I express my warmest thanks to Dr. Hugh Felkin (CNRS), in whose laboratories much of this work was done, for his help and generosity throughout. I also thank gifted students and coworkers, Dr. George Morris, Dr. Tauqir Khan, and Jennifer Quirk, for their key contributions. I also wish to acknowledge helpful conversations with Drs. Malcolm Green and Melvyn Churchill and to thank Dr. Roald Hoffmann for communicating some of his results prior to publication. We also acknowledge funding from the CNRS, a DuPont Young Faculty Grant, the Petroleum Research Fund, administered by the American Chemical Society, the National Science Foundation, and a loan of part of the precious metals from the Compagnie des Mêtaux Prêcieux (Ivry).

Transport of Ions

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Cells have an ionic composition that differs drastically from that in their environment. This is true for both simple creatures in the ocean and for complex cells that are nourished by blood. The cytoplasm of cells harbors mostly K^+, a little Na^+, some Mg^{2+}, and virtually no Ca^{2+}. On the outside there is much more Na^+ than K^+, and about 1000 times more Ca^{2+}. Moreover, there are many ions like copper and zinc which are needed by the cell in trace amounts but are highly toxic when present in excess. To maintain this ionic inbalance, both energy and controls are required. In most instances adenosine triphosphate (ATP), the universal energy currency of living cells, is used either directly or indirectly to perform the work. Few biologists (not to speak of chemists) realize how expensive these transport processes are. Indeed, in resting cells they consume a major portion of the energy the cell utilizes. Controls are established by imposing specificity and directionality. For example, a pump is installed in the plasma membrane which exports 3 Na^+ for every 2 K^+ that are imported.[1]

Export and import of ions are, however, only part of the story. Cells contain organelles which are busily engaged in the ion-transport business. For example, there are intracellular compartments that accumulate Ca^{2+}. In muscle this compartment is called sarcoplasmic reticulum. It plays an important role in muscular contraction. When an appropriate nerve impulse comes in, Ca^{2+} is released from the sarcoplasmic reticulum, initiating a series of complex reactions which lead to the contraction of muscle. Then the ATP-driven pump starts working and collects the released Ca^{2+} back into the safe compartment of the organelle and the muscle relaxes again.[2]

For the maintenance of life, a most important organelle is the mitochondrion. It contains an outer and inner membrane (Figure 1). The latter is the seat of the process called oxidative phosphorylation which generates most of the ATP the cell requires. Mitochondria have been called the powerhouses of the cell and, as I shall describe later in much greater detail, the key process that drives the generation of ATP is translocation of protons. The mitochondria contain enzymes which oxidize (via the Krebs cycle) the food substrates that are delivered to these organelles, thereby generating $NADH_2$ (see Figure 1). The hydrogens of $NADH_2$ (also written as NADH + H^+), instead of interacting directly with oxygen to form water, are channeled into an enzyme assembly located in the inner membrane of the mitochondria. These enzymes separate electrons from protons. The electrons are transported within the membrane through several catalysts of the electron-transport chain to cytochrome oxidase, the terminal enzyme, which combines the electrons and protons with oxygen to form water (Figure 2). The protons derived from $NADH_2$ are transported across the membrane, thereby generating an electrochemical ion gradient which is utilized to generate ATP.[3] The generation of ATP takes place in the inner compartment of the mitochondria (the matrix) which is surrounded by an ion-impermeable membrane, so that an ion gradient can be maintained. As a consequence, however, the two ions that are required for the generation of ATP, namely P_i and ADP, must be imported. Since most of the ATP is used by the cell outside of the mitochondria, it must be exported. Thus, these specific transport processes must take place through the inner mitochondrial membrane, as illustrated in Figure 3.

There are many other cellular membranes that participate in ion transport. Gastric cells excrete H^+ and Cl^-; adrenal cells transport adrenalin; nerve cells have specific channels for Na^+ and Ca^{2+}. All these processes are catalyzed by multiple devices, which I shall discuss later.

Physiologists speak of active transport and facilitated diffusion. Active transport takes place against a gradient and requires an input of energy, whereas facilitated diffusion does not require an energy input and runs downhill until equilibrium is attained. The

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(1) (a) In order to limit the number of references I have often quoted reviews where additional references can be found. (b) (R) I. M. Glynn and S. J. D. Karlish, *Annu. Rev. Physiol.*, **37**, 13 (1975).

(2) (R) Y. Tonomura and A. Inoue in "Energy Transducing Mechanisms", Vol. 3, E. Racker, Ed., Butterworths, London, 1975, p 121.

(3) P. Mitchell, *Biol. Rev. Cambridge Philos. Soc.*, **41**, 445 (1966).

