permselective membranes.

The *rates of exchange of anions and cations,* and thereby the *ionic selectivities* were described elsewhere in detail [43-45], also *the influence of the concentration of the liquid ion exchanger* in the membrane phase and of the concentration of the aqueous solutions on the rates of exchange of anions across strong base anion exchange membranes. Most important in the present context is the fact that the cross-membrane transmissivities of liquid ion exchanger membranes for the permeable species of ions is larger by several orders of magnitude than that of porous ion exchanger membranes of the same resistances. With the liquid membranes the diffusive transmembrane movement of the permeable ions occurs while these ions are essentially in a bound, undissociated state. In this respect the situation with liquid ion exchanger membranes is profoundly different from that prevailing with porous ion exchange membranes. With the latter membranes, the cross-membrane movement of ions both by diffusion or in an electrical field occurs with the ions in the dissociated state. Here, the numerical correlation of these two processes is defined by the NERNST-EINSTEIN equation, as was shown recently [51]. With the liquid ion exchanger membranes the NERNST-EINSTEIN relationship by definition cannot hold true. Here the diffusive cross-membrane transportation of ions occurs essentially by a

physically entirely different process than the (additional) transportation of the sparsely present ions by an applied electric field.

It might be added here that contrary to the situation prevailing with the porous permselective membranes, the ionic selectivity of the liquid ion exchanger membranes does not decrease with increasing concentrations of the adjacent solutions, a result of the absence of "non-exchange electrolyte". At all but the lowest solution concentrations, the ionic selectivities of the liquid membranes exceed by one to several orders of magnitude those of even the most highly ion selective porous permselective membranes [21-25] referred to before.

After this disgression on the nature and main electrochemical properties of liquid ion exchanger membranes we turn now to some macro-mosaic membrane model systems in which liquid ion exchanger membranes are used.

We begin with the consideration of the theoretically anticipated electromotive and ion permeability properties of charge-mosaic membranes whose, say, anion permeable parts consist of a liquid ion exchanger of very high electrical resistance and a high transmissivity for anions, while their cation selective parts are of the nature of porous permselective membranes of moderate electrical resistance and a correspondingly moderate transmissivity for cations, as defined by the NERNST-EINSTEIN equation.

As in some previously discussed instances the physicochemical behavior of such liquid-porous charge-mosaics is most readily clarified by reference to a conceptual macro-model in which like membrane parts are assumed to be assembled in two macro-membranes, one liquid one porous, which are mounted side by side between the two experimental solutions.

What *electromotive behavior* is to *be expected from liquid-porous mosaic membranes or from two macro-membrane model systems* of the stated nature? We shall discuss here only the simplest possible case, concentration cells.

With respect to the diffusion of ions from the more concentrated to the more dilute solution the two membranes operate in parallel. From the electrical point of view, the model is a closed circuit system with two sources of E.M.F.s arranged in series and a current flows. The potential difference between any two points in the system is defined by KIRCHHOFF's law. The current which flows will be very weak because of the high resistance of the liquid ion exchanger membrane part of the circuit. For the same reason, the potential drops within the two solutions will be minimal, and the magnitude of the potential difference between the two solutions will approach, more or less closely, that which would arise if only the porous membrane were present, depending on the relative resistances of the several component parts of the system.

The electromotive behavior of such porous-liquid charge-mosaic models in more complicated situations, e.g., cells with solutions of two different electrolytes, three and four ionic cells, can be reasoned out readily in an analogous manner.

Preliminary, still unpublished experiments by the one of us (G.M.S.) strongly support the outlined reasoning.

We turn now briefly to the *permeability properties of porous-liquid charge-mosaic membranes* of the before outlined nature. Suppose such a membrane were mounted between two solutions of the same concentration of the electrolyte A^+B^-, with tracers A^{+*} and B^{-*} added to the one solution. According to our premises the rate of selfexchange of the anions, B^-, across the high transmissivity high resistance liquid ion exchanger membrane will be much higher than the rate of self-exchange of A^+ across the porous, negative membrane element of moderate resistance and relatively low cation transmissivity.

If we were to carry out the just described experiment with the same porous-liquid charge-mosaic membrane but with solutions of different concentration of the same electrolyte, i.e., in a concentration cell, the following can be predicted safely.

A comparison of the rates of selfexchange of anions and cations as determined by the tracers would indicate that the mosaic is preferentially anion permeable; at the same time, however, it would act electromotively as a preferentially cation permeable membrane. The measurable E.M.F. would approach the value of the E.M.F. which would arise if only the cation permeable membrane were present. Preliminary experiments by the one of us (G.M.S.) indicate the correctness of the outlined reasoning. This whole area needs much further exploration.

In concluding our discussion of mosaic membranes we return briefly to the problem of electrolyte accumulation against concentration gradients.

From the biological point of view, the accumulation model of Figure 8, wbich is based on the use of two porous permselective membranes, is rather unrealistic because its high resistance part is the "inside" solution which represents the cell sap, while the resistances of the two membranes are low. In a more realistic model this situation should be reserved.

The question arises how to design a model which on the one hand facilitates higb rates of cross-membrane exchange of ions, the process which leads to the accumulation of electrolyte in the "inside" solution, and, on the other hand minimizes the flow of a discharging current which counteracts accumulation. This end should be achieved without the use of some artificial, contrived arrangements, such as the high resistance pumping device shown in Figure 8.

The solution of this problem, as pointed out some time ago [45], was found in the use of the liquid ion exchange membranes which on the one hand have very high resistances, and on the other hand facilitate a rapid cross-membrane exchange of ions by an essentially nonionic process.

Figure 15 is a schematic drawing of an accumulation model of this type with two liquid highly ion-selective membranes, one being readily permeable to cations, the other one to anions. The functional identity of this model and that with two analo-

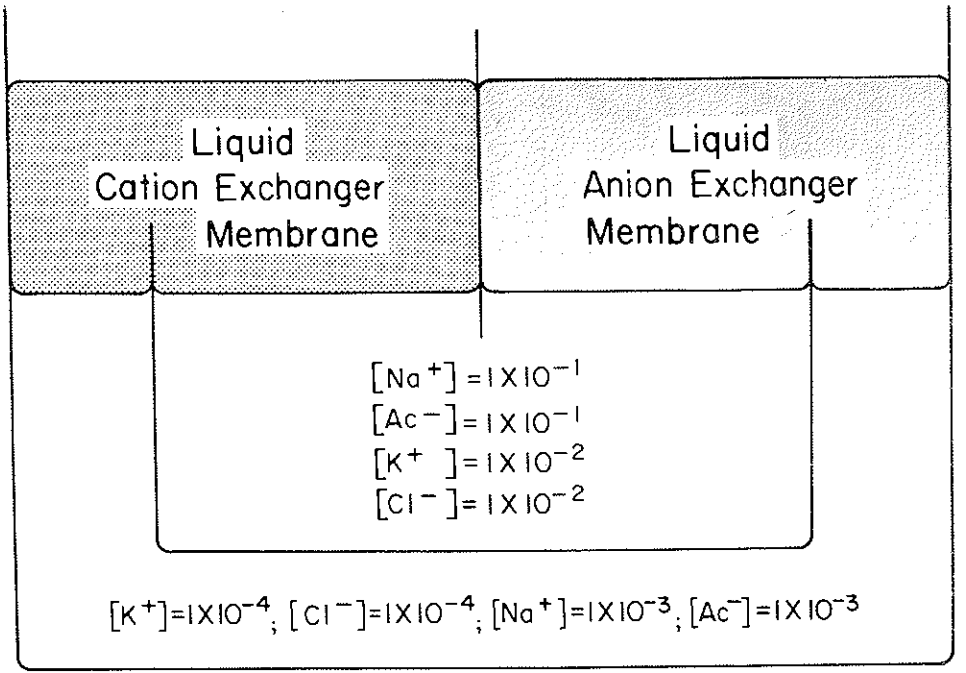

FIG. 15 — The simultaneous accumulation of cations and anions against concentration gradients in a system with two essentially nonconducting liquid membranes, one exclusively cation-permeable (left side) and the other exclusively anion-permeable (right side) in which ideally no discharging current flows. The concentrations of the ions in the inside solution are the equilibrium concentrations calculated for ideal leakage-free systems.

gous porous membranes shown in Figure 8 is obvious. In the model of Figure 15 only a very small current can flow because of the extremely low conductivity of the two liquid membranes, whereas in the model of Figure 8 the flow of current was prevented by the specially arranged circulation pump mechanism. In both models the cations and the anions exchange across the two membranes until, in theory, the degree of accumulation described by the Donnan equation is nearly fulfilled. The current, however weak, which flows in the model of Figure 15 together with the unavoidable leakiness of the two membranes are the main factors which prevent reaching the theoretical accumulation ratio fully.

The condensed protocol of a successful test experiment reads as follows: cation permeable membrane, 0.1 *N* dinonyl naphtalene sulfate salts in o-xylene; anion permeable membrane (about) 0.1 *N* Aliquat 336 (General Mills), consisting mainly of trioctyl methyl ammonium salts also dissolved in o-xylene; "inside" solution, 10^{-2} KCl, whose Cl^- ion concentration was monitored continuously and kept constant by means of a potentiostat and automatic injecting device; "outside" solution, 10^{-4} KCl and 10^{-3} NaAc, these concentrations being maintained constant by a copious flow of this solution through the "outside" compartment of our model.

For experimental reasons, the concentrations of the driving electrolyte, KCl, as the reader may note, both in the "inside" and the "outside" solutions were ten times higher than the corresponding concentrations of driving electrolyte, HCl, in the model of Figure 8; the ratio of the drivers, accordingly, was the same in the two instances.

Theoretically, both Na^+ and Ac^- should accumulate in the "inside" solution up to 0.1 *N*. Experimentally a stationary state was reached when the "inside" concentration of Na^+ was 0.028 *N* and that of Ac^- 0.032 *N*, in other words, when the Na^+ and Ac^- accumulations had reached 28% and 32% respectively of the value predicted by theory for an ideal, leakage

free system. Further work along this line is in progress; still higher degrees of accumulation should be obtained when better membranes, particularly better cation exchanger membranes become available.

