

## CURRENT PROBLEMS IN RESEARCH

## The Mitochondrion and Biochemical Machines

Mitochondria or their equivalents are the principal energy transducers in all aerobic organisms.

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Living systems are capable of effecting a wide variety of energy transformations. Even the less-than-complete list of such transformations provided in Table 1 shows that nature has experimented successfully with a considerable number of transducing possibilities. These transformations or transductions of energy are accomplished by highly specialized and complex biochemical systems which can best be described as machines. There is often a reluctance on the part of biologists to speak of parts of living systems as machines because of the connotation of mechanical principles. But if a machine is looked upon merely as a structured device for converting energy from one form to another regardless of the details of mechanism, there need be no objection to the designation of biological transducing systems as machines.

Rarely is there one giant transducing machine in living systems; rather, there are assemblies of many small, identical machines arranged in parallel and in series. To the extent that our present knowledge permits any conclusions, it would appear that the mechanism of biological transducing machines has to be sought at the molecular level. That is to say, the transduction process takes place at the level of a single molecule

or macromolecule. Indeed, there is now a growing body of knowledge about some of the molecules which are specialized for implementing a particular transduction (see Table 2). In a sense, biochemical machines may be looked upon as structured devices in which the transducing action of specialized molecules is facilitated or made possible. The structure is designed, as it were, for the optimum performance of the molecular transducer, and the structure has to be explained and interpreted from the standpoint of the physicochemical requirements or necessities of the transducing molecules.

Thus, the study of cellular machines is in essence topological enzymology. Function and structure are inextricably intertwined. Before the enzymology (in the sense of catalytic function) can be meaningful, there has to be an adequate and precise biochemical description of the structure of a given machine.

When we say that the transduction takes place at the level of a single molecule, it is implied that the transducing substance accepts energy in one form and transmits or stores energy in another form. For example, retinene (1) is photochemically transformed to a *trans*-isomer and is thus the molecular instrument for converting radiant energy to some form of chemical energy. Actomyosin (2) undergoes either molecular modification in size and shape or

positional displacement when acted upon by adenosine triphosphate, thus serving as a molecular instrument for converting chemical energy to mechanical energy. Chlorophyll (3) is the molecular device for converting radiant energy into some as yet undefined form of chemical energy. Thus the operation of cellular machines must involve the integration of the performance of many hundreds or thousands of transducing molecules.

The key problems posed by biochemical machines are covered by the following questions: What are the component parts? How are they arranged? Which components are the molecular energy transducers and how do they work? In this article we try to give a bird's eye view of the state of progress in the study of one cellular machine—namely, the mitochondrion. The experience which is being gathered in the study of this machine may well be a guide to the problems and pitfalls which can attend exploration of other cellular machines.

### Function of the Mitochondrial System

Aerobic cells of animals and plants contain a subcellular body of characteristic shape, size, structure, and staining properties, known as the mitochondrion (4) which serves as the principal generator of chemical energy in utilizable form (5). The corresponding particle in microorganisms (6) is functionally and chemically very similar to the mitochondrion, but it is generally of smaller size.

The universal function of all mitochondria or their structural equivalents in bacteria is to couple the aerobic oxidation of some substance (usually pyruvic acid) to the synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate. That is to say, these are devices for liberating chemical energy by oxidation and converting or harnessing this energy in the form of the bond energy of ATP (7). In general, the oxidation of pyruvic acid to CO<sub>2</sub> and H<sub>2</sub>O

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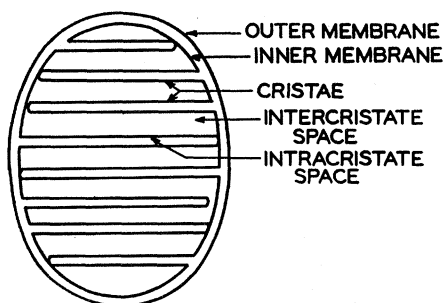


Fig. 1. Idealized structure of the mitochondrion.

by way of the citric acid cycle is the principal, if not the only, oxidative process involved. For each of the five atoms of oxygen used up in the oxidation of one molecule of pyruvic acid, three molecules of ATP are synthesized from ADP and inorganic phosphate. In other words, the complete combustion of one molecule of pyruvic acid to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  leads to the synthesis of 15 molecules of ATP (8).

Mitochondria can oxidize substances other than members of the citric acid cycle. Some of these substances, such as fatty acids (9) or amino acids (10) ultimately give rise to members of the citric acid cycle, while other substances, such as  $\alpha$ -glycerophosphate (11) and  $\beta$ -hydroxybutyrate (12), have no direct connection at all with the citric cycle. In bacteria even inorganic substances can be oxidized by the particles concerned with terminal respiration (13). The universal element in all mitochondria is the coupling of oxidation to synthesis of ATP—that is, oxidative phosphorylation. The variable element can be the nature of the substances oxidized.

In addition to the basic coupling function, some, but not all, mitochondria catalyze various ATP-dependent synthetic processes, such as synthesis of phospholipid (14), protein (15), hippuric acid (16), and citrulline (17). These are secondary mitochondrial functions which apparently are not involved in the exercise of the primary coupling function.

The terminal electron transport system is one of the invariant universals of aerobic living systems. Whether isolated from heart muscle, *Azobacter*, or mung beans, its basic function is the same; it contains essentially the same catalytic components and exhibits similar, if not identical, structural patterns in all three cases. There is certainly no evidence of convergent evolution as a factor in the development of the mitochondrial system in animals, plants, and microorganisms. It would appear that once the problem of coupling electron flow to ATP synthesis had been solved in a particular fashion early in evolutionary history, no major modification in the underlying principles was introduced thereafter.