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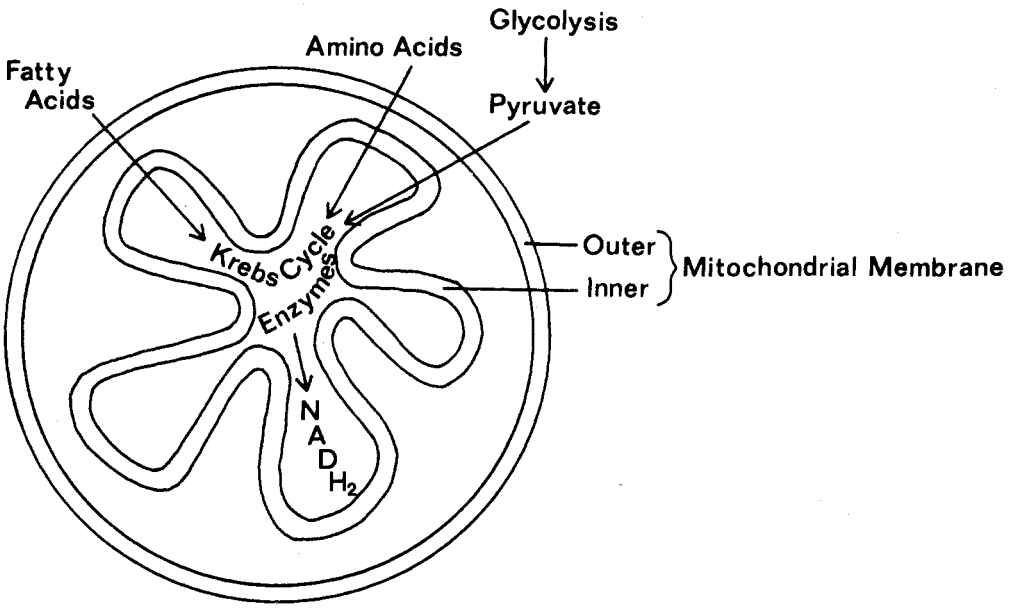

Figure 1. The mitochondrion.

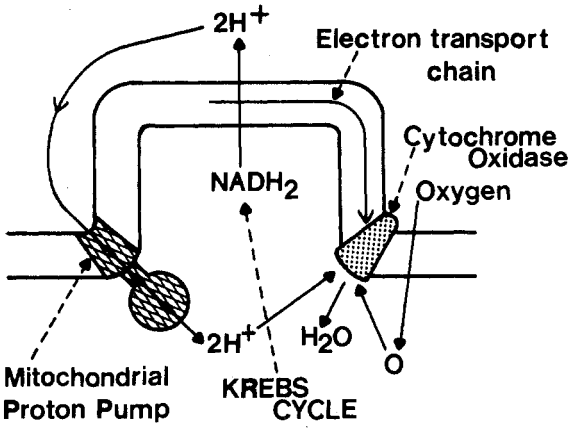

Figure 2. Inner mitochondrial membrane.

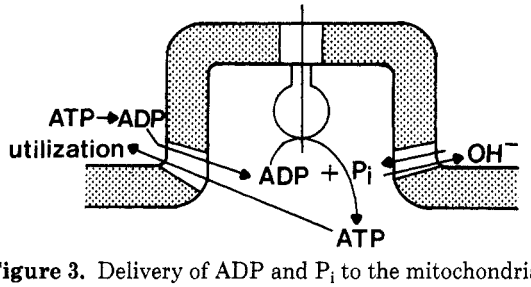

Figure 3. Delivery of ADP and P_i to the mitochondrial proton pump.

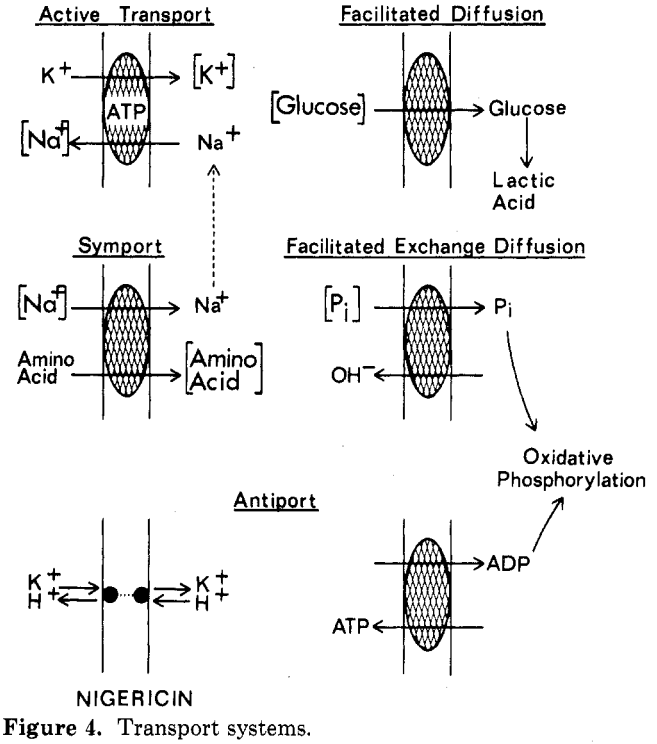

Figure 4. Transport systems.