In concluding, it may be added that there is no reason to doubt that accumulation models can be constructed in which the one membrane is, say, an exclusively anion-permeable liquid ion exchange membrane, and the other one an exclusively cation-permeable porous, permselective membrane (or *vice versa*). Such models would be just one more step forward into the still wide open field of basic studies on complex macromodel membrane systems.

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DISCUSSION

Chairman: Prof. TEORELL

TEORELL

Well, Professor SOLLNER, it was a fine presentation. As you mentioned, you get your inspiration from NERNST and other giants. Perhaps some people in the future will tell that they have been inspired by meeting you at the Vatican in 1975.

SOLLNER

Thank you very much Prof. TEORELL. I think you exaggerate. However, if anyone will mention me, he will not forget the work of my good friend TORSTEN TEORELL.

TEORELL

I invite the floor to comment on Dr. SOLLNER's presentation.

KEDEM

I am sure Professor SOLLNER is aware that the mosaic membranes he envisaged are now being investigated for desalination,

though not under the name of mosaic. The unusually high salt permeability of mosaics and the coupling to water, are used for desalting by negative, reverse osmosis. In reverse osmosis through mosaics filtration creates more concentrated salt solution. This process is currently called piezodialysis.

SOLLNER

Thank you for mentioning this. I really forgot only to mention that the work of Professor KEDEM and her associates is in my opinion currently the most productive work on membranes. They have a very good balance; from membranes to organic chemistry and polymer chemistry.

TEORELL

Any more questions? Professor MONNIER.

MONNIER

I feel that our young collaborators should sometimes believe their elders and believe what KARL SOLLNER has done in the years since his early days. I know he continues most actively his work. There is one point which should be stressed in his results. It is the very high diffusibility of electrolytes when separated by mosaic membranes, that is the extraordinary effect at the junction of the patches of cation and anion selective membrane. At these junctions the diffusion of an electrolyte is very intense. It is a very important phenomena in membrane biophysics.

SOLLNER

Yes, of course; thank you for mentioning this point. If you

have a mosaic membrane it can be very dense but the anions and cations go through the mosaic and inorganic molecules cannot go through.

TEORELL

Are there any more comments? Again, many thanks for your presentation.

THE FEED-BACK BETWEEN BIOLOGY AND MEMBRANE TECHNOLOGY

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Uppsala - Sweden

The early periods up to World War I

Experiments with membranes, earlier called " diaphragms ", began long before the biologists created the concept of cell membranes. Already in 1748 NOLLET made the observation that various solutions could pass a diaphragm at different speeds. He seems to have coined the term " osmosis " for this phenomenon. Experiments with the passage of electrical current through diaphragms were reported by REUSS in 1883, showing that the solvent was carried across diaphragms by an electrical current. The name for this phenomenon " electro-osmosis " was proposed by PORRET, 1816. It took some time before the first quantitative measurements of this phenomenon were made by WIEDEMANN in 1852. QUINCKE seems to be the first one who draw attention to the wall charges in the membrane pores. This was apparently the start of the history of fixed charge membranes, which is one of the focal points of this Conference. The experimental materials of these early days consisted of pig bladder, parchment or clay etc. The real membrane technology took its first steps with TRAUBE who in 1867 described the so called " precipitation membranes " of copper-ferrocyanide, deposited in clay cylinders. This type

of membrane was employed by PFEFFER for studies of the osmotic pressure of sugar solutions, utilizing the fact that these membranes were semipermeable, allowing only the solvent to pass the membrane; the sugar was retained. Some time later, 1887, VANT HOFF formulated his wellknown law, which was applied to semipermeable membranes. It is conceivable that these early men may have been partly inspired by the contemporary animal and plant physiologists, who had by that time introduced the cell membrane concept, mainly from physiological, microscopical observations. The physiologists brooded over the mechanisms which produced water transport, swelling and shrinkage of cells (" plasmolysis "). Another reason for their interest in membranes was certainly derived from the phenomenon of " animal electricity " which was first studied by GALVANI around 1790. Many theories as to the mechanism of observed " injury currents " and " negative Schwankungen " were discussed. It was not until the presentation of the electrolyte dissociation theory by ARRHENIUS in 1884 that the bioelectrical phenomena could be ascribed to ion diffusion processes (Arrhenius was only 25 years old when he presented this thesis). The theories of electrolytic membrane and boundary potentials by NERNST and PLANCK were readily accepted by the physiologists towards the turn of the century. On the other hand, the biologists had demonstrated that cell membranes were not a simple sieve or pore system, but they contained lipoids in which lipoid soluble material could be transported by " solubility " or " partition ". Now the interest among physical chemists turned from parchment and clay membranes to non-aqueous " oil membranes ". The popularity of the pore membranes and the solubility membranes has shifted over the years. At the present time, both types are accepted as representative for a cell membrane. The solubility concept has recently gained much attention due to new techniques of making ultrathin bimolecular membranes (" bilayers "). The dominating role in the model making of cell membranes has been played by the porous membrane materials. However,

the lipoid membrane theory or the solubility theory has remained popular among physiologists. OVERTON suggested that narcosis, for example by ether, was due to the solubility in cell membranes.

The period after 1914

In the early decades of this century the lipoid cell membrane concept was re-inforced by model experiments of which we only cite the names BEUTNER and COLLANDER. About this time another cell membrane model came into more common use, it was the collodion membranes. JAQUES LOEB at the famous Rockefeller Institute of New York gave an enormous impetus to the biology. He studied the behaviour of gelatin as a model for an animal colloid protein by the use of collodion membranes with regard to the influence by salts and hydrogen ion concentration. Above all, he introduced the Donnan equilibrium (published in 1911) into modern biology. Loeb's book " The Colloidal Behaviour of Proteins " (1921), had a profound influence on cell biology. As an anecdote of this " feed-back " it may be mentioned that the present author was once told by Professor Donnan, " I woke up one morning in 1921 and found myself famous due to Jaques Loeb ". DONNAN was a physical chemist in London and LOEB was a general physiologist. Another great impact on biology was made by MICHAELIS, also of the Rockefeller Institute, who used dried collodion membranes and studied the membrane potentials. MICHAELIS was aware of some earlier concepts of BETHE and TOROPOFF on acidity changes in membranes, but he was interested in the potential effects and placed the emphasis on the " fixierte Ladungen ". His publications in the " Kolloid Zeitschrift " gave much inspiration to physiologists who were concerned with the permeability riddles of the red blood cells. These cells show a remarkable selectivity for potassium ions and further studies on red cells have been

influenced by Michaelis early publications up to this very day. The present author, who at that time worked in biomedical laboratories, received much inspiration from Donnan's and Michaelis' papers. I was attracked by the problem of the formation of the gastric acid in mammals and man. This is the most conspicuous case of " ion accumulation ", a popular term in the thirties. The gastric acid formed in the stomach is about 1/10 normal hydrochloric acid (stomach juice pH can be near 1 or 2, while the blood plasma and cell has a pH around 7.6.). This is a case of an accumulation ratio of hydrogen ions of about one million! We did not solve the main problem, but as a spin-off we tried to reconcile Beutner's observation on the so called " concentration effect " (i.e. the membrane potential as dependent on the surrounding salt concentration ratios) with explanations offered by OSTERHOUT (known fo his studies on bioelectrical phenomena in plant cells) and MICHAELIS' concept of selective cation or anion permeability. Our experiments, performed on a presumably " old fashioned " cellophane, gave unexpectedly high transference numbers for the cations of different electrolytes tested. According to Osterhout-Michaelis this might be ascribed to a mobility ratio cation/anion different from that in hulk water. We attempted another line of explanation. Assuming that the membrane behaves as " its own electrolyte " owing to presence of fixed charges (assumed to arise from adsorption or dissociation), the question was put, how would these fixed charges influence the membrane potential if the mobility ratio of cations/anions were assumed to remain *unaltered* within the membrane? As the fixed charge in the membrane could be considered as a " colloid electrolyte " in Loeb's sense, we applied the Donnan equilibrium separately to each of the boundaries of the membrane. Indeed, it turned out to be a fruitful concept, because it gave immediately a good explanation for the above mentioned concentration effect. What the fixed charges in the membrane " did " was to alter the " concentration profiles " within the membrane. The theory was publish-

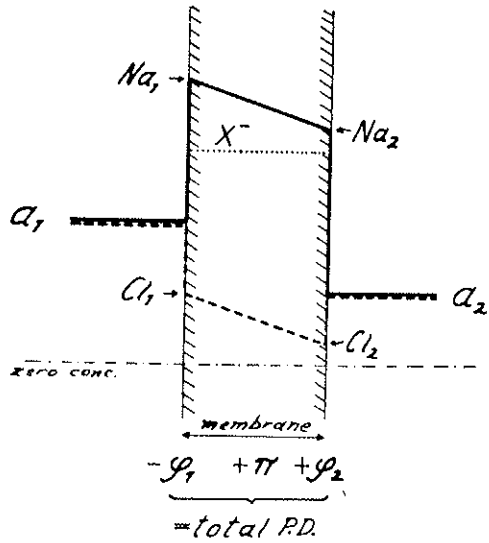


FIG. 1 — Consider a membrane which carries electrical charge. This charge may be due to an electrolyte character of the membrane substance itself, or the charge may be caused by "adsorption" or polar groups, etc. For simplicity we may represent the membrane by X^- behaving as an *immobile*, negative ion uniformly distributed within the membrane. Then NaCl is placed on both sides of the membrane. What will happen? The very beginning is hard to account for, but ultimately an ionic distribution according to the sketch may be attained. Two Donnan distributions are obtained, one at each phase boundary (where Na > Cl). At the same time two potential jumps appear, ϕ_1 and ϕ_2 (each being of the form $58 \cdot \log \frac{\mathrm{Na}_{\mathrm{membr.}}}{\mathrm{Na}_{\mathrm{bulk}}}$). Besides this two boundary potentials, there must arise a common diffusion potential, π. This can be easily calculated according to Henderson's formula, because all concentration gradients are likely to be linear. The complete formula for the total membrane P.D., *i.e.*, the "mixed" potential, is written

$$\text{Total P.D.} = 58 \left[\underbrace{\log \frac{a_1 \cdot \mathrm{Na}_2}{a_2 \cdot \mathrm{Na}_1}}_{\text{Donnan}} + \underbrace{\frac{u - v}{u + v} \log \frac{\mathrm{Na}_1(u+v) - X \cdot v}{\mathrm{Na}_2(u+v) - X \cdot v}}_{\text{Henderson}} \right]$$

TEORELL, T. 1937 (Gen. Discussion, Farad. Soc. *33*, 1054).

