Energy Transformation in Biological Systems

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Energy Transformation in Biological Systems
FRITZ LIPMANN

[Photograph by Heka]
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Opening remarks

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The Ciba Foundation describes its symposia as multidisciplinary international meetings but, since all symposia nowaday tend to be multidisciplinary, we may claim this one to be ‘more multidisciplinary than thou’. The objective of such a symposium is cross-fertilization between the distantly related subjects represented here. The analogy of cross-fertilization is drawn from animal breeding and seems to be appropriate for the description of progress in a science. Although cross-fertilization is important for the improvement of, say, a breed of sheep, other analogies may be drawn between scientists and sheep: both have tendencies to follow *en masse* when a new idea gets a head. Darwin pointed out that crossing, though necessary, is a minor matter in improving the breed in the face of the slow *selection* over long periods. This is true for scientific progress; despite the occasional bright idea from outside a discipline, 99% of progress is the steady work of demolishing the many theories that unfortunately turn out to be wrong and of establishing, as far as is possible, facts by experiment—there is no royal road towards progress. In each subject that we shall deal with, the number of possible mechanisms is almost unlimited, but, for example, the great majority of the many mechanisms of muscle contraction that have been put forward independently in the last 20 years have little contact with what is known experimentally.

How far should we speculate and how far should we stick to ideas which are to some degree based on experimental evidence? A pervasive feeling, undoubtedly to some extent justified, is that similar processes are followed in similar ways in different biological contexts. Thus, we hope that an understanding of how energy is transformed from a photon into the making or breaking of a chemical bond may help us to discover how energy is transformed from some chemical event into, say, movement of a muscle. Although we accept partially the uniformity of nature, we do not know the circumstances
in which the uniformity will appear until after the event. Until a problem has been resolved it is possible to make the most terrible mistakes; the history of science is full of such mistakes. For instance, that both smooth muscle and striated muscle contract is a perfectly valid statement but, according to the principle of the uniformity of nature, contraction must then be the same in both cases and so the striations cannot be of essential interest. Such arguments were popular around the turn of the century, before which time the emphasis had been largely on microscopy. They were made explicitly, for example, in the statements of Verworn (1892) and Bernstein (1901), according to whom striations were crude and irrelevant and contraction was clearly something molecular, below the level of what could be seen with the microscope. Attention moved away from the micrometre scale of what was visible with the microscope towards the objective of finding the molecular explanation directly. As it happened, sliding filaments were proposed twenty years ago and showed that this approach had been in a sense wrong. No doubt, however, smooth muscle has essential analogies with striated muscle, but we still do not know just what form these analogies take.

In the contractile field, Weis-Fogh has recently demonstrated a conspicuous example of the non-uniformity of nature. Organelles of Vorticella and related protozoa work by a mechanism which 25 years ago was an attractive candidate for the mechanism of muscle contraction. The interest of muscle physiologists in these mechanisms disappeared with the advent of sliding filaments, but the work of Weis-Fogh and others has provided a 'substrate' for these theories which now apply to this structure, the spasmoneme. So, here is a case where, despite the theory originally having been inappropriate for the structure to which it was intended to apply, it has turned out to be applicable in another related area. Clearly, we cannot predict how ideas from one discipline will affect progress in another.

References


VERWORN, M. (1892) Die Bewegung der Lebendigen Substanz, Fischer, Jena
The roots of bioenergetics

FRITZ LIPMANN

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Abstract Understanding metabolic energy transformation began with the realization of an 'intrusion' of phosphate into the mechanism of alcoholic fermentation. The discovery of an analogous participation of phosphate in muscle glycolysis connected the metabolic generation of energy-rich phosphate bonds fed into a common transmitter, adenosine triphosphate (ATP), with the production of mechanical energy through the finding that the phosphoryl group of creatine phosphate transferred to ATP could supply the energy for muscle contraction. In this way, a functional applicability of the energy of the phosphate bond was first shown. This observation was soon followed by the recognition that the phosphoanhydride bond of ATP provided the driving force in biosynthetic reactions; in this type of bond, metabolic energy apparently collects before it is transmitted for functional and biosynthetic use.

The storage of energy in ATP was first detected in anaerobic energy-yielding reactions but soon was also found in respiratory and photosynthetic energy production. However, the mechanism by which energy derived from metabolites was converted into phosphate-bond energy in the latter processes appeared to differ from that of anaerobic energy transmission. Whereas phosphorylated compounds mediate the latter in homogeneous solutions, aerobic phosphorylation and photophosphorylation in prokaryotes seem to require special submembranous structures; and in eukaryotes, energy conversion is a function of special organelles, the mitochondria and chloroplasts.

