Mechanism of Energy Transformations in Biological Membranes*

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One of the most fundamental problems of biology is how energy is transduced in living systems. It is a problem that has fascinated and absorbed a great many distinguished chemists, biochemists and physicists. I believe we have reached a stage in the development of our knowledge of the mechanism of energy transduction which now permits us to see the direction which leads to the final solution. It is with this development that I shall be concerned in this evening's lecture.

All of us are aware that "energy" in some way is garnered by the "burning of food." The oxidations which underlie this so-called burning of food take place within the cells of living organisms and within organelles called mitochondria. In plant cells there are comparable organelles which are called chloroplasts. Both the mitochondrion and the chloroplast are concerned with the transduction of oxidative energy into a special form of chemical energy, and in this regard they are first cousins at the molecular level.

In the mitochondrion a molecule related to sugar (pyruvate) is oxidized to CO_2 and water by molecular oxygen in a series of discrete steps. At each step, the oxidation leads to the liberation of what we may loosely call "hot" electrons, i.e., electrons with high reducing potential. These hot electrons traverse a chain known as the electron transfer chain and are transferred from one component to another in this chain in such a way that the fall in energy is gradual as the electrons proceed through the chain. In the chloroplast, the hot electrons are generated by the action of light on chlorophyll, and here again these hot electrons traverse an electron transfer chain rather similar to the chain of the mitochondrion. The passage of the hot electrons through the electron transfer chain, be it of the mitochondrion or the chloroplast, is linked to the synthesis of ATP. That is to say, in the oxidative reactions involved in the transfer of electrons through the chain, free energy is released, and this energy is coupled to the formation of a bond between inorganic phosphate and ADP, leading to synthesis of ATP. The burning of food by molecular oxygen involves this electron transfer chain and the passage of hot electrons throuh the chain, ultimately, to molecular oxygen. The capture of energy by the burning of food is then the coupling of the energy released by oxidation to the formation of a bond between the two molecules which have to be united to make ATP. The problem of energy transduction in a nutshell reduces to the question of how the released oxidative energy can be utilized for the synthesis of ATP.

The transduction of energy from one form to another (in this case from oxidative to bond energy) requires a machine. We are all familiar with the innumerable devices by which electric current can power appliances which cook, blow air, refrigerate, translate radio waves into sound, stir, give off light, etc. All these appliances are devices which transduce energy from one form to another. It would appear obvious from our experience with energy transformations in our kitchens and homes and factories that some kind of machine or contrivance would underlie the transformation of energy in the mitochondrion or the chloroplast. But, strange to relate, the biochemist has for at least two decades proceeded on the assumption that there is no need for a machine to account for the coupling of oxidation to synthesis of ATP in mitochondria. The evidence that appeared to justify this assumption was the fact that there is indeed a secondary system also in mitochondria that links the oxidation of α-ketoglutarate to synthesis of ATP, and this system operates by purely chemical principles. If the transduction achieved by the secondary system can be apparently accounted for without invoking a machine, then why invoke a machine for the primary system?

In point of fact, a machine is always required for energy transductions, but since the enzyme that catalyzes the synthesis of ATP coupled to oxidation of α-ketoglutarate is the machine, the crucial point was missed. The question was not whether a machine was required but whether the particular transducing device peculiar to the α-ketoglutarate dehydrogenase was the prototype for the primary transducing systems of mitochondria and chloroplasts. It took 20 years of unremitting failure to convince the

* Presented to the Sigma Xi Society, Medical College of Virginia, February 8, 1968.

MCV QUARTERLY 4(3): 96-100, 1968

biochemist that his original assumptions about the form of the mechanism were not correct.

It will be my thesis this evening that the mitochondrion and the chloroplast are biological machines specialized for transducing oxidative energy into the bond energy of ATP. The gross and detailed molecular structure of these organelles have provided the crucial clues to the nature of the transducing machine and to its mode of operation. It is, thus, to the structure of the mitochondrion that we must turn for the insights that eventually led to the first realistic definition of the transduction problem.

The mitochondrion is in its general form a rod-shaped organelle made up of two boundary membranes (usually referred to as outer membranes), which enclose the organelle, and a set of inner membranes (cristae), which are approximately at right angles to the bounday membranes (Fig. 1). The cristae are tubes which are closed at one end and, at the other end, open into and connect with the inner of the two boundary membranes.