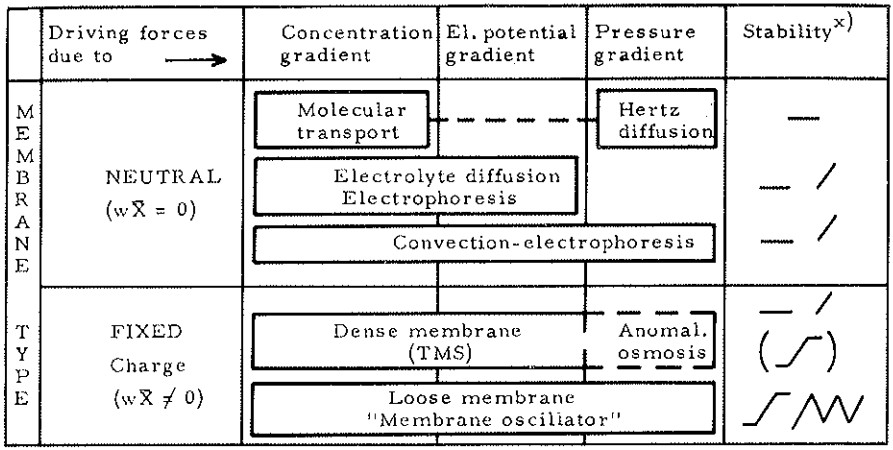
ed in its essence in 1935 and a similar concept was published the following year of 1936 by MEYER and SIEVERS at a great length. After the war Professor Meyer and I agreed to denote the concept " TMS ". At this occasion I should like to reproduce the first diagram, as far as I know, of the concentration profiles within a fixed charge membrane, Fig. 1. In 1951 (1953) the present author published a more complete description of this, admittedly simple model, which also included membrane conductance, rectification and other concepts which were relevant to neurobiology. In the mean time it had been clarified that the living membranes were not only sites for action potentials, they also showed equally conspicuous changes of ion conductance and in many cases a very pronounced electrical rectification. OSTERHOUT had taken up some older studies by European workers on the behavior of certain algae (Nitella, Chara, Valonia), which displayed large action potentials, of up to several hundred mV at a convenient speed for recording, about one spike/minute. He and his collaborators had also paid attention to the resistance change and his pupil BLINKS had observed electrical rectification in these plant cells. These observations became modestly recognized by the animal physiologists. However, they were awakened by K.S. COLE's beautiful demonstration in 1939 of the electrical impendance changes in an isolated nerve fiber from the inkfish, Loligo. This is a real giant fiber, up to 1 mm in diameter, and was conveniently accessible by the glass micro-electrodes, which were introduced about this time by PETERFI and BUCHTHAL in Europe and by GERARD and LING in the USA. One has to remember that in this period of the thirties the first vacuum-tube amplifiers and the new cathode oscilloscope had become available. All this technical advancements stimulated the study of bio-electrical potentials, particularly the nerve action potentials, enormously. With the wealth of miles of moving film recordings the biologists now could start to apply straight forward physical chemistry in their search for the mechanism of the nerve potentials. At this point intelligent physiologists realized the value of the

profound studies carried out by SOLLNER and his many collaborators on the elementary properties of model membranes which showed what Sollner called " perm-selectivity ". Sollner used the common membrane model, collodion. By chemical treatment or adsorption he could create a great variety of changes of the properties which affected the ion transport, the water transport and the electrical conductance. He devoted much attention to so called " bi-ionic " potentials (a system of the type KCl//NaCl with equal concentrations on both sides of the membrane). It is just this type of system which occur in biology (The K-ions are accumulated in the cells while the sodium ions remain outside of the cell membranes). Sollner's membrane technology, his definitions and his observations were now transformed into the biological realm. Just before and right after World War II a group of young neurophysiologists in Cambridge (HODGKIN, HUXLEY and KEYNES) went back to the Neernt equations for diffusion potential and succeeded in describing the nerve events in a somewhat extended Nernst-Planck formalism which was developed by GOLDMAN (1943). The Goldman equation applies to an extended bionic case and is one of the most frequently employed concepts also in the present treatments of the biological membrane events. It suffers, however, from one deficiency. The electrolyte behaviour of the membrane structure itself, the fixed charge contributions, are not explicitly present in the Hodgkin-Huxley-Goldman concepts. (It should be noted that Goldman in his paper considered the presence of fixed charges but he neglected the implications of it). This was perhaps a pity, because the acknowledgement of fixed charge influence in biological membranes was neglected for a long time. Only in the last decade have the neurophysiologists considered this concept. The spokesman for fixed charge in nerve membranes has been the Japanese-American I. TASAKI. The later followers of the Hodgkin group have, however, considered fixed charge layers as attached unsymmetrically to one side of an excitable membrane, perhaps a reasonable assumption.

The greatest stimulation of biological membrane research came from the introduction of isotope marking, which came into common use after World War II. One could now study transport kinetics of ions and of water in greater details. Isolated pieces of surviving frog skin became a fine object for the study on the in-and outflux relations by the USSING school in Copenhagen. KEYNES of the Cambridge nerve group could study the movements of Na and K during nerve actions. Similar kinetic studies were performed also on the classical membrane material collodion or cellophane. The results corroharated the soundness of the Nernst-Planck concepts that the driving force for ions was the gradient of the electrochemical potential.

A new era

Up till now we have mainly dealt with the feed-back between membrane *physicists* and biologists rather than the influence excerted by membrane *technology*. In fact, it was only about 1950 that the polymer chemists could manufacture new types of membranes which could be tailor-made with regard to porosity and fixed charge content. Extended studies, both experimental and theoretical, were now possible. The new polymer membranes could have a fixed charge density far larger than the Michaelis-Sollner membranes of the collodion type. The TMS hypothesis could now be put to a more severe test in the hands of many biophysicists of which I here only will mention the names of SCHLÖGL, SCHATCHARD, HELFFERICH, U. FRANCK and in particular MEARES. By and large the basic ideas of the TMS hold up but important extensions and corrections were made. In parallel with this technological advancement there was also the availability of a new approach to membrane transport phenomena by the *irreversible thermodynamics*. This new science was introduced by Belgium and Dutch theoreticists (PRIGOGINE, DE GROOT, STAVERMAN,



x) Stability conditions: _ / stable or monotonic time variation
∫∿ unstable (bistability or oscillations)

FIG. 2

OVERBEEK, MAZUR and others) and became known in the years after World War II (S. SPIEGLER employed it quite early). Again a feed-back occurred in the direction to biology, particularly with regard to osmotic phenomena. STAVERMAN'S " reflection coefficient " is now widely used in experiments with living membranes. The ONSAGER " cross coefficients " were perhaps not readily appreciated by biologists, but the new formulated laws for electrokinetic phenomena, e.g. electroosmosis and streaming potentials, could be tested on biological material. These included the earlier mentioned plant cells, the algae Chara and isolated gall bladder epithelium. It must be admitted, however, that these methods of probing the presence of fixed charges in living tissues have been less successful. Indirect methods based on chemical analyses of acid and basic amino residues give indications that the chemical constituents of living membranes may give a rise to a charged density of not less than 1 mole/liter. This is a figure which matches well with the fixed charge density in the artificial ion exchange

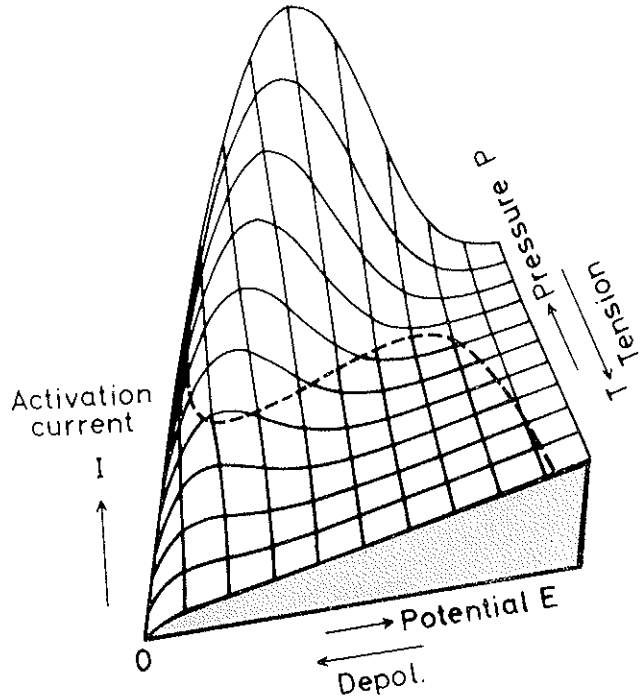


FIG. 3 — 3-dimensional representation of the parameters of the excitation-contraction theory (computer calculation). "The IEP-space".

membranes of the polymer type. At the present state purely chemical data indicate the presence of fixed charges in the biological membrane structures, but the dilemma is that their existence is difficult to prove. The present author has developed a membrane model which displays rhythmical potential oscillations and conductivity changes, which in many respects are similar to the ones which can be observed in nerve membranes or heart muscle cells, the "membrane oscillator" (cf. Fig. 2). It is built up around a porous fixed charge membrane which gives rise to electroosmosis. Without going into details some pictures will be presented which may demonstrate the model's capability of simulating features of