ATP-driven Na^+–K^+ pump shown in Figure 4 is an example of an active transport system. The transport of glucose in the red blood cell is an example of facilitated diffusion. When we examine certain other transport systems they appear more complex. Transport of some amino acids is a mechanism in which Na^+ is transported along with the amino acid.[4] If we have a Na^+ gradient (which we maintain by the ATP-driven plasma membrane pump), we can transport amino acids uphill against a gradient.

The transport of P_i into mitochondria takes place by facilitated diffusion: a P_i is exchanged for a OH^-. Nevertheless the process can be driven uphill by creating a H^+ (or OH^-) gradient. Similarly, the exchange of K^+ or Na^+, which is catalyzed by a mobile carrier called nigericin, can be used for active transport if a proton gradient is available. The ADP/ATP exchange mechanism in the mitochondrial membrane (Figure 3) is complicated by the fact that there is a difference in the electrical charge, the ATP being more negative than ADP. Thus another ion (probably a proton) has to move to facilitate the process. An interesting example is the excretion of lactate which is driven by a proton gradient.[5] This means that a proton leaves the cell together with a lactate. If the cell becomes acidic, e.g. during glycolysis, the excretion of lactate against a concentration gradient is possible. Until recently, this

(4) (R) H. N. Christensen, "Biological Transport", 2nd ed., W. A. Benjamin, New York, 1975.

(5) T. L. Spencer and A. L. Lehninger, *Biochem. J.*, 154, 405 (1976).

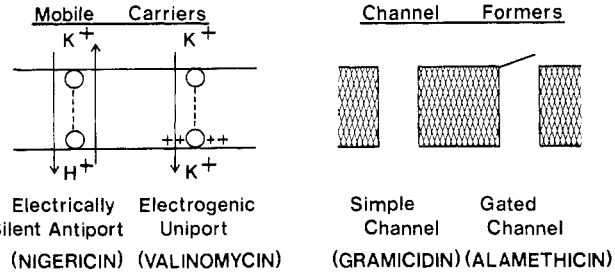

Figure 5. Mobile carriers and channels.

was not recognized as being an active transport system.

Facilitated diffusion may also be more complex than in the case of glucose transport in red blood cells. In some instances the removal of the transported sugar from the plasma membrane, e.g. by hexokinase in the presence of ATP, accelerates the rate of transport. Another example is the case of Ca^{2+} entry into the cell which is coupled to a sequestering of Ca^{2+} by mitochondria or microsomes. Thus, the overall process is energy driven, although the translocation itself may not be. This has also led to confusion.

Because of these complexities, the simple concepts of active transport and facilitated diffusion have lost some of their attractiveness. Indeed, there are other ways of characterizing transport processes, based on differences in modes of action. Most of these new concepts have been derived from studies with simple model compounds which are mainly antibiotics excreted by microorganisms.[6] As shown in Figure 5, they either act as mobile carriers or as channels.

Carriers, Channels, and Pumps

I previously mentioned nigericin, which functions as a mobile carrier exchanging H^+ against K^+ or Na^+. In a medium containing Na^+ or K^+ salts, it can be used to collapse a proton gradient. Nigericin therefore interferes with oxidative phosphorylation (which is dependent on an electrochemical proton gradient). This feature is probably responsible for its toxicity. Another antibiotic, valinomycin, is an electrogenic mobile carrier. It specifically interacts with K^+ to form a charged complex which traverses the membrane and thereby generates a positive membrane potential (or collapses a negative membrane potential). Valinomycin has been a valuable tool in studies of ion transport, as I shall illustrate later. Although most mobile carriers are bacterial excretion products, there is at least one class of mobile carriers that is present in mammalian cells. Long-chain fatty acids are natural proton ionophores. They are known to be "uncouplers" of oxidative phosphorylation, and they probably play an important role in the balance of our energy budget.

Most of the transport systems of mammalian cells contain channels. Models of simple channels are once again antibiotics of microbial origin. When two molecules of gramicidin interact, they form a channel which is wide enough to allow the flux of monovalent cations. An example of a more complicated channel is alamethecin. The formation of the channel requires the interaction of several alamethecin molecules (8 to 10) as well as an appropriate membrane potential. It was proposed[6] that the vertical orientation of the molecule

(6) (R) "Carriers and Channels in Biological Systems", *Ann. N.Y. Acad. Sci.*, **264** (1975).

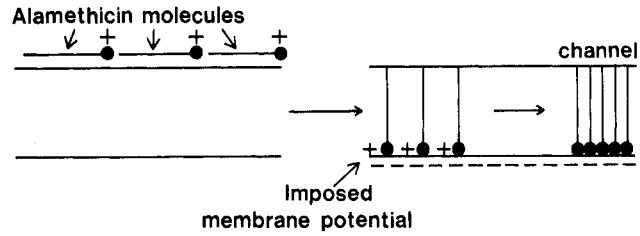

Figure 6. Model of a gated channel.

