2 November 1956, Volume 124, Number 3227 SCIENCE

Bioenergetics

Albert Szent-Györgyi

The most basic property of the heart is that it is a muscle, and the chief property of muscle is that we do not understand it. The more we know about it, the less we understand and it looks as if we would soon know everything and understand nothing. The situation is similar in most other biological processes and pathological conditions, such as the degenerative diseases. This suggests that some very basic information is missing. The story of myosin may illustrate this point.

Energetics of Myosin

Myosin is the main contractile protein of muscle. It converts the chemical energy of adenosine triphosphate (ATP) into motion. From the work of Edsall, Weber, and their associates, we knew that the myosin molecule was a rodlet (*1*), as is indicated in Fig. 1*a*. We had little doubt that contraction, essentially, was a folding, as is symbolized in Fig. 1*b*. We supposed that the ATP produced this folding by attaching itself at a point to myosin and producing there a change which, sooner or later, could be described in terms of classical chemistry.

The first disturbance in this happy state of affairs was caused by the studies of Gergely, Perry, and Mihalyi, who found that myosin has a complex structure and is built of two kinds of subunits (*2*), which were isolated subsequently by Andrew G. Szent-Györgyi and called *meromyosins*. The one sedimenting faster was called H (heavy) and the other L (light). There were twice as many L's as H's, and they were arranged in series, in a row, as is symbolized in Fig. 1*c*. What was disturbing about this finding was the fact that the L seemed to be involved in contraction, while the H alone interacted with ATP. How could the energy released on the H support work done by the L's? How could a bond-energy locked up in a chemical link produce work somewhere else? It was still possible to make theories to save the situation and suppose, for instance, that ATP produced some local change on the H, whereupon the L folded back on the H, but these theories began to look rather artificial.

The real difficulty arose when A. G. Szent-Györgyi and Borbiro (*3*) made the discovery that the meromyosins themselves are built of a great number of much smaller subunits, *protomyosins*, which are held together only by secondary links, such as H-bonds, van der Waals forces, and electric attractions. The myosin "molecule" was thus no molecule at all, if we call a molecule a structure of atoms held together by covalent links. The myosin particle was but a regular heap of still smaller particles, as is symbolized in Fig. 1*d*.

Contraction and Electronic Excitation

Secondary links have no fixed valency angles, which must give a great pliability to the structure. It became difficult to see how such a structure could "fold." It seemed more probable that contraction consisted of a rearrangement of protomyosins, which went into a more compact heap, as is symbolized in Fig. 1*e*. There must be strong attractive force between protomyosins to enable the myosin particle to withstand strain, and these forces must tend to pull the protomyosins closer together. The force of muscular contraction could thus be due to these attractive forces, in which case we would need force to stretch the particle out again. This theory of contraction is simple and attractive. The difficulty is with ATP, which induces contraction and foots the energy bill. In order to induce a rearrangement of protomyosins, an ATP molecule would have to influence many weak links, but how could a "bond energy," locked up in a molecule, influence many links, especially if that molecule is far away? To bridge this gap, we would have to suppose that the bond energy of the ATP molecules is transformed into some more mobile and active form of energy when it has to go into biological action. Such an energy, on the molecular level, could hardly be anything other than the energy of electronic excitation. Vibrational energies would, probably, be dissipated too easily. Practically all molecules are excitable by light of one wavelength or another, but most of them immediately dissipate their excitation energy and would thus be unfit to partake in biological energy transmissions. Only a relatively small number of specifically built molecules do not dissipate their excitation energy immediately. But, as a rule, no electron can stay in the excited state longer than 10^{-8} second, and, if the molecule is unable to dissipate its excess energy, the electron will drop back to its ground level in 10^{-8} second, shooting out its excess energy in the form of a photon. This means that the molecule is fluorescent. Fluorescence thus becomes an indicator of qualities that may have a major biological importance, indicating that the molecule can accept energy without dissipating it.

It could be objected that such excitation energies can play no role in biological energy transmissions because 10^{-8} second is too short a time to allow their utilization. However, there is always a small but definite chance that the excited electron may revert its spin, which would greatly prolong its lifetime. With its spin reverted, the electron is in the "triplet state," from which it cannot drop back to its original energy level, because the single energy levels of an atom can be occupied by no more than 2 electrons, and only by 2 electrons of opposite spin. The reverted spin of the excited electron having become parallel to that of its earlier partner, its return to its ground level is "forbidden" by quantum rules. So the electron has to remain in its excited triplet state till a thermic collision dissipates its energy.