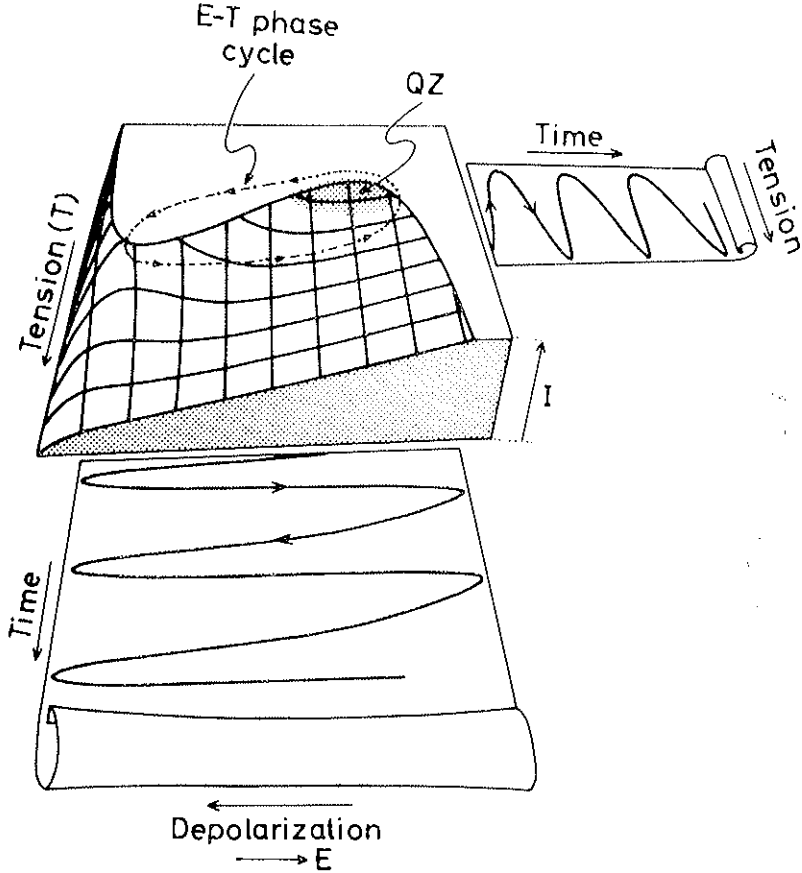


FIG. 4 — Generation of rhythmical variations of potentials (E) and tension (T) at a constant activation current (I). This is a model of a heart muscle contraction.

excitable, biological membranes (figs. 3-4). In essence, this model operates according to the laws of irreversible thermodynamics for coupling between electrochemical potentials and hydrostatic pressure gradients. Perhaps new possibilities of measurements of intracellular pressure would help in the search for operation of a fixed charge factor in living models.

A diagrammatic review of the relations between membrane technology and biology

So far this paper has been a more or less historical account of the mutual interactions between membrane research of a physical chemical nature and biological membrane phenomena and transport processes. Of course, it is very difficult to discern a special pattern in the historical sequence of these events. But beyond any doubt there has existed and still exists psychological undercurrents in the minds of the investigators which drive them to interact. Many physical chemists have often alluded to biological implications of their theoretical conceptions. Donnan, for instance, in his very first paper mentioned that his ion equilibrium system might be applicable to the secretory problems of the mammalian stomach. Nernst was more or less directly glancing at diffusion and phase boundary potentials as sources for bioelectrical currents. On the other hand, the biologists have often shown an ambition to be familiar with physics and physical chemistry and wanted to impose " laws of nature " on their observations. The present author confesses that he more than often prefers to be labelled a biophysicist rather than a physiologist, which was his official profession. The human leanings of the scientist most certainly shapes the science! But such reflections are outside the scope of this Meeting, although we all wish, in one way or another, to contribute to a human problem, the need for pure water.

Let us instead attempt to summarize diagrammatically " in terms of processes " how membrane technology and biology interact. Very schematically the figure 5 represents a feedback system. From the point of view of our Conference we find that there is a common denominator for the desalination technologists and the biologists, who deal with transport processes and excitability. It is the transport of ions and of water. The scheme may roughly define the salient problems. It deals with

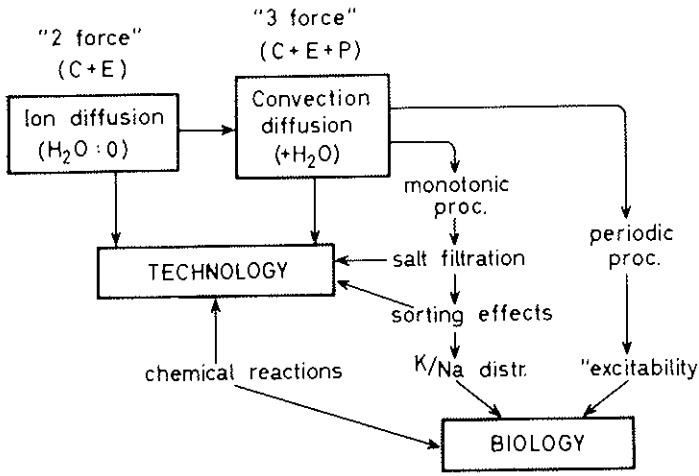


FIG. 5

separation of salt to obtain the solvent, the water. The biologists might say that he wants to separate the ions without operating on the water to achieve the specific ion distributions of potassium and sodium across living cell membranes. Thus the " sorting effect " becomes important for the biologists, while it might be a nuisance for the water purification man. Both parties have to focus their attention on the chemical and physical build up of their membranes. Indeed, membrane technologists and biologists should have a great deal in common!

One may pose the question, what can the membrane technologist and the biologists learn from one another? The answer is obviously " a great deal ". However, there are certain " communication difficulties " due to the difference in approach and the ensuing difficulties with the different scientific languages. This creates a certain fear of a mutual approach. Although the present author has been very much impressed by the achievement of the water purification scientists of this Conference I should like to invite this group to consider a biolog-

The Chief Constituents of Blood and Urine

Constituent.	Per cent. in blood plasma.	Per cent. in urine.	Degree of concentration.	
Water	90–93	95	— *)	
Colloids	7–9	0	0	
Creatinine	0·001	0·1	100	"ash" product accumulation
Urea	0·03	2	60	
Sulphate	0·003	0·18	60	
Phosphate	0·009	0·27	30	
Uric acid	0·002	0·05	25	
Potassium	0·02	0·15	7	"salt-retention"
Calcium	0·008	0·015	2	
Chloride	0·37	0·6	2	
Sodium	0·32	0·35	1	
Glucose	0·1	0	0	
Ammonia	0·0001	0·04	400	

*) Out of 120 ml/min pressure filtrate from the blood plasma only 1–2 ml/min is voided in urine.

FIG. 6

ical desalination and salt separation factory, namely the kidney. This organ has the responsibility of maintaining the proper balance between water and salt ions for the maintenance of tbe living processes. It also has to remove waste products or ash from the chemical metabolism of the body. A great deal of the daily intake of water should be removed through the kidney without dangerous loss of the precious cations. At the same time the ash products should be concentrated and effectively removed. The following table (fig. 6) is a balance sheet of the separation process between blood plasma and urine. Although we do not know all the details in this salt retention machinery, it is widely accepted that it involves a cooperation between *hyperfiltration* in special anatomical entities, called the glomeruli, a *countercurrent multiplier system* and *ion pumps* as indicated by the next figure 7. The counter current mechanism (invoked by HAGGERTY and WIRTZ, who were inspired by the physical chemist R. KUHN) seems to be a clever solution.

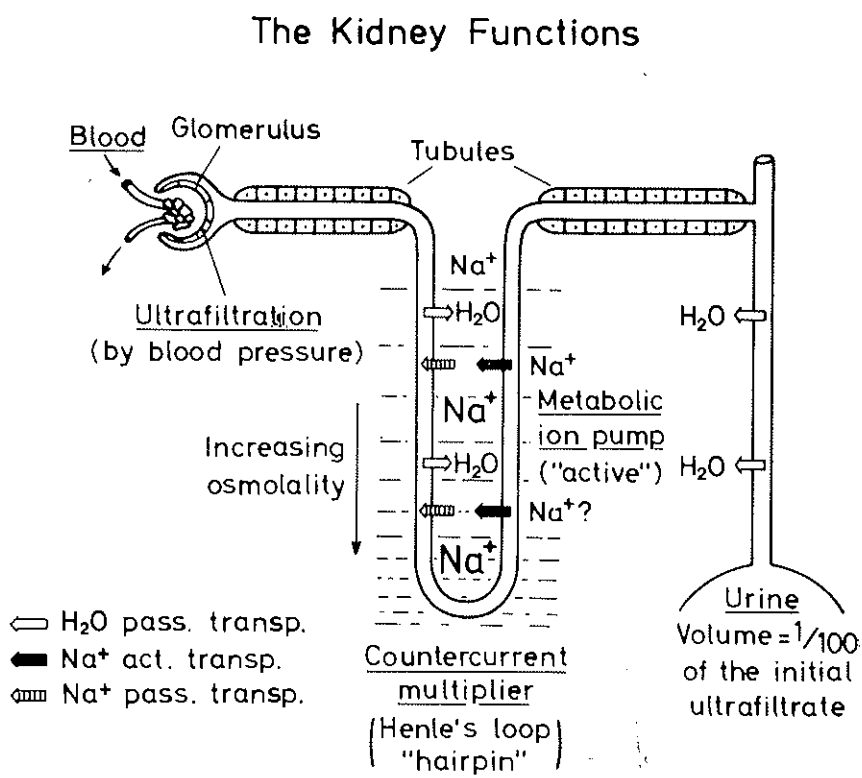


FIG. 7

Incidentally, the same kind of counter-current is used in the feet of water birds on order to prevent undue heat losses when they move in cold water. Here we see an achievement by Nature which has anticipated many counter-current constructions hy human engineers. The operation of " active " ion pumps (mainly for sodium) still remains an ad hoc assumption. This much we know, that ion pumping must utilise chemical (metabolic) energy derived from the combustion of carbohydrates with the aid of enzymatic systems. Many details of such systems have been reasonably well explored but

much remains to be done. Membrane technology has a long way to go to be able to mimic the salient features of the living kidney membranes.

Artificial membrane " matrices " and medicine

May be one can expect important leads for biology from promising recent attempts to incorporate enzymes and other catalysts in artificial membranes. The coupling of chemical energy or chemical reactions to membrane transport processes have been elucidated in brilliant papers emerging from the late ARON KATCHALSKY and ORA KEDEM and their collaborators. Based on irreversible thermodynamics they have published theories on *composite membranes* of " mosaic " or " sandwich " structure. They have also considered what incorporation of chemical reactions in the membrane might do to the transport phenomena. This is the most interesting approach from the point of view of biologists. It is now known that a great deal of the vital processes are mediated by enzyme systems bound to, or operating near, membranes (DE DUVE, STOECKENIUS, PALADE). We are probably facing a new epoch in membrane technology which may have very important feed back on biology and medicine. " Matrix-bound " enzymes or catalysts have already found its use in medical research and in patient treatment. In heart surgery artificial " heart-lung machines " have been used for many years. Oxygenation of the blood supplied to the patient has been accomplished by " artificial lungs " where the natural lung epithelium has been replaced by an artificial membrane material, readily permeable to oxygen. Improvements of the rate of oxygen exchange has been attempted by SELEGNY (by incorporation of haemoglobin-like substances in the membrane matrix). A carrier mechanism is thus accomplished which creates a " facilitated transport " of the oxygen (fig. 8).