#### Mitochondrial Form and Structure

Mitochondria of animal and plant sources share a characteristic form and organization (4) (see the idealized representation in Fig. 1). The long dimension of the rod-shaped mitochondrion may be greater than 10 microns or less than 1 micron, depending upon the source. Surrounding the mitochondrion is an envelope with a double-membrane structure. Within the mitochondrion

Table 1. Biological transductions.

Energy transduction	Biological transducing system
Sonic to electrical energy	Ear
Radiant to electrical energy	Eye
Mechanical to electrical energy	Skin
Chemical to electrical energy	Nerve
Radiant to chemical energy	Chloroplast
Chemical to radiant energy	Luminescing organisms (firefly)
Chemical to osmotic energy	Kidney, cell membrane
Chemical to mechanical energy	Muscle
Chemical to sonic energy	Vocal cords
Chemical to electrical energy	Electric organs in electric fish
Oxidative to utilizable chemical energy	Mitochondrion

there is an array of double-membrane structures which usually are at right angles to the long axis of the particle and which may be looked upon as invaginations of the inner membrane of the envelope. These internal structures are known as cristae. External and internal membrane systems form one continuous network. The light area between the electron-dense, twin membranes of cristae and envelope probably is the locus of the internal fluid medium of the mitochondrion. If this is the case, it follows that the internal medium would bathe all the membrane structures of the mitochondrion, and furthermore, that the structured elements would interpose a mechanical barrier to the flow of solutes and solvents either from the external medium inwards or from the internal medium outwards. Thus, there are barriers within barriers which interdict the free flow of substances between the mitochondrion and the external milieu.

The mitochondria of heart muscle are packed with cristae, while those of liver contain relatively few. The greater the number of cristae per unit area, the greater the oxidative rate of the mitochondrion and the fewer are the accessory enzymatic activities—that is, activities other than or unconnected with the primary mitochondrial function. Liver mitochondria surpass all others in wealth of auxiliary functions, and this wealth is accompanied by a low density of cristae in the mitochondrion.

Sonic vibrations comminute mitochondria to much smaller particles which no longer show mitochondrial form and which represent fragments of the mitochondrial cristae and envelope

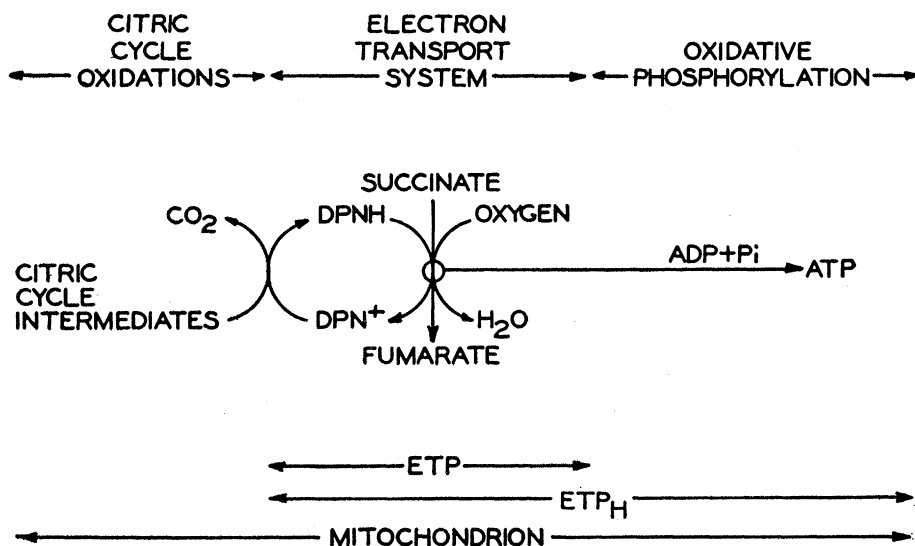


Fig. 2. Schematic representation of reactions catalyzed by mitochondria versus  $\text{ETP}_H$  versus ETP.

(18, 19). Some important correlations have been found between the structure of these derivative particles and the extent to which the original mitochondrial functions are preserved. Let us consider only the three basic functions: citric cycle oxidations, oxidative phosphorylation, and electron transport. As soon as mitochondrial form disappears, the capacity for implementing the complete citric cycle is lost. But as long as the derivative particle retains double membrane structure, even though mitochondrial form has been lost, the capacity for coupling oxidation to synthesis of ATP can be preserved. Finally, when the comminution produces a particle with a single membrane structure, then only the capacity for electron transport is retained. The particle with an intact electron transport chain but lacking the coupling capacity is referred to in our laboratory as the electron transport particle, or ETP (20), whereas the comparable particle with coupling capacity is known as ETP<sub>H</sub> (19) (Fig. 2).

Thus, mitochondrial function can be eliminated or whittled down seriatim, and by similar tactics the structure of the mitochondrion can be reduced in complexity step by step. This technique of seriatim degradation and modification has proved to be the solution to the dilemma of studying a system too complex for direct analysis.

#### Mitochondrion as a Complete Enzymic Unit

It cannot be emphasized too strongly that the mitochondrion, when properly isolated, contains the complete repertoire of enzymes, coenzymes, and cofactors required for implementing its wide spectrum of catalytic activities (21). Moreover, the relative proportions of these many components are of a comparable order of magnitude and appear to be fixed. Thus, without any supplementation the mitochondrion is a complete operational unit, in which all the component parts in the proper proportions are fitted together.

Enzymes which are localized outside the mitochondrion are not found in the mitochondrion. That is to say, only enzymes pertinent to the exercise of mitochondrial function are present in any significant amount in the mitochondrion. There is thus no encouragement for the idea that the mitochondrion is a random, heterogeneous particle in which each of a large number of miscellaneous enzymes becomes oc-

Table 2. Components involved in biological transductions.

