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THE REDOX PUMP IN THE BIOLOGICAL PERFORMANCE OF OSMOTIC WORK

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SVMMARIVM — Investigans de fonte ex quo provenit « energia libera » illa, quae necessaria est ad iones per membranam active vehendos, Auctor concludit eam « energiam » aut ex phosphatibus provenire, qui magna polleant energia (quales sunt ATP vel eius complexus), aut directo ex energia quae liberatur cum electrones transeunt per antliam « redox ». Ex principiis autem quibus machina « redox » operatur, Auctor explicat hosmosim in ionum hydrogenii liberatione et translationem activam cationum inorganicorum.

The term Redox Pump signifies as regards the pump action, the active transport of ions across membranes, and redox indicates the interaction of redox systems in the pumping action (vide CONWAY, 1953a, 1955 and 1959).

The release of the free energy of the foodstuffs or their hydrolysed products proceeds in the cells through a series of oxidative stages, which imply in effect the transference of H atoms, or of electrons (the H atoms may be formally regarded as made up of electrons and H ions) across a series of catalysts. These may be termed redox systems with redox potentials. The transference is naturally from higher to lower energy levels (the smaller the energy level the higher the redox potential in accordance with conventional procedure). If one step in electron

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transfer between two redox systems, 1 and 2 (No. 2 having the higher potential) be considered, then the free energy change in the passage of one milliequivalent of electrons is given by:

$$-\Delta G = F(E_{h2} - E_{h1}) \qquad 1$$

This free energy change, when it occurs, is at once converted into heat.

Unless this free energy change and similar such changes with difference in redox potentials are available no work can be done, and yet when they do occur and the electron jumps across the potential difference, the free energy is lost as heat. The question then arises as to how this apparently paradoxical position is resolved and it is resolved as follows.

The performance of chemical work

If chemical work such as the formation of ATP be done as one equivalent of electrons pass from system 1 to 2, then one may write:

$$-\Delta G = F(E_{h2} - \acute{E}_{h1}) + \tfrac{1}{2}\sim P \qquad 2$$

($\sim$P representing energy-rich phosphate)

As the value of $-\Delta G$ remains the same and also the redox potential of the acceptor system, then the redox potential of the donator system must increase to $\acute{E}_{h1}$. In short the available free energy change is used up by the performance of chemical work. This process of doing work with the use of the free energy initially available goes on until $\acute{E}_{h1}$ becomes equal to E_{h2}. At this stage no further electrons can pass, and the process comes to a stand still. The continued doing of work at appreciable rates must mean then at the same time some measure of electron transference and waste of free energy.

THE PERFORMANCE OF OSMOTIC WORK BY THE REDOX PUMP

In this the doing of chemical work within the cell is not considered but rather the pumping of ions across the cell membrane by a somewhat analogous process.

THE EXCRETION OF HYDROGEN IONS

This may be instanced by the excretion of H ions by the oxyntic cells of the gastric mucosa or by yeast fermenting under certain conditions.

Two systems in the cell are considered which can be represented by the following equations:

$$\tfrac{1}{2}\,RH_2 \rightleftarrows \tfrac{1}{2}\,R + H \rightleftarrows \tfrac{1}{2}\,R + H + e \qquad 3$$

$$\text{and } \bar{M} \rightleftarrows M + e \qquad 4$$

The first may be thought of as a flavine and the second as a cytochrome system. If, for simplicity of presentation, it is assumed that the reductant and the oxidant concentrations are the same in each system one may write:

$$E_{h1} = E_{o1} + \frac{RT}{F} \ln [H^+]_{cell} \qquad 5$$

$$\text{and } E_{h2} = E_{o2} \qquad 6$$

where E_{h2} and E_{o2} are the potentials of the donator and acceptor systems respectively. If it be now considered that one equivalent of H atoms is transferred from system 1 to 2, then H

atoms are converted into H ions and electrons, the electrons being retained on the metal catalyst until they pass on to some other or final acceptor.