The evolutionary aspects of the transition from prokaryotes to eukaryotes are of considerable interest. In conclusion, the relevance of an apparent prokaryotic origin of the energy-transforming organelles in the eukaryotes will be commented on.

I shall begin with an eye-witness account of my years in Meyerhof's laboratory, from 1927 until I left in 1930. While there, I was only slowly drawn into the work on bioenergetics; I was observing and learning rather than doing any work of importance myself. Later, I did get into other problems, but eventually when I had leisure to work on my own in the Carlsberg Laboratory in Copen-
hagen (Lipmann 1934a, 1935), I drifted back into the exciting developments in the mechanism of fermentation and glycolysis. Subsequently, as I became more and more involved in the mechanism of energy transformation, I graduated from an onlooker to a participant (Lipmann 1939, 1940, 1941). In Meyerhof's laboratory I had the good fortune to live through the time when attention was first turning to the importance of phosphate in intermediary metabolism through the discovery of two key compounds, creatine phosphate (Cr-P; see p. 7) (Eggleton & Eggleton 1926; Fiske & Subbarow 1927) and adenosine 5'-triphosphate (ATP) (Lohmann 1929; Fiske & Subbarow 1929).

I intend to trace the circuitous and slow development of the understanding of metabolic energy transformation through the analysis of the intermediary reactions in cell-free fermentation in yeast extracts and in cell-free glycolysis in muscle extracts. For such an analysis, it was essential to have cell-free extracts available for the bulk conversion of glucose into ethanol and carbon dioxide as well as a conversion of glucose into lactic acid. The first preparation of this kind, a cell-free extract of yeast that promoted vigorous fermentation, was made by the brothers Büchner in 1896. This extract has yielded all the enzymes that catalyse the intermediary steps of metabolic energy conversion, present there mixed in a homogeneous solution. I dare to call this isolation of a solution containing a normally-functioning metabolic system the dawn of what we now call molecular biology.

THE DISCOVERY OF PHOSPHATE AS PARTICIPANT IN FERMENTATION

Harden and his colleagues used this cell-free fermentation to probe into its mechanism. Early in this century, Harden & Young (1906) published on the role of phosphate; they discovered that inorganic phosphate was necessary to promote rapid fermentation and that about one mole of phosphate was esterified for every mole of alcohol and carbon dioxide formed. The product was a hexose diphosphate. In other words, for every pair of (alcohol + CO₂) formed, two phosphates were fixed to the hexose molecule (equation 1).

\[ 2\text{glucose} + 2\text{P} \rightarrow 2(\text{ethanol} + \text{CO}_2) + \text{hexose diphosphate} \]

The primary observation on the participation of phosphate is illustrated in the rate curve (Fig. 1), taken from a study on yeast fermentation in the middle thirties (Lipmann 1934a). The curve shows the characteristics of cell-free fermentation; the autocatalytic period of the extract, which contains phosphate and glucose, can be abolished (see later) by the addition of phosphate donors. Once the fast period of fermentation ensues, phosphate is fixed according to the Harden–Young equation. Some glucose 6'-phosphate is formed with the
hexose diphosphate, which was later identified as fructose 1',6'-phosphate. When the inorganic phosphate is exhausted (after 60 min in Fig. 1), fermentation slows dramatically. Then, large amounts of fructose diphosphate are present but are fermented slowly. However, when new inorganic phosphate is added (not shown) fast fermentation starts again. The puzzle posed by the need for inorganic phosphate in fermentation, as well as the relative inactivity of the hexose diphosphate, even though it impressed as being an intermediary in alcoholic fermentation, had to wait for almost 30 years to be fully resolved.

THE PARALLEL BETWEEN FERMENTATION AND GLYCOLYSIS

When I arrived at Meyerhof's laboratory, glycolysis was being studied with a muscle extract which was prepared by the extraction of rabbit muscle with distilled water (Meyerhof 1927); it contained practically no glycogen and only poorly glycolysed free glucose. In order to obtain a glucose metabolism analogous to that in yeast extracts, Meyerhof prepared from yeast autolysates a fraction which he called glucose activator and later found to be hexokinase (Meyerhof 1930) that was virtually absent from these muscle extracts. With the combination of muscle extract and hexokinase, he obtained exact parallels between glycolysis and fermentation with regard to fast rate in the presence of an excess of inorganic phosphate and its fixation as fructose diphosphate: in other words, the glycolytic equivalent of a Harden–Young reaction with lactic acid as product rather than ethanol and carbon dioxide.