A membrane is a closed system (no open ends) built up from repeating units which nest together to form a continuum like the surface of a hollow ball. The thickness of the membrane is *always* equal to the thickness of one particle. The repeating particles which make up the boundary membranes are different from the repeating particles that make up the cristael tubes. The boundary membranes have a vesicular or spherical form; the cristael membranes have a tubular form. In any membranes, all the repeating units have the same geometry, but there are usually multiple species, each chemically and functionally distinct. Thus we have, on the one hand, geometric uniformity; on the other, chemical diversity among the repeating units of a given membrane. The fit of repeating units one to another is of paramount importance, because membranes must act as barriers to the movement of ions and molecules; and this can only be achieved by close to perfect fit of the nesting repeating units.

The transducing elements in the mitochondrion are localized exclusively in the cristael membranes, and the transducing elements have been identified with the repeating units in these membranes. The electron microscope has revealed in detail the fine structure of the repeating unit of the cristael membranes (Fig. 2). This repeating unit has a tripartite structure—a cylindrical stalk which connects at one end to a cuboidal basepiece and, at the other end, to a spherical headpiece. All the repeating units of the cristael membrane have this tripartite structure.

In our laboratory we have been able to localize some of the enzymic functions of the mitochondrion in the different sectors of the

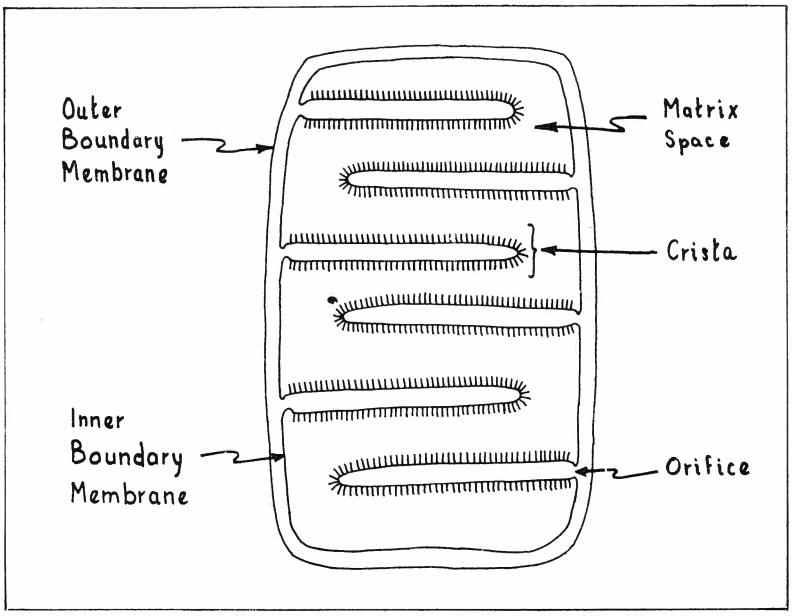

Fig. 1—Diagrammatic representation of the mitochondrion and its system of membranes.

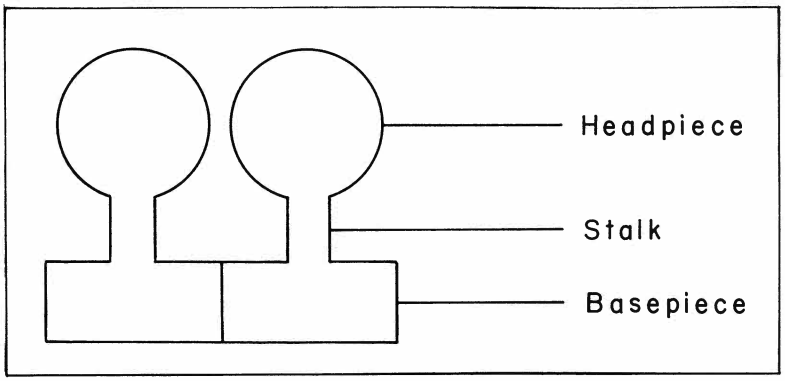

Fig. 2—Diagrammatic representation of the tripartite repeating units of the inner (cristael) membrane of the mitochondrion. Two repeating units are shown in alignment—the basepiece of one nesting with the basepiece of the other.

tripartite unit. The electron transfer chain is localized in the basepiece (the membrane-forming sector); the capacity for forming or hydrolyzing ATP is localized in the headpiece; the stalk is some kind of communication link which transmits change from headpiece to basepiece and from basepiece to headpiece.

The picture we must form is that of thousands of identical machines (repeating units) nesting together to form a membrane continuum. Each crista is a large collection of identical machines; the mitochondrion is in turn a large collection of cristae. The individual machine works best when part of a membrane continuum, and the individual crista works best when part of an organized, intact mitochondrion. We are dealing with "collectivized" machines that are intrinsic parts of membrane systems and membrane networks.

