# Bioenergetics

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# Foreword

There is but one safe way to avoid mistakes: to do nothing or, at least, to avoid doing something new. This, however, in itself, may be the greatest mistake of all. The selected, who are able to open new roads to science without erring, are very few and the author, certainly, does not belong to them. The unknown lends an insecure foothold and venturing out into it, one can hope for no more than that the possible failure will be a honorable one.

One of the most characteristic features of present-day biochemistry is the coexistence of highlights with darkness, knowledge with ignorance. While we can perform reactions that amount to a "miracle" and, here and there, even improve on nature, we cannot answer many of the simplest and most fundamental questions. We have, for instance, detailed information about the structure of the protein molecules but cannot tell why nature has put those atoms together in that highly specific way, what was the quality she wanted to achieve by doing so. The same holds true for nucleic acids and nucleoproteins. We know most hormones, and many of them we can build ourselves outside of the living body. In a few cases we can even produce more active agents than nature did. But how hormones act, what they do on the molecular level, we do not know; we have not gone beyond symptomatology in the analysis of their action. The same holds true for most of our drugs.

The same duality exists also in our knowledge relating to the high-energy bonds, the main representative of which is the high-energy phosphate bond P—O—P, "~P." Their discovery belongs, undoubtedly, to the most brilliant achievements of modern biochemistry. We know how, at the expense of one ~P, another

endergonic bond is established. We know how, in fermentation, the bonds in hexose or triosephosphate are shifted around till the P's become ~P's which, transferred on to ADP, can support endergonic syntheses. We have an astounding knowledge about the processes in which our foodstuffs are used to build our body, erect the edifice of life, construct its machinery; but how energy is moving this machine, how work, *w*, is done, be it motion, mechanic, osmotic, or electric work, in a word, how energy is *driving* life, we do not know. Dazzled by our successes we even forget to ask.

This *"chiaroscuro,"*[1] "clear-obscure," is one of the most characteristic traits of current biochemistry. Such a schism between the known and unknown suggests that some basic information is missing. This book represents a guess about its nature.

There is one reason why the inquiry into this duality is urgent and imperative. Corresponding to the big lacunas in our understanding there are equally big lacunas in medical science. Most human suffering, at present, is caused by the so-called "degenerative diseases"—the name standing for "diseases we don't understand and, consequently, can do nothing about." The existence of such a closed group of diseases also points towards some major gap in our basic knowledge. Possibly, all these gaps, may they relate to normal function or to disease, have one common denominator, some process which, hitherto, eluded detection. Some fundamental fact, if not a whole dimension, is missing from our biological thinking.

Shortcuts, in science, mostly turn out to be blind alleys and the only safe approach to fundamental questions is that on the basic level. Cures for disease flow out of progress in understanding as

[1] "Chiaroscuro," in painting and the graphic arts, denotes the mixture of highlights and darkness, as often found, for instance, in Rembrandt's etchings.

the natural fruits of knowledge. This will be the leitmotif of this book which contains an attempt to identify the missing link in our knowledge and open alleys to its approach.

*Woods Hole, Massachusetts*
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ALBERT SZENT-GYÖRGYI

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PART I

# General Considerations

*"There are more things in heaven and earth,*
*Horatio, than are dreamt of in your philosophy."*
(Hamlet)

# 1. The Problem Is Stated

The problem is: how does energy drive life? How does it move the living machine? This is one of the most basic problems of biology and, at present, there is no answer to it. So it is possible that the "oscuro," alluded to in the Introduction, is due to our inability to answer this question.

In order to avoid losing ourselves in generalities, we have to take a specific example. I will take a little experiment I made a few years ago. In this experiment I took a strip of muscle (I chose the *musculus psoas* of the rabbit), put it into diluted glycerol, and kept it in the glycerol for a few days in the refrigerator and for a few weeks in the deep freeze. Then I suspended it in 0.1 *M* KCl at room temperature, added a little Mg, and added ATP in the same concentration as the muscle contained it *in vivo*. The muscle contracted and developed the same tension as it developed maximally in the living animal. If we identify life with motion we could say: the muscle came to life again. In this process the ATP was split, losing its terminal phosphate which was linked to it by a P—O—P. Since we know that this link is a so-called high-energy phosphate bond, ~P, and no other energy donor was present, it is evident that the energy which moved the muscle was the energy of this ~P, and so we can narrow our problem down and ask how did the energy of the ~P move the muscle?

