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EX AEDIBVS ACADEMICIS IN CIVITATE VATICANA

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THE SIGNIFICANCE OF INORGANIC LEVELS IN THE INTERNAL MEDIUM OF HIGHER ANIMALS

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SVMMARIVM — Ut investigetur cur talis sit in interiore superiorum animalium medio rerum inorganicarum status, qualem esse constat, non possunt elementa desumi ex comparatione cum palaeozoici oceani compositione. Auctor contra huius inorganicae compositionis originem quaerit in physiologicis corporis necessitatibus et utilitatibus.

THE INORGANIC PATTERN COMPARED WITH THAT OF THE PALAEOZOIC OCEAN

The term « milieu interieur » was introduced by CLAUDE BERNARD (1879) to signify the environment directly surrounding the cells of higher animals. This « milieu interieur » or internal medium may be taken here as having practically the same inorganic composition as that of the blood plasma.

In answering the question of the origins of such inorganic composition, answers for the individual constituents will be given in terms of the physiological advantage for the organism, or as something necessarily arising out of its structure. How-

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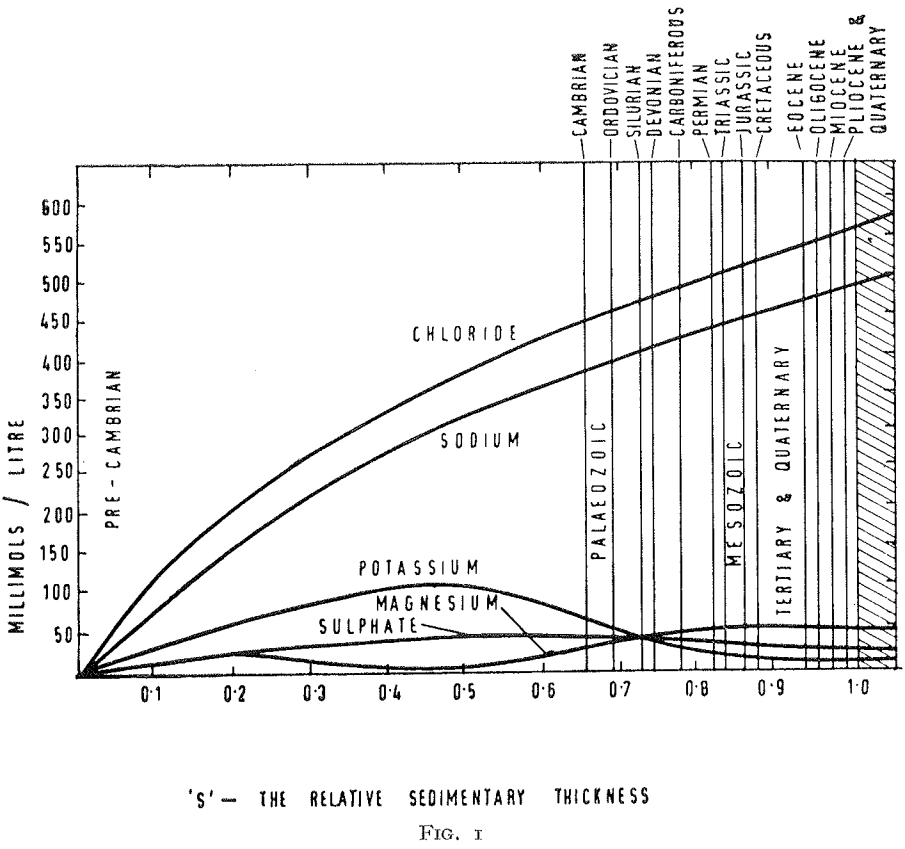

FIG. 1

The cross-hatched area gives an extrapollation into the next one hundred million years.

ever, at the outset it is proposed to deal briefly with those views which attribute an early marine origin to the inorganic levels of the internal medium and that such origin imprinted a sustained quantitative pattern on the internal medium of higher animals. Such views were in vogue at or near the beginning of the present century. The most widely known and best documented was that of MACALLUM (1904, 1926) developing suggestions previously made by BUNGE (1889) and QUINTON (1897).