Several years ago David Slautterback of the University of Wisconsin published a remarkable paper on the electron microscopy of the mitochondria of canary heart muscle *in situ* (Fig. 3). The remarkable feature of these electron micrographs is that they show three kinds of structures which the cristael membranes can assume. Within the mitochondrion there are domains in which a set of cristae show one of these three structural forms. The three forms are set forth in Figure 4. These are, respectively, the cristae with linear structure (parallel membranes); cristae with vesicular structure; and finally, cristae with zigzag structure. It became obvious to us that the cristael membranes of mitochondria were going through a cycle of ultrastructural changes, and that each of these three domains represented a different stage in this cycle. The process of transducing oxidative energy into the bond energy of ATP had to be related to these ultrastructural changes in the cristael membranes.

We may summarize the various ways in which energy can be manipulated in the mitochondrion by means of a simple diagram (Fig. 5). Electron transfer or hydrolysis of ATP can give rise to an energized state. This is a reversible process. The energized state can lead to the synthesis of ATP or drive the electron transfer process in reverse. In addition to driving the synthesis of ATP from ADP and Pi, the energized state can be linked to a set of work performances—translocation of monovalent ions, translocation of divalent ions, and transfer of a hydride from DPNH to TPN^+ to an extent that is far beyond the equilibrium point for the non-energized transhydrogenation. Thus, there is a cycle of generating and discharging the energized state which underlies synthesis of ATP or translocation or transhydrogenation.

The critical question was whether the energized state could be equated

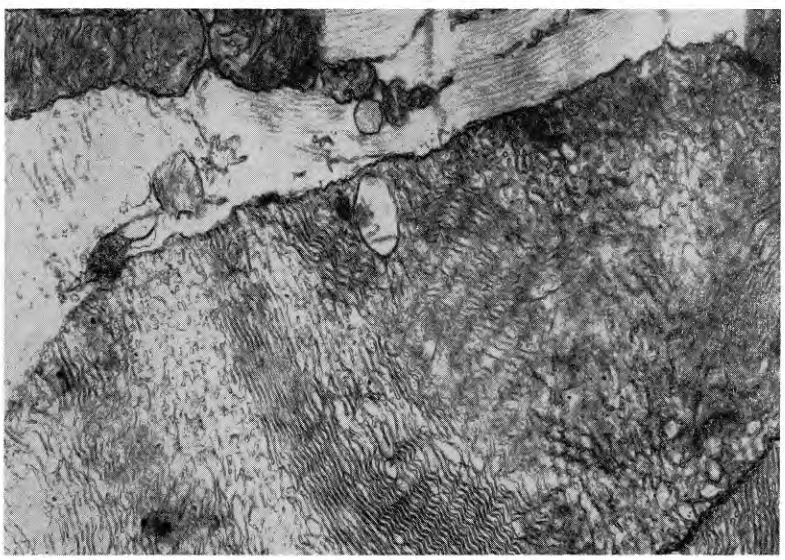

Fig. 3—Electron micrograph of a canary heart mitochondrion *in situ*. The cristae are arranged in sets (domains) which show the same configuration. Three configurational patterns are readily recognized.

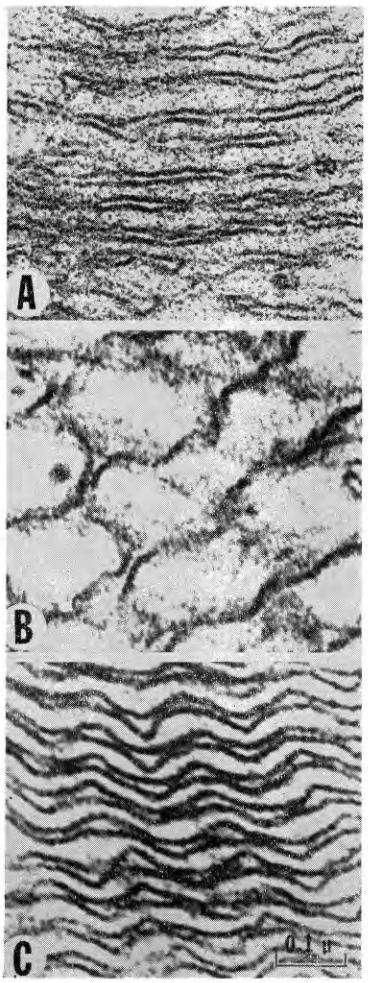

Fig. 4—The three configurations of the cristael membrane in canary heart mitochondria: (A) nonenergized, (B) energized, (C) zigzag (energized-twisted).

with one or more of the three ultrastructural states of the cristael membrane seen in Slautterback's electron micrographs. Before we consider the evidence that equated the energized state of the mitochondrion with one or other of the ultrastructural states, let us digress to take up two points relevant to and crucial to the argument. There is evidence that there are in fact two energized states: (1) the state induced by electron transfer or ATP *in the absence* of inorganic phosphate, and (2) the state induced by electron transfer or ATP *in the presence* of inorganic phosphate. The zigzag state of the canary mitochondrion corresponds to the phosphate-induced energized state; the vesicular state of the canary mitochondrion corresponds to the energized state in absence of added inorganic phosphate.