The physicist who wants to study triplet states has to protect his excited elec-

Dr. Szent-Györgyi is a member of the staff of the Institute for Muscle Research at the Marine Biological Laboratory, Woods Hole, Massachusetts. This article is based on a lecture given at the heart symposium of the 20th international Physiological Congress in Brussels on 31 July 1956. The underlying studies will be published in their entirety by Academic Press in a monograph of the same title.

trons from thermic agitation. This he can do, to some extent, by enclosing the excited molecules in a rigid medium—for instance, by dissolving his substances in melted borax, which he then allows to solidify. He can also use liquid solvents, like glycerol, which he subsequently freezes to a "glass" in Dry Ice, or liquid nitrogen. The more electrons there are in the triplet state, the greater the chances that some of them will drop back to the ground level, sending a message to the observer in the form of an emitted photon. Such light emission from the triplet state is called, after the pioneering studies of G. N. Lewis and his associates (*4*), *phosphorescence* to distinguish it from *fluorescence*. It can be identified as phosphorescence by measuring the time that elapses between excitation and light emission. This can be done in a phosphoroscope, and if the lifetime is found to be of the order of 10^{-3} second or more (instead of 10^{-8} or 10^{-9}), then it is phosphorescence.

Excitations and Water

But here again the physicist may object to biological implications, because a transition into the triplet state has a very small probability of occurring, and a transition that is improbable can have no major biological meaning. I wasted 4 years in futile attempts to break through these difficulties. Lately it occurred to me that the physicists, in their studies on this line, never used water as solvent. They had a good reason to avoid it, for ice at low temperatures cracks up and becomes optically inhomogeneous, making exact measurements impossible. But the biologist is inseparably linked to water, which is the matrix of life, and water has many exceptional properties that may have played a hand also in the generation of life and might also alter the probability of electronic transitions. The most notable quality of water is its strong dipole character with its resulting high dielectric constant. I thus reinvestigated the problem, dissolving fluorescent substances in water, freezing my solutions in Dry Ice, and looking at them under an ultraviolet lamp mounted with a filter that passed only ultraviolet light that could excite the fluorescent matter without interfering with visual observation. All substances behaved in an extraordinary fashion, going, if excited, into the triplet state, opening up a new and colorful world. This may be illustrated by two examples.

If a glycerol solution of the dye rhodamine B is frozen, freezing makes no difference in the behavior. Frozen or unfrozen, the solution shows the same brilliant orange fluorescence. The situation is similar if the solvent is water with 10-percent glycerol. However, if pure water is used as solvent, then, on freezing, all fluorescence disappears. That this disappearance of the light emission is the result of the electrons' going into the triplet state can be shown by cooling the solution to lower temperatures. Below $-40°C$, the system begins to emit a red light, and the lifetime of the underlying excitation exceeds 10^{-3} second. If 3-percent glucose is present, the light emission is very intense, and the lifetime is around 1 second. Hence, if the illumination is suddenly disconnected, the solution shows a strong afterglow.

Riboflavin phosphate, one of the most important pieces of the oxidative machinery of the cell, is known for its brilliant yellow-green fluorescence, which it displays under the ultraviolet lamp if it is present in a watery solution or if it is frozen in 10-percent glycerol. If the molecular oxygen present is eliminated and a watery solution of riboflavin is frozen, its fluorescence disappears. If a trace of oxygen is admitted, the system assumes a red-brown phosphorescence (*5*, *6*). Because there are many more riboflavin than oxygen molecules present, this change cannot be due to a direct interaction between riboflavin and oxygen. What happened, probably, was the following: on freezing, the excited riboflavin molecules went into the triplet state, from which they could emit no light, the transition from the triplet to the ground state being forbidden. This situation was altered by the oxygen, which, as a paramagnetic molecule, perturbed the electromagnetic field; in this perturbed field the transition to the ground state became possible, and thus electrons emitted their excess energy in the form of the red-brown phosphorescence. Oxygen thus profoundly alters the reactivity of riboflavin and it is possible that riboflavin fulfills its role in such an altered state *in vivo*, oxygen not merely being an electron acceptor but also acting by tuning the reactivity of the molecules that cater to the electrons it finally accepts. This may have far-reaching biological implications, and it may also explain the mysterious interrelations between oxidation and fermentation (Pasteur reaction).