In Macallum's theory the cationic pattern or the concentration of Na, K, Ca and Mg are the same in the internal medium of higher animals as they were in the external ocean when the developing organisms formed a closed circulation independent of the external environment. This theory was dealt with in detail by the present author (CONWAY, 1942, 1943). In dealing with the chemical evolution of the ocean, it was pointed out that four basic possibilities could be entertained with respect to the origins of the oceanic volume and its halogen content. The one most favourable for Macallum's views may be termed the constant volume-volanic halogen hypothesis. The course of the oceanic evolution from such a view is outlined in Fig. 1. The time factor is here given in terms of relative sedimentary depths and in accordance with principles outlined elsewhere (CONWAY, 1943).

From the curves so obtained, the following approximate values have been made out for the inorganic composition of the ocean in the early Ordivician period, the earliest period in which a circulation independent of the external marine environment could have been established. These values are compared with those of human blood plasma and those of the present ocean in Table I.

TABLE I

Ion	Early Ordivician Ocean	Ocean to-day	Vertebrate Extracellular fluid (Hman Plasma)
	mM/l	mM/l	mM/l
Na	379	478	144
K	51	10	5
Ca	7	11	5
Mg	38	55	2
Cl	441	559	103
SO_4 . . .	40	29	1
HCO_3 . . .	(5)	(2)	28
PO_4 . . .	—	(Trace)	2

It appears that even with a scheme of oceanic evolution most favourable to the Macallum theory there is no kind of quantitative relation between the ionic levels in the early Ordivician ocean and that of the internal medium of higher animals. Writing from Yale University in the *American Journal of Science* concerning the author's studies on the « Chemical Evolution of the Ocean » HUTCHINSON (1944) states that they "dispose once and for all of the hypothesis that the body fluids of homoiosmotic animals represent samples of sea water collected at the time when their body walls become impermeable to electrolytes". The following reflections may now be added. Prior to the establishment of an independent circulation, the internal medium may be regarded as having an inorganic pattern practically identical with the external ocean, and over which the organism could not have control. With the advent of an independent circulation, the pattern of the internal medium changed profoundly, not only in a general osmolar sense but for each constituent. While it is true that sodium and chloride are the predominant ions, as they were in the Paleozoic ocean and in the ocean of to-day, their quantitative values and the ratios of same are quite different. Assigning an ancient marine origin with quantitative significance to the inorganic levels of the internal medium is hence outmoded (in effect, since 1943) and one is obliged to consider what are the more real and more interesting physiological interpretations of such inorganic levels.

THE PATTERN INTERPRETED PHYSIOLOGICALLY

The pH of the blood plasma

This is first chosen for physiological interpretation, because it is a key value for other constituents, and illustrates so well the physiological approach.

It may be asked, why the pH of the blood plasma should be 7.35 (or very close thereto) and not 6.4, or 8.4 or some other figure. This question can be answered precisely in the sense that 7.35 gives the maximum advantage to the organism. This may be shown briefly by the following considerations. The efficacy of the blood as a carrier of carbon dioxide from tissues to the lungs may be measured by the derivative-dpH/dS where 'S' is the addition of total carbon dioxide from the tissues into the blood being mostly converted into the bicarbonate ion.

Assuming firstly, a mixture more simplified than blood, but having the inorganic composition of plasma, and the same total buffering power of blood ('b' about 25 m.moles/1), produced it may be assumed by the requisite inclusion of protein and the same tension of CO_2 producing 1.2 mM free CO_2 in solution. One can then write (CONWAY, 1945)

$$-\mathrm{dpH/dS} = \frac{1}{b(1 + [\mathrm{H}]K'_1 + 2\,3\,[\mathrm{CO_2}])} \qquad (1)$$

where b is the buffering power as given above, and K^1 the first apparent ionization constant of H_2CO_3 ($K^1 = 10^{-6.1}$ at an ionic strength of 0.16 and temperature of 38°C) [H] being the hydrogen ion concentration. Plotting the value of $-\mathrm{dpH/dS}$ against the pH of the mixture there is obtained curve A in Fig. 2. From this curve it appears that from a pH of 7.4 the value of $-\mathrm{dpH/dS}$ declines in value but there is no defined minimum. Instead of the simplified mixture mentioned above one may chose one simulating the effect more closely of whole blood and in which the protein present has a special ionizing group, like haemoglobin, which changes on oxidation its ionizing constant from $10^{-8.04}$ to $10^{-6.76}$.

