phonuclear leukocytosis ($P < 0.01$) 3 hr after the injection of epinephrine. A significant eosinopenia follows the injection of epinephrine in the saline-pretreated group ($P < 0.01$), but there is a significant eosinophilia in the vitamin-pretreated group after the injection ($P < 0.05$). All cellular elements, except the eosinophils of Group II, are back to normal by 24 hr after the epinephrine treatment. A slight eosinophilia still persists in the vitamin treated group.

Histological examination indicated that epinephrine stimulated the adrenals of the saline-treated (Group I) animals. Steroid depletion and sinusoidal depletion of ascorbic acid from the inner zones of the cortex are indicative of the alarm reaction (*9*). The adrenals of Group II animals were completely normal in appearance except for their increased content of ascorbic acid.

The eosinophil and histological tests indicate that ascorbic acid pretreatment prevented signs of the alarm reaction in animals under the stress of epinephrine. It is noteworthy that the lymphocyte picture indicates stimulation of the adrenal cortex in both groups of animals. This is not substantiated by the histological tests. We have concluded that, in this experiment, the eosinophil test of adrenal activity proved to be more accurate than the lymphocyte test. This conclusion is supported by the observation that the lymphocyte response is not under complete regulatory control of the adrenal cortex; Dury (*10*) observed that the lymphopenia of stress does not occur in the splenectomized animal; in the same paper he stated that "the eosinophil therefore seemed most unequivocally, of the leukocytes studied, responsive to adrenal cortical activity alone."

Although the action of ascorbic acid observed in this experiment is very similar in some respects to that of some adrenal cortical hormones, there are certain points of dissimilarity that should be considered. Dugal and Therien observed that the vitamin prevented the hypertrophy of stress but did not cause atrophy of the adrenal; we have observed that pretreatment with the vitamin did not lead to changes caused by adrenal cortical hormones, e.g., lymphopenia, eosinopenia, and polymorphonuclear leukocytosis. In this latter respect the vitamin closely imitates the action of DCA, but it is not known whether this similarity is complete. It is expected that the DCA pretreated animal submitted to stress will not show the lymphopenia which we have observed in the ascorbic acid treated animals. It seems reasonable, therefore, in view of the findings of this experiment to assume a close relationship between the eosinophils and the amount of ascorbic in the organism. Whether this relationship involves transportation of the vitamin or some mass action process is not known. The problem is under further study in our laboratory.

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The Biological Performance of Osmotic Work. A Redox Pump

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There is now much evidence for the view that when yeast in short-period fermentation produces a high degree of acidity the H^+ ions derive from a change such as

$$CtH_2 + 2M^{n+} \rightleftarrows Ct + 2M^{(n-1)} + 2H^+,$$

and the same would appear to hold for the gastric parietal cell, as suggested by Conway and Brady (*1*) and followed by similar views advanced by Crane and Davies (*2*), by Patterson and Stettin (*3*), and by Rehm (*4*).

For yeast it also appears that the immediate process leading to the H^+ ion formation takes place in an outer region which has been identified as the cell wall.

The process occurs cyclically, which means that when the metal catalyst is again oxidized it has either to pass electrons through the inner membrane into the cell or else to pass there physically or to rotate in the membrane; and similarly with the cyclical reduction of Ct. The present communication is not concerned with the exact redox process that may occur, the main object being to indicate a relation between electrical and osmotic energy under such conditions, and the manner in which the relation might be used more generally than in the process of secreting H^+ ions.

Relation between electrical and total energy change when a redox system of type $CtH_2 \leftrightarrows Ct$ *transfers hydrogen atoms to a metal system which retains only the electrons.* It will be assumed that the two systems are in solution in different half cells, with liquid junction:

$$Pt \mid CtH_2,\ Ct,\ HCl \parallel M^+,\ M,\ HCl \mid Pt,$$

and that in each half cell of one-liter capacity there is the same HCl concentration. The platinum electrodes are joined to a source of emf which can be varied so as to allow no current to pass, or to pass very slowly and reversibly.