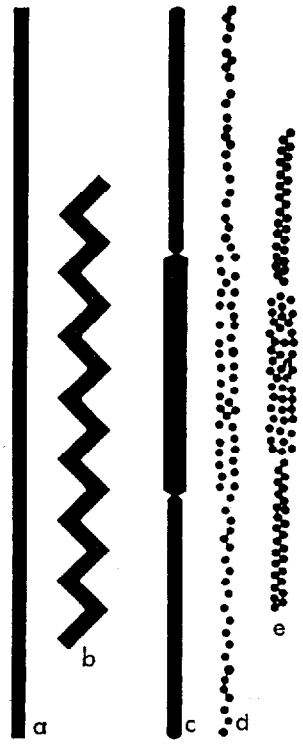

Fig. 1. The myosin molecule.

This simple experiment not only shows that water can stabilize triplet states but also shows that the further reactions of the triplets are accessible to regulatory influences. This can also be shown by freezing a riboflavin solution in the presence of air and $10^{-3}M$ potassium iodide. Potassium iodide completely abolishes the phosphorescence. This may evoke biological associations, for iodine, in the form of thyroxin, is one of the main regulators of the energy household, and Kasha (*7*) has shown that its influence on triplet transitions is independent of its state, being due to its high atomic number—that is, to its heavy nucleus. The great number of its electrons may enable it also to take over energies from other excited molecules. In any case, in our frozen medium collisions of molecules are very limited, and so we can suppose that iodine did not exert its action by a direct collision but *par distance*, being coupled to the excited molecules by the interlying electromagnetic field.

Structured Water

The main question is: Why does freezing introduce such a change? The answer is evident: Ice is not just solid water. It is a crystalline solid with a regular structure. Modern physics places less emphasis on the idea of "liquid" and "solid" than on "random" and "regular." Glass has no crystalline regularity, and thus to the physicist it is a liquid of high viscosity, like frozen glycerol.

The biologist may feel inclined to reject the biological implications of all this, there being no "ice" in the body. But probably there is. It seems even likely that our cells contain but very little or no random water at all, but do contain ice, or, more exactly, water which acquires an ordered structure around surfaces or molecules. Observations on this line were published, for example, by Palmer, Cunliffe, and Hough (*8*), who found that around mica plates water behaved

as "liquid ice" and showed dielectric properties that pointed to an ordered state. Dramatic developments were precipitated by the observation that gas pipes carrying natural gas may freeze up even in the summer. The studies of Buswell and Rodenbush, summed up lately in a fascinating article (*9*), showed that water forms cubic lattices around nonpolar substances. In Sweden, B. Jacobson (*10*) showed that electropolar groups on surfaces may also induce order in the adjacent water. Hence, there is every reason to believe that most of the water in the closely packed protoplasm of cells is in an ordered state that may render triplet excitation probable and stable.

The introduction of the electromagnetic field and water structures into biology as a matrix could not fail to have far-reaching consequences, and so we may do well to look around for evidence. Naturally, such a theory cannot be proved by any single observation and can gain weight but gradually by suggesting useful experiments or making biological reactions appear in a new light, offering an explanation for unexplained phenomena.

Unsolved Problems

Where competitive inhibition or complex formation are excluded, the molecular mechanism of drug actions is obscure. Hormones and drugs produce violent reactions in the body even though they are chemically inert, and the problem is how can a molecule that does nothing chemically produce a reaction? Hence, the question arises whether they do not act by influencing electronic excitations in one way or another. This assumption could be supported by showing that drugs are capable of influencing electronic excitation *in vitro* in the same concentration in which they produce reactions *in vivo*. Very specific drugs may be accumulated by their target, and so we do not know in what concentration they actually act, but in the case of less specific drugs we can calculate with a random distribution and thus compare biological action and *in vitro* activity. For example, 2,4-dinitrophenol produces marked changes in metabolism in man in doses of 100 milligrams. This corresponds to a random concentration of the order of $10^{-5}M$. If this action is due to an action of the excitation of riboflavin, then $10^{-5}M$ dinitrophenol should affect these excitations also *in vitro*. This is actually the case, and it is easy to show that 2,4-dinitrophenol actually quenches the phosphorescence of riboflavin in this concentration. Two more drugs have been analyzed in an analogous fashion with similar results—2,4-dichlorophenoxyacetic acid and chloropromazine. The first is known to produce myotonia in mice in doses of 200 milligrams per kilogram. In this concentration it greatly shortens the lifetimes of various triplet excitations. Chloropromazine has other, very colorful actions on electronic transitions in concentrations in which it affects the basal metabolic rate and induces hypothermia in experimental animals. So have serotonin and lysergic acid, while alcohol upsets triplet excitations in concentrations in which it makes us tipsy.