VAN SLYKE in 1926 described the changing ionizing constant as passing with oxygenation from $10^{-8.18}$ to $10^{-6.62}$ though the

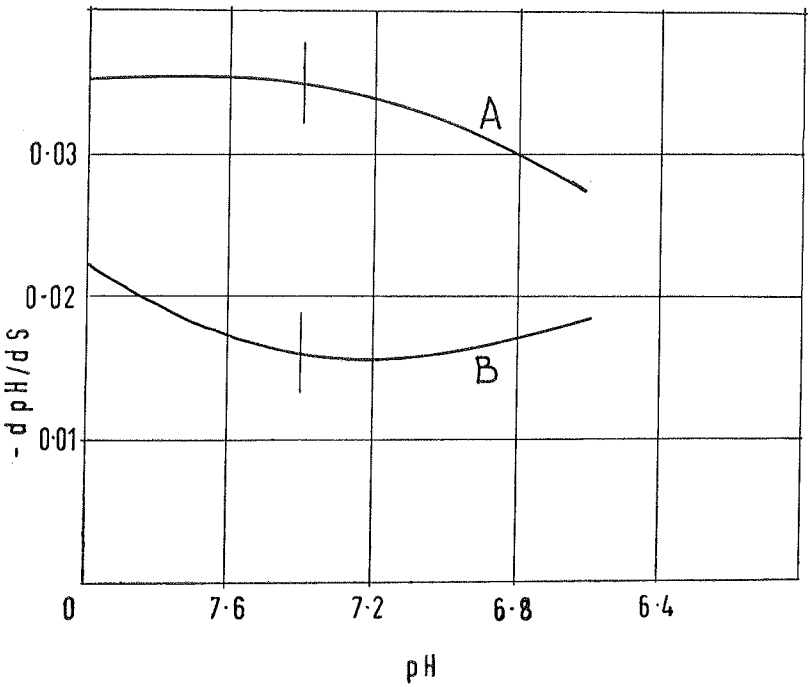

FIG. 2

figures given above appear to fit slightly better Van Slike's curve. With such a mixture one can write

$$\frac{dS}{d[CO_2]} = +1 - \frac{bK_1' + 1{\cdot}84fK_1'[CO_2]}{[H]b + 1{\cdot}84f[H][CO_2] + 2\,3[CO_2]K_1'\beta'} \qquad (2)$$

where $$f = \frac{1}{1+[H]K_O} - \frac{1}{1+[H]K_R}$$

$$(K_O = 10^{-6{\cdot}76} \text{ and } K_R = 10^{-8{\cdot}04})$$

and $$\beta = (1 - 0{\cdot}8f[H]K_1' - 0{\cdot}8f),$$

From equation 2 curve B has been obtained. In this curve not only is the value of - dpH/dS markedly lower than in

curve A, but a definite minimum appears at or very close to the value of 7.35. (The derivation of the equations is given in the Appendix to the article by the author in the *Biological Reviews,* 1945). It appears then that the pH of 7.35 in the blood gives maximum advantage to the organism, in the vital matter of the carriage of Carbon dioxide in the blood stream.

The significance of the HCO_3 content of blood plasma

The bicarbonate concentration is given by the equation

$$[HCO_3] = \frac{K^1 [CO_2]}{[H]} \qquad (3)$$

The value of $10^{-7.35}$ may be introduced for $[H]^+$ and $10^{-6.1}$ for K. Concerning the concentration of CO_2 in the blood, this corresponds to a tension of 38 mm Hg. or to about 5% carbon dioxide partial pressure in the alveoli in the lungs. To account for this figure basically, it may be said to be necessarily related to the difference in oxygen tension between the atmosphere and the alveoli, and this tension difference is determined by the volume of the metabolism and the degree of ventilation. These figures are necessarily related to optimal data for the organism.