The electrode reactions are

$$A)\ CtH_2 = Ct + 2H^+ + 2e \quad (1)$$

$$B)\ 2M^+ + 2e = 2M. \quad (2)$$

The electrical work done, when a relatively very small

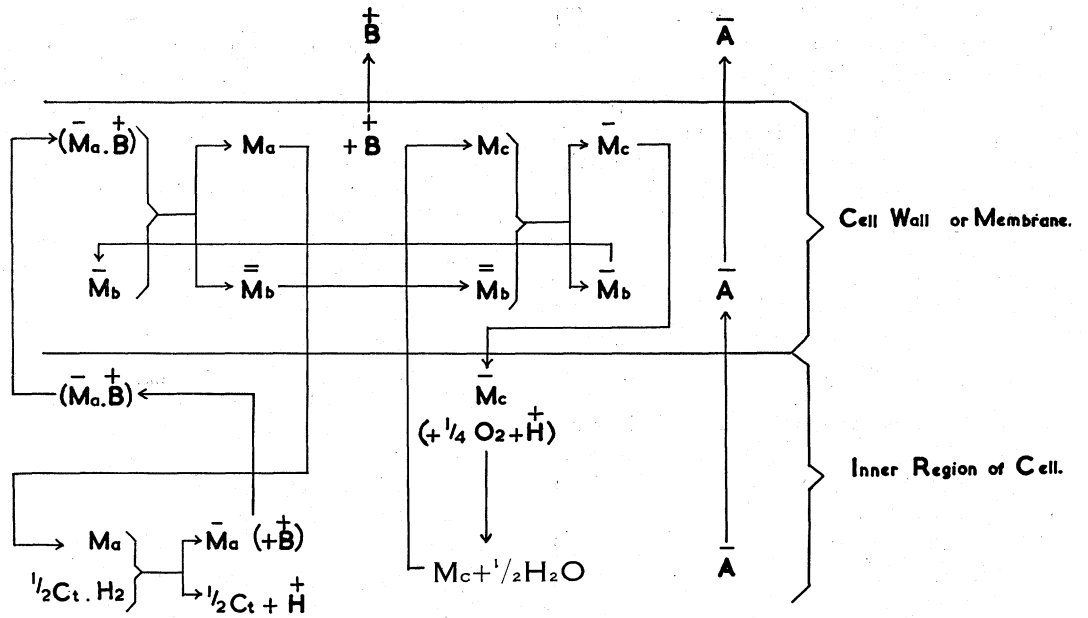

FIG. 1. The diagram indicates 3 oxidation-reduction cycles of the metal containing catalyst systems, Ma, Mb, and Mc. Ma is the carrier system for the cation B^+ (the oxidized form Ma being reduced by a catalyst, CtH_2, of flavoprotein type, and binds the cation B^+. The catalyst system Mb oxidizes the reduced Ma and liberates B^+. The Mb cycle is completed in the membrane or outer cell region, transferring its electrons to the system Mc, which transfers them finally to the oxygen system within the cell. The anion A^- is carried out by the pd established by the active carriage of B^+. The diagram is one of many that could be used to illustrate the working of the redox pump, with active carriage of anions or cations, with and without H^+ ion change.

amount of electrons (δn), in relation to a large amount of reactants, pass from Chamber A to B, may be written:

$$\delta n\mathrm{F}\ (\mathrm{E}_{B1}-\mathrm{E}_{A1}).$$

At the same time δn equivalents of H^+ ion is liberated into the half cell A, and there is an increase of $\delta n\mu_{H^+1}$ in the chemical potential of the H^+ ions.