Progress in the chemistry of muscle made it possible to simplify the problem even further. I showed almost two decades ago that contraction in muscle is, essentially, the interaction of actomyosin (a complex formed of two proteins, actin and myosin) with ATP and ions. Of the two proteins, myosin is responsible for the elementary act of contraction and so we can simplify our proposition

by considering myosin instead of muscle, and ask how the energy of ~P moves myosin?

We know from the studies of Edsall and Weber that the myosin molecule is a thin filament. So without knowing any more details about it, we can form two different pictures of the process in which the energy of the ~P is transferred to this filament and produces contraction. The one would be to suppose that the molecule carrying this ~P, in our case ATP, enters into some chemical reaction with the myosin, as the result of which a local change is produced in the protein which leads to its folding. An ATP-myosin complex would have to be formed which then splits up, leaving behind phosphate, ADP, and the altered myosin. Such a reaction finds many analogies in the "group transfer reactions" of the intermediary metabolism and, in principle, could be described with symbols of classical chemistry.

The alternative picture is based on the supposition that the ATP molecule does not enter into any such local reaction, but the bond energy of its ~P's becomes released in a more active and mobile form which then is transferred to the myosin molecule, moves through it, and produces in its wake changes which, somehow, lead to contraction and could adequately be described only in terms of quantum mechanics. Compared to the first, this picture is vague, has no analogies in intermediary metabolism, and one may ask why make such hazy pictures if we can make clear ones with deep roots in existing knowledge?

The inadequacy of the earlier classical pictures was brought out by the advances made in the chemistry of myosin. The more we learn about myosin the less we understand it, which suggests that we are looking at it in the wrong way. Continuing some studies made by Gergely, Perry, and Mihalyi, Andrew Szent-Györgyi showed the myosin molecule to be built of two kinds of subunits, "meromyosins" which, within the molecule, stand in a row in series (Lauffer and Andrew Szent-Györgyi). If Laki and Caroll's value of the molecular weight of myosin is correct, one molecule of

myosin contains three meromyosins, while if Weber's value is correct, it contains six. Assuming the smaller value to be correct, the myosin molecule would look something like Fig. 1. Of the two kinds of meromyosins one is somewhat plumper and sediments faster and has been called H-meromyosin, the H standing for "heavy." In Fig. 1, arbitrarily, it is placed into the middle. The

Fig 1

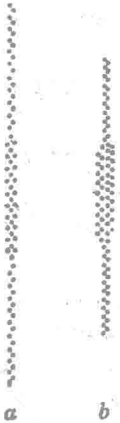


Fig 2

FIG. 1. Schematic representation of a myosin molecule, consisting of one H and two L meromyosins. Sequence of meromyosins arbitrary.

FIG. 2. a: Schematic representation of the myosin molecule of Fig. 1, as consisting of protomyosins. b: Possible rearrangement of protomyosins in contraction.

other two are more slender and have been called L-meromyosins, L standing for "light," these having a lower sedimentation constant. There are two L's for every H.

What makes it difficult to bring this discovery into agreement with earlier concepts is that only the H interacts with ATP, releasing the energy of its ~P's, while there is every reason to believe that the L's are involved in contraction, do the work, and use the energy. The energy would have to get, somehow, from the H's to the L's and it is difficult to see how a bond energy could

do this. There are still possibilities for bringing this structure into line with our earlier concepts. One could suppose, e.g., the ATP to produce some local change on the H which would make the L's fold back on it, producing thus a shortening, or contraction. So there are ways out, though not nice or good ones.

