Complete hydrolysis of *B* with 2 *N* hydrochloric acid for 3 hr. at 100° yielded equimolar amounts of mannose and glucosamine hydrochloride. These results suggested that *B* was an *O*-mannosyl glucosamine hydrochloride. *N*-acetylation[13] of *B* (250 mgm.) yielded a product (250 mgm.; $R_{glucosamine}$ 0·6) the chromogen of which exhibited 26 per cent of the absorption at 590 mμ of an equal weight of *N*-acetylglucosamine in the Morgan–Elson determination[14]. Reduction of *N*-acetyl *B* (200 mgm.) with sodium borohydride (200 mgm.) in water (20 ml.) at room temperature for 5 hr. yielded a crystalline *O*-mannosyl 2-acetamido 2-deoxy glucitol (43 mgm.; from methanol–acetone), m.p. 202°–203°; $[\alpha]_D$ − 50·6° (*c*, 1·0 in water). Found: C, 43·5; H, 6·9; N, 3·6. Calc. for $C_{14}H_{27}O_{11}N$: C, 43·6; H, 7·1; N, 3·6 per cent. Hydrolysis with 2 *N* hydrochloric acid for 3 hr. at 100° gave D-mannose and D-glucosaminol, establishing that the glucosamine-moiety formed the reducing portion of the original disaccharide. On periodate oxidation, the *O*-mannosyl 2-acetamido 2-deoxy glucitol consumed 3·9 moles of periodate and liberated 1·8 moles of formic acid per mole of alcohol. No formaldehyde was liberated. Reduction of the periodate oxidized disaccharide alcohol followed by hydrolysis with 0·5 *N* hydrochloric acid at 100° for 1 hr. yielded *inter alia* 2-amino 2-deoxy glyceritol. Hence the disaccharide alcohol was 6-*O*-D-mannopyranosyl 2-acetamido 2-deoxy D-glucitol. Application of Hudson's rules in the manner used by Stavely and Fried[15] to assess the configuration of the mannosyl residue in mannosido-streptomycin gives a value (*A*) of *c.* − 18,000 for the contribution of carbon atom one of the mannose residue to the molecular rotation. The negative value of *A* (cf. A_{OMe} = 14,230) indicates a β-linkage in the disaccharide alcohol. The rotation of the 6-*O*-mannosyl glucosamine hydrochloride, that is, + 55°, compares favourably with the rotations of related 6-*O*-β-D-mannosyl disaccharide derivatives[16].

The disaccharide probably arises from a condensation between 1 : 6-anhydro-D-mannopyranose, formed during the pyrolysis, and D-glucosamine hydrochloride. Indeed, a component with the same R_F value as the β-anhydro sugar has been detected in the pyrolysis mixture, which is not unexpected since pyrolysis is used in the preparation of the anhydro sugar from ivory nut mannan[17]. It is noteworthy that a heated mixture (1 : 1) of this anhydro sugar and D-glucosamine hydrochloride gave a 20 per cent greater yield (by weight and by Elson–Morgan reaction[6]) of *O*-mannosyl glucosamine type disaccharides than a heated D-mannose D-glucosamine hydrochloride mixture. Furthermore, L-rhamnose (6-deoxy-L-mannose), which cannot form a 1,6-anhydro derivative, did not yield Elson–Morgan-positive[6] disaccharides.

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COUPLING OF PHOSPHORYLATION TO ELECTRON AND HYDROGEN TRANSFER BY A CHEMI-OSMOTIC TYPE OF MECHANISM

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At present, the orthodox view of the coupling of phosphorylation to electron and hydrogen transfer in oxidative and photosynthetic phosphorylation stems from knowledge of substrate-level phosphorylation[1,2]. It is based, consequently, on the idea that water is expelled spontaneously between two chemical groups, *A* and *B*, by the formation of a strong bond (of low hydrolysis energy), and that subsequent or simultaneous oxidation or reduction of *A*–*B* to *A*–*B** can result in a weakening of the bond, popularly written $A \sim B^*$, so that adenosine triphosphate (ATP) can be synthesized by coupling the opening of $A \sim B^*$ to the closing of the 'high-energy bond' between adenosine diphosphate (ADP) and phosphorus through group transfer systems of appropriate substrate and oxido-reduction-carrier specificities[3–5]. There are a number of facts about the systems catalysing oxidative and photosynthetic phosphorylation that are generally acknowledged to be difficult to reconcile with this orthodox (chemical) view of the mechanism of coupling. For example: (*a*) The hypothetical 'high-energy' intermediates (for example, reduced diphosphopyridine nucleotide (DPNH) ~ ?, reduced flavin adenine