Component	Transducing system
Retinene 1 and 2	Eye (retina)
Rod opsin	Eye
Cone opsin	Eye
Chlorophyll	Chloroplast
Cytochrome <i>f</i>	Chloroplast
Cytochromes	Mitochondria
Coenzyme Q	Mitochondria
Flavin	Mitochondria
DPN	Mitochondria
Myosin	Muscle
Actomyosin	Muscle
ATP	Muscle
Luciferin	Firefly ( <i>Photinus pyralis</i> )
Luciferase	Firefly
ATP	Firefly

cluded. On the contrary, the weight of evidence supports the view of the mitochondrion as a highly precise, organized mosaic of a strictly determined number of enzymes and coenzymes arranged in a repeating and invariant pattern.

#### Unit of Mitochondrial Function

Let us consider only one parameter of mitochondrial function—the electron transport chain, or at least that segment thereof which includes all the catalysts between reduced diphosphopyridine nucleotide (DPNH) or succinate at one end and oxygen at the other end. What is the smallest common denominator for the exercise of electron transport?

The mitochondrion, or at least the structured portion thereof, may be looked upon as a polymer of several thousand monomeric repeating units each of which contains a complete electron transport chain as defined above. In theory, then, it should be possible to comminute the electron transport particle to a size which would accommodate only a single unit. Sonic fragmentation of ETP leads to the formation of particles of very small dimensions. This may be a strong indication for the existence of such units. On the other hand, the smallest unit that can couple electron transport to the synthesis of ATP is more complex than the unit which is limited to the exercise only of the electron transport function. The minimal requirement for a particle to exercise the coupling function appears to be double membrane structure.

We may interpret this in the following way. Coupling requires a group of enzymes and factors other than those of the electron transport chain. To conserve these auxiliary enzymes, or per-

haps to facilitate their action, it is necessary to have, in effect, a tube with the two ends sealed off to prevent excessive leakage out of the loosely held components in the interior. According to this interpretation, the unit for coupling is merely the unit for electron transport supplemented with a group of easily detachable factors. The double-membrane arrangement may just ensure this close association of the particulate electron transport unit with the complex of enzymes and factors essential for the coupling process.

In the same way, the loss of the capacity for citric cycle oxidations which attends the comminution of mitochondria may be a consequence merely of damage to the cristae and the subsequent leaching out of enzymes and cofactors from the interior of the cristae. In fact, when supplemented with the appropriate enzymes, ETP or ETP<sub>H</sub> can carry out the complete citric cycle.

Thus, we may think of the electron transport chain as the ultimate unit. When separated from the milieu of the crista, it is restricted to the electron transport process. When supplemented with some of the cofactors and enzymes, it can couple electron flow to synthesis of ATP, as in ETP<sub>H</sub>, and when supplemented with all the enzymes and cofactors, it can couple the oxidations of the citric cycle to synthesis of ATP, as in the mitochondrion.

#### Strategy for Study of Mitochondrial Function

By virtue of the complexity (both structural and functional) of the mitochondrion, observations of a gross character cannot penetrate very deeply its underlying chemical and physical principles. The strategy has been to trim down the mitochondrion to a point where the complexity is more manageable (22). In practice this has meant that the starting point for structural and functional studies has been not the mitochondrion but the less complex particle, ETP. Two of the three main facets of mitochondrial function—namely, citric cycle oxidations and the coupling of electron flow to synthesis of ATP—have been set aside, and experimental emphasis has been placed on the elucidation of the electron transport chain. Without a precise knowledge of the electron transport chain it would be difficult to decipher the principles underlying

Table 3. Components of the electron transport system.

Component	Minimal molecular weight	Absorption bands (m $\mu$ )	Prosthetic group
$f_s$	$\sim 230,000$	450 (oxidized)	Flavin dinucleotide of unknown structure
$f_D$	$\sim 70,000$	450 (oxidized)	Flavinadenine dinucleotide
Coenzyme Q	863.4	405, 275 (oxidized in ethanol), 290 (reduced)	
Cytochrome <i>a</i>	$\sim 110,000$	444, 517, 605, 835* (reduced)	<i>a</i> heme
Cytochrome <i>b</i>	$\sim 30,000$	429, 530, 562 (reduced)	<i>b</i> heme
Cytochrome $c_1$	$\sim 38,000$	418, 540, 554 (reduced)	$c_1$ heme
Cytochrome <i>c</i>	12,000	415, 521, 550 (reduced)	<i>c</i> heme

\* Present only in the oxidized enzyme.

coupling. Once the chain is defined, the road will be clear to achieve this objective.

There is the obvious danger that when the electron transport process is no longer coupled to synthesis of ATP it may not faithfully represent the counterpart process which operates in a coupled system. This possibility has been examined, and it appears that the same electron transport process operates whether coupling takes place or not, in the sense that the sequence of components and the nature of the oxidoreductions are the same. Certain oxidoreductions can take place either with or without phosphate esterification, but there does not seem to be an essential qualitative difference between coupled and uncoupled electron transport processes (23).

### Sequence of Components in the Chain

The electron transport particle (ETP) catalyzes the oxidation of succinate to fumarate and of DPNH to DPN<sup>+</sup> by molecular oxygen. Involved in these oxidations are two flavoprotein dehydrogenases (24, 25), four cytochromes (26–28), iron (29) (in a form other than that of porphyrin-bound iron), copper (29, 30), and a benzoquinone derivative known as coenzyme Q (31, 32).

Electrons originating from either succinate or DPNH are transferred eventually to oxygen through a chain involving the cytochromes, coenzyme Q, nonheme iron, and copper. Our present state of knowledge about the sequence of components in the chain is summarized in Fig. 3. Although the idea of a single chain from coenzyme Q to oxygen is more generally accepted,

several pieces of information are more consistent with the concept of two interconnecting chains, one for succinic flavoprotein dehydrogenase ( $f_s$ ) and one for DPNH flavoprotein dehydrogenase ( $f_D$ ). The bracket in the figure is used to indicate this possibility. Like coenzyme Q, cytochrome *b* is situated between the flavoproteins and cytochrome  $c_1$ . However, its position relative to coenzyme Q is not clear (33).