The progress of the combined studies on alcoholic fermentation and muscle glycolysis seemed to justify the expectation that one might open the 'black box' wherein one hoped to find the then mysterious transformation that would
yield out of the initial substrate, glucose, the end products of fermentation, \( \text{C}_2\text{H}_5\text{OH} + \text{CO}_2 \), and of glycolysis, \( \text{CH}_3\text{CH(OH)}\text{COOH} \) (lactic acid).

**THE LINK OF GLYCOLYSIS TO MUSCLE CONTRACTION**

Meyerhof proposed that lactic acid production was directly connected with muscle contraction (Meyerhof 1925). He reached this conclusion by finding "... as a general rule that a fixed relation exists under normal conditions between the lactic acid formed (anaerobically) upon stimulus, and the developed isometric tension...". This he called the isometric coefficient of lactic acid and concluded that it indicated that the production of lactic acid is mechanistically connected with mechanical response. Meyerhof was aware that, in order to understand the physiological significance of metabolic reactions, it was essential to know, in addition to its chemical equation, the change of energy during the reaction. Calorimetry was used for such estimates (Meyerhof & Lohmann 1928). Thus, when two new phosphate-containing compounds, \( \text{Cr} \sim \text{P} \) and ATP, were discovered in muscle, although far from realizing their bioenergetic importance, Meyerhof & Lohmann determined their heat of hydrolysis. They found it to be surprisingly high, about 50 kJ/mol \( \text{Cr} \sim \text{P} \) (12 kcal/mol) and about 50 kJ/mol for each of the two terminal \( \sim \text{P} \) in ATP. In 1927, the Eggletons, in a short note on the significance of phosphate in contraction (Eggleton & Eggleton 1927), reported briefly some data which they rightly interpreted to mean a connection between breakdown of creatine phosphate (they called it phosphagen) and muscular action. However, in view of the convincing link established with lactic acid formation, such a connection was not seriously considered in Meyerhof's laboratory when I joined it in 1927 (cf. Meyerhof & Nachmansohn 1928).

**A MUSCLE CONTRACTION CAUSED BY BREAKDOWN OF CREATINE PHOSPHATE**

Lundsgaard (1932) studied the contraction of iodoacetate-poisoned muscle from rats. Muscles which had contracted for a brief period were entirely free of the lactic acid normally formed (Fletcher & Hopkins 1907; Meyerhof 1925). In view of this, Lundsgaard tested for breakdown of \( \text{Cr} \sim \text{P} \) and found that it closely paralleled tension; after exhaustion of the small store of creatine-bound phosphate, the muscle went into rigor.

In the light of these results, the significance of Meyerhof's and Lohmann's finding (1928) of the unusually large heat obtained by hydrolysis of the \( \text{N} \sim \text{P} \) bond in \( \text{Cr} \sim \text{P} \) became obvious. Lundsgaard called the contraction of an
iodoacetate-poisoned muscle an alactacid contraction, after proving that its glycolysis was completely blocked. This work created a great disturbance in Meyerhof's laboratory, shattering the confidence in a direct connection of lactic acid to the mechanical work done. The rationalization of the relation between breakdown of Cr~P and contraction was greatly furthered when Lohmann (1934) connected Cr~P with phosphorylation of ADP by showing in muscle extract that ADP acted as a cofactor since, after dialysis, Cr~P was not split in the absence of ADP to which its ~P was transferred to yield ATP. In other words, phosphoryl transfer was catalysed by the intermediate acceptor ADP. This feature was to become the prototype of a general event in intermediary metabolism, namely, that metabolically generated energy-rich (Lipmann 1941) phosphate bonds in nearly all cases were converted into ATP for metabolic use. I introduced the wiggle sign (~) to distinguish energy-rich or high-energy phosphate bonds with a free energy of hydrolysis of around and above 42 kJ (10 kcal) from the energy-poor bonds in ordinary phosphate esters. It marked the ~P derivatives as energy-carriers, on account of what I began to call their high phosphoryl potential. The metabolic cycling I proposed from inorganic-P to ~P through energy utilization back to inorganic-P, generalizing to other energy-carrying combinations, is illustrated in Fig. 2.