dinucleotide (FADH) ~ ?, cytochrome ~ ?) are elusive to identification[4,6]. (*b*) It is not clear why phosphorylation should be so closely associated with membranous structures[7–9]. It has sometimes been assumed that digitonin-treated mitochondrial 'particles' can couple oxidation to phosphorylation without membranes[10]; but it is doubtful whether this assumption can be justified, for such 'particles' give only poor respiratory control and contain much lipid[11], which may well exist as a leaky membrane[12]. (*c*) Coupling may vary with the stress, causing variation of respiratory control without corresponding variation in phosphorus/oxygen quotient[4,13]—a phenomenon difficult to explain in terms of molecular stoichiometry. (*d*) Hydrolysis of external ATP by mitochondria causes reduction of internal DPN, accentuated by the oxidation of succinate[14]. There is disagreement as to what complex assumptions offer the better explanation[15]. (*e*) Uncoupling can be caused at all three hypothetical oxido-reduction sites in mitochondria by agents that do not share an identifiable specific chemical characteristic[4] (for example, dinitrophenol, dicoumarol, salicylate, azide). (*f*) Unexplained swelling and shrinkage phenomena accompany the activity of the phosphorylation systems[7,9a,11,16].

Structural features have been invoked as the causes of the departure of the phosphorylation systems from 'ideal' behaviour[4,14,17]; but, as Green has pointed out in a far-sighted paper[18], although the structural features have been recognized as playing an important part in the catalytic activity of multi-enzyme systems, they have so far been treated rather conservatively. The structural (or supramolecular) features have, in fact, generally been regarded only as modifiers of the basic chemical type of coupling process outlined above. The general conception of enzyme-catalysed group translocation that we have been developing in my laboratory for some years[19] offers a more radical approach to the problem; but this has not, so far, been made use of by those working in the field of oxidative and photosynthetic phosphorylation.

The purpose of this article is to suggest that in view of the difficulties confronting the orthodox chemical conception of coupling in oxidative and photosynthetic phosphorylation, one might now profitably consider the basic requirements and potentialities of a type of mechanism that is based directly on the group translocation conception. This type of mechanism differs fundamentally from the orthodox one in that it depends absolutely on a supramolecular organization of the enzyme systems concerned. Such supramolecularly organized systems can exhibit what I have called chemi-osmotic coupling[19c,20], because the driving force on a given chemical reaction can be due to the spatially directed channelling of the diffusion of a chemical component or group along a pathway specified in space by the physical organization of the system[20]. We shall consider chemi-osmotic coupling between the so-called ATPases on one hand and the electron and hydrogen transfer chain on the other: mediated by the translocation of electrons and the elements of water across the membrane of mitochondria, chloroplast grana, and chromatophores.

The first basic feature of the chemiosmotic coupling conception is a membrane-located reversible 'ATPase' system[20b] shown very diagrammatically in Fig. 1. This system, which may include lipids and other components as well as proteins, is assumed to be anisotropic so that the active centre region (indicated by the dotted circle) is accessible to OH′ ions but not H^+ ions from the left (inside organelle), and to H^+ ions but not OH′ ions from the right (cytoplasm side of organelle). The active centre, like that of phosphokinases in general, is assumed to be relatively inaccessible to water as water. To illustrate the hydrolytic activity of the system, the OH′ ion is depicted as diffusing from left to right and finally combining at the active centre region with the terminal phosphorylium (P^+) derived from the ATP (ADP—O′—P^+) giving inorganic phosphate (POH) and ADP having a terminal ionized oxygen (ADP—O′), assuming the right-hand phase to be at about *p*H 7 and at electrical neutrality. The elegant conception of the transfer of phosphoryl as phosphorylium ion is due to Lipmann[21]. The system, of course, catalyses hydrolysis equilibrium, and the reverse or phosphokinetic activity of the 'ATPase' is indicated by the single barbs on the arrows of Fig. 1. In the case of the phosphokinetic activity, the OH′ ion is depicted as passing down a free-energy gradient towards the left from an inorganic phosphate group (POH) that passes to the active centre region from the right. The phosphorylium ion (P^+), created by the withdrawal of the OH′, is attacked by the negative atom ($-R'$) in the active centre region, and the phosphorylium ion is then donated to the terminal oxygen of ADP—O′ to give ATP. The chemi-osmotic coupling hypothesis depends thermodynamically on the fact that in such an anisotropic ATPase system, the electrochemical activity of the water at the active centre ($[H_2O]_c$), which determines the poise of the hydrolysis equilibrium in the ATP/ADP system, would be given, not by the product of $[H^+] \times [OH']$ in the aqueous phases *L* or *R*, but by the product $[H^+]_R \times [OH']_L$ (where [] stands for electrochemical activity, and *R* and *L* for right- and left-hand phases respectively). The ratio of the electrochemical activity [ATP]/[ADP] (including all ionic forms) can be raised, consequently, and the ATPase activity can be reversed to give an ADP phosphokinase activity proportional to the lowering of $[H_2O]_c$, in accordance with the mass-action law for hydrolysis equilibrium, written to include the elements of water as follows:

$$\frac{[ATP]}{[ADP]} = \frac{[P]}{K_1[H_2O]_c} \qquad (1)$$

The electrochemical activity of a component in a certain place defines absolutely the escaping tendency of the particles of the component due both to the

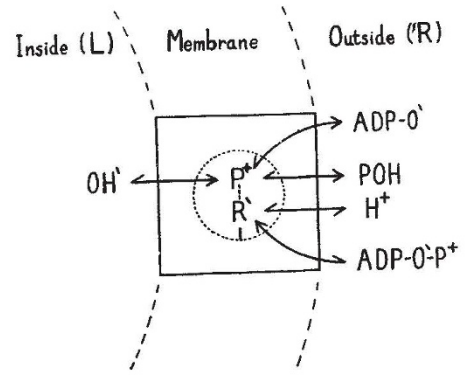

Fig. 1. Anisotropic reversible 'ATPase' system located in an ion-impermeable membrane between aqueous phases *L* and *R*

chemical and to the electrical pressure to which the particles are subject in that place at equilibrium. Since

$$K_2 = \frac{[OH']_L \times [H^+]_L}{[H_2O]_L} \quad (2)$$

and K_2 is independent of the medium because we are using electrochemical activities, we can describe the electrochemical activity of the water at the active centre of the 'ATPase' system as follows:

$$[H_2O]_c = [H_2O]_{aq.} \times \frac{[H^+]_R}{[H^+]_L} \quad (3)$$

where $[H_2O]_{aq.}$ stands for the electrochemical activity of water in the aqueous physiological media of phases L or R, and is equivalent to about 55·5 M water.

By the definition of the electrochemical activity:

$$\frac{[H^+]_L}{[H^+]_R} = 10^{pH_{R-L}} \times 10^{(mV._{L-R})\frac{F}{2303\,RT}} \quad (4)$$

where pH_{R-L} is the pH of phase R minus that of phase L; $mV._{L-R}$ is the membrane potential in millivolts, positive in phase L; R is the gas constant; F is the faraday; and the factor $\frac{F}{2303\,RT}$ is approximately 1/60. It can be seen from equation (4) that the ratio $[H^+]_L/[H^+]_R$ is multiplied by a factor of 10 for each pH unit more negative on the left, relative to the right, and for each 60 mV. membrane potential, positive on the left. Equations 1 and 3 show that the ratio [ATP]/[ADP] at equilibrium is determined by $[H^+]_L/[H^+]_R$ as follows:

$$\frac{[ATP]}{[ADP]} = \frac{[P]}{K_1[H_2O]_{aq.}} \times \frac{[H^+]_L}{[H^+]_R} \quad (5a)$$

When the right-hand phase (representing the cytoplasm) is the region of the zero or reference potential, the electrochemical activity ratio of total ATP to ADP will be nearly the same as the corresponding concentration ratio, and [P] will correspond approximately to the inorganic phosphate concentration. Thermodynamic data[22] show that at pH 7 and at physiological temperatures the 'hydrolysis constant' as usually defined or the product $K_1[H_2O]_{aq.}$ is approximately 10^5; and when [P] is at the physiological level of 10^{-2} M, equation (5a) can be written:

$$\frac{[ATP]}{[ADP]} \simeq 10^{-7} \frac{[H^+]_L}{[H^+]_R} \quad (5b)$$

Thus, the [ATP]/[ADP] equilibrium can be poised centrally through the anisotropic 'ATPase' by making the ratio $[H^+]_L/[H^+]_R$ about 10^7. Equation 3 shows that this could be done, for example, by poising the left side 2 pH units below and 300 mV. above the right side. Such is the basic thermodynamic conception of the mechanism of reversal of the 'ATPase' activity. In kinetic terms, the reversal of the 'ATPase' activity can be understood by regarding the electrochemical activity gradient of hydrogen and hydroxyl ions across the active centre region of the 'ATPase' as the cause of the donation of the phosphorylium ion to the negative acceptor atom ($-R'$) by the simultaneous withdrawal of an OH′ ion from the inorganic phosphate down a steep gradient to the left and of an H^+ ion from $-RH$ to the right to ionize $-RH$ or to prevent H^+ from competing with phosphorylium ion for the acceptor $-R'$. Dehydration is accomplished by using the high $[H^+]$ region as a sink for OH′ and the high [OH′] region as a sink for H^+. It should be understood that the phosphorylation of ADP can be strictly described as dehydration only when the standard state is less than pH 6 (see Lipmann[21]). Since, however, the hydrogen atom of the terminal hydroxyl group of ADP, involved in phosphorylation, is dissociated reversibly as an H^+ ion, when the standard state is taken as pH 7, the phosphorylation process is mainly that of dehydroxylation. From the kinetic point of view, the fundamental processes involved are dehydroxylation + deprotonation, or dehydroxylation. It is relevant to note that phosphorylation is not directly caused by raising $[H^+]$, but is due to depression of [OH′]. For this reason, the hydrogen ion depicted in phase R of Fig. 1 (and correspondingly in Figs. 2 and 3) is shown as equilibrating with the active centre region of the 'ATPase' system, but not as being withdrawn stoichiometrically in relation to the withdrawal of OH′.

The second basic feature of the chemi-osmotic coupling conception is the electron and hydrogen translocation system, which is assumed to create the gradient of electrochemical activity of H^+ and OH′. Unlike the conception of the anisotropic 'ATPase', the idea of the anisotropic o/r system is not new, but stems from the work of Lund[23], and Stiehler and Flexner[24], and was first stated explicitly by Lundegårdh[25] more than 20 years ago. Lundegårdh's idea was more exactly defined in relation to ion transport by Davies and Ogston[26], and was elaborated by Conway[27]. It has been excellently reviewed by Robertson[28].

Fig. 2 illustrates how the electron transfer can affect the ratio $[H^+]_L/[H^+]_R$. The electron translocation and 'ATPase' systems are depicted as being placed in opposition in a charge-impermeable membrane. The hydrogen ions generated on the left and the hydroxyl ions generated on the right by the electron translocation system dehydrate ADP and inorganic phosphate (now simply denoted by P) to form ATP by withdrawing hydroxyl ions to the left and hydrogen ions to the right through specific translocation paths in the 'ATPase' system as described here. Conversely, of course, the effect of the back pressure of ATP hydrolysis is to force the o/r system towards reduction on the left (inside the

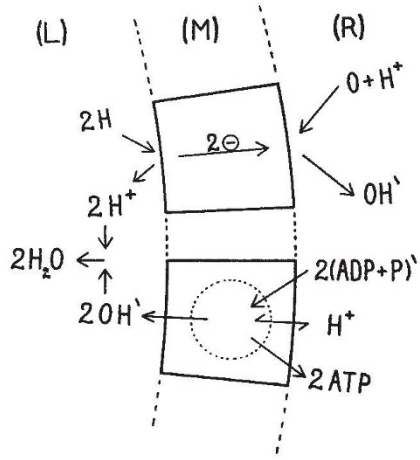

Fig. 2. Electron transport system (above) and reversible 'ATPase' system (below) chemiosmotically coupled in a charge-impermeable membrane (M) enclosing aqueous phase L in aqueous phase R

organelle). Note that the stoichiometry is 2 ATP per O, as in succinate oxidation by mitochondria. As shown in this simple diagram, the dehydrating force driving the phosphokinetic activity of the 'ATPase' system would be due largely to the chemical potential differential of the H^+ and OH' ions across the membrane, which would have to show as a pH difference of some 7 units across the membrane, acid on the left when the [ATP]/[ADP] equilibrium was poised centrally (see equations (5*b*) and (4)). We assume, however, that exchange diffusion carriers, as defined by Ussing[29], are present in the membrane and that they will allow strictly coupled one-to-one exchange of H^+ against K^+ or of OH' against Cl', for example. The pH differential would thus tend to be reduced to a relatively small figure and would be equivalently replaced by a membrane potential as described by equation (4).