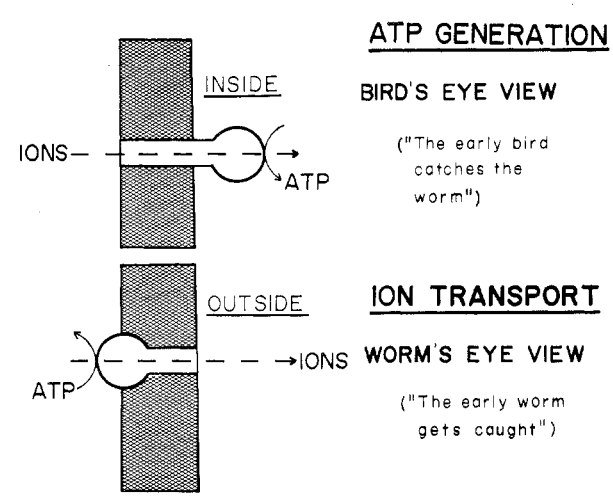

Figure 7. ATP generation and ion transport.

within the membrane is dependent upon the electrical charges on the membrane, as illustrated in Figure 6.

How can we tell whether we are dealing with a mobile carrier or a channel? This is not always easy. Mobile carriers traverse the membrane from one side to the other and are therefore much more dependent on membrane fluidity than rigid channels which span the membrane. Thus, a mobile carrier cannot function in a frozen membrane (below the transition temperature of its phospholipids), while a channel may be unperturbed. On the other hand, a mobile carrier is less influenced by the thickness of the membrane. For example, the dimer of gramicidin forms a channel which is only 28 Å long.[6] If the membrane is too thick it becomes ineffective, while mobile carriers can traverse even multilammelar membranes. A most important difference between mobile carriers and channels is their potential kinetic capacity. If a transporter is capable of operating at a rate exceeding 10^4 ions/s, we can confidently rule out a mobile carrier mechanism because the rate of diffusion limits its kinetic potential.

ATP-driven pumps are channels linked to an energy transformer which usually also serves as a gate. One of the key questions in bioenergetics is the mechanism of action of the transformer. How is ATP utilized to generate an ion gradient? Since most of the ATP-driven pumps are reversible, we can rephrase the question by asking how an ion gradient can be used to generate ATP. Since the generation of ATP in mitochondria is driven by a proton gradient (generated during oxidation of $NADH_2$), this also becomes the key issue in our understanding of oxidative phosphorylation. Figure 7 emphasizes this relationship. Whether we look at Na^+ transport driven by ATP or at ATP generation driven by a proton gradient, the basic phenomenon is the same and equally mysterious. These are the problems we shall discuss in this Account.

The Mechanism of Oxidative Phosphorylation

Encouraged by the editorial policy of this Journal, I shall give an account of the research trail that we have followed. I have come to the study of ion transport through the back door, actually through two back doors. Twenty years ago my laboratory was engaged in two major projects: the mechanism of oxidative phosphorylation and the control of the high aerobic glycolysis rates characteristic of cancer cells. Today these two problems have channeled into one: the mechanism and control of ion movements.

Two decades ago the mechanism of oxidative phosphorylation was believed to be strictly chemical. Models were proposed that were based on the mode of action of glyceraldehyde-3-phosphate dehydrogenase. This enzyme catalyzes the key reaction in glycolysis (the oxidation of an aldehyde to an acid) and it is coupled to phosphorylation. In other words, the energy of oxidation is utilized to form from inorganic phosphate and a carboxylic acid an anhydride which has been identified as 1,3-diphosphoglycerate. This compound is capable of donating its acyl phosphate group to ADP to generate ATP. Many investigators searched for such "high-energy compounds" in mitochondria—without success. I should say without reproducible success. Actually, repeated claims were made for such intermediates which either turned out to be side products, unrelated to oxidative phosphorylation, or artifacts. Unfortunately, this led to a great deal of confusion and did not help the reputation of the field.

Then in 1961, Peter Mitchell,[7] working in a private laboratory in England, proposed a new model based on a chemiosmotic mechanism. The driving force for ATP formation, he said, is not a chemical intermediate but an electrochemical proton gradient. A few years later[3] he presented experimental evidence for his formulation. He showed that the oxidation processes which take place in mitochondria are associated with the translocation of protons. He also showed that the mitochondrial ATPase, which was known to participate in ATP formation, is an ATP-driven proton pump. As illustrated in Figure 2, the respiratory chain creates an electrochemical proton gradient during electron transport; the mitochondrial proton pump, by operating in reverse, like a turbine, utilizes this gradient to generate ATP from ADP and P_i. Although osmotic mechanisms had been proposed earlier (cf. ref 3), Mitchell's proposal for the function of the respiratory chain as a proton translocating system was novel and testable.

Many objections were raised to this concept. It was agreed that mitochondria pump protons, but the opponents pointed out that mitochondria also pump K^+ and Ca^{2+}. What is the evidence, they asked, that the proton pump was part of the main pathway of ATP generation and not on a side pathway as in the case of K^+ or Ca^{2+} transport?