Putting in the value of 1.2 mM in equation 3 for the $[CO_2]$ the $[HCO_3]$, concentration turns out to be 21.4 mM and this is close to such a figure as 22.4 mM determed for rat plasma (CONWAY, 1947).

The significance of the Na^+ and K^+ concentrations of the internal medium or of the blood plasma

This significance goes deep into the physiology of the cells and of the excitable tissues.

It may be approached firstly through the concept of the standard permeability for ions (CONWAY, 1947). This permeability which is applicable to muscle, nerve and gland tissue may be described thus.

a) Below a certain size level solute particles will penetrate the cell membrane freely, independent of their lipoid solubility.

b) Small cations and anions can in general pass the cell membrane with various degrees of freedom.

c) The critical size in general for rapid passage of cations is at the potassium level (hydrated ion) or between it and that of the sodium ion, and the critical size for the anions is at or near the dimensions of the chloride anion. In short for free entrance of cations or anions, it is approximately 4 Å in radius. Thus, while K, Rb and Cs ions can enter the cell at appreciable rates over short periods, Na and Li ions are virtually excluded; and while Cl, Br and NO_3 ions enter freely HCO_3, and CH_3COO ions diffuse very slowly, and SO_4 ions are practically excluded.

That the size of the hydrated ion in free solution is not the sole determinant of the entrance is shown by the fact that with muscle, caesium enters much less rapidly than potassium (*The same would appear also for nerve though the difference is less marked,* HODGKIN, 1947). It may be noted, however, that the hydrated caesium ion contains very few attached water molecules compared with potassium, and the outer water molecules of the hydrated K ion may be relatively easily detached as they pass through membrane pores.

Besides the permeability characteristics mentioned above, there is the added mechanism of the sodium pump which can actively transport Na ions out of the fibres or cells, maintaining a steady low concentration of Na within; but the cell membrane may be treated as virtually impermeable to the Na ions outside, and Na ions, in any case, enter very slowly, much more slowly as it may be shown, than has hitherto been believed.

Two important quantities may now be defined. The first symbolised as "η" represents the total molarity of the non-diffusible constituents within the fibre or cell, and given as per

litre of cell water in the normal and resting condition. If the cell water changes in volume from this standard η value changes to η/V. The second quantity (symbolised as "ε") is the sum of electric charges on the non-diffusible constituents (this sum is always negative), and may be represented as the m. equivalents of univalent cations which will balance the total of charges on "η". The physiological problem for the cell may be said to be the osmotic balancing of the non-diffusible constituents inside the cell by a non-penetrating substance outside, on the one hand, and as well the supplying of an adequacy of univalent penetrating cations to balance "ε". This is effectively done by using Na externally as the non-penetrating ion species, and the external penetrating K ion to balance the internal value of "ε". This may be put in equational form as follows. An equilibrium condition of the cell may be taken to involve the following three equilibria: osmotic, electrical and Donnan. From these the basic equations:

$$\eta/V + b_1 + d_1 = C \qquad (4)$$

$$\varepsilon/V = b_1 - d_1 \qquad (5)$$

$$\text{and } b \times d = b_1 \times d_1 \qquad (6)$$

may be written where the penetrating cations and anions are represented for convenience as single species b, , and d, , and similarly with the non-penetrating ions. From these, various other equations suitable for experimental testing may be formed, and which have, in fact been used to confirm by experimental evidence the theoretical basis (BOYLE and CONWAY, 1941). One of the simplest of such equations is equation 17 a in the paper of BOYLE and CONWAY

$$[Na_o] = \eta/V \qquad (7)$$

or when V is 1.0 as in the normal resting condition then $[Na_o]$ = or the external sodium concentration is equal to the sum of the non-diffusible material within the cell.