GYCOLYSIS IDENTIFIED AS ATP DONOR TO MUSCLE

To return to the slowly developing identification of the intermediate steps in
glycolysis and fermentation, one now began to realize that glycolytically derived ATP—but not lactic acid—is used to drive the muscle normally, and Meyerhof's results, thus re-interpreted, proved correct: they now implicated the delivery of ATP with the production of mechanical energy. In the meantime, the sluggishness of glycolysis and fermentation of hexose diphosphate in extracts metabolizing glucose rapidly at optimal conditions had raised doubts about the insertion of phosphate in fermentation and glycolysis. This was chemically uncomfortable since so little was known then about organic phosphates. It was seriously proposed that the formation of hexose diphosphate was an unphysiological side reaction. The road to its true appreciation was paved by two observations on the reactivity of fructose diphosphate. Nilsson (1930) found with dry yeast that in the presence of fluoride ions (a fermentation inhibitor) fructose diphosphate reduced acetaldehyde rather easily to ethanol and, as the equivalent oxidation product, he found, to everybody's surprise, phosphoglyceric acid, which appeared for the first time in a fermentation experiment. This acid was well characterized by crystallization as the barium salt.

It is surprising now that nobody—including myself—seemed to connect this with the early realization that at some stage in the course of fermentation and glycolysis the hexose molecule had to be split into two C₃ compounds to yield CH₃·CH(OH)·COOH or C₂H₅·OH + CO₂. After the three-carbon phosphoglyceric acid appeared on the horizon as intermediary, it still took quite a while to associate this oxidation of fructose diphosphate with the oxidation of a triose phosphate.

The second observation that led eventually to the understanding of the oxidoreductive events was made by Lohmann (1930). He found that muscle extract, in the presence of fluoride ions, converted the ester phosphate in fructose diphosphate into a form which was more difficult to hydrolyse with acid. He asked me to help him find out if fluoride was necessary (Lipmann &

| TABLE 1 |
| Conversion of fructose diphosphate into an acid-stable phosphate ester in muscle extract of winter frogs after incubation at 20 °C |

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Phosphate bound (mg P₁)</th>
<th>Phosphate hydrolysed by acid in 3 h (mg P₁)</th>
<th>Converted phosphate (mg P₁) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.48</td>
<td>0.48</td>
<td>0.12 (25)</td>
</tr>
<tr>
<td>20</td>
<td>0.50</td>
<td>0.39</td>
<td>0.27 (33)</td>
</tr>
<tr>
<td>60</td>
<td>0.50</td>
<td>0.25</td>
<td>0.27 (33)</td>
</tr>
<tr>
<td>120</td>
<td>0.49</td>
<td>0.19</td>
<td>0.33 (68)</td>
</tr>
</tbody>
</table>

From Lipmann & Lohmann (1930).
Lohmann 1930). If not, the reaction would be more likely physiological. I found with some muscle extracts that essentially the same conversion took place without fluoride ions. Such a conversion is illustrated by Table 1.

The reactivity of fructose diphosphate in muscle extract prompted Embden et al. (1933) to similarly incubate the diphosphate with muscle preparations, but they then proceeded to analyse more thoroughly for the compounds formed. They discovered that fructose diphosphate was not converted into a hexose ester which was difficult to hydrolyse, as presumed, but, rather, was broken down to C₃ compounds which were difficult to hydrolyse, since they found phosphoglyceric acid and some phosphoglycerol as products. With Nilsson's results in mind, they then concluded that this could only mean a triose phosphate split followed by a dismutation.