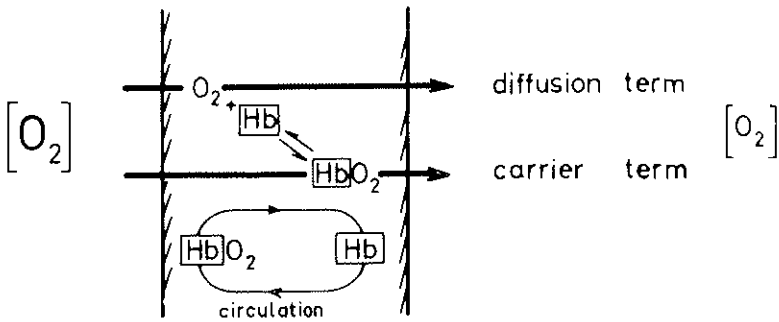


FIG. 8 — Example of facilitated transport. Carrier transport of oxygen (diffusion with chemical reaction).

As a further example of the impact of membrane technology on medicine we will present a few pictures from a recent review article by MOSBACH (*Sci. Amer.* 224 [3], 26, 1971). The first one shows the utilization of a membrane coupled " enzyme electrode " (fig. 9). Some attempts have been made recently to build implantable such glucose sensitive electrodes on patients, which could automatically control the insulin supply to diabetic patients for the maintenance of a healthy, normal blood sugar level. In clinical medicine special dialysis membranes have been used in the " artificial kidney machine ", which is used in the life saving treatment of kidney failure. These membranes are somewhat akin to the desalination membranes. Incorporation of catalysts in the polymer can greatly add to the efficiency of the blood purification. This is illustrated in another figure, Fig. 10 (also cited from Mosbach).

Conclusions

There are new ways in which membrane technology can " feed back " on biology and medicine. First, by facilitating model studies of membrane-enzyme systems which may elucidate

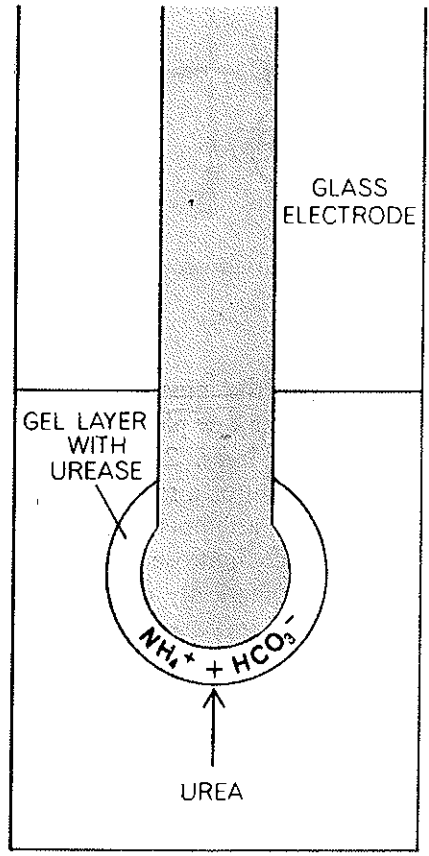


FIG. 9 — Measurement of urea in body fluids can be accomplished by coating the end of a glass electrode with a thin gel layer, about 1 millimeter thick, to which the enzyme urease is kept bound. The enzyme catalyzes the reaction of urea and water into ammonium ions (NH_4^+) and bicarbonate ions (HCO_3^-). The potential of the electrode is altered by the buildup of ammonium ions, providing a direct measure of the amount of urea present in the sample.

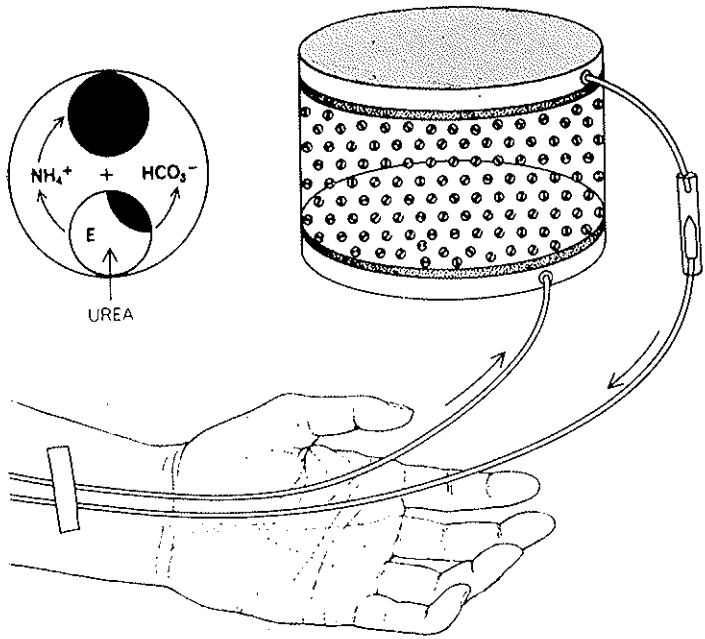


FIG. 10 — Removal of urea from body fluids could be achieved by a new kind of artificial kidney consisting of a vessel filled with microcapsules containing the enzyme urease. The enzyme would convert urea and water into ammonium and bicarbonate ions. The microcapsules would also have to contain either an ammonia absorbent or an additional enzyme for the removal of ammonium ions. Charcoal has been used as an absorbent in clinical trials.

what may occur at the biological cell membrane levels and secondly by furnishing material of great importance for medical analyses and medical procedures for treatment of patients. Also in the future there will be a continual cross-fertilization between the men who work with the chemical and physico-chemical membrane technology and the biologists who cope with the challenging problems offered by the living cell membranes.

X

GENERAL DISCUSSION AND CONCLUSIONS

GENERAL DISCUSSION

Chairman: Professor TEORELL

TEORELL

Ladies and gentlemen. — We have now to resume for the last session. I indicated this morning that we would not try to make a « one person » concluding remarks. With the consent of the Presidium we have instead formed a small panel here which will attempt to induce a summarizing discussion on the conclusions, or the new knowledge, we have acquired this week. Our transactions have covered a broad spectrum. As indicated here on the blackboard it spans from the ultramicro-morphology of the cell membranes and the protoplasmic constituents to the physiological behavior of, in particular, so called excitable membranes of the nerves, heart, plant cell etc. Then comes the physical chemistry of artificial membrane systems in general leading over to the other extreme, the technological applications of artificial membranes in desalination and desalination problems in general. This may be a somewhat artificial subdivision, but we hope it will be useful for our purpose. The main subject of this conference is, after all, desalination. In view of this, it would be very nice if you all from various angles adjust the focus on that title.

The panel sitting with me here represents our topic spectrum. WALTER STOECKENIUS, to my left, represents of course the « ultramorphologists ». To my right is SAM SPIEGLER, a physical chemist

on membrane and transport, who also has a profound personal knowledge about desalination technology (unfortunately Professor PASSINO was prevented from joining our panel). I myself will mainly act as a mediator between the two extremes, and also to some extent represent the biophysical physiology. Now for the sake of the game, we on the panel intend to start to ask one another questions and then hy degrees ask for comments or challenge our esteemed colleagues in front of us. In this way one question will induce another and the general discussion will be soon, we hope, in full hloom. In view of the short time available we would very much value if you could concentrate of the major issues rather than asking for clarification of some colleague's paper, which may be straightened out in private. Please, try to spell out the main results, positive or negative, you may have got from our conference. It sounds a difficult task, but you are such experienced people all of you that you will be able to do it.

I will now open the discussion by challenging Professor STOECKENIUS. WALTER, you may perhaps remerber a conference in the US more than 10 years ago when the « membrane transport workers » had a friendly fight with the « ultramorphologists ». It was at the time when the shadowing technique of your preparations had been introduced. There was some suspicion, not the least among the picture producers themselves, that many slides showed beautiful artefacts, which had little to do with the real living structures. I said, that you behaved like archeologists who fly in a helicopter above Sahara at sunset and observe some hills which may reveal ancient ruins. In fact, they do not see ruins, but shadows of ruins, and yet, they try to describe how people once lived below. Of course, this was an exaggeration from my point and I would not have been so impertinent to-day. But nevertheless, I should like to ask you about the quality of the pictures you present to-day with many new, refined methods. Are they trustworthy, how far can one stretch the conclusions as to the living state — or are you still labouring with aerial archeology? Please, WALTER, defend your profession and illuminate us.

STOECKENIUS

Well, it seems to me archeologists still may obtain some important information from aerial observation — but that may be beside the point. I think the main contribution of morphology to the membrane field is that morphology has focussed attention on the lipid bilayer and has produced convincing evidence for its existence in biomembranes not only by electron microscopy but as much or even more by X-ray diffraction and some modern spectroscopic techniques. This led to the study of lipid bilayer model systems which would not have been developed if morphology had not shown that such lipid bilayers form the backbone of biomembranes. These studies have clearly shaped our recent view about the function of cell membranes and intercellular membranes. The other point I would like to bring up is that we have heard very little here about the energetics of processes in biological membranes. And that really is one of the central questions. Is it worthwhile for people who would like to work on desalination to actually look at the processes which occur in the typical cell membrane? Which as we see now are quite different from the processes occurring in the artificial membranes used for desalination. They are dealing with rather large channels which have a large number of fixed charges on their inner surface, whereas in cell membranes we are dealing with transport processes which occur by carriers or through very small pores where apparently the solute is usually stripped of its solvation shell before it can move through the membrane. We should consider the question: what is the efficiency of these mechanisms for moving solutes and solvents? To make one contribution, last night I quickly calculated how much solute we could extract with a square metre of membrane when we illuminate it with saturating light intensity. My rough calculations show we could move about one mole in an hour from the water phase on one side of the membrane to the other side. And I would like to ask Professor SPIEGLER what he thinks of that kind of efficiency?