Reconstitution of Oxidative Phosphorylation

Our approach to the problem of oxidative phosphorylation has been that of classical biochemistry: First purify, then think. This approach of resolution and reconstitution of the individual components of multienzyme pathways had been successful in the elucidation of many pathways catalyzed by soluble enzyme systems. Why should it not be successful with membrane-associated processes? Indeed, several enzymes of the mitochondrial electron-transport chain had been successfully isolated in many laboratories and studied in great detail, e.g., cytochrome oxidase, succinate dehydrogenase, etc. Even oxidizing enzyme packages, the so-called respiratory complexes, had been separated from each other.[8] Complex I catalyzes the transfer of electrons between $NADH_2$ and coenzyme Q, complex III between QH_2 and cytochrome *c*, complex IV (cytochrome oxidase) between reduced cytochrome *c* and oxygen. When these three complexes were mixed, the entire electron-transport chain from $NADH_2$ to oxygen was operative. However, the energy of oxidation was lost in heat, and all attempts in our and other laboratories to couple the oxidative process to ATP formation failed.

At this point we started to consider seriously Mitchell's formulation which required reconstitution with a new dimension: formation of an ion-permeable compartment and an asymmetric assembly of the catalysts capable of generating an electrochemical proton gradient. Before embarking on this task, we had to convince ourselves that Mitchell's assumptions were correct. Is there an ATP-driven proton pump that can operate independently in the absence of respiratory enzymes? Or is an interaction between these components obligatory, as required by the chemical and conformational hypotheses? Is there an asymmetric distribution of all the respiratory components as postulated by Mitchell? We approached the latter problem by both chemical and immunological methods and could show that the two faces of the inner mitochondrial membrane are indeed different.[9] Thus cytochrome *c* and cytochrome c_1 were localized on the C side of the membrane facing the cytosol; succinate and $NADH_2$ dehydrogenases were localized on the M side of the membrane facing the mitochondrial matrix. These studies were made possible by the curious fact that when mitochondria are disrupted by sonic oscillations, the resulting submitochondrial particles are "inside-out". For example, succinate dehydrogenase faces the *m*atrix side of the mitochondria and the *m*edium side in submitochondrial particles (this is why we call it the M side of the membrane).

We then successfully isolated and reconstituted the mitochondrial ATPase and showed that it functions independently as a reversible ATP-driven proton pump. Two observations aided us in these experiments. The first was made by William Arion[10] who found that submitochondrial particles, which cannot synthesize ATP after treatment with cholate, recover this activity after dialysis. The second was made by Yasuo Kagawa[11] who found that, after extraction of mitochondria with cholate and fractionation with ammonium sulfate, the precipitated proteins were inactive in $^{32}P_i$–ATP exchange (a convenient assay for the energization of the membrane) but could be reactivated by addition of the dialyzed supernatant that remained after precipitation of the protein. The supernatant

(7) P. Mitchell, *Nature (London)*, **191**, 144 (1961).

(8) Y. Hatefi, A. G. Haavik, L. R. Fowler, and D. E. Griffiths, *J. Biol. Chem.*, **237**, 2661 (1962).

(9) E. Racker, *Essays Biochem.*, **6**, 1 (1970).

(10) W. J. Arion and E. Racker, *J. Biol. Chem.*, **245**, 5186 (1970).

(11) Y. Kagawa and E. Racker, *J. Biol. Chem.*, **246**, 5477 (1971).

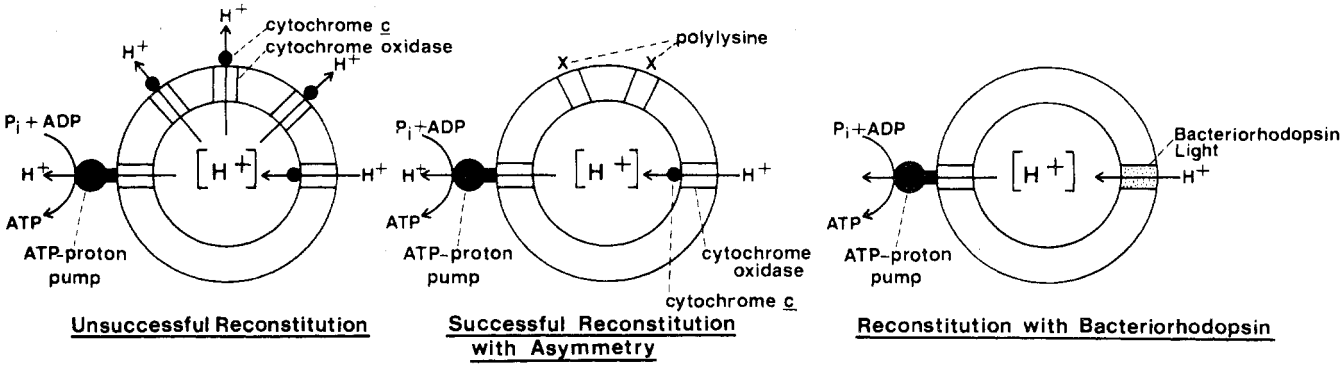

Figure 8. Reconstitution of oxidative phosphorylation. (Left) Reconstitution of cytochrome oxidase together with cytochrome *c* gave rise to phospholiposomes in which the orientation with cytochrome *c* outside predominated. This did not permit creation of the electrochemical proton gradient required by the ATP proton pump. (Middle) Cytochrome *c* on the outside was displaced with polylysine, thus preventing the outward movement of protons. Now the electrochemical proton gradient generated by cytochrome oxidase was available for the ATP-proton pump. (Right) Bacteriorhodopsin in the light pumps protons into the vesicles generating the electrochemical proton gradient required for ATP formation by the ATP-driven proton pump.