### Components of the Electron Transport Chain

The two flavoprotein dehydrogenases (succinic and DPNH dehydrogenase) as well as the four cytochromes are proteins which have now been isolated in homogeneous state and defined with respect to molecular weight, spectrum, prosthetic group, and gross composition (see Table 3). Coenzyme Q is not found in close association with any of the six proteins and can be readily extracted and isolated as a crystalline product of molecular weight 863.4 (32, 34).

The succinic dehydrogenase ( $f_s$ ) (24) and DPNH dehydrogenase ( $f_D$ ) (25) are both flavoprotein enzymes. The flavin prosthetic group of  $f_D$  is flavinadenine dinucleotide, while that of  $f_s$  is a dinucleotide as yet uncharacterized. The flavin group is readily split

off by acid from the protein of  $f_D$  but not from the protein of  $f_s$ , and this difference in behavior is the basis of the method for estimating the proportion of  $f_s$  and  $f_D$  in a mixture. According to Kearney (35), the flavin prosthetic group of  $f_s$  is linked to the protein by a peptide bond which can be ruptured by proteolytic enzymes. Nonheme iron appears to be closely associated with both  $f_s$  and  $f_D$  in the ratio of at least four atoms of iron per molecule of flavin.

The four cytochromes are hemoproteins which differ in respect to both the protein and heme moieties. The heme group of cytochrome *b* (36) is protohemin, while that of cytochrome *a* is a heme derivable from protohemin by replacement of three of the ring substituents with a formyl group and two long-chain carbon residues (37). The heme group of cytochromes *c* and  $c_1$  is also protohemin, but the heme group of cytochrome *c* (and probably that of cytochrome  $c_1$ ) is attached to cysteine residues in the apoprotein by thiol ether links (36). The heme groups of cytochromes *a* and *b* are readily extracted from the respective apoproteins by acid acetone, whereas the link of the heme groups of cytochromes *c* and  $c_1$  to the apoproteins is unaffected by this reagent.

Cytochrome *b* exists in two forms; one of these forms occurs in close association with succinic dehydrogenase (38) while the other can be isolated as a discrete protein with no functional group other than the heme (28). The succinic dehydrogenase has been isolated by Ziegler and Doeg (39) as a soluble complex containing one molecule of *b* heme per molecule of flavin, and also lipid in the amount of 18 percent by weight. At present, it is not certain whether the same protein moiety is the bearer of all three functional groups (the flavin, nonheme iron, and heme) or whether the succinic dehydrogenase as isolated is a complex of two or more proteins each with a separate prosthetic group, as has been found to be the case for the component

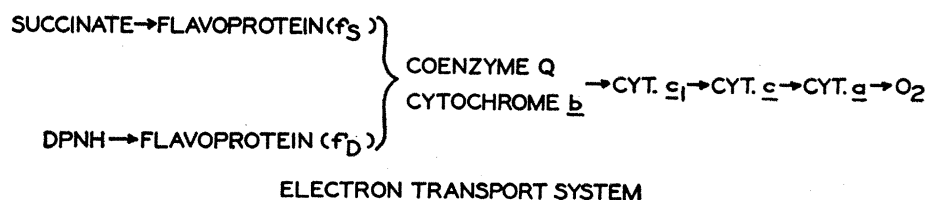
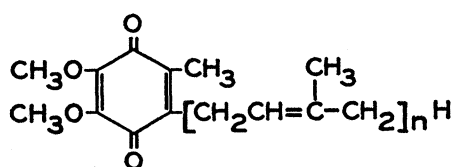


Fig. 3. Sequence of components in the electron transport system.



	SOURCE
$n=10$	BEEF HEART
$n=9$	RAT, TORULA
$n=8$	AZOTOBACTER
$n=7$	TORULA
$n=6$	SACCHAROMYCES CEREVISIAE

Fig. 4. Structure of coenzymes Q.

proteins of the pyruvic (40, 41) and  $\alpha$ -ketoglutaric dehydrogenase (41, 42) complexes.

Cytochromes *a* and *b* are isolated as water-insoluble, polymeric particulates. These can be solubilized in water with bile salts and depolymerized to monomers by reagents such as thioglycollate (43) and cetyldimethylethyl ammonium bromide (28). Cytochromes *c* and *c*<sub>1</sub> are water-soluble as isolated, but *c*<sub>1</sub> occurs in polymeric forms (aggregates of six or more molecules) which can be depolymerized to the monomer by reagents such as thioglycollate (27, 44). Cytochromes *a* and *c*<sub>1</sub>, as isolated at the highest purity level, contain lipid (27, 45) (about 26 and 10 percent, respectively), whereas *b* and *c* can be isolated in lipid-free forms. However, cytochrome *c* can also be shown to exist in the particle in close association with lipid (46).

Cytochrome *a* is isolated in the form of a hemoprotein which contains copper (47, 48), the molecular ratio of copper to heme being 1:1 (45, 48). This hemoprotein-copper complex catalyzes the oxidation of reduced cytochrome *c* by molecular oxygen (45, 49, 50).

Coenzyme Q is a tetrasubstituted benzoquinone with a side chain containing ten isoprenoid units (see Fig. 4) (32, 51). Animal tissues generally contain coenzyme Q<sub>10</sub>—that is, the coenzyme with a side chain containing ten isoprenoid units—though Q<sub>6</sub> has recently turned out to be the preferred homologue in the tissues of the rat (52). In microorganisms, coenzymes Q<sub>6</sub>, Q<sub>7</sub>, Q<sub>8</sub>, Q<sub>9</sub>, and Q<sub>10</sub> have been found to occur under physiological conditions (32, 53). Coenzyme Q is water-insoluble and is probably solvated in areas of high lipid concentration in the mitochondrion.