SPIEGLER

I would like to make some remarks about the general problem of this meeting, and then try to answer the specific desalination question which was asked.

TEORELL

Please, go on Dr. SPIEGLER.

SPIEGLER

I am sorry that Professor PASSINO is not here, he was going to do this but I understand that he couldn't come back on time. What I want to concentrate on first are the common points of biologic membranes and membranes which desalination engineers use. Before I do this I would like to thank Professor CHAGAS and Father ROVASENDA for having chosen exactly this topic — biological membranes and desalination, and brought us all together here so we can have such a discussion, and so we can learn from each other.

When I asked myself what are the common points it seemed sometimes during this meeting as if there were two divergent streams here in this room, and we would have to find common points. I would like to go back to Professor TEORELL's lecture this morning. He demonstrated that in the history of this field, physical chemists laid the groundwork for the understanding of some of the functions of biological membranes. If this was only a matter of historical interest, it wouldn't be worth while having a meeting in 1975 which brings us together here. Let me just add one other point that shows the historical connection very well. Dr. STAVERMAN who very modestly didn't talk about this, is actually responsible for much of the early development of electrodialysis in Europe. He was head of a group in Holland which tried after the war to develop electrodialysis. In the course of his work he commissioned an

historical review of membranology as applied to practical processes. I am the proud possessor of a copy of this thorough review, which shows how much these two fields were actually intertwined many years ago, namely membrane biology and practical membranology. So there is no question that in the past the two fields went together and helped each other. But in the future it is quite possible that this will happen too. The first thing that comes to mind is of course methodology. There was a cross fertilization in methodology. Microelectrodes, which physical chemists developed are being used by biophysicists in order to study biological membranes, and vice versa. Much of the instrumentation that was developed for tiny biological cells is now being used by physical chemists. Electronic instrumentation developed by physicists and engineers which is now being used by biophysicists. Moreover, I believe that there has been considerable progress in applying membrane engineering to *medical* problems. One cannot think of an artificial kidney or a membrane blood oxygenator without the progress that was made in membrane engineering. One other application that seems to be quite important at the present, and will no doubt be more important in the future, is the controlled release of pharmaceutical materials through membranes. It has been argued that it doesn't really make much sense to swallow an active material and over-saturate the blood with it, and then pretty soon afterwards not to have a sufficient level of it in the blood. Controlled-release agents which are already made with an astounding degree of sophistication involving the study of transport of pharmaceutical materials through membranes occupy some place in the field of chemotherapy today, and will certainly be more important in years to come. Recently I had a chance to visit a Company which develops such materials, and most of them are not yet on the market. But one cannot think about such development without a good understanding of membranes and their physical chemistry, because you have to know exactly how much of the active material is transported through the membranes per hour.

We have these types of interaction: methodology and medical application of membrane engineering. I'll say some words about

Professor STOECKENIUS' question later, but I think even the few things which are mentioned so far are of considerable importance.

TEORELL

Thank you SAM SPIEGLER for your nice review. I think there is much more to learn this evening and I will pass the word to somebody else outside of the panel. I suggest that we address a senior member of our assembly, who is a general physiologist and biophysicist. Professor MONNIER, would you like to indicate what you have learnt at this meeting. You have learnt us so much, but yet you may reveal more of your thoughts, could you kindly do that?

MONNIER

Mr. Chairman, I would like to delinate the frame of mind of the physiologists who, as you and I, by profession, have had to teach general physiology to many students. We had a multi-oriented curiosity or polyvalent curiosity. All the aspects of the living organisms, the physical and chemical laws which preside to all their functions, have tempted our curiosity. This has given us, through long efforts, a wide knowledge of all science, but the wideness of our knowledge is mitigated by its superficial quality, its absence of depth. In recent history many physiologists went into the field of politics because this wide but superficial knowledge gives to the physiologist an impression, an illusion of universal competence. But to turn to your question Mr. Chairman, about what I learnt here, well, I have learned that, more than ever, a physiologist should be a polyvalent intellectual having some familiarity with many systems and many things outside his own field. And that is why this temporary cohabitation between people dedicated to widely different fields such as desalination, technology and cellular morphology, has been most fortunate. Indeed we have learnt that those two apparently distant domains merge together most profitably. Problems

have identical aspects when considered at the molecular scale. Wether we consider industrial membranes of which area is measured in square metres, or artificial or natural membranes, after all both types of membranes deal with the transfer, molecule by molecule, cation by cation. Thus what the specialists of desalination physics teach to all of us is invaluable and also of immense value to tbe methodologist because it shows that basic laws apply to both systems in spite of their difference in scale. I have also learned that we cannot dissect (as we are tempted to do when we build artificial membranes) the functions of a membrane — function by function — we cannot consider a living membrane as a collection of separate functions. These form an integrated system. We know some of the mechanisms of this system, but we still ignore many more. They do not compete but are integrated, associated for the maximum economy of each function. And this, I think, is an invaluable lesson. For instance, we have learned that active transport proceeds according to the same laws, in spite of minor differences in detail, in animal cells and in plant cells. We have learned also, and this is perhaps most important, that we have to look for possible but not yet established processes. For instance it has just been shown to us that ATP has to transfer the membrane. We have been shown that this is an absolute necessity. How this transfer occurs, that will be a most important problem of which the solution will soon appear. I think that during the Study Week we have acquired an integrated knowledge coming from widely different methods, widely different processes and widely different questions even if they are a long distance apart, from living membranes to desalination problems. Well, all this knowledge, integrated in our receiving brain will certainly lead to progress. Especially when these merging knowledges have been given to us in this most wonderful setting of the Pontifical Academy.

TEORELL

Thank you Professor MONNIER. It comes to my mind that we

have one person here who really has an interest covering the whole spectrum of our conference. May I address, more accurately, Professor ORA KEDEM. Would you kindly comment on what has been said already and also bring up other aspects which you might find worth while?

KEDEM

I would like to react to the numbers given by Dr. STOECKENIUS. Meanwhile my neighbour, Dr. HASSELBACH, and I have had time to go over these numbers. The comparison of the systems is indeed illuminating: in your hypothetical bacterial membrane you are passing a current of the order of mA per square centimeter or not much less comparable to that customary in electrodialysis. Of course, the energy input may be quite different. On the other hand, Dr. HASSELBACH'S numbers show that calcium-flow in the sarcoplastic reticulum is much lower: 5mA per square meter. The remarkable fact is however, that one square meter is contained in a few milligrams: the effective functioning of many biological systems is based on their extreme compactness. This compactness comprises not only a large area per volume, but also highly organized spatial integration of consecutive functions. Thus the purple patches in the halophilic bacterium are built into an ATP-ase containing membrane (as we heard in Dr. STOECKENIUS' lecture), every nephron contains a « desalting » and a concentrating as well as a filtering system etc. etc.

I believe an important lesson for those of us interested in the technological application of membrane systems is to be learned in this general conceptual area. After all, polymeric materials can be produced in arbitrary, even complicated shapes. Maybe one could achieve better performance or even unify functions by a careful spatial coordination of elements. An interesting start in this direction is the current development of enzyme membranes as biochemical reactors. Consecutive reactions through sensitive intermediates, separations following reactions, may be carried out in the near future

by one membrane system. On a much simpler level, we are now trying to improve the performance of electrodialysis stacks by an ion-conducting spacer of a specific shape facilitating ion-removal.

STOECKENIUS

I didn't tell you one essential number and that is what saturating light intensity is. It would probably be rather bright sunlight in a sub-tropical region, 10^6 ergs/cm^2 . sec.

TEORELL

I think it is about time to switch back to the desalination aspects, particularly the energetics. Professor STAVERMAN would you like to comment on this?

STAVERMAN

A point which I would like to ask is to what extent can we learn from morphology or from nature in forming a tremendous area into a small volume. How large is the area in the kidney, the total area used in the kidney for desalination? Does anybody know this?

STOECKENIUS

Many square metres.

KEDEM

I was referring to the filtering area alone, i.e. glomeruli. This is about 2 m^2 per kidney.

SOLOMON

The glomerular filtrate in the human kidney is about 120 ml/min and 99% of that is pumped back. With a moment or two of thought one can figure out what the transport is.

TEORELL

May I ask a question myself. We have just discussed some surface area aspects within the biological systems. It makes me think also of the surface forces. By and large, the cell membranes and also the intracellular structures, represent more of a two-dimensional system than of a three dimensional one. Hence surface forces, or rather interfacial forces, must be considered. May be that the chemical reactions which furnish the energy become coupled to surface tension gradients. Perhaps this kind of coupling acts behind the drift of vesicles and fusion on membranes which has been so much discussed in relation to the interesting pictures of Professors DE DUVE, PALADE and STOECKENIUS. I have a notion that the « transport workers » should supplement their « cross-membrane » studies with, if I may say so, « along the membrane » kinetics. I mean, we must try to define the driving forces for aggregates of giant molecules and larger structures in general, including the much discussed vesicles. How to get a grip on these problems? Is there anybody willing to comment on this difficult question? Perhaps Professor PALADE would embark on this task.

PALADE

The answer is easy at present: we don't know anything about the forces involved in this type of movement. I presume that Dr. TEORELL's question refers to the movement of membrane-bound containers, i. e. intracytoplasmic vesicles of different sizes. In the absence of actual data, the hypotheses entertained at present follow

two different lines of thinking. The first line I already mentioned. Starting from certain premises, it has been estimated that simple diffusion (or Brownian movement) of a vesicle through the cell sol at the temperature of the cell or body would be sufficient to account for the rate of transport effected by the vesicles. What remains completely unknown is the time and forces involved in the interactions (fusion, opening or detachment) of the vesicle membrane with the plasmalemma. This hypothesis has been advanced to explain primarily the transport of macromolecules by vesicles (called plasmalemma vesicles) from one side to the other of the vascular endothelium in blood capillaries.

The second hypothesis implicates microtubules and microfilaments as guiding and contractile elements, respectively, in the movement of vesicles within the cytoplasm. It is assumed that the movement is vectorial and leads the vesicles to the « right » terminus (another intracellular compartment or the plasmalemma). Microtubules and contractile proteins organized in microfilaments have been detected in a wide variety of cells. In this case, guided active movement replaces the random process of diffusion implicated in the first hypothesis. In both cases, the common, still unexplained part concerns membrane interaction between the vesicle and the terminus. As you remember, energy is needed to effect vesicular transport, but the energy requiring steps are still unknown. It could be membrane interaction in the first hypothesis, or intracellular transport and membrane interaction, in the second hypothesis.