The total free energy of the H atoms in the system $CtH_2 \rightleftarrows Ct$. An H atom in the system may be considered as made up of an electron and an H^+ ion, both at certain energy levels. The reference standard for the electron energy may be chosen as the standard hydrogen electrode and a solution containing H^+ ion at unit activity for the H^+ ion reference. The total energy of δn atoms of hydrogen may then be written:

$$\delta n(St-\mathrm{FE}_{A1}+RT\ln a_{H^+1}),$$

where a_{H^+1} is the activity of the H^+ ions in the solution and St is a constant representing the sum of the two energy standards.

If δn electrons are now considered to pass through the cell with the half cells at any potentials E_{A1} and E_{B1} and H^+ ion activity of a_{H^+1}, the total energy of the H atom previously in the $CtH_2 \rightleftarrows Ct$ system appears as

$$\delta n\{St-\mathrm{FE}_{B1}+\mathrm{F}(\mathrm{E}_{B1}-\mathrm{E}_{A1})+RT\ln a_{H^+1}\}.$$

Since E_{B1} may be regarded as a constant figure, unaffected by the H^+ ion level, nothing being varied in the systems but the H^+ ion concentration or activity, then the expression

$$\mathrm{F}(\mathrm{E}_{B1}-\mathrm{E}_{A1})+RT\ln a_{H^+1}=\text{a constant}. \qquad (3)$$

It appears therefore that, for the passage of unit quantity of electrons through such a cell, when the H^+ ion concentration is increased the electrical work falls and the chemical potential of the H^+ ions is increased in equivalent amount, to reach a maximum when the potentials of the half cells approach each other.

Application to the H^+ ion excretion by yeast or parietal cells of the gastric mucosa. In this it is supposed that a system $CtH_2 \rightleftarrows Ct$ exists in the membrane and is capable of acting alternately in the outer and inner regions, with a metal receiving system similarly placed. A steady state is considered in which the electrons are carried through the outer region, from which they travel to the inner part of the cell and to a final acceptor. The terminal stage of electron transference is associated with an uptake of H ions from the cell, hydrogen atoms being thus transferred to the acceptor. In the outer region the H^+ ions formed in the passage to the metal system (which transfers only electrons) accumulate with an equivalent amount of anions removed from within the cell by the pd established, or by active carriage.

In a steady state, if the passage of the electrons from the lower redox system to the higher were to occur wholly through the central region, on the one hand, or through the outer region on the other, the electrons beginning and ending in the same redox systems, then

$$\mathrm{F}(\mathrm{E}_M-\mathrm{E}_{Ct1})+RT\ln a_{H^+1}=\xi+RT\ln a_{H^+o} \qquad (4)$$

where ξ is the electrical work done on passing through the outer region.

Hence, it follows that

$$F(E_M - E_{Ct1}) - \xi = RTln a_{H^+o}/a_{H^+1}. \quad (5)$$

When the value of ξ approaches zero the maximum concentration of H^+ ions is reached in the outer region. In other words, as the H^+ ion concentration rises therein, the potential of the system $CtH_2 \leftrightarrows Ct$ also rises, the electrical energy of the reaction of the two systems falls toward zero, and the chemical potential of the H^+ ions reaches a maximum. A kind of see-saw effect is thus produced between the electrical energy and the osmotic.

In this we obtain an intimate picture of a transfer of energy by the cell, and the manner in which it can achieve a high concentration level for the cell constituent, which in this case is the H^+ ion.

In the parietal secretion not only are H^+ ions secreted but also Cl^- ions. The enery required is relatively very small and may be assumed to derive from the same energy source.

Wider applications. It is in the extension of this idea of the redox pump, here treated in an exploratory way, that a wider insight into certain secretory processes would appear possible. Thus, instead of hydrogen ions and hydrogen atoms, we may consider as a possibility the excretion of an inorganic cation, such as sodium ion, and that this is actively excreted from a cell where it is in low concentration into a medium in which it is in much higher concentration, the cation excretion being accomplished by Cl^- ions.