For the frog the value of $[Na_o]$ is 104 mM and that of 'η' from direct assessment of the analytical data is 100, while indirectly ($\eta = c - b_1 - d_1$) it is 105.

The significance of the potassium concentration in the internal medium

With the isolated sartorius of the frog immersed at about 0°C in Ringer fluid of constant Na concentration, the volume remains constant (when equilibrium is attained) even when the external K^+ is considerably increased (as KCl). Much of K^+ can be taken up by the fibres along with Cl ions. One may consider that the internal muscle K^+ is present as two quantities, one which balances the ε value, the other being associated with diffusible Cl. As the external K^+ is decreased the first quantity remains but the second can leave the fibres corresponding to very low K_o values. As K_o declines the membrane potential increases. This potential is given by the equation

$$E_m = \frac{RT}{F} + \ln \frac{[K]_1}{[K]_0} = 25 \ln \frac{[K]_1}{[K]_0} \qquad (8)$$

at 18°C.

At low $[K]_o$ values, $[K]_1$ approximates to ε and one may write

$$[K]_0 = \varepsilon \times 1^{-Em/25} \qquad (9)$$
$$(= \text{about } 120 \text{ mV})$$

The maximum value of E_m (= about 120 mV) is observed when $[K]_o$ is zero, and as $[K]_o$ approaches zero it may be supposed that equation 8 is no longer valid. Apart from the maximum value of E_m there is the normal *in vivo* figure for frog skeletal muscle of 99 mV, corresponding to a $[K]_o$ figure of 2.5 mM. The normal figure is thus close to the maximum, and may be considered as the optimal figure. The matter may be interpreted thus, that as high a potential as possible is reached consonant with the maintenance of a normal permeability. As $[K]_o$ falls below the value of 2.5 mM, Na begins to enter the fibres. As the membrane potential determines the local energy available for excitation, it will be seen why this arrangement appears to be the optimal.

Ca, Mg, and PO_4 content

In this short article, only some of the most outstanding factors producing the concentration of these ions can be suggested. (For a fuller treatment see CONWAY, 1945). The inorganic content of bone may be regarded as containing solid calcium phosphate and carbonate, and the internal medium in contact with these solid phases must be at least saturated with respect to them. Since they are sparingly soluble the constancy of the solubility products for the ions may be considered.

The *minimal* value for the Ca ion is given by the following equation (after RONA and IAKAHASHI, 1913)

$$[Ca] = \frac{k_1 [H]}{K_2' [HCO_3]} \qquad (10)$$

where k_1 is the solubility product of $CaCO_3$ (μ, the ionic strength being 0.16) and K'_2 the second adjusted ionization constant of $H_2 CO_3$.

Besides this equation for the minimal value of Ca ion there is also a relation with protein of the form:

$$\frac{[\mathrm{Ca}] \times [\mathrm{Prot.}]}{[\mathrm{Ca\ Prot.}]} = \mathrm{K} = 10^{-2.22} \qquad (11)$$

(Mc Lean and Hastings, 1935).

With regard to the total inorganic content of P in plasma, account is taken of the solubility product of calcium phosphate (expressed as k_2) and the various ionization constant of phosphoric acid. Finally P may written

$$P = \frac{[\mathrm{H}]^2 + [\mathrm{H}]K'_{2p}}{K'_{2p}\,K'_{3p}} \times \frac{k_2^{0.5} K_2'^{1.5} [\mathrm{HCO_3}]}{k_1^{1.5} [\mathrm{H}]^{1.5}}. \qquad (12)$$

(For values of these various quantities see Conway, 1945, and Sendroy and Hastings, 1926 *a* and *b*). The actual value of P from this complex equation turns out as 3.0 mg P/100 ml. which is not far removed from the observed value of 3.5 mg.P/100 ml. in human plasma.

Mg. ion concentration

The factors which determine a minimal value for the Ca ion concentration will in turn determine that of the Mg concentration. The minimal Mg ion concentration may be expected to be 1.15 times that of the Ca value (expressed as mg./100 ml.) from observations on powdered bone in fluid stimulating the salt concentration of blood plasma at 38°C.

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