Such a conclusion really was indicated, the more clearly so from Nilsson's experiments where fructose diphosphate was oxidized by acetaldehyde to the well characterized phosphoglyceric acid. But the connection was not made. Only Embden saw the light: without actually having the full chemical evidence, he concluded that such a triose split of the diphosphate would yield glyceraldehyde phosphate (Fig. 3) and dihydroxyacetone phosphate, and that these two would interact to yield glycerol phosphate, with the glyceraldehyde phosphate being oxidized to phosphoglyceric acid (Fig. 4). His observation suggested in one sweep many of the intermediary steps that had to be postulated for glycolysis and fermentation. However, at that stage, the explanation reached only the level of an oxidation-reduction (and the dismutation was really not on the main path of glycolysis); it did not extend to the energy transformation being the true implication of the insertion of phosphate. This, however, soon developed.
It had been observed in Embden’s experiments that phosphoglyceric acid was readily split into pyruvate and inorganic phosphate in muscle extracts (Fig. 5). Furthermore, other experiments (Parnas et al. 1934) suggested that phosphoglyceric acid yielded ATP on incubation in muscle preparations. These results began to indicate that in some manner the energy-poor phosphate ester in phosphoglyceric acid was converted into an energy-rich form. Some
of my own observations convinced me of the truth of such an assumption. Addition of phosphoglyceric acid with glucose to yeast autolysate eliminated the induction period (see Fig. 6; Lipmann 1934b). Such an effect, as mentioned earlier, indicated the facilitation of phosphate transfer to glucose. The phosphoglyceric acid eliminated the sluggishness with which the energy-rich phosphate was 'found' to prime glucose by conversion into glucose phosphate. Because phosphoglyceric acid was one of the best inducers that I could find, its conversion into a $\sim$P-containing compound donating phosphate to glucose via ATP and hexokinase was strongly indicated. This was elaborated in the work of Lohmann & Meyerhof (1934), who analysed the chain of reactions leading from 3'-phosphoglyceric acid to a $\sim$-phosphoryl group, transferable to ADP; the first step was conversion of 3' into 2'-phosphoglyceric acid, then by dehydration into energy-rich phosphoanhydride-like phospho$\sim$enolpyruvate (cf. Lipmann 1941) and thence to ATP by transferase to ADP.

$$\text{HO-CH}_2\text{CH(O-PO}_3^{2-})\text{COOH} \xrightarrow{\text{H}_2\text{O}} \text{CH}_2\text{CO}(-\text{PO}_3^{2-})\text{COOH}$$

Again, the metabolically available $\sim$P was fed into the ATP pool.
There still remained the second part of the puzzle: why is inorganic phosphate needed? This was solved by Warburg & Christian (1939): the inorganic phosphate was essential in the oxidation of glyceraldehyde 3'-phosphate where it was fixed as an energy-rich carboxy phosphate anhydride in 3'-phosphoglyceraldehyde phosphate. Thereby, the energy of oxidation was saved in the ~P link to the carboxy group. This ~P was transferred to ADP and thereby another type of ~P was shown to be generated in the course of glycolysis and fermentation.

Thus, by 1935–1936, all the intermediary steps in glycolysis had been established, and it became evident that the phosphate acted in the mechanism of fermentation and glycolysis as the energy carrier in the form of energy-rich phosphoryls developed in the metabolic transformations. As shown in Fig. 7, for each half glucose, one phosphoglycerate ~phosphate and one phospho~enolpyruvate are generated and collected in two ATPs. Therefore, for every mole of glucose, four ATPs are yielded; two are returned into the reaction to form fructose diphosphate and two are the net yield of glycolysis. So, here we have the answer to the question of why glycolysis can drive the muscle machine that depends on the transfer of energy-rich phosphate to myosin (Engelhardt & Ljubimova 1939; Needham et al. 1941; Szent-Györgyi 1941–1942). Obviously, all the energy that can be derived from glycolysis flows into energy-rich phosphate bonds to be delivered into the ATP pool (Fig. 7). In the absence or insufficiency of metabolically formed ~P, as for instance when glycolysis is inhibited, the ~P in Cr~P, by producing ATP, as ~P buffer causes contraction (cf. Eggleton & Eggleton 1927).