When the oxido-reduction and phosphorylation systems are in chemi-osmotic equilibrium, one ATP molecule will be produced per electron translocated across the membrane. The relationship between the o/r potential span (ΔE) of the electron and hydrogen translocation system and the poise of the [ATP]/[ADP] equilibrium will be given as follows:

$$\frac{[ATP]}{[ADP]} = \frac{[P]}{K_1[H_2O]_{aq.}} \times 10^{\frac{\Delta E.F}{2303\,RT}} \qquad (6a)$$

or approximately:

$$\frac{[ATP]}{[ADP]} = \frac{[P]}{10^5} \times 10^{\Delta E/60} \qquad (6b)$$

ΔE being in millivolts. It should be understood that ΔE, as defined here, is equivalent to the free-energy change in the electron-translocating system per electron translocated. Assuming that ATP synthesis were occurring when the [ATP]/[ADP] ratio was poised centrally at pH 7 and in the presence of 10^{-2} M inorganic phosphate, the o/r span, ΔE, would have to be about 420 mV. This, of course, being the equilibrium potential, represents the minimum o/r span of the electron and hydrogen translocation system required to drive ATP synthesis under the conditions specified above. The span between the succinate–fumarate couple and oxygen at 76 mm. mercury pressure is about 750 mV.—well above the minimum ΔE of 420 mV.

In practice, the mitochondrial or chloroplast membrane across which the chemi-osmotic coupling may be organized would allow a certain amount of ion leakage, and the translocation paths for H^+ and OH', connecting the internal and external phases (L and R) with the active centre region of the 'ATPase', would not be expected to have absolute specificity for H^+ and OH' respectively. Consequently, equations (6*a*) and (6*b*) would represent the practical state of affairs in the most tightly coupled systems. On 'loosening' the membrane system, or if 'uncoupling' were effected by catalysing the equilibration of H^+ and charge across the lipid of the membrane with reagents like dinitrophenol or salicylate, the relationship between the poise of the [ATP]/[ADP] ratio and ΔE in the steady state would be described by the inequality:

$$\frac{[P]}{K_1[H_2O]_{aq.}} \leqslant \frac{[ATP]}{[ADP]} \leqslant \frac{[P]}{K_1[H_2O]_{aq.}} \times 10^{\frac{\Delta E.F}{2303\,RT}} \qquad (6c)$$

The outer terms of equation (6*c*) represent the extreme values of the [ATP]/[ADP] ratio from complete uncoupling on the left to complete coupling on the right.

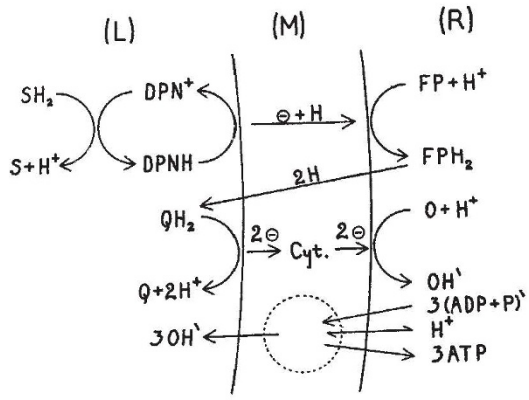

Fig. 3. Diagram of chemi-osmotic system for coupling phosphorylation to the oxidation of substrate (SH_2) through DPN, FP (tentatively identified with flavoprotein), Q (tentatively identified with a quinone) and the cytochromes (*Cyt.*). The other conventions are as in Figs. 1 and 2

Fig. 3 shows, in principle, a rather fuller description of oxidative phosphorylation, in which I have included the o/r components FP and Q, tentatively identified with flavoprotein and quinol–quinone systems respectively. The main aim is to illustrate how a stoichiometry of 3 ATP per O can readily be obtained for substrate (SH_2) oxidation through DPN, by the obligatory transport of one (net) hydrogen atom inwards per O, owing to the spatial arrangement of the electron and hydrogen transfer chain and the zero, one, or two hydrogen-transfer characteristics of the carriers involved. In this system, the span of both parts of the o/r chain across the membrane would have to be poised against the same ratio $[H^+]_L/[H^+]_R$ at equilibrium, according to equation (5*a*). Using the conventions of Dixon[2], the $-\Delta F$ value for the DPN/DPNH couple at about pH 5 would be some 3,000 cal. and the $-\Delta F$ for FP (corresponding to an E'_0 (pH 7) of -60 mV.) (ref. 30) would be about 17,000 cal., giving a span of 14,000 cal.; equivalent to a ΔE of 600 mV. Assuming the $-\Delta F$ of the Q system to be about 24,000 cal., corresponding to an E'_0 (pH 7) of + 100 mV., as in the ubiquinone system[31], the span from the Q system to oxygen at 76 mm. mercury pressure would be equivalent to a ΔE of about 750–100 = 650 mV. The tendency of the two o/r values to drift together and the exact magnitude of the composite o/r potential would, of course, depend on many factors that it would be premature to consider here. It will suffice to point out at present that the value of 600–650 mV. for ΔE is appropriately above the required minimum of 420 mV. and that the proposed system is thus in accord with the thermodynamic facts.