fraction was heat stable and replaceable by a crude mixture of soybean phospholipids. We could show that the phospholipids were responsible for the formation of an ion-impermeable compartment and that the proteins provided the heat-labile catalysts required for proton pumping.

We were confident that we could now also reconstitute the entire pathway of oxidative phosphorylation without much difficulty. As it turned out, we were overconfident. All initial attempts to bring together the respiratory complexes and the proton pump into a functional unit failed, until we realized that our clumsy method of reconstitution did not provide the asymmetry required for the operation of the chemiosmotic mechanism. By chemical displacement of cytochrome *c* with polylysine from the M side of the membrane (Figure 8), we finally achieved asymmetry and reconstitution of oxidative phosphorylation.[12]

A Bacterium Comes to the Rescue

In spite of this success the opponents of the chemiosmotic hypothesis were still not satisfied. Could we rule out, they asked, that in the reconstituted phospholipid vesicles the ATPase complex and the respiratory chain interacted to form a functional intermediate? Meanwhile, Stoeckenius and his collaborators in San Francisco had studied a curious salt-loving purple microorganism, *Halobacterium halobium*. They observed that when these bacteria were illuminated, protons were excreted by the cells and the intracellular ATP content rose. The membrane component, which responded to light, was a small molecular weight protein of 26 000 daltons with properties remarkably similar to those of the mammalian visual pigment rhodopsin. It was named bacteriorhodopsin. I invited Stoeckenius to our laboratory at Cornell University to collaborate in attempts to reconstitute bacteriorhodopsin into phospholipid vesicles. These experiments were successful beyond our expectations. Bacteriorhodopsin incorporated into liposomes not only pumped protons when illuminated, but they pumped protons in rather than out. Compared to bacteria, these vesicles were "inside out", just like submitochondrial particles. This was exactly what we needed since all our reconstitutions of oxidative phosphorylation were in the inside-out configuration which did not require the transport of inorganic phosphate and adenine nucleotides. Now we could show that bacteriorhodopsin can substitute for the respiratory complexes and catalyze, in conjunction with the mitochondrial proton pump, light-driven ATP formation[13] (Figure 8). This was a convincing experiment because the light-driven proton pump of bacteriorhodopsin did not involve an oxidation process and thus no direct transfer of energy via a chemical oxidoreduction intermediate could be responsible for ATP formation.

How Do ATP-Driven Pumps Work?

These experiments provided strong evidence in favor of Mitchell's chemiosmotic formulation for the transduction of energy from the oxidation process to the mitochondrial ATPase, but they provided no insight into the problem of how the proton gradient is used to generate ATP. Since, as mentioned earlier, other ATP-driven ion pumps are known to be reversible and thus capable of using an ion gradient to generate ATP, the problem has a much broader experimental base. How can ATP generate an ion gradient? How can an ion gradient generate ATP in the case of three established systems: (1) the mitochondrial H^+ pump, (2) the Na^+K^+ pump of the plasma membrane, and (3) the Ca^{2+} pump of sarcoplasmic reticulum?

The mitochondrial ATPase is structurally the most complex of these pumps. It contains at least nine polypeptide components that are required for its function. The Ca^{2+}-ATPase is a single polypeptide chain of about 100 000 daltons,[14] and the Na^+K^+-ATPase[15] contains two polypeptide chains. These latter two ATPases have a special attraction to chemically oriented biochemists. When exposed to ATP, these proteins form a phosphorylated intermediate which has been identified as an anhydride between phosphate and the carboxyl group of an aspartic acid residue in those proteins. Furthermore, it has been observed in several laboratories that the same anhydride can be formed by the purified ATPases (without a compartment) on exposure of the enzyme to Mg^{2+} and inorganic phosphate.[16] This was rather surprising since such an-

(12) E. Racker and A. Kandrach, *J. Biol. Chem.*, **248**, 5841 (1973).

(13) E. Racker and W. Stoeckenius, *J. Biol. Chem.*, **249**, 662 (1974).
(14) D. H. MacLennan, *J. Biol. Chem.*, **245**, 4508 (1970).
(15) (R) P. L. Jørgensen, *Quant. Rev. Biophys.*, **7**, 239 (1975).
(16) (R) E. Racker, *Trends Biochem. Sci.*, **1**, 244 (1976).