USE OF ATP IN BIOSYNTHESIS

Some of my own observations helped to generalize on the function of the phosphoryl in ATP as a donor not only in the production of mechanical energy
but also in biosynthesis. During work on the oxidation of pyruvate with extracts of *Lactobacillus delbrueckii* (Lipmann 1939), I accidentally observed that oxidation of pyruvic acid in this cell-free system required the presence of inorganic phosphate. Just at that time Warburg had discovered that the role of inorganic phosphate in alcoholic fermentation is, so to say, to preserve redox energy by transfer of the activated carboxy group to form an energy-rich phosphate. With oxidation of pyruvate to acetate and CO$_2$, the phosphate again prevents energy dissipation by connecting phosphate to the activated carboxy group of acetate. In this case, the acetyl phosphate seemed to be a candidate for the 'active' acetate postulated by Schoenheimer (1942) from his work with $[^2]H$acetate. [$^2]H$Acetate had been shown to be a precursor of many essential components of the living organism, its deuterium atom appearing in cholesterol, steroids, fatty acids and many amino acids. The production of an activated acetate in the form of acetyl phosphate made one argue that the acetyl phosphate could not only act as a phosphoryl donor to ADP but also as an acetyl donor (Fig. 8). Subsequently, this acetyl was found not to be donated directly but after intermediate transfer to a specific acetyl carrier, coenzyme A. Stadtman & Barker (1950) had discovered in bacteria an enzyme that exchanged inorganic phosphate with acetyl phosphate which they called transacetylase and which was found to be CoA-dependent (Stadtman *et al.* 1951). This made us conclude that the enzyme reversibly transferred the acetyl group from phosphate to CoA. An acetyltransferase activity of mammalian phosphoglyceraldehyde dehydrogenase reversibly connecting acetyl phosphate and CoA was found by Harting & Chance (1953). Its physiological significance, however, was considered problematical.

Thus, a metabolite that was linked in an energy-rich bond to phosphate was shown to be primed for transfer to CoA from which it catalysed condensation to fatty acids and steroids. This showed for the first time that ATP could promote biosynthetic reactions by way of a compound like acetyl phosphate.
A host of biosynthetic pathways were soon discovered in which the mere addition of ATP to extracts in combination with the ingredients for a condensation initiated that condensation, so that the generalization seemed to be warranted that one can consider the phosphoryl of ATP as a general energy donor. This is also to be concluded from the ability of a yeast to grow anaerobically with ATP-producing fermentation as the only source of energy.

It cannot be considered an accident that the sequence of reactions leading, in metabolic energy generation, to the formation of energy-rich phosphate bonds, was discovered in anaerobic fermentations. These could be analysed in homogeneous solutions where the enzymes responsible for the intermediate steps could all be separated and the initial phosphoryl carriers donating phosphate to ATP could be identified.

AEROBIC PHOSPHORYLATION AND PHOTOPHOSPHORYLATION

For a short while, it was argued that perhaps this manner of energy transformation occurred only in anaerobic metabolism, but soon phosphorylations connected with the respiratory chain were found in kidney homogenates by Kalckar (1938) and in muscle by Belitser & Tsybakova (1939). In addition, a photophosphorylation was found in bacterial extracts (Frenkel 1954) and in chloroplasts (Arnon et al. 1954); it became clear, furthermore, that in photosynthesis the synthesis of carbohydrate from CO₂ was coupled with phosphorylation of intermediates. Aerobic phosphorylation occurs in submembranous lamellae of bacteria and in mitochondria (Lehninger 1964), presumably originating from bacterial symbionts (Stanier 1970). Photophosphorylation is observed in photosynthetic bacteria and in blue-green algae in submembranous lamellae, and in plants in chloroplast-organelles presumably originating from symbiont blue-green algae (Stanier 1970).

The reactions will not proceed in homogeneous solutions; they require the intervention of the membranes. After a long and seemingly unsuccessful search for phosphorylated intermediates in aerobic and photophosphorylations, Mitchell (1961) proposed a mechanism quite different from that in anaerobic 'extract' phosphorylation in that protons and hydroxy ions are separated, creating a membrane potential. In this way, the theory of respiration proposed by Lundegardh (1945) (see Fig. 9) was revived. Mitchell presented preliminary evidence that a membrane potential thus set up might be converted into phosphate bond energy by a dehydration between ADP and inorganic phosphate that was catalysed by the ATPase connected with these membrane systems (Fig. 10) (Fernandez-Moran et al. 1964). Although it is taking some time for the Mitchell interpretation to be generally accepted, it appealed to many from
Fig. 9. Charge separation by respiration.

Fig. 10. The membrane-linked, ATPase-containing globules seen in the electron micrograph of mitochondria.
Fig. 11. Conversion of electron flux energy into \( \sim P \). The bracketing of the formation of three \( \sim P \) (36 kcal \( \approx \) 150 kJ) by the transfer of substrate hydrogen to oxygen through several redox levels indicates that it occurs by a uniform mechanism of \( \sim P \) transfer to ADP. This is probably an oversimplification.

the beginning as a welcome way of explaining the absence of phosphorylated intermediates in aerobic phosphorylation. Influenced by the all-or-nothing effect of uncouplers such as dinitrophenol (Loomis & Lipmann 1948) on respiration and phosphorylation, this indicated a common mechanism at different redox levels and for quite a while I have come to indicate this in lectures by a scheme for aerobic phosphorylation represented in Fig. 11. There now appear to be good indications that the steps generally assigned to the three phosphorylation steps in the transfer of electrons do produce ATP instead by a common mechanism.