It will be supposed, but only for illustration, that this active excretion is being effected by a series of metal systems in the following way. The metals involved with their total protein complexes will be referred to as *Ma, Mb* and *Mc* (as in Fig. 1).

When *Ma* is reduced it may be assumed to expose a free or extra negative charge, and upon this Na^+ is specifically adsorbed. The transit of the electrons may first be considered to occur within the cell from the *Mb* metal catalyst, on which there is no specific Na adsorption, finally to *Mc,* also within the cell and not in the wall or membrane; alternatively, the second metal catalyst may be assumed to exist in an outer region (cell wall), with the electrons passing through this system to reach the same destination as before; one may write

$$F(E_b - E_a) + F(E_c - E_b) + \mu_{Na^+1} = \xi + F(E_c - E_b) + \mu_{Na^+o}, \quad (6)$$

and thus

$$F(E_b - E_a) - \xi = 2RTln(Na)_o/Na)_i, \quad (7)$$

and a similar result follows as treated above for H^+ ion secretion, namely, the maximum value of $RTln\ (Na^+)_o/(Na^+)_i$ is $F(E_b - E_n)$. But here, instead of the relative concentration of Cl^- ions being negligible compared with the Na^+ ions, it may be assumed that it is of the same order and if, for example, they are equal in concentration then equation (7) becomes

$$F(E_b - E_a) - \xi = 2RTln(Na^+)_o/Na^+)_i, \quad (8)$$

or, when ξ approaches zero, then

$$F(E_b - E_a) = 2RTln(Na^+)_o/Na^+)_i$$

If a computation be made of the value of $(E_b - E_a)$ a difference of only 0.12 V is required for a 10 times concentrating effect. Such activity, however, would be counteracted by the entrance of NaCl from without, and the actual internal concentration of Na^+ would come to depend on the balance of entrance and of active excretion.

In this cycle of events there occurs no change in H^+ ions in the regions discussed.

The fractional passage of the electrons through metal catalyst systems other than through $(Ma^- \cdot Na^+)$ will lessen the efficiency of the process, which may thus have values ranging from the theoretical maximum to zero.

In the above treatment it is necessary only to suppose that the ion adsorbed on the catalyst is no longer osmotically active. Also, though specificity of adsorption has been assumed in presentation, actually this need be but little marked.

The system could be applied in turn to an arrangement whereby the ion is bound to the complex by the sharing of an electron, but since such an electron may be presumed to have an energy level of the same order as the sodium atom the process of transfer would pass over a very considerable energy barrier.

In the active absorption of K^+ ions into yeast during short-period fermentation, the specificity of the absorption is about 20 times that of Na^+ ions at a pH near 2.0, but only about 4–5 times at a pH of about 5–6. That sodium ions can be actively transferred in a more or less specific manner has been shown by Krogh (*5*), and Ussing (*6*), for the frog skin; and the above mechanism may be applicable to this transfer in yeast.

Active transport of anions. Lundegardh (*7*) has presented much evidence for the conclusion that anions are actively absorbed across root hairs by a redox mechanism, similar to that considered above, for active cation transport, and such views have been furthered by the experiments of Robertson and Wilkins (*8*). Apparently little specificity is associated with such anion transport in contrast to the high degree of specificity for active absorption in yeast.

Preceding such redox views were those of Lund and Kenyon (*9*) explaining the currents drawn from the surface of plant root and those of Stiehler and Flexner (*10*) for the movement of basic and acid dyes in the choroid plexus secreting cerebrospinal fluid.

In such hypotheses the movement of free electrons is required, and this, as many have pointed out, introduces a serious difficulty when dealing with nonmetallic conductors, though the movement is possible over molecular distances.

In Lundegardh's view, as in the mechanism described above, free electron passage is not required, and the catalysts may operate on each side of a membrane by rotation therein.

Interpreting the energetic association of electron energy with osmotic work in the manner described above for cations, a similar mechanism could be

readily outlined, and will be described elsewhere.