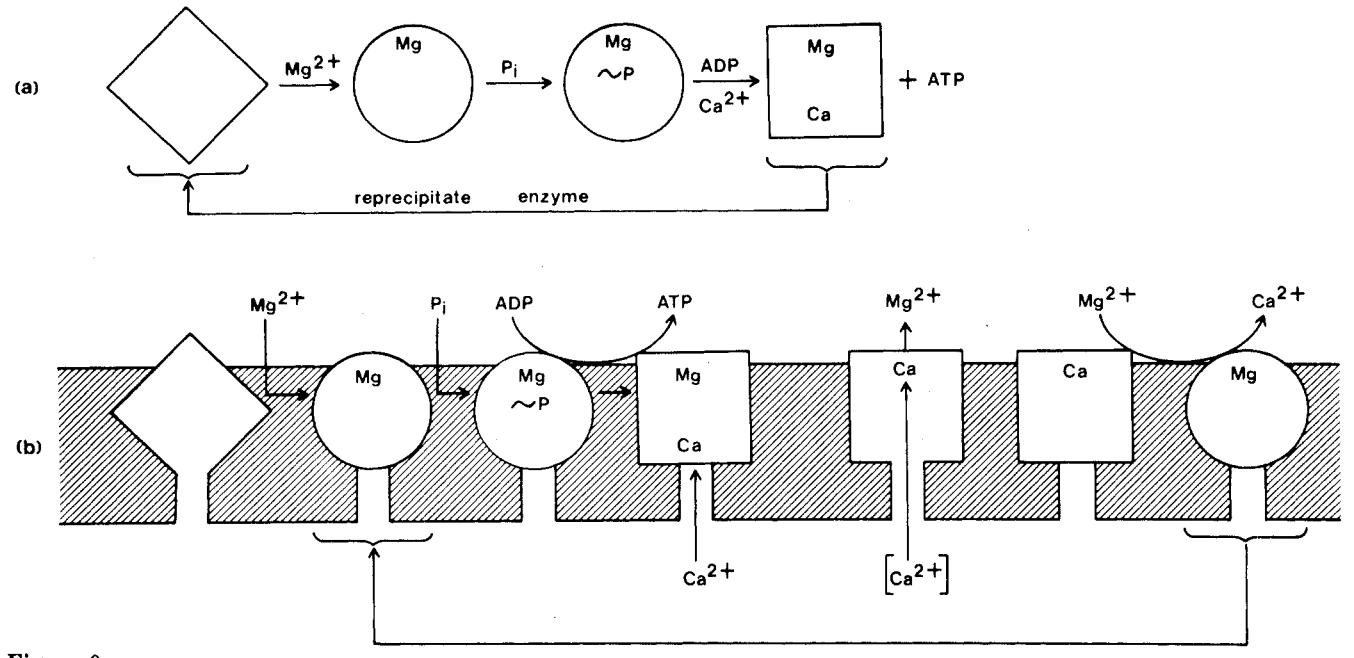

Figure 9.

hydrides are viewed by biochemists as "high-energy compounds". What is the source of energy for anhydride formation when the only interacting species are proteins and inorganic ions? The biochemists (including myself) faced with this problem sought refuge in the hydrophobic pockets of the protein in answer to the challenge of thermodynamics. That retreat was, however, no longer honorable when we discovered that the phosphoenzyme formed with inorganic phosphate was capable of donating the phosphoryl group to ADP to yield ATP.[17] This ATP was soluble, not protein-bound, and fully accessible to an enzyme such as hexokinase. Moreover, we could repeat the cycle of ATP formation provided we precipitated the protein and removed the inorganic ions.

Calorimetric measurements[18] performed in collaboration with Biltonen at the University of Virginia yielded some startling results. The interaction of these ATPases with the divalent inorganic cations gave rise to heat changes of a magnitude not previously encountered in protein chemistry. For example, on mixture of the Na^+K^+-ATPase with Mg^{2+}, 40 kcal of heat was released per mole of enzyme. These findings suggested that major conformational changes take place in these proteins, and we demonstrated this by other methods. Based on these findings, we proposed the model for the reaction sequence shown in Figure 9a. The interaction of the protein with Mg^{2+} changes the conformation of the protein so that it can interact with inorganic phosphate to form the acyl-enzyme. When Ca^{2+} is now added, the phosphoryl group becomes accessible to ADP, resulting in ATP formation. Repetition of the process requires the removal of the cations from the enzyme as indicated in the figure. What bearing do these formulations have on the ATP-dependent transport of ions through membranes, or on ATP formation that takes place during dissipation of an ion gradient? Now we again enter the realm of speculation which may help us to design future experiments. In Figure 9b, I have formulated the major new idea that has emerged from these experiments. Basically, it is visualized that a Ca^{2+} flux from the inside of the vesicles, via an open channel, displaces Mg^{2+}, thereby achieving the same net result as the reprecipitation of the enzyme in the experiments shown in Figure 9a. This step is followed by the closing of the channel, thereby allowing the Mg^{2+} to once again displace the Ca^{2+} which is now released into the medium on the other side of the membrane. Although not included in this basic scheme, it seems likely that during conformational changes of the protein the relative affinities of Ca^{2+} and Mg^{2+} to the enzyme change as well. It is not difficult to read this scheme from right to left and to see how ATP-driven ion transport might take place. It is equally easy to imagine (though much more difficult to prove) that the principle shown in Figure 9b is operative in mitochondria during oxidative phosphorylation. All we need to do in this scheme is to replace Ca^{2+} by H^+. We thus propose that the major function of the proton gradient is to displace Mg^{2+} from the active site which cycles back after closure of the channel.