In the type of ATPase attached to the ends of myosin threads in Fig. 12 (Slayter & Lowey 1967) I like to see some similarity to the ATPase attached by a thread to the mitochondrial membrane that catalyses aerobic phosphorylation and I wonder if a new look at the energy transfer from ATP to myosin might not uncover parallels between it and the mechanism of aerobic phosphorylation.
FIG. 12. Electron micrograph of heavy meromyosin: the two ATPase globules at the end of the double-stranded meromyosin are histologically similar to the ATPase globules on the mitochondrial membrane in Fig. 10. Both ATPases catalyse energy transformation.

EVOLUTIONARY ASPECTS

A generally accepted dogma says that the early Earth had a reducing atmosphere. The early prokaryotic (i.e. bacterial) organisms had to rely, therefore, on an anaerobic energy supply, and many present-day bacteria still do. More recently, when atmospheric oxygen had become available, prokaryotes developed the more economical respiration, and eventually transition to eukaryotes took place (Stanier 1970; Gel'man et al. 1967). One outstanding difference between these two basic lines of organisms lies in the fact that the eukaryotes possess organelles in which the main energy transducing systems are located: the mitochondria for respiration and the chloroplasts for photosynthesis. These mitochondria and chloroplasts appear to have originated from prokaryotes symbiotically associated with a 'host' cell. The prokaryotic origin presumably as symbiont was first strongly suggested by the finding of a DNA in organelles (Ris & Plaut 1962; Nass & Nass 1963; Nass 1969) that was different from nuclear DNA in composition and that was, as in prokaryotes, of a circular nature.
The presumably prokaryotic origin of the mitochondria came to my attention when, in the course of extensive work on prokaryotic and eukaryotic ribosomal protein synthesis (Lipmann 1969a; Lucas-Lenard & Lipmann 1971), we investigated the type of protein synthesis peculiar to mitochondria (Krisko et al. 1969; Richter & Lipmann 1970; Richter 1971) and also chloroplasts (Sy et al. 1974). We found then that the complements to ribosomes, the so-called supernatant factors in mitochondrial protein synthesis, were prokaryotic, whereas the cytoplasmic ribosomes, which are more abundant in the eukaryotic cell, were complemented by a set of truly eukaryotic factors (Richter & Lipmann 1970).

The most interesting aspect of this is our ignorance about the origin of the receiving part, the host for these prokaryotic organelles (Margulis 1971). It is significant that the prokaryotes remained single-celled and seemed to be unable to evolve in the sense we tend to use the word 'evolution'. Rather, they were 'inventive' and created an amazing metabolic diversification. They prepared evolution by the synthesis of nucleotides, amino acids and coenzymes, and, most importantly, they constructed the chain for transfer of genetic information, DNA $\rightarrow$ RNA $\rightarrow$ protein, creating for the last step the universal genetic code. The prokaryotes possess an enormous potential to develop different metabolic pathways, including anaerobic use of all kinds of inorganic compounds such as nitrate and sulphate as hydrogen acceptors and molecular hydrogen as donor, systems which, among many other metabolic features, the eukaryotes have discarded, thereby simplifying their metabolic repertoire.

One of the most drastic metabolic simplifications was eventually adopted by the higher organisms when they abandoned their own manufacture of eight of the twenty amino acids (Fruton & Simmonds 1958) and of nearly all the complex metabolic accessories we call vitamins (Lipmann 1969b). To concentrate on progressive evolution, eukaryotes unburdened themselves by importing these essentials and, furthermore, by adopting a rather standard energy metabolism. This eased the way for the orientation towards, adaptation to, and eventual domination of the environment. Part of the advantage gained by eukaryotes must be sought in their enclosure of energy transduction into organelles. Thereby, the energy-generating system was taken out of the cell proper and appropriately prepared energy carriers were delivered to it for use, just as in daily life we plug into dispensers of appropriately transformed energy.

We can draw a speculative plot of 'evolution' against the age of the Earth, assuming it to be about 5000 million years old (Fig. 13). The first prokaryotes probably appeared about 3500 million years ago; then came the metabolic plateau from which evolution was to take off. It took about 2000 million years