TEORELL

We would appreciate if Professor DE DUVE would comment something on membranes, structures and biochemistry. In several speaches it has been indicated that one can incorporate enzymes in model membranes. Do you think that you can use models of this type for your particular purpose? Of course, we would also appreciate your general personal impressions of this Conference.

DE DUVE

Many of the communications in this study week were outside my field of competence. I have listened to them with great interest and have learned a great deal. In one area particularly, with which I have very little familiarity, it seems to me that theoreticians, physical chemists, physiologists and biophysicists have moved very close together. This is the area which concerns ion transport under the influence of either chemical or electric potentials. I have been very much impressed by the fact that one can, with relatively simple artificial systems, such as have been used by Professor MONNIER and by Professeur MUELLER, practically duplicate the kinetics of hiological ion movement upon excitation. So here it seems that the fine dynamics and kinetics of action currents can be reproduced by a model system. These highly advanced experimental and theoretical studies that we have heard about have therefore been very valuable. In addition there is of course their important practical application to the problem of desalination.

To biochemists, that is to biochemists of my particular brand, two other aspects of ion transport are particularly interesting. One was touched upon by Professor KEYNES and by Professor MUELLER. It concerns the actual mechanism of gating, or, conversely, of opening tbe gates, of depolarization of the membranes. The other aspect on which we heard a great deal of interesting information is the mechanism that actually generates the potential, i.e. the electrogenic pumps, which the physiologists have been less concerned with, but on which the biochemists have worked a great deal. From what we have heard, especially from Professors HASSELBACH, DE MEIS and POST, it appears that the biochemical analysis of the systems has already progressed considerably. This brings up the more general problem of energy transduction in membranes, which involves not only electric potentials, ion transport and chemical energy transformation from and into ATP, but also in some cases, as we heard from Professor STOECKENIUS, light energy. On this topic, the com-

munications of Professor STOECKENIUS and of Professor BOYER have been particularly illuminating and interesting.

Perhaps we should keep in mind that there are several other interesting transport processes besides ion transport, which were hardly touched upon at our meeting. One is the transport of small molecules, such as sugars or amino acids. This was briefly alluded to with respect to the coupling of some of these transport systems with the sodium pump. Another form of transport, which could easily occupy a whole conference of this type, is filtration, such as occurs across basement membranes or in kidney glomeruli. Here we would need a different kind of theoretical analysis, dealing with gel filtration. Finally, there is bulk transport, that is the transport of large aggregates. This is the phenomenon that was briefly mentioned by Professor PALADE and to some extent by myself. It is a completely different kind of transport, which relies on translocation by means of vesicles. It involves a key phenomenon of membrane fusion, the dynamics of which are completely mysterious at the present time. Our own contribution here has been largely methodological, dealing with the actual characterization of the membranes. I am afraid so far it bas thrown no light on the processes, or perhaps has made them even more obscure, by emphasizing the lack of biochemical similarities between membranes which nevertheless are known to fuse together in a very specific manner.

One last remark and then, I will stop. I have heard allusions to the possible medical application of some of these techniques especially the use of artificial membranes and artificial containers. Those who are involved in this kind of work should keep in mind the possible toxicity of their materials. Biologically inertness is not a sufficient criterion of innocuousness, since cells may take the materials up and suffer through their inability to handle them. Several man-made diseases created by the injection of inert natural or artificial polymers are known. In this respect, and also in a more general way as a measure against pollution, a greater effort should be made on the part of the polymer chemists to develop bio-degradable materials.

TEORELL

Thank you very much for this comprehensive study.

TEORELL

Maybe it is time for a more «person to person» discussion and forget about the panel operations?

SPIEGLER

I think we'll have to try to answer one of the previous points before we ask further questions.

TEORELL

Very well, go ahead.

SPIEGLER

I would like to comment on the problem that Prof. STOECKENIUS has raised.

TEORELL

Please, go on, Professor SPIEGLER.

SPIEGLER

When I heard the question asked in this way I could not but

remember the famous painting of the desalination plant at the seashore which borders the desert. The desalination plant spouts forth large amounts of water which make the desert bloom. Presumably wheat and other cereals are grown there to feed the people who live around the desalination plant. I am asking myself whether one should pose the problem in these terms. The question that you posed and the question that occupied the artists mind was: can I produce enough water to feed more and more people by growing cereal crops with desalinated water? If asked in this manner, the problem of water desalination is *quantity* problem and I'd be inclined to say that this can in general not be done at this time. But water desalination is primarily a *quality* problem. It may improve the quality of a very essential raw material for our present civilisation. I remerber that at the beginning of this study week it was pointed out that here exists a correlation between health and the quality of the water supply. Humanity has known this for a long time, I suppose: the *quality*, not the *quantity*. It seems to me that the problem of water desalination has to be posed in terms of quality of water, quality of life, quality of health, and not in terms of quantity. If we want to produce more grains, corn, rice, soyabeans and so on by artificial irrigation with desalinated water we have lost before we have begun our endeavour. But let us not forget that we live in an age of increasing urbanization, in which another aspect of development is extremely important, namely, to make life in crowded cities bearable, to make it possible for people to be happy and enjoy a high quality of life even if they do not live in the country. We might think about the middle ages when pestilence was the most feared scourge of mankind; pestilence was particularly felt in the crowded cities. Look at romanticism in the 18th and 19th centuries — if you read George Sand's letters for instance. Why did people want to get away from the cities, why did they crave the « wonderful life in the country » when country life was really very primitive? Because the quality of life in the cities had already begun to deteriorate at least 200 years ago. Water is just one of the important factors

of life in crowded quarters, and it is important to produce good water to make life in an urbanized world bearable and even pleasant, and in this way to contribute to the quality of life and make men and women raise their sights. Look at water desalination as a problem of this kind, as a method for the production of a high-quality product which should be conserved and not wasted. If you include the raising of certain amounts of high value crops which we have in California by desalted water, but not of grains and similar bulk crops, then you have something to build on and something that makes not only scientific sense but also economic sense in our time. To summarize, I don't want to play a game in which I've lost before I make the first move: I believe the problem of water desalination should be posed in terms of quality rather than in terms of quantity.

STOECKENIUS

Well, I don't disagree with Dr. SPIEGLER, of course, the quality of life is most important; however, I had asked a much simpler technical question: is there some process in biological membranes that could actually be used for desalination of sea water? If you want to do this, you would try to do it as efficiently as you can, I suppose. So this was my question. But I have something else in mind. I think I got from this meeting the impression that something has fundamentally changed since I attended a meeting of this kind for the first time about 15 years ago. Then we knew much less about the functioning of biological membranes. We are only now beginning to unravel the detailed mechanism by which they transport solutes. At that time we thought it possible that biological membranes might use the same principles on which ion exchange membranes and similar devices operate. It is becoming obvious now that the typical cell membrane and the membranes of cell organelles use quite different mechanisms. However, there are in higher organisms a number of other processes, for instance filtration in the glomerula

of the kidney and also the passage of solutes through the capillary walls in general that may be more relevant to the mechanisms of the artificial membranes. I think this is a topic that has not been sufficiently discussed here. I know that Dr. PALADE for some time has worked on the permeability of the capillary wall and I wonder if he would care to comment.

PALADE

To begin with, I should point out that the term « membrane » is used at present to designate a number of different and unrelated structures. The most common type of membrane is, of course, the bilayer of polar lipids modified by interaction with proteins which is found at the periphery of the cell (plasmalemma) or within the cytoplasm (intracellular membranes of various kinds). This type of membrane is so commonly encountered, so prevalent, and so intensely studied at present, that we tend to forget that the term « membrane » (more precisely « basement membrane ») is also applied to laminar, extracellular feltworks of fibrillar proteins (collagen) and glycoproteins which do not have a significant hydrophobic component and which are regularly found at the base of every epithelium, vascular endothelium included. Among membranes of this type, the basement membrane of renal glomerular capillaries occupies a special place: it appears to he the only membrane in the body used for ultrafiltration under normal physiological conditions. It selects molecules according to size-retaining plasma albumin and all molecules larger than it, and possibly according to charge, although this property is not yet fully documented. (There is still some discussion concerning the structural identity of the glomerular filter but most of the evidence identifies the basement membrane as the main filter).

There are basement membranes around other types of blood capillaries in the body, but they are thinner and less selective than

those of glomerular capillaries. They function not as primary filters, but as coarse secondary filters which retain large particles ($d > 700$) that have accidentally crossed the endothelium (in this type of capillaries, the latter functions as primary filter). It should be pointed out that the size-limiting structures (« pores ») in the endothelium do not involve the plasmalemma, but appear to be extracellularly located (in the diaphragms of endothelial fenestrae, in transendothelial channels or — according to some investigators — in intercellular spaces).

Basement membranes come much closer (than modified bilayers) to the artificial membranes used in the past by physical chemists for a variety of experimental studies, desalination included. In this respect, and for such special purposes, the stress put on modified bilayers and the neglect of basement membranes are not only unjustified, but they may prove to be misleading.

TEORELL

At this point I think we should give room for a general open discussion. I leave the floor free now — I recognize ARTHUR SOLOMON.

SOLOMON

I was just wondering about a numerical comparison of desalination permeation with that of biological membranes. The permeability of the red cell membrane is normally measured in units of 10^{-5} cm sec^{-1} and water which is the most permeable molecule has a permeability coefficienty by filtration of about, 800×10^{-5} cm sec^{-1}, that is about 10^{-3} cm sec^{-1}. I was wondering how that kind of permeability coefficient fits with the filtration coefficients characteristic of your filtration beds.

SPIEGLER

It is quite interesting that synthetic membranes as used in desalination technology have water permeabilities of the same order as some of the much thinner biological membranes, or not more than one order of magnitude different. You would think that a biological membrane which is so much thinner should have a much higher water permeability. However, it is a much tighter membrane and therefore the permeability is within one order of magnitude of the permeability of the most used synthetic membranes. We have looked into the literature about animal and also vegetable membranes, and found that the water permeabilities are within one order of magnitude of some of the synthetic membranes used in technology.