### Stepwise Fragmentation of the Chain

The sequence of components in the electron transport chain has been deduced from the information obtained by fragmenting the chain into smaller units and determining the composition and the catalytic activity of the derivative segments (see Table 4).

The fragments derived by the cleavage of the mitochondrion or ETP are usually particles, though some of the simple and uncomplicated. The diffusion separation of mixtures of particles which a priori might have been expected to pose a very difficult technical problem has proved to be relatively simple and uncomplicated. The difficulty has been predominantly that of finding the reagent and the conditions which would permit the selective fragmentation of one specific bond. Once this has been achieved, the separation of particles by ultracentrifugation and salt precipitation has been straightforward.

The few reagents which have been found to be efficacious for rupturing the bonds which hold together the components of the chain (see Table 5) are soluble in both water and lipid.

### Lipid in Relation to Mitochondrial Structure and Function

Lipid accounts for about 30 percent of the total dry weight of the mitochondrion and of ETP. Recent studies of Fleischer and Klouwen indicate that the composition of the lipid, regardless of the segment of the chain with which it is associated, varies little if at all (54). Phospholipid (55) accounts for the bulk of the lipid (> 90 percent). In addition to coenzyme Q, vitamin E (56), carotenoids (57), and substantial amounts of cholesterol (55) are found in the neutral lipid fraction. The mitochondrial lipids are characterized by fatty acid residues with a high degree of unsaturation (55, 58, 59) and by an unusually high proportion of plasmalogen (55, 59).

Mitochondrial lipid readily assumes, and probably exists, in a state of orientation which, in effect, makes the lipid "soluble" in water (60). This property may be attributed to the phospholipid molecules which constitute the bulk of the mitochondrial lipids. While the role of lipid (except coenzyme Q) in electron transport is far from clear, the close association of lipid with most of

Table 4. Subfractions of ETP. In addition, cytochromes *b*, *c*, and *c*<sub>1</sub> and coenzyme Q have been isolated in pure form. Fe, non-heme iron; Q, coenzyme Q; cyt., cytochrome.

Enzymic activity of subfraction	Components
Succinic dehydrogenase	$f_s$ , Fe
DPNH dehydrogenase	$f_D$ , Fe
Succinic-coenzyme Q reductase	$f_s$ , Fe, cyt. <i>b</i>
Succinic-cytochrome <i>c</i> reductase	$f_s$ , Fe, Q, cyt. <i>b</i> , cyt. <i>c</i> <sub>1</sub>
DPNH-cytochrome <i>c</i> reductase	$f_D$ , Fe, Q, cyt. <i>b</i> , cyt. <i>c</i> <sub>1</sub>
Coenzyme Q oxidase	cyt. <i>c</i> <sub>1</sub> , cyt. <i>c</i> , cyt. <i>a</i> , Cu
Cytochrome <i>c</i> oxidase	cyt. <i>a</i> , Cu

the protein components of the chain and its activating effect in some of the catalytic functions of these components (50, 61) are significant indications for the participation of lipid in electron transport and oxidative phosphorylation.

### Mechanism of Electron Flow

Electron flow in the mitochondrion or in ETP is an extremely rapid process. Recent studies in our laboratory indicate, for example, that a DPNH oxidase system can be reconstructed from three purified segments [DPNH-cytochrome *c* reductase (62), cytochrome *c*, and cytochrome oxidase] and that this system is capable of catalyzing the oxidation of DPNH with a  $Q_{10}$  of more than 20,000 (20,000 microliters of O<sub>2</sub> per hour per milligram of protein at 38°C).

This rapid flow of electrons does not seem to be the result of simple molecular collision between the components of the electron transport system. The somewhat rigid structure of the electron transport chain does not allow free movement of the electron carriers. The components with larger size, such as the flavoproteins and the cytochromes, seem to be fixed in place and capable of only restricted movement (cytochrome *c*, which is a hemoprotein of relatively small molecular weight, may be an exception). Other components,

Table 5. Fragmenting reagents.

Cholate	Thioglycollate	Ethanol
Deoxycholate	<i>t</i> -Amyl alcohol	Cetyldimethylethyl ammonium bromide
Duponol	<i>n</i> -Butanol	Triton Tweens

such as coenzyme Q, nonheme iron, and copper, which have smaller dimensions, facilitate electron transfer between the less mobile carriers.

The sum of the protein contributions by succinic dehydrogenase, DPNH dehydrogenase, and the four cytochromes, as calculated from the purified form of each, represents no more than 25 percent of the total protein of ETP. This is also true for many of the subfractions of ETP. In other words, about 75 percent or more of the protein of ETP cannot be accounted for. Perhaps this excess protein serves a multitude of functions in the mitochondrion which we are as yet unaware of. But it seems that an additional, if not the principal, function of this excess protein is to provide a framework, as it were, for the spatial arrangement of the electron transport components. We may conceive of the electron transport chain as imbedded in a matrix of "framework" proteins and lipid, and the structural arrangement of the unit of the electron transport chain is thus an expression of the close interdigitation of the "framework" proteins with the oxidation-reduction proteins.

It may well be that such an arrangement is common to transducing systems other than the mitochondrion, and the remarkable similarity of the underlying structure of transducing systems suggests that the design of a "framework" protein-lipid continuum has broad applicability (62a).

### Oxidative Phosphorylation

Reduced to the most elementary considerations, oxidative phosphorylation may be looked upon as a consequence of three principal reactions, shown in Fig. 5. The first reaction is the passage of electrons from a reduced carrier of lower oxidation-reduction potential to an oxidized carrier of higher potential. This process involves a negative free-energy change, which is utilized in converting inorganic phosphate to a so-called "high-energy" organic phosphate (reaction 2). In the third reaction, the "high-energy" phosphoryl group ( $\sim P$ ) is transferred to adenosine diphosphate, resulting in the formation of adenosine triphosphate.