One may write:

$$-\Delta G = F(E_{h2} - E_{hi}) + RT \ln [H^+]_{cell} \qquad 7$$

In this equation it appears that the total free energy change is made up of two components, an electrical and an osmotic. Instead of a direct transference of the equivalent of H atoms or electrons within the cell, this process may be considered as diverted to the cell membrane, in which case the flavine enzyme receives metabolic H atoms from a donator and transfers them to the metal respiratory system. If this be assumed to occur at the outer edge of the membrane, one may regard the resultant H ions formed as passing out into the lumen while the electrons pass on through the metal respiratory system.

In a steady state:

$$-\Delta G = F(E_{o2} - \acute{E}_{h1}) + RT \ln [H^+]_{lumen} \qquad 8$$

where $\acute{E}_{hi}$ is the increased potential of system 1, due to the increased H ion concentration in the lumen.

As the change of free energy due to the passage of one equivalent of H atoms from system 1 and 2 is the same no matter what the route is, it will appear from equations 5 and 6, that

$$F(E_{h2} - E_{h1}) - F(E_{h2} - \acute{E}_{h1}) = RT \ln [H^+]_l / [H^+]_c \qquad 9$$

where $[H^+]_l$ and $[H^+]_c$ are the hydrogen ion concentrations in lumen and cells respectively. When the H ion concentration in

the lumen increases sufficiently high the second expression on the left approaches zero, and

$$F(E_{h2} - E_{h1}) = RT \ln \frac{[H]_l}{[H]_c} \qquad 10$$

In this way the whole of the electrical free energy available from the redox potential differences between the two systems within the cell can be converted into osmotic work. If the pH within the oxyntic cell be taken as 7.0 and the pH in the lumen during acidic secretion as 0.9 and the frog's gastric mucosa at 20°C be considered, the expression on the right of the equation may be evaluated as 8220 calories approximately. Inserting the value of the FARADAY constant on the left of equation 8 as 23,060 then the value of $(E_{h2} - E_{h1})$ becomes 0.36 of a volt.

ACTIVE TRANSPORT OF INORGANIC CATIONS BY TBE REDOX PUMP

As pointed out (CONWAY, 1953) such a theory of H ion secretion could be generalized to include active transport of inorganic cations across membranes and also of inorganic anions, the latter in accordance with LUNDEGARDH's views.

If, for example, one considers such a system as

$$\overset{-}{M}.\ \overset{+}{B}. \rightleftharpoons M + \overset{+}{B} + e \qquad 11$$

where the reduced form of a metal catalyst becomes associated with B^+ a univalent cation. This is formally similar to equa-

tion (3), and pursuing the same considerations as for the secretion of H ions above, one reaches the equation

$$F(E_{h2} - E_{h3}) - F(E_{h2} - E_{h3}) = RT \ln [B]_1/[B]_c \qquad 12$$

and when, with increasing levels of $[B]_1$, $(E_{h2} - E_{h3})$ becomes zero then

$$F(E_{h2} - E_{h3}) = RT \ln [B]_1/[B]_c \qquad 13$$

An essential principle in the conversion of the available redox electrical energy into work - Osmotic or chemical

The essential principle in the conversion of redox energy into work, is that the potential of the donor system moves towards that of the acceptor. This process could go on until the potentials of the donor and acceptor systems approach each other and no further appreciable work can be done.

The above principle is stated in its simplest terms, but in fact there exists a certain elasticity in the values both of the donor and acceptor potentials. It has been assumed above that the reductant and oxidant levels are the same within the two systems, but as the flow of electrons becomes lessened as the potential of the donor system increases it can no longer be assumed that the reductant and oxidant forms for each system remain equal. A consequence of the restriction in flow of electrons is that the reductant concentration of the donor system becomes greater than its oxidant form and the reverse holds for the acceptor system. It is hoped to present elsewhere a more detailed consideration of the effects described in the above paragraph. With the simpler statement of the principle described, the following may be mentioned:

a) The rate of passage of electrons across a redox couple may be considered to depend largely on the difference $F(E_{h2} - \acute{E}_{h1})$.