We can extend such preliminary experiments in various directions. A simple experiment is the following: acridine orange is frozen in water, whereupon it shows a weak red-brown phosphorescence coming from a relatively short-lived excitation. If a small quantity of cortisone is dissolved in the water, the lifetime becomes very long, of the order of seconds. A trace of a narcotic, such as chloroform or ether, cuts down the lifetime again to its original value, inviting speculation about the nature of narcosis. Similarly, the lifetime of the excitation of rhodamine in frozen water is relatively short, of the dimension of 10^{-3} second. Addition of glutathione in physiological concentrations prolongs it 1000-fold, which may give a clue to the role of sulfur in biological systems. One of the oldest mysteries of biology is why, in our bodies, potassium is kept inside our cells and sodium outside of them. The dimensions of the potassium ion are such that it just fits nicely into the water lattice, while sodium (which has a bigger hydrate shell) does not do so and has to cause a disturbance. So, if the regularity of water structures is important for life, then the presence of sodium in greater quantity would be incompatible with it. A watery rhodamine solution, if frozen in Dry Ice in the presence of $0.1M$ KCl, shows practically no light emission under the ultraviolet lamp, while, if it is frozen with $0.1M$ NaCl, it shows a splendid red glow, which indicates disturbed water structures. It is very impressive to see the difference of the two ions demonstrated visually in such a simple and striking manner. Muscle, if stained with acridin orange, shows at room temperature a red phosphorescence showing that within the tissue the dye behaves in the same way as it does in ice. (The dye seeping out of the tissue into the underlying filter paper shows the usual green fluorescence.) These examples may suffice to indicate that it seems likely that triplet excitations play a major role in biology and invite extensive experimentation.

To return to muscle, my point of departure, a theory of its contraction suggests itself. It may be that muscular contraction, essentially, is a rearrangement of protomyosins, consecutive to a destruction of their surrounding water structures, while relaxation is the reestablishment of this hydrate envelope. It is possible that the destruction is done by the energy of ATP, released in the form of a triplet excitation which interacts with the water structures (*11*).

Conclusion

All this suggests that biological phenomena, such as muscular contraction, cannot be described in terms of classical chemistry but belong to the domain of quantum mechanics, to "quantum biology."

The experiments described here indicate that we will have to introduce three new factors into our thinking if we want to understand biological reactions: water structures, the electromagnetic field, and triplets or some other unusual form of excitation made possible by water structures. As Chargaff says in a recent article (*12*), we have forgotten how to say "don't know." The three factors mentioned may limit the number of questions to which we have to give this answer.

References and Notes

1. H. H. Weber and H. Portzehl, *Ergeb. Physiol. biol. Chem. exptl. Pharmakol.* 47, 369 (1952).
2. A. G. Szent-Györgyi, *Advances in Enzymol.* 16, 313 (1955).
3. ——— and M. Borbiro, *Arch. Biochem. and Biophys.* 60, 180 (1956).
4. Th. Förster, *Fluorescenz Organischer Verbindungen* (Vandenhoeck and Ruprecht, Göttingen, 1951).
5. The red-brown light emission of a frozen riboflavin solution had been observed earlier by Dhéré and Castelli (*6*), who interpreted it justly as phosphorescence.
6. Ch. Dhéré and V. Castelli, *Compt. rend.* 206, 2003 (1938).
7. M. Kasha, *J. Chem. Physics* 20, 71 (1952).
8. L. S. Palmer, A. Cunliffe, J. M. Hough, *Nature* 170, 796 (1952).
9. A. M. Buswell and W. H. Rodenbush, *Scientific American* 194, No. 4, 77 (Apr. 1956).
10. B. Jacobson, *J. Am. Chem. Soc.* 77, 2919 (1955).
11. Such a release of energy would take place when the membrane of the fiber is depolarized and brings myosin-ATP together with the other contractile protein, actin. The theory does not necessarily involve electronic excitation and could be upheld even if contraction were only a precipitin reaction in which actin and myosin-ATP mutually discharge each other.
12. E. Chargaff, in *Essays in Biochemistry*, S. Graff, Ed. (Wiley, New York, 1956).