The span in the oxidation-reduction potential ( $\Delta E'$  at pH 7.0 and 25°C) between DPNH and oxygen is about 1.12 volts and between succinate and oxygen, about 0.8 volt. These values

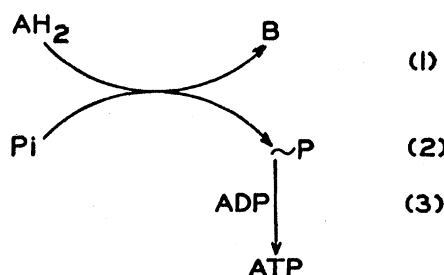
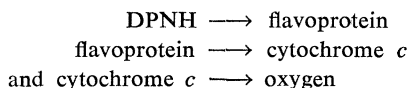


Fig. 5. Schematic representation of principal reactions in oxidative phosphorylation.

for  $\Delta E$  correspond to  $-\Delta F$  values of about 52,000 and 37,000 calories, respectively. In round numbers, we may say that the synthesis of 1 mole of ATP from ADP and inorganic phosphate requires 12,000 calories (62b). Since the oxidation of 1 mole of DPNH by oxygen is accompanied by the esterification of 3 moles of inorganic phosphate, only about 69 percent of the total free energy change involved in the oxidation is recovered in the form of the bond energy of ATP. The oxidation of succinate by oxygen shows a P/O ratio of 2, which would correspond to about 65 percent efficiency of conversion of oxidation energy to phosphate bond energy.

The experiments of Loomis and Lipmann (63), Copenhagen and Lardy (64), Nielsen and Lehninger (65), Slater (66), and Maley and Lardy (67), as well as those of Chance and Williams (68) and Chance *et al.* (69), have shown that the phosphorylation occurs in three separate segments of the electron transport chain. These segments are responsible for the following oxidoreductions:



Since these early studies were carried out, a new member of the chain—namely, coenzyme Q—has been discovered. With a better knowledge of the components involved between flavoprotein and cytochrome *c*, it may now be possible to define more accurately the segment concerned with the second phosphorylation.

Oxidative phosphorylation as a physiological mechanism for the recovery of useful energy was first realized by Kalckar in 1937 (70) (see also 71). It may seem to have taken too long to get where we are today in our understanding of the mechanism of electron transport and oxidative phosphorylation.

Admittedly, we do not know all the answers yet, but we know most of the questions that remain to be answered, and we can ask them in great detail (72).

### References and Notes

- G. Wald and R. Hubbard in *The Enzymes*, P. D. Boyer, H. Lardy, K. Myrback, Eds. (Academic Press, New York, 1960), vol. 3, pt. B, p. 369.
- A. Szent-Gyorgyi, *Chemistry of Muscular Contraction* (Academic Press, New York, 1951); J. Hanson and H. E. Huxley, *Symposia Soc. Exptl. Biol.* 9, 228 (1955); M. F. Morales and J. Botts, *Arch. Biochem. Biophys.* 37, 283 (1952); M. F. Morales in *Enzymes: Units of Biological Structure and Function* (Academic Press, New York, 1956), p. 325.
- H. Gaffron, A. H. Brown, C. S. French, R. Livingston, E. I. Rabinowitch, B. L. Strehler, N. E. Tolbert, Eds. *Research in Photosynthesis* (Interscience, New York, 1957); D. I. Arnon, *Science* 122, 9 (1955); M. Calvin, *Revs. Modern Phys.* 31, 147 (1959).
- G. E. Palade, *Anat. Record* 114, 427 (1952); *J. Histochem. and Cytochem.* 1, 188 (1953); in *Enzymes: Units of Biological Structure and Function* (Academic Press, New York, 1956), p. 185; F. S. Sjöstrand in *Fine Structure of Cells: Symposium, Eighth Congress on Cellular Biology, Leyden, 1954* (Interscience, New York, 1955), pp. 16, 222; F. S. Sjöstrand, *Revs. Modern Phys.* 31, 301 (1959).
- L. Ernster and O. Lindberg, *Ann. Rev. Physiol.* 20, 13 (1958); O. Lindberg and L. Ernster, *The Chemistry and Physiology of Mitochondria and Microsomes* (Springer, Vienna, Austria, 1954).
- S. Mudd, L. C. Winterscheid, E. D. Delamater, H. J. Henderson, *J. Bacteriol.* 62, 459 (1951); S. Mudd, A. F. Brodie, L. C. Winterscheid, P. E. Hartman, E. H. Buettner, R. A. McLean, *ibid.* 62, 729 (1951).
- No distinction is being made between biological transducers which convert energy from one type to another and transducers which convert chemical energy from one type to another.
- S. Ochoa, *J. Biol. Chem.* 151, 493 (1943).
- A. L. Grafflin and D. E. Green, *ibid.* 176, 95 (1948); W. C. Schneider and V. R. Potter, *ibid.* 177, 893 (1949); A. L. Lehninger and E. P. Kennedy, *ibid.* 179, 957 (1949).
- J. V. Taggart and R. B. Krakaur, *ibid.* 177, 641 (1949); J. L. Still, M. V. Buell, D. E. Green, *Arch. Biochem.* 26, 406 (1950); *ibid.* 26, 413 (1950).
- R. L. Ringer and T. P. Singer, *J. Biol. Chem.* 234, 2211 (1959).
- D. E. Green, J. G. Dewan, L. F. Leloir, *Biochem. J.* 31, 934 (1937); A. L. Lehninger, H. C. Sudduth, J. B. Wise, *J. Biol. Chem.* 235, 2450 (1960).
- S. Taniguchi, R. Sato, F. Egami, in *Inorganic Nitrogen Metabolism*, W. D. McElroy and B. Glass, Eds., (Johns Hopkins Press, Baltimore, 1956), p. 87; M. Ishimoto, J. Koyama, Y. Nagai, *J. Biochem. (Tokyo)* 41, 763 (1954).
- E. P. Kennedy, *J. Am. Chem. Soc.* 75, 249 (1953); — and H. M. Bergoff, *J. Biol. Chem.* 201, 393 (1953).
- H. M. Bates, V. M. Craddock, M. V. Simpson, *J. Biol. Chem.* 235, 140 (1960).
- P. P. Cohen and R. W. McGilvery, *ibid.* 166, 261 (1946); N. K. Sarkar, H. Beinert, M. Fuld, D. E. Green, *Arch. Biochem.* 37, 140 (1952).
- P. P. Cohen and M. Hayano, *J. Biol. Chem.* 170, 687 (1947).
- W. McMurry, G. F. Maley, H. Lardy, *ibid.* 230, 219 (1958); W. W. Kieley and J. R. Bronk, *ibid.* 230, 521 (1958); D. M. Ziegler, A. W. Linnane, D. E. Green, M. S. Dass, H. Ris, *Biochim. et Biophys. Acta* 28, 524 (1958).
- A. W. Linnane and D. M. Ziegler, *Biochim. et Biophys. Acta* 29, 630 (1958).
- F. L. Crane, J. L. Glenn, D. E. Green, *ibid.* 22, 475 (1956).
- D. E. Green, *Biol. Revs. Cambridge Phil. Soc.* 26, 410 (1951); — and J. Järfelt, *Perspectives in Biol. and Med.* 2, 163 (1959).
- D. E. Green, *Harvey Lectures, Ser. 52* (1956–57) (1958), p. 177.
- A. W. Linnane and E. B. Titchner, *Biochim. et Biophys. Acta* 39, 469 (1960).