If an uncoupling agent such as DNP is used and only chemical work resulting in ATP is being done, then the value $\acute{E}_{h1}$ reverts back to the original and lesser value of E_{h1}. The result can be a large increase in the electron flow and it may be noted that upwards of ten times the increase in the uptake of o_2 can occur in skeletal muscle when DNP in concentration 0.027 mM is used in the external fluid in which frog sartorii are immersed.

b) What is happening at what CHANCE (1961) terms the « cross over » points (i.e. at regions where ATP is being produced) is not due to the effect of an inhibitor in the usual sense of the word. From the above standpoint what is happening is a reduced electron gradient due to the performance of chemical work.

c) The effect of the uncoupling agent with resulting increase in o_2 uptake operates on the very first stage of high energy intermediates requiring the performance of chemical work from electrical free energy.

d) Considering the applicability of the redox pump theory to the secretion of Na^+ from Na-loaded sartorii, when certain conditions are established in the immersion fluid, relating to the Na^+ and K^+ concentrations so that the energy barrier to Na section expressed by

$$-\Delta G = RT \ln \frac{[Na]_1}{[Na]_o} + E_m F \qquad 14$$

(being $[Na]_o$ and $[Na]_i$ the concentrations outside and inside the muscle fibres and E_m the membrane potential) exceeds the available redox energy, then the secretion of Na^+

should not arise. This has in fact been very fully demonstrated with Na loaded muscles. This critical energy barrier is far below the value of the free energy change available from the relation

$$ATP = ADP + P_i \qquad 15$$

being only one-fourth or less.

Evidence for the application of the Redox Pump theory in the secretion of H ions or inorganic cations

Such will here be only touched upon and with respect to the main evidence.

The redox pump has special application to the secretion of H ions by the oxyntic cells of the gastric mucosa or by yeast fermenting under certain conditions (in which latter case the pH of the suspending fluid can reach to as low as 1.5); also to active transport of inorganic cations in yeast or muscle.

1) *H ion secretion by the oxyntic cells of the gastric mucosa.*

a) Outstanding evidence here is the fact that, basing the matter chiefly on the numerous results of Davenport (1952) and of Davenport and Chavré (1952) the ratio of $\frac{\Delta[H^+]}{\Delta O_2}$ has the value of 4.0 as an upper limit. Davies has argued against this (but vide the results by the present author 1959 and Robertson's review (1960). Also it may be noted that Davies supports the redox pump theory but considers the applicability of a modified from (Davies and Ogston, 1950).

b) The parietal or oxyntic cell secretion is almost pure hydrochloric acid. It contains no appreciable organic

material and the only inorganic ion apart from H^+ and Cl^- is a small amount of K^+. This accords with the theory.

2) *H^+ ion secretion by yeast.*

a) When washed yeast (*Saccharomyces cerevisiae*) is suspended in 5 % glucose solution containing 0.1 N KCL, K^+ ions are exchanged for H^+ ion and a pH of near 2.0 is reached after about 30 mins. at room temperature. Some succinic acid is secreted at the same time.

If the yeast suspension is oxygenated (prior to glucose introduction) and the volume of the suspending solutions reduced to a 2:1 ratio, the pH of the external fluid can reach 1.5, while the organic acid secretion can almost disappear. The only inorganic cations present in the external fluid are H^+, K^+ and Cl^-.

b) *The effect of redox dyes* (CONWAY and KERNAN, 1955).

When yeast is fermented anaerobically at a pH of 4.5, and the effect of a series of redox dyes are examined with concentrations in the suspending fluid as low as 0.0005 M, the secretion of H ions and the uptake of K ions are effected so that there is produced almost complete inhibition by dyes with characteristic redox potentials of +19 or lower .

With increasing characteristic potentials of the redox dyes used the secretion of H ions and uptake of K ions are increased in a linear relation. With dyes having high redox potential the effect on H^+ ion secretion and uptake of K ions can be much above the level in the absence of dyes.

c) *The redox potential of fermenting anaerobic yeast suspension* (1:2) *and the effect of pH.*

The effect of a change of pH from 4.0 to 6.0 causes

a change expressed as $\frac{\Delta Eh}{\Delta pH}$ of only 3 or 4 millivolts. (CONWAY, KERMAN and DUGGAN, 1963). The expected change if two H atoms were involved in the determing reaction would be 57 millivolts per unit of pH change and about 28 if one H atom were involved. As the change per unit pH is only 2.5 between pH of 4.0 and 6.0, this is in agreement with the view that a metal catalyst is involved.