We are now engaged in studies of the proton pump of mitochondria and chloroplasts. The complete resolution and characterization of the individual polypeptide components are in sight. It has taken a long time.

The High Rate of Aerobic Glycolysis in Cancer Cells

The story of the aerobic glycolysis in cancer cells is a curious chapter in the history of biochemistry. When Warburg[19] reported this discovery he also offered an explanation which was simple and ingenious but proved to be incorrect. He said that cancer cells glycolyze because they are undifferentiated, primitive cells that have lost the power of generating respiratory energy and therefore use the fermentative pathway as a major energy income. Later biochemists discovered that this

(17) A. F. Knowles and E. Racker, *J. Biol. Chem.*, **250**, 1949 (1975).
(18) Y. Kuriki, J. Halsey, R. Biltonen, and E. Racker, *Biochemistry*, **15**, 4956 (1976).

(19) O. Warburg, "Uber den Stoffwechsel der Tumoren", Springer Verlag, Berlin, 1926.

cannot be correct, because many tumor cells have good respiration and oxidative phosphorylation. Subsequently the phenomenon was pushed aside and virtually no attempts were made to elucidate its mechanism. This negative posture was supported by observations that some normal cells, e.g. retinal cells, glycolyze rapidly under aerobic conditions. Yet high aerobic glycolysis is still the most generally accepted property shared by all rapidly growing cancer cells.

What is the reason for this rapid rate of fermentation? Our systematic studies over the past 20 years have revealed that a membrane lesion appears to be responsible. In some tumor cells the lesion is in the plasma membrane and affects the Na^+K^+-ATPase which generates an excessive amount of ADP and P_i. This, in turn, increases glycolysis because the regeneration of these cofactors of glycolysis is a rate-limiting step in the process of lactic acid formation. In some other tumor cells the lesion is in the mitochondrial membrane leading to an increased ATPase activity. In a third group of tumor cells there is also an increased rate of ATP hydrolysis, but the responsible catalyst has not been identified. We are attempting to analyze the lesion of the plasma membrane which appears to render the operation of the Na^+K^+ pump less efficient.

We are also trying to characterize the transport system which controls the excretion of lactate together with protons. What would happen to cells in which this transport is blocked by a specific inhibitor? Would they drown in their own acid? We have synthesized some reactive analogues of lactic acid and find that some interfere with lactate transport and some inhibit growth of cells in vitro and in vivo. However, thus far there seems to be no correlation between the effects on transport and on growth. We are intrigued and stimulated, and we continue the search for a solution to this puzzle.

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Preparation and Analytical Uses of Immobilized Enzymes

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Enzymes are polypeptides which serve as catalysts for chemical reactions in biological systems. These catalysts are usually very efficient, and are capable of increasing the rate of many complex chemical reactions, upon which depends the very existence of life as we know it, by several orders of magnitude at or near room temperature and at atmospheric pressure. Furthermore, enzymes are often very specific, and catalyze reactions of substates at low concentrations. Because of their specificity and sensitivity, enzymes continue to enjoy widespread use as analytical tools especially in biochemical and clinical laboratories. During the past few years alone, scores of books and review articles have been published describing the use of enzyme-catalyzed reactions for the trace analysis of substrates, activators, inhibitors, and enzymes themselves.[1-4]

The use of enzymes for analytical purposes, however, has been limited because of certain disadvantages, such as their instability, poor precision, and lack of availability. Moreover, aqueous solutions of enzymes often lose their catalytic ability fairly rapidly, and the enzymes can neither be recovered from such solutions, nor their activity regenerated. These difficulties have now been removed or minimized by the development of enzyme immobilization techniques. The free enzyme is immobilized (insolubilized) by trapping it in an inert matrix such that the immobilized enzyme retains its catalytic properties for a much longer time as compared to the free enzyme and can be used continuously for many more analyses.

The science and technology of immobilized enzymes have experienced phenomenal growth in the recent past. Consequently, there has been a very rapid accumulation of scientific literature describing various aspects of this subject.[5] Also, there are more specific reviews of the

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(1) G. G. Guilbault, "Enzymic Methods of Analysis", Pergamon Press, Oxford, England, 1970.
(2) G. G. Guilbault, "Handbook of Enzymic Analysis", Marcel Dekker, New York, 1977.
(3) G. G. Guilbault and M. H. Sadar, "Analytical Uses of Enzymes", Proceedings of the Analytical Division of the Chemical Society, London, 1978.
(4) M. M. Fishman, *Anal. Chem.*, **50**, 261R (1978).
(5) K. Mosbach, *Methods Enzymol.*, **44** (1976).

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