24. T. P. Singer and E. B. Kearney in *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1957), vol. 4, p. 312.
25. B. De Bernard, *Biochim. et Biophys. Acta* **23**, 510 (1957); D. M. Ziegler, D. E. Green, K. A. Doeg, *J. Biol. Chem.* **234**, 1916 (1959).
26. D. Keilin and E. F. Hartree, *Nature* **176**, 200 (1955); E. Yakushiji and K. Okunuki, *Proc. Imp. Acad. (Tokyo)* **16**, 299 (1940); —, *ibid.* **27**, 263 (1941); D. Keilin, *Proc. Roy. Soc. (London)* **98B**, 312 (1925); I. Sekuzu and K. Okunuki, *J. Biochem. (Tokyo)* **43**, 107 (1956).
27. D. E. Green, J. Järnefelt, H. D. Tisdale, *Biochim. et Biophys. Acta* **31**, 34 (1959); *ibid.* **38**, 160 (1960).
28. R. Bomstein, R. Goldberger, H. Tisdale, *Biochem. Biophys. Research Comm.* **2**, 234 (1960).
29. D. E. Green in *Enzymes: Units of Biological Structure and Function* (Academic Press, New York, 1956), p. 465; F. L. Crane, J. L. Glenn, D. E. Green, *Biochim. et Biophys. Acta* **22**, 475 (1956).
30. B. Eichel, W. W. Wainio, P. Person, S. J. Cooperstein, *J. Biol. Chem.* **183**, 89 (1950).
31. F. L. Crane, Y. Hatefi, R. L. Lester, C. Widmer, *Biochim. et Biophys. Acta* **25**, 220 (1957); Y. Hatefi, R. L. Lester, F. L. Crane, C. Widmer, *ibid.* **31**, 490 (1959); R. A. Morton, *Nature* **182**, 1764 (1958).
32. R. L. Lester, Y. Hatefi, C. Widmer, F. L. Crane, *Biochim. et Biophys. Acta* **33**, 169 (1959).
33. Y. Hatefi, P. Jurtshuk, A. G. Haavik, in preparation.
34. F. L. Crane, R. L. Lester, C. Widmer, Y. Hatefi, *Biochim. et Biophys. Acta* **32**, 73 (1959).
35. E. B. Kearney, *J. Biol. Chem.* **235**, 865 (1960).
36. K. G. Paul in *The Enzymes*, P. D. Boyer, H. Lardy, K. Myrbäck, Eds. (Academic Press, New York, 1960), vol. 3, pt. B, p. 277.
37. M. Morrison, J. Connelly, J. Petix, E. Stotz, *J. Biol. Chem.* **235**, 1202 (1960).
38. K. A. Doeg, S. Krueger, D. M. Ziegler, *Biochim. et Biophys. Acta* **41**, 491 (1960).
39. D. M. Ziegler and K. A. Doeg, *Biochem. Biophys. Research Comm.* **1**, 344 (1959).
40. V. Jagannathan and R. S. Schweet, *J. Biol. Chem.* **196**, 551 (1952); R. S. Schweet, B. Katchman, R. M. Bock, V. Jagannathan, *ibid.* **196**, 563 (1952).
41. I. C. Gunsalus, in *The Mechanism of Enzyme Action*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1954), p. 545.
42. D. R. Sanadi, J. W. Littlefield, R. M. Bock, *J. Biol. Chem.* **197**, 851 (1952); D. R. Sanadi, M. Langley, R. L. Searls, *ibid.* **234**, 178 (1959); D. R. Sanadi, M. Langley, F. White, *ibid.* **234**, 183 (1959); V. Massey, *Biochim. et Biophys. Acta* **32**, 286 (1959); M. Koike, L. J. Reed, W. R. Carroll, *J. Biol. Chem.* **235**, 1924 (1960); M. Koike and L. J. Reed, *J. Biol. Chem.* **235**, 1931 (1960); M. Koike, P. C. Shah, L. J. Reed, *ibid.* **235**, 1939 (1960).
43. K. S. Ambe and A. Venkataraman, *Biochem. Biophys. Research Comm.* **1**, 133 (1959).
44. R. S. Criddle and R. M. Bock, *ibid.* **1**, 138 (1959).
45. D. E. Griffiths and D. C. Wharton, unpublished studies.
46. C. Widmer and F. L. Crane, *Biochim. et Biophys. Acta* **27**, 203 (1958).
47. C. V. Wende and W. W. Wainio, *J. Biol. Chem.* **235**, PC11 (1960); S. Takemori, I. Sekuzu, K. Okunuki, *Biochim. et Biophys. Acta* **38**, 158 (1960).
48. R. H. Sands and H. Beinert, *Biochem. Biophys. Research Comm.* **1**, 175 (1959).
49. D. Keilin and E. F. Hartree, *Nature* **141**, 870 (1938); L. Smith and E. Stotz, *J. Biol. Chem.* **209**, 819 (1954); K. Okunuki, I. Sekuzu, T. Yonetani, S. Takemori, *J. Biochem. (Tokyo)* **45**, 847 (1958).
50. Y. Hatefi, *Biochim. et Biophys. Acta* **30**, 648 (1959).