Isolated mitochondria undergo a cycle of ultrastructural change that is similar to but not identical with that seen *in situ*. The difference in appearance can be accounted for in terms of the presence of sucrose in the media used for isolating mitochondria. In the presence of sucrose, the cristael membranes of mitochondria undergo geometric deformation, and it is these deformations that underlie the different ultrastructural appearance of the nonenergized and energized states of mitochondria *in situ* as compared to mitochondria in isolation. The three ultrastructural states of beef heart mitochondria are shown in Figure 6. The snake-like forms (energized-twisted configuration of the membrane) correspond to the zigzag forms seen in the mitochondria of canary heart muscle. The compressed tight membranes (nonenergized configuration of the membrane) correspond to the linear forms seen in the mitochondria of canary heart muscle. The expanded membranes with a lumen (energized configuration of the membrane) correspond to the vesicular forms seen in mitochondria of canary heart.

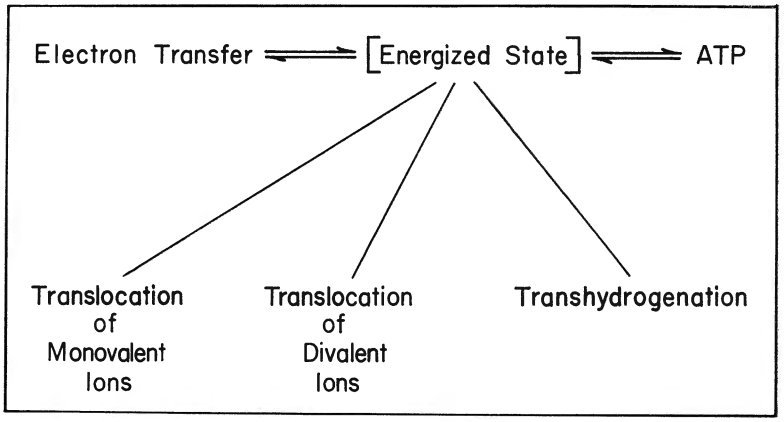

Fig. 5—Diagram showing how the energized state of mitochondria is generated and utilized to do work.