If a theory is good, then any newer knowledge should support it and contribute to clarification, as was the case with intermediary metabolism. With myosin things are going the other way. Andrew Szent-Györgyi and Borbiro showed that the meromyosins also are built of subunits, protomyosins. The protomyosins are of equal size and rather small. Their MW is about 4500 g, which means that one meromyosin is built of a greater number of them, the L of about 20, the H of about 50. These protomyosins are held together by secondary forces only, such as H-bonds, and van der Waal's and electrostatic attractions. If we call a molecule a structure of atoms held together by covalent bonds, then the myosin particle is no molecule at all, only an aggregate. The structure is symbolized in a very crude way in Fig. 2a. It is difficult to see how such a structure could fold; it seems more likely that contraction is not a folding at all, but a rearrangement of protomyosins within the particle, which rearrangement leads to a more rounded, shortened form, as symbolized in Fig. 2b. In order to produce such a rearrangement, many weak forces must be disturbed which keep the protomyosins together. It is impossible to see how a bond energy, enclosed in a ~P, could cause such a disturbance, especially if that ~P is far away, on the H-meromyosin.

We can thus sum up the situation by saying that we do not know how muscle contracts, how it uses bond energy to produce work, and the more we know about its structure the less we understand its function. We might have arrived here at the edge of the chasm which seems to extend through medicine and biology and may be responsible for its "chiaroscuro."

## 2. A Theory of Energy Transmission

It often happens that, unconsciously, our thinking becomes dominated by certain pictures which we have met too often to question their correctness. In my opinion, our difficulty in approaching the problem of energy transformation in muscle is due to our having been misled by the formalism of our thermodynamic bookkeeping. When making up the energy balance sheets of reactions we usually express both the "potential energy" of a bond and the kinetic forms of energy in calories and so, unconsciously, accept their identity. But there is a very great difference between the two, at least in their biological activity, which we can illustrate by comparing it with the difference between sitting on top of an atomic bomb while its potential is a potential, its bonds are bonds, and its energy is locked up inside its atoms, and then trying to remain sitting on it when these bonds are exchanging their potential for more active, kinetic forms of energy. Though mechanics may find both forms of energy essentially identical we will sense a very considerable difference in their biological activity. The situation with the "energy" of the ~P is analogous to that of the A-bomb. While its energy is enclosed in the bonds of the molecule as a potential, it can be expected to have no outward action (except showing a little extra weight which we could find if our balances were more sensitive). This bond energy may be transferred, as such, from molecule to molecule and from bond to bond in the group transfer reactions of our intermediary metabolism. But if this potential has to go into biological action, produce work or motion, an analogy to the A-bomb, it might be exchanged for more active and mobile forms of energy. Such active and mobile forms of energy, on the molecular level, could hardly be anything

else than some form of molecular excitation, be it electronic, vibrational, or rotational. So what we biologists can safely do without getting into an argument with statistical mechanics is to use different symbols for bond energies which are linked to molecules and have no outward action, and excitation energies which are mobile and may interact with their surroundings. The former I will denote by $(E)$, meaning by $E$ energy and symbolizing by the parentheses that this energy is enclosed within a molecule. Excitational energy I will denote $E^*$. So I can formulate our problem by asking whether, in muscle the $(E)$ of the ~P in ATP is not exchanged for $E^*$ when it has to go into biological action and produce contraction? Group transfer reactions of intermediary metabolism could be symbolized by writing:

$(E_n) \rightleftharpoons (E_3) \rightleftharpoons (E_2) \rightleftharpoons (E_1)$ where $(E_n)$ stands for the energy of reserve food as fat and carbohydrate while $(E_1)$ stands for the energy of the substance which is directly fed into the muscle machine, in our case ATP. In this row of reactions the potential energy is transferred from bond to bond, from substance to substance. Bond creating bond, these reactions can be expressed by symbols of classical chemistry. The question is whether our inability to understand muscle is not due to the fact that what happens further belongs to a different group of reactions which can no more be described by these symbols, in which $(E)$ is turned into $E^*$? This duality may hold for all reactions in which work, $w$ is produced, be it mechanical, osmotic, or electric work, etc. While $(E)$ may be the core of reactions in which substances are synthetized and the living machinery is *built*, $E^*$ may be the core of reaction in which this machinery is *driven* and work is produced. This could explain why our notions, derived from intermediary metabolism, did not lead us to a better understanding of muscular contraction.