The above basic chemi-osmotic conception can be applied to photosynthetic phosphorylation with the difference that the electron and hydrogen translocation are seen as being driven, not by the affinity of oxygen for the hydrogen atoms and electrons, but by the energy of the absorbed photons, according to the type of mechanism described by Calvin[9b]. It can readily be shown that in a chemi-osmotically coupled system for non-cyclic photophosphorylation, the photon-activated movement of 2 electrons and 2 hydrogen atoms outwards through the membrane of the grana would produce one O_2 and 2 ATP

molecules. Similarly, in non-cyclic photophosphorylation, the skew of $[H^+]_L/[H^+]_R$, and thus the synthesis of ATP, could be caused by the photon-activated passage of equal numbers of hydrogen atoms and electrons in opposite directions across the membrane of the grana or chromatophores.

The facts that were listed at the beginning of this article as being difficult to reconcile with the orthodox chemical conception of the mechanism of coupling phosphorylation to electron and hydrogen transfer can now be reconsidered in relation to the chemi-osmotic coupling hypothesis: (*a*) The elusive character of the 'energy-rich' intermediates of the orthodox chemical coupling hypothesis would be explained by the fact that these intermediates do not exist. (*b*) According to the chemi-osmotic coupling hypothesis, the differential of the electrochemical activity of the hydrogen and hydroxyl ions across the membrane, generated by electron transport, causes the specific translocation of hydroxyl and hydrogen ions from the active centre of the so-called ATPase system, thus effectively dehydrating ADP + P. The charge-impermeable membrane would therefore be an absolute requirement for tight coupling. (*c*) Coupling would be expected to vary with the extent of leakiness or strain in the membrane, determined, of course, by the osmotic and electrical stress. (*d*) The internal components of mitochondria such as DPN would tend to be reduced by the high electrochemical activity of H^+ caused by hydrolysis of external ATP, which would withdraw OH' ions from the inside in competition with the electron-transport system. This effect would be accentuated by oxidation of succinate (which would raise the internal value of $[H^+]$) but not, of course, by substrates such as acetoacetate that directly oxidize DPNH. (*e*) Uncoupling would be caused by lipid-soluble reagents, such as DNP, salicylate, azide, and ammonia, catalysing equilibration of H^+ or OH' and charge across the membrane. (*f*) According to the chemi-osmotic type of hypothesis, the coupling of phosphorylation to electron and hydrogen translocation would cause considerable electrical and mechanical stress in the membrane across which coupling was effected. Complex swelling and shrinkage effects would therefore be expected to accompany the activity of the system.

It is evident that the basic features of the chemi-osmotic coupling conception described here and elsewhere[20] are in accord with much of the circumstantial evidence at present available from studies of oxidative and photosynthetic phosphorylation. This simple hypothesis also has the merit that it represents the result of carrying to its logical conclusion the present trend towards recognizing the equivalent status of supramolecular and molecular features in the channelling of chemical processes in living organisms[18]. Further experimental support for the chemi-osmotic coupling conception may best be sought by attempting to characterize separately each of the three hypothetical basic elements of which the system is thought to be built: (1) the anisotropic 'ATPase' system which I have defined above; (2) the anisotropic *o/r* system of the type originally defined by Lundegårdh; (3) the specific charge-impermeable membrane in which the systems 1 and 2 are supposed to be orientated in opposition. Work along these three lines is proceeding in my laboratory.

In the exact sciences, cause and effect are no more than events linked in sequence. Biochemists now generally accept the idea that metabolism is the cause of membrane transport. The underlying thesis of the hypothesis put forward here is that if the processes that we call metabolism and transport represent events in a sequence, not only can metabolism be the cause of transport, but also transport can be the cause of metabolism. Thus, we might be inclined to recognize that transport and metabolism, as usually understood by biochemists, may be conceived advantageously as different aspects of one and the same process of vectorial metabolism[20a,32].

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