3) *The secretion of Na ions from skeletal muscle.*

When frog sartorii are loaded with Na ions overnight in K-free RINGER fluid containing 120 mM Na and then the usual and unfailing secretion of Na ions is demonstrated by re-immersing by re-immersing for two hours at room temperature in a RINGER-CONWAY fluid containing 104 mM Na and 10 mM K, the average of this secretion amounts to about 18 m.eqs./kg.

With regard to the two theories advanced, to account for such secretion of Na from skeletal muscle, namely the redox pump theory and the ATP-ase theory (in particular that of SKOU, 1960) that the evidence in favour of the first is practically conclusive for this tissue.

The chief evidence may be briefly summarised as follows:

a) 2.4 dinitrophenol has a marked stimulating effect on the Na secretion (CONWAY, 1960). It has no stimulating action on SKOU's ATP-ase. In vivo the effect of DNP in uncoupling phosphorylation from oxidation also extends to anaerobic metabolism (CONWAY, 1960).

b) Iodoacetate (2 mM) cuts off completely and practically instantly the active secretion of Na from the Na-loaded muscles (CONWAY, 1960). It has in turn no effect on SKOU's ATP-ase (DUGGAN, 1962).

In this context it is to be noted that when the secretion of Na is cut off at the beginning of the recovery period, there is still a total concentration of ~P (ATP + PC) of about 10 mM/kg. In the classical experiment of LUNDESGAARD (1930) the remaining value of ~P (under anaerobic conditions) can cause continued muscular contractions until ~P is exhausted.

In the case of Na loaded muscles on the contrary the ~P in the fibres is quite unable to cause any secretion of Na.

c) Proceeding from the condition of the critical energy barrier to Na secretion from Na-loaded muscles and altering the Na - K concentrations of the external or suspending fluid to decrease the energy barrier then it has been shown, that the ratio of $\frac{\text{Na secreted}}{O_2 \text{ uptake}}$ is approximately 4.0 and when the secretion occurs anaerobically the ratio of $\frac{\text{Na secreted}}{\text{lactate formed}}$ is approximately 2.0.

REFERENCES

CONWAY E.J. (1953), *The Biochemistry of Gastric Acid Secretion*. Chas. C. Thomas, Illinois.

CONWAY E.J. (1953-a), « Int. Rev. Cytology », *2*, 419.

CONWAY E.J. (1955), « Int. Rev. Cytology », *4*, 377.

CONWAY E.J. (1959), *1er Colloque de Biologie de Saclay*. Pergamon Press. London.

CONWAY E.J. (1960), « Journ. Gen. Phy. », *43*, No. 5.

CHANCE B. (1961), « J. Biol. Chem. », 235, 5.

DAVIES R.E. and OGSTON A.G. (1950), « Biochem. J. », *46*, 324.

DAVENPORT H.W. (1952), « Fed. Proc. », *11*, 715.

DAVENPORT H.W. and CHAVRÉ (1952), « Amer. J. Physiol. », *171*, 1-6.

CONWAY E.J. and KERNAN R.P. (1955), « Biochem. J. », *61*, 1, 32.

DUGGAN P.F. (1962). Unpublished work.

LUNDESGAARD E. (1930), « Biochem. Zeit. », *217*, 162.

SKOU J.C. (1960), Symposium on « Membrane Transport and Metabolism », Prague, 228, ed. by Kleinzeller A., and Kotyk A.

CONWAY E.J. (1960-a), Symposium on « Membrane Transport and Metabolism.

LUNDEGÅRDH H. (1954), *S.E.B. Symposium*, VIII, 262.

ROBERTSON B.N. (1960), *Biological Reviews 35*, 231.