When supposing a transformation of $(E)$ into $E^*$ we are not lost in the marshes of speculation, for the reaction on which all life is built *is* essentially such a transformation. This reaction is

photosynthesis, in which the solar energy enters into the living world to drive it. In this reaction the radiation is captured by dyes, mostly chlorophyll, in which it produces an electronic excitation. This $E^*$ is then stabilized in the form of $(E)$. Subsequently $(E)$ is shifted from one bond or molecule to another until, eventually, it is stored away in the form of the $(E)$ of carbohydrates or fats. The process of photosynthesis could thus be symbolized by:

$$h\nu \rightarrow E^* \rightarrow (E_1) \rightarrow (E_2) \rightarrow (E_3) \rightarrow (E_n)$$

The reverse process occurs in photoluminescence when, for instance, the firefly emits light:

$$(E_n)^* \rightarrow (E_3) \rightarrow (E_2) \rightarrow (E_1) \rightarrow E^* \rightarrow h\nu$$

Looking at this row of reactions one cannot fail to notice its identity with that of photosynthesis. Only the order is reversed. If we look upon the production of light by the firefly only as upon an example of production of work, $w$, then we arrive at the conclusion that the energetics of the living world consist of only two processes: photosynthesis and its reversal.

In muscle $E_1$, which is directly fed into the contractile mechanism, is the $(E)$ of ATP, and the recent work of Arnon and his associates indicates that ATP plays a very intimate role in the first steps of photosynthesis, while Strehler and Arnold, and Arnold and Davidson have shown photosynthesis to be reversible.

In the above reactions $(E_n) \rightarrow (E_1)$ is what is called "intermediary metabolism." The problem to be dealt with in this book is whether $(E_1) \rightarrow E^* \rightarrow w$ does not represent the reaction which *drives* the living machine and, belonging in the realm of quantum mechanics, can be expressed only in terms of the latter.

Such a question cannot be answered by any single experiment. Only the accumulation of data on various lines can make such a theory acceptable. If correct, this theory should lead us to a better understanding of various biological structures and phenomena, should open new views and suggest new experiments.

## 3. The Mobility of $E^*$ and Organization

No form of energy can be mobile if there is nothing to conduct it. So if we are looking for mobile forms of energy which could take part in biological energy transmissions we have to consider not only the energy itself, but also the mechanisms which have to conduct it. In this chapter I will review instances of mobility of energy and discuss the qualities demanded of the medium, leaving open the question of which of these mechanisms play a role in living systems. That such transmissions do occur was shown by photosynthesis in which many chlorophyll molecules collaborate in the reduction of one $CO_2$ molecule (Arnold and Meek).

### CONJUGATED SYSTEMS, $\pi$ ELECTRONS, AND n,$\pi$ TRANSITIONS

If a molecule contains a system of conjugated double bonds, then it also has $\pi$ electrons—which are no longer bound to any single atom but belong to the conjugated system as a whole, within which they have a more or less free mobility. If such a $\pi$ electron accepts energy and is excited to a higher $\pi^*$ energy level, then its $E^*$ belongs to the whole conjugated system and may produce changes at any of its points. The purine in ATP has such an extensive conjugated system, and so have pyrimidins, isocyclic aromatic compounds, or carotenes with their long chain, built of isoprene units.

Biological catalysts and cofactors often contain N, O, or S atoms in their conjugated system or linked to it. These atoms have their "nonbonded" "lone pair" of electrons which can be excited to the $\pi^*$ levels and thus contribute to the pool of $\pi^*$ electrons. Those so-called n,$\pi$ excitations discovered by McMurry and Mulliken, have specific qualities: their lifetime is considerably longer than