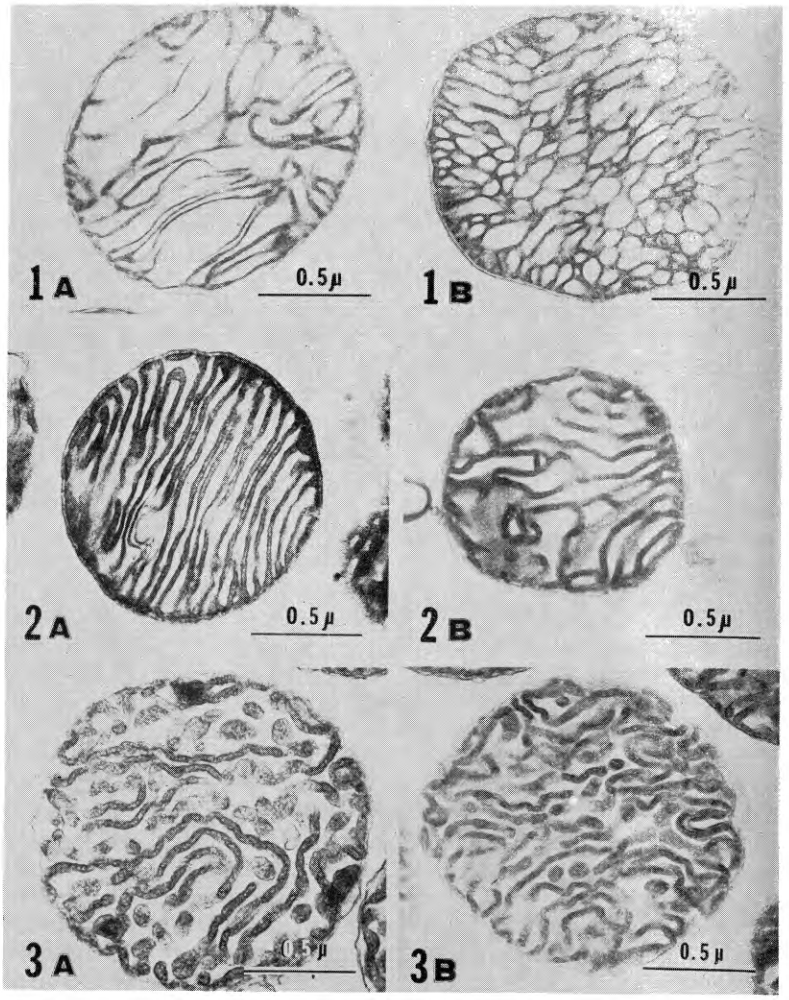

Fig. 6—Electron micrographs of beef heart mitochondria in three configurational states: 1A and 1B, nonenergized; 2A and 2B, energized; 3A and 3B, energized-twisted. Osmium-fixed sections.

Now we may return to the question of how the energized states of the mitochondrion can be identified with the different ultrastructural states. If the two are identical, then the generation of the ultrastructural changes should be inhibited by inhibitors of electron transfer when powered by electron transfer, by inhibitors of ATP hydrolysis when powered by ATP, and by uncouplers when powered by either electron transfer or ATP. Furthermore, the ultrastructural form of the energized state should be discharged by reagents which can lead to any of the work performances. Lastly, the speed of the ultrastructural changes should be of the same order of magnitude as the speed of electron transfer in ATP hydrolysis. Experiment has shown that all these predictions of the energy diagram can be verified. In other words, there is an exact correlation between the energized state and the configurational state of the cristael membrane. Thus, we can identify the ultrastructural changes in the membrane with the formation and dissipation of the energized state.

The repeating units of the cristael membrane have a particular geometry in the nonenergized state. It is this geometry of the repeating units that determines the ultrastructural form of the membrane. Suppose that the geometry of each repeating unit were to change in a specified way, e.g., the basepieces would undergo a transition from the cuboid form to a spherical form. What would happen? The whole membrane would undergo a change in configuration to accommodate to the geometric change in each of the repeating units. In other words, a change in geometry of the repeating unit would be reflected in a change in geometry of the membrane itself. Therefore, when changes in geometry of the membrane are observed, such as the changes from tubular to vesicular to zigzag forms, we are dealing not only with gross changes in the membrane but also with the molecular changes in the repeating units which determine the gross changes in the membrane. In other words, the molecular changes in a single repeating unit are magnified enormously because of the interaction and packing of multiple repeating units within a membrane continuum.

The ultrastructural changes arising from the energy cycle can be demonstrated not only in mitochondria but also in other membrane systems. When chloroplasts are illuminated, the internal structure of the membranes is profoundly different than the internal structure of the membranes in the dark. The granae, which are the membranous analogies of the cristae in the chloroplast, contract in the light and expand in the dark. Similar ultrastructural changes can be recognized when retinal rods are illuminated or when the plasma membrane of the red blood corpuscle is energized by ATP or deenergized by discharge of ATP. All the indications are that we are dealing with a universal mechanism of energy conservation in membrane systems—a mechanism whereby the transduction involves conformational changes in the membrane itself. That is to say, the membrane is the transducer. When energized by electron transfer or ATP or light, depending on the membrane, it undergoes an appropriate molecular convulsion. In this convulsed state the energy is trapped and conserved, and in turn there are ways by which this conformational energy can be utilized to do physiological work.

Electron transfer or hydrolysis of ATP triggers the configurational changes in the membrane. Does this mean that oxidative energy is directly transduced into conformational energy? We have good reason to believe that this is a two step transduction. Electron transfer leads to the generation of electrostatic energy by the delivery of two electrons to molecular sites which are very close together. It is then the conformational change compelled by the buildup of electrostatic energy that leads to the transduction of electrostatic energy into conformational energy.

The question has been raised whether enough conformational energy can be packed away to drive the synthesis of ATP. The answer is that there is no limit to the amount of conformational energy that can be stored. The more pertinent question is whether the extent of the configurational change is compatible with the energy that has to be invested in synthesis of ATP from ADP and Pi. Present indications are that there is no basis for worry on that score. The magnitude of the configurational changes is very great—extending over 100 A within a single repeating unit. But, most important, the exact correspondence between the configurational changes and the energized state effectively rules out the possibility that these changes are not the primary changes underlying the transducing events.

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