The efficiency of the redox pump. Franck and Mayer (*11*) in their theoretical development of the "osmotic diffusion pump" found that under the most favorable conditions there would be about a 30% efficiency, and a concentrating power of about 1.3 times, higher concentration ratios being possible with a layered series.

For the active secretion of H ions by the redox pump it will be seen from the above treatment that the immediate efficiency can approach 100%. Insofar as the electrons must be carried to some final acceptor, free energy may be lost in this process, and the overall efficiency much reduced. At the same time, the energy change involved in the further passage of the electrons could be negligible or utilized in another but quite different system.

Apart from its possible very high efficiency, the most attractive feature of the "redox pump" is the fact that the active carrier and the energy source are one and the same system.

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Effect of Aureomycin on the Respiration of Normal Rat Liver Homogenates

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Recently Loomis (*1*) published a note to the effect that aureomycin specifically depresses phosphorylation without inhibiting respiration of normal mitochondria. Concurrently a study of the effect of aureomycin on enzyme systems of whole rat liver homogenates was in progress in this laboratory. In the course of this study it was found that the addition or omission of certain components in the basal medium of the system profoundly influenced the oxygen consumption in the presence of aureomycin. The basal medium used was essentially that of Pardee and Potter (*2*), with minor changes.

The most marked effect was caused by the omission of citrate from the medium (Fig. 1). Without citrate

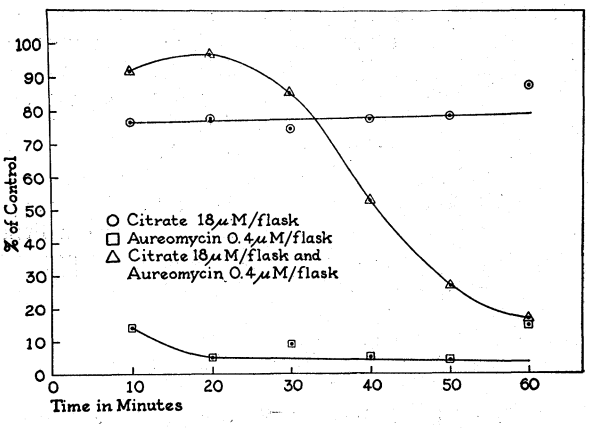

FIG. 1. Inhibition of respiration of rat liver homogenates by aureomycin.

in the presence of aureomycin, respiration is virtually brought to a halt within the first 10 min of the incubation. By contrast in the presence of citrate, the rate of oxygen consumption does not start to decline until after 30 or more min of incubation. An additional 30 min must elapse before the oxygen uptake approaches the level of the citrate-free aureomycin preparation.

This suggests that a possible mode of action of aureomycin may be through blocking some part of the Krebs cycle. Studies to determine the probable sites of action are in progress in this laboratory.

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A Synthesis of 2-Desoxy-D-Ribose[1]

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Recently we synthesized the 2-desoxy-D-ribose by the following route: Glucose → calcium gluconate → D-arabinose → D-acetobromoarabinose → D-diacetylarabinal → D-arabinal → 2-desoxy-D-ribose.

2-Desoxy-D-ribose.—D-arabinal was prepared according to Karrer and Becker (*1*), mp 81°.

$$[\alpha]_D^{22^\circ} = \frac{+3.92^\circ \times 100}{1 \times 2} = +196^\circ \text{ (in water)}.$$

1.1 g of crystalline D-arabinal was dissolved in 18.3 ml of ice-cold 1.0 *N* sulfuric acid, and the solution was allowed to stand at 0°. It gradually became faintly yellow, and after 2½ hr a faint turbidity occurred, accompanied by a flocculent precipitate. At this time the solution was soon neutralized with barium hydroxide and finally with barium carbonate. After removing the precipitate and barium carbonate, the clear and less colored filtrate was concentrated to a thick syrup under reduced pressure without heating.

[1] Aided by a grant from the Scientific Research Fund of the Department of Education.