51. R. A. Morton, G. M. Wilson, J. S. Lowe, W. M. F. Leat, *Chem. & Ind. (London)* **1957**, 1649 (1957); D. E. Wolf, C. H. Hoffman, N. R. Trenner, B. H. Arison, C. H. Shunk, B. O. Linn, J. F. McPherson, K. Folkers, *J. Am. Chem. Soc.* **80**, 4752 (1958).
52. R. E. Olson and G. H. Dialameh, *Biochem. Biophys. Research Comm.* **2**, 198 (1960); D. E. M. Lawson, E. I. Mercer, J. Glover, R. A. Morton, *Biochem. J.* **74**, 38P (1960).
53. R. L. Lester, F. L. Crane, Y. Hatefi, *J. Am. Chem. Soc.* **80**, 4751 (1958).
54. S. Fleischer and H. Klouwen, unpublished studies.
55. R. E. Basford, *Biochim. et Biophys. Acta* **33**, 195 (1959).
56. A. Nason and I. R. Lehman, *J. Biol. Chem.* **222**, 511 (1956); J. Bouman and E. C. Slater, *Biochim. et Biophys. Acta* **26**, 624 (1957).
57. F. L. Crane, R. L. Lester, C. Widmer, Y. Hatefi, *Biochim. et Biophys. Acta* **32**, 73 (1959).
58. R. T. Holman and C. Widmer, *J. Biol. Chem.* **234**, 2269 (1959).
59. E. V. Marinetti, J. Erbland, J. Kochen, E. Stotz, *ibid.* **233**, 740 (1958); G. D. Joel, M. L. Karnovsky, E. G. Ball, O. Cooper, *ibid.* **233**, 1565 (1958).
60. S. Fleischer and H. Kouwen, *Federation Proc.* **19**, 33 (1960).
61. W. W. Wainio and J. Greenlees, *Science* **128**, 87 (1958); Y. Hatefi, *Biochim. et Biophys. Acta* **34**, 183 (1959).
62. Y. Hatefi, A. G. Haavik, P. Jurtshuk, in preparation.
- 62a. H. Fernández-Morán, *Revs. Modern Phys.* **31**, 319 (1959).
- 62b. L. L. Ingraham and A. B. Pardee in *Metabolic Pathways*, D. M. Greenberg, Ed. (Academic Press, New York, 1960), vol. 1, p. 1.
63. W. F. Loomis and F. Lipmann, *J. Biol. Chem.* **179**, 503 (1949).
64. J. H. Copenhaver, Jr., and H. A. Lardy, *ibid.* **195**, 225 (1952).
65. S. O. Nielsen and A. L. Lehninger, *J. Am. Chem. Soc.* **76**, 3860 (1954).
66. E. C. Slater, *Nature* **174**, 1143 (1954).
67. G. F. Maley and H. A. Lardy, *J. Biol. Chem.* **210**, 903 (1954).
68. B. Chance and G. R. Williams, *ibid.* **217**, 383, 395, 409, 429 (1955).
69. B. Chance, G. R. Williams, W. F. Holmes, J. Higgins, *ibid.* **217**, 439 (1955).
70. H. Kalckar, *Enzymologia* **2**, 47 (1937).
71. —, *Biochem. J.* **33**, 631 (1939); V. A. Belitzer, *Enzymologia* **6**, 1 (1939); — and E. T. Tsiwakowa, *Biokhimiya* **4**, 516 (1939).
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## An Experiment in the History of Science

With a simple but ingenious device Galileo could obtain relatively precise time measurements.

Thomas B. Settle

On the "Third Day" of his *Discorsi* (1) Galileo described an experiment in which he had timed a ball accelerating along different lengths and slopes of an inclined plane. With it he believed he had established the science of nat-

urally accelerated motion. To get a better appreciation for some of the problems he faced I have tried to reproduce the experiment essentially as Galileo described it. In the process I found that it definitely was technically

feasible for him, and I think I gained a good idea of the type of results he probably looked for and of how well they turned out.

He described the experiment because, in his words: "in those sciences where mathematical demonstrations are applied to natural phenomena, as is seen in the case of perspective, astronomy, mechanics, music, and others [,] the principles, once established by well-chosen experiments, become the foundations of the entire superstructure" (1, p. 171). In this case his aim was to establish a science based on two principles: (i) a general definition of uniform acceleration, "such as actually occurs in nature" (1, p. 154), as that motion in which equal increments of velocity are added in equal times and (ii) an assumption that "the speeds acquired by one and the same body

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