MONNIER

Referring to Dr. KEDEM's slides one can calculate, if I am correct, that desalination with the new digitated membranes can be carried at the rate of 300 amperes per sq. metre. Well, that is a very large figure and shows the excellency of a progress certainly brought about by a technical trick and a new idea, a new technical idea. There is another question I would like to ask. When several years ago I had the privilege to visit the reverse osmosis plant in Beersheba, a nice and big flow, of absolutely pure water, was rined with a small pump activated by a motor, something like 3 or 4 h.p. not more. I asked what happens when there is an accident to those large cellulose acetate membranes when there is an accidental small perforation of the membrane. Well, I was told and I was very much surprised that, if the hole is not too large, the membrane self-repairs. That impressed me very much. I would like to know how an inert cellulose acetate membrane can self-repair. To put the question on a more general basis I would like to ask if someone could comment on the repair of the living membranes, when they are disrupted by injury. After all our wounds repair and many of our disrupted mem-

branes stick together again. If someone could comment on that I would enjoy it very much.

SOLOMON

I can comment on red cells. It is well known that if you hemolyze red cells so that they lose most of their haemoglobin and leave them alone, they reseal themselves and will also continue to pump ions if they sealed under proper conditions. Resealing membranes that are composed largerly of lipids seems to be a general phenomenon that is to be expected because lipids don't like to have broken ends. Free energy considerations make them form close structures which I would expect to be a general phenomenon.

KEDEM

The cause of healing in the artificial membranes is of an entirely different nature. They are simply clogged up, as long as pores are small enough. Very effective use of this is made by the so-called dynamic membranes, you start with porous material and filter a colloid solution of suitable properties. The colloid is adsorbed in the pores and creates a selective membrane. Sometimes a dirty enough feed will clog the pores by itself!

TEORELL

I regret that we have been unable to invite everybody to give a remark but I am quite certain that many of you have valuable viewpoints still not exposed to us. In this programme what can plant physiology contribute to the purification of water? Professor MACROBBIE, would you kindly comment on that?

MACROBBIE

On Wednesday I suggested that in the membranes of vacuolate plant cells we have a desalination system. The use of intact cells as a kind of filtering column is probably not practical on a large scale. But after listening to some of the later discussion on enzymes adsorbed on solid surfaces, I wonder whether an analogous adsorption of membrane transport proteins is a possibility for the future. Asymmetric adsorption of the ion transport proteins of the plant cell membrane might provide a biological desalination system, to be used in much the same way as the non-biological systems now in use.

SOLOMON

Dr. MACROBBIE's comment reminds me of another effective biological desalination system. We used to occasionally get K-free water in the laboratory using *E.coli* which are marvellous for scavenging potassium out of water. They can remove it, like Dr. MACROBBIE's carrot roots, better than any other way. But *E.coli* have the great advantage that they multiply readily and one wonders if anyone has ever thought of the use of bacteria for desalination systems.

TEORELL

Prof. WEIDMANN, does a heart specialist have an opinion?

WEIDMANN

Since nobody of the pre-1950 Cambridge group is here any more

I might as well lift a professional secret. When in 1948 I came to the Cambridge lab I was horrified to see that supply of distilled water contained algae. I told ALAN HODGKIN that we should take the algae out. He smilingly commented « no, please not, we are distilling the water in copper vessels and if we remove the algae we have heavy metal ions in the distilled water ».

MEARES

You and I, Mr. Chairman, have been under the personal influence of the late Sir ERIC RIDEAL in forming our views in surface chemistry and I would like to make some remarks related to that subject. Colloid scientists have now shown lipids and water can co-exist in many equilibrium phases of different geometrical forms and it has become clear that, despite the large surface areas which the lipids in membrane expose to their surroundings, the structures found in biological cell membranes are not far from thermodynamic stability. The small size and high curvature of such cells is an important feature in securing their relative stability and ability to recover from damage.

The question has been raised by Professor STOECKENIUS as to whether biological membranes might have a role to play in desalination. However high the permeability per m^2 of the purple membrane, it does not seem feasible to mount it or other biological membranes in the form of large areas suitable for a commercial desalination process. One might imagine using either vesicles or lyposomes to accumulate salt but their capacity would be very limited and regeneration after saturation would present a great problem. Nevertheless desalination scientists can learn much from biologists as the following example shows.

For a number of years a desalination process, known as the Sirotherm process, has been under development in Australia. It

makes use of weak base and weak acid ion-exchange resins in bead form the functional groups of the resin being so designed that ions taken up from cold water are released again into moderately hot water. The chemistry of the process works well but uptake and release from normal resin beads was too slow for a commercial process. Very recently the inventors have produced an ingenious new idea which they call « plum pudding » resins. In these the ion-exchange resins are in the form of extremely small particles comparable in size with biological cells. Thus the specific area for ion-exchange is greatly increased. To maintain good hydrodynamic conditions in beds of these ion-exchangers the tiny particles have been incorporated into larger beads of porous gel in which diffusion is rapid and between which bulk flow is not greatly impeded. In the desalination process a circulation system can be used in which the « plum puddings » are continuously circulated between the cold desalinating compartment and the hot regenerating compartment thus giving a continuous process conceptually not unlike the blood circulation system.

While listening this week two matters have come to my mind regarding the movement of vesicles in cells. There is some danger in discussing the various transport processes within a cell as though each could be considered independently and then in expressing them relative to the plasma membrane as fixed reference frame when one wishes to make deductions about the mechanisms of movement. The passive and active (metabolically mediated) transport of small molecules within the cell gives rise to local mass and volume flows while the overall system is subject to external constraints applied via the plasma membrane. The combination of these requirements may give rise to directed relative motions of the vesicles within a cell without the need to invoke the random Brownian motion of the vesicles which will, in any case, be superimposed on these local mass currents.

Where vesicles are surrounded by their own lipid membranes

the interfacial energy of the outer surface of a vesicle will be a function of the composition of the surrounding cytoplasm and of the vesicle membrane composition. If there is a metabolically maintained gradient of composition in the endoplasm the vesicle will experience a variation of interfacial energy over its surface. The interfacial tension related to this energy will influence the shape of the vesicle and give rise to an unbalanced force in the direction of decreasing interfacial energy. Because the membrane composition is equally as important as the cytoplasm composition in fixing interfacial energies, it seems possible to have different types of vesicles experiencing forces in different directions as long as the composition gradients are maintained by cellular functions.

TEORELL

Thank you very much Prof. MEARES, this was a good answer to a vague question I made earlier in this discussion. Well, I think we are moving towards the end of the afternoon and I would like you to fire the last questions or problems.

STOECKENIUS

I did find a mistake meanwhile in my calculation by a factor of 10 and so the amount is now down to 0.1 mole of salt per hour per square meter of membrane. I would like to answer one particular point of Dr. MEARES comments. With the purple membrane you have no difficulty in forming a large flat surface, because the membrane actually is a planar hexagonal crystal, the problem the cell has is rather to adapt it to its curved surface. We can incorporate the purple membrane into a curved surface by adding additional

lipid and form vesicles. Suspensions of such vesicles perhaps held in a porous support could also be used.

TEORELL

There seems to be no further question. If this is the end of the general discussion it ought to be my duty to summarize it, but it is impossible. Nevertheless, I hope that the discussion we just have finished may be a summary in itself. If I may single out a few high-lights of this discussion which I appreciated myself, which may have perspectives for the future, they are as follows.

In order to understand the dynamic features of the cell membranes and the cell constituents we have to go back to study surface chemistry. The biological cell is partly only a three dimensional world, in other parts it seems indeed more like a two-dimensional microcosmos, which we realize from the achievements told by the morphologists. However, the laws of two-dimensional systems are also very strict and they have to be studied again, as pointed out by Professor MEARES. By such studies we may perhaps get an understanding of another aspect of living cells, namely the *structure formation*. It is interesting to note that some advanced people within irreversible thermodynamics have begun to define these problems — the birth of the living matter is structure formation. We are in the beginning and it is a long way to go.

Another aspect which has cought my interest is the problem of *energy transduction*. From chemical (metabolic) energy derive the electrical phenomena displayed by excitable tissues like the nerves or the heart. At the present time we tend to explain the bioelectrical phenomena in terms of different ion distribution and different ion selectivity. One invokes various sorts of pumps for sodium, proton-pumps etc., in which ATP, among other things, takes part in the energy supply. But the term « pump » is still very symbolic and

much remains to be done, although it is true that many new observations have been presented this week relating permeability to metabolism. Personally, I was much impressed by the presentation of Professor SLAYMAN, who related the cell potentials in *Neurospora* to « current sources » instead of to specific ion batteries. As far as I see, this is not a difference in formalism but rather a change in philosophy of the origin of bioelectrical phenomena, in particular with regard to the rhythmical phenomena displayed by the excitable tissues. One can venture that most of the living membranes systems are metastable, in other words they tend to exist in either of two « preferred » states. The action potential of nerves is an oscillation between these two states. I believe that we in the future have to devote more efforts to the study of concepts like metastability, instability, transitory state, rhythmicity. As you know, it is now evident that pure chemical systems, even in test tubes, can display rhythmical reactions. Chemical oscillations certainly exist. But purely physical oscillations also can exist at artificial membrane as I indicated in my own talk, if you have a « current source », just of the type mentioned by Dr. SLAYMAN. And couplings between chemical and physical oscillations are not farfetched. Maybe the current source concept can aid us to find biological « fuel cells », that is devices which can convert chemical energy directly into electrical energy. Such devices are known in human technology as you know.

I think I have briefly sketched some of the thoughts which have come to my mind during this very fine week. It remains now for me to express, on the behalf of all my colleagues, our thanks for a profitable and generously hospitable conference. In the first place, I wish to address myself to our President CARLOS CHAGAS and Father DI ROVASENDA, and express our deep obligations. We also wish to include Professor PASSINO, who had the burden of the earlier part of the organization of this conference. Furthermore we wish to thank for the secretarial work during the meeting, carried out by our nice

ladies in the background. Due to all these efforts this Study Week has become a great success. We all participants will remember the week in the Papal Academy of Science in 1975 also in the future. Thank you all!

(With an unanimous consent a telegram was sent to His Holiness the Pope summarizing the features of the Study Week and the participants gratitude).