IN SEARCH OF NEW BIOLOGICAL DIMENSIONS

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When I was a young man, passing through Chicago, Carlson invited me to give a seminar. After my talk, the secretary handed me a check for fifty dollars, which was, then, much more than today and seemed to me a very great lot. I refused to accept it, but the secretary said that I could not refuse it, as Carlson would feel very badly about it. On my question as to where the money was coming from, I was told that it was Carlson's private money. "Why does he do this?" I asked. "Because he is a prince," was the secretary's answer. It was then I learned that there are princes who wear their crowns on their heads and princes who wear their crowns in their hearts. Carlson was one of the latter, and ever since it was of great encouragement to know that we all can be princes without having been reared in a golden cradle. Carlson's cradle must have been very simple indeed.

Though we do not know what life is, we can distinguish between life and death, and will not have the least doubt that the cat is alive when it moves, has reflexes, and excretions. What is underlying these age-old signs of life is the transformation of chemical energy into mechanical, electrical, or osmotic work. We do not understand the mechanism of any of these transformations. This ignorance is in sharp contrast to the brilliant successes that biochemistry has achieved in other fields—which suggests that there is something wrong, somewhere.

Present biochemistry is based on the experience that if we pull a living system to pieces we arrive at atoms or molecules. So we have to study atoms and molecules to understand the living organism. No doubt atoms

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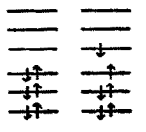
and molecules are the bricks of the building of life, and we have to know about bricks if we want to understand a building. But will the study of bricks ever tell us what a Greek temple was, and will the study of molecules ever tell us what the temple of life is, which is our body? In order to understand the temple, we must attempt to connect the bricks to higher units—walls, columns, and the like—in the hope that eventually we may even approach the sanctuary.

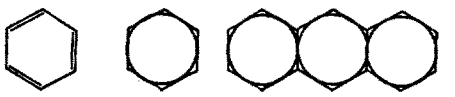
What I propose to do is to throw a fleeting glance on atoms and molecules and then try to connect them. In this first effort we will have to be very modest, limiting ourselves to first neighbors.

Atoms are built of a nucleus and electrons around it. The paths, orbitals, on which these electrons can move have different shapes and correspond



(a) (b)

FIG 1



(a) (b) (c)

FIG. 2

to different "allowed" levels of energy, separated by "forbidden zones." We usually symbolize these energy levels by horizontal lines. The lower ones are occupied, as a rule, by a pair of electrons spinning in opposite directions, while the higher ones are unoccupied (Fig. 1*a*). What interests us biochemists are the outer electrons on the highest occupied levels, which are involved in chemical reactions. Of the empty levels, the lowest one will be of the greatest interest. If an electron on the highest occupied orbital absorbs a photon, then it is "excited" by the absorbed energy, i.e., raised to a higher level (Fig. 1*b*).

Most molecules are formed by atoms sharing a pair of the outer electrons. We can describe the structure of these molecules with fair accuracy, by the current symbols of chemistry, by various letters (standing for the various sorts of atoms), while their link we symbolize by dashes. Atoms

can also be connected by sharing two pairs of electrons, in which case we write two dashes and call it a "double bond." If, in a system, every second bond is a double bond, as in –C=C–C=C–C–, then we call the double bonds "conjugated." In this case, the second pair of electrons does not know whether it belongs to its right or left neighbor. It belongs to both. In fact, these "π" electrons are "delocalized" and belong to the whole system. Kékulé, who could not then know about π electrons, wrote his benzene formula as in Figure 2*a*. Today we would rather write it as in Figure 2*b*. Anthracene could be written as in Figure 2*c*. The π electrons, not belonging to any C atom, but to the whole molecule, we call their orbitals "molecular orbitals." These molecular orbitals interest us especially, partly because many of the most important biological catalysts contain such extensive systems of π electrons, partly because the energy levels of these molecular orbitals can be relatively easily calculated.

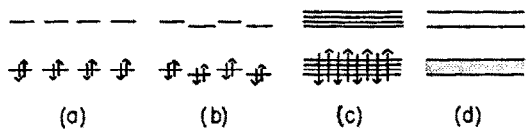


FIG. 3

It was the "solid-state physics" which broke first through this strict individuality of atoms or molecules. It has shown that if atoms, or molecules, are closely packed with a high regularity (Fig. 3*a*), their electrons perturb each other's levels (Fig. 3*b*), which then fuse to one single band containing many levels (Fig. 3*c*). Such an energy band (Brillouin zone) can also be looked upon as a continuous layer of electron gas, extending over the whole system (Fig. 3*d*). The system symbolized in Figure 3*d* will not conduct electricity since the upper band is empty and the lower band is full, every atom having contributed two electrons, the maximum number allowed by quantum mechanics. In such a saturated band, electrons cannot move, as people cannot move in a room completely filled. The system could be made conductant either by placing electrons on the empty band, where these electrons would have a free mobility, or could be made conductant by taking electrons off from the filled band, creating "holes" in it.

Another "breakthrough" came about by the study of molecular complexes. In the thirties we find data here and there suggesting that in certain

complexes an electron may pass from one molecule to the other. It was J. Weiss, in 1942, who gave a definite formulation to this "charge-transfer." In such a transfer an electron passes, as a rule, from the highest occupied orbital of one of the two molecules to the lowest empty orbital of its partner, the former being thus the "donor," *D*, the latter the "acceptor," *A*, as symbolized in Figures 4*a* and 4*b*. Under favorable conditions, *D* and *A* can part, *D* having lost, *A* having gained an electron. Both of them will then have an unpaired electron, will thus be what is called a "free radical," which can be expected to give a signal in the ESR, the electron spin resonance experiment. We could write this sequence of reactions as:

$$D + A \rightleftharpoons D^+A^- \rightleftharpoons D^+ + A^- . \qquad (1)$$

Oxido-reduction is often defined as an electron transfer. So what, then, is the difference between "charge-transfer" and "oxido-reduction"? The difference is an important one. It is this: in most organic molecules the single orbitals are occupied by pairs of electrons forming a "closed shell." In an oxido-reduction, as a rule, a pair of electrons is transferred from one molecule to another, with the consecutive rearrangement of orbitals,[1] leaving behind, thus, two closed-shell molecules. Such a transfer of two electrons involves a major rearrangement of molecular structure. In charge-transfer, *one* electron only is transferred, with the formation of two radicals, which may remain coupled or may separate. This transfer does not necessarily involve a major rearrangement of structure.

Does this charge-transfer open any new possibilities for our thinking about biological processes? It does. Let us imagine, for instance, that an electron would be transferred onto the acceptor from a filled band, as represented in Figure 3*d*. Then, a "hole" would be created and the band would become conductant. Similarly, we could transfer an electron by charge-transfer onto the empty band, where the electron would have a free mobility and could thus transport energy. We could also imagine that the electron, after having been transferred from *D* to the empty level of *A* (Fig. 4*b*), would be transferred from here onto the empty level of a third and fourth molecule, losing energy in every step. This would be a sort of "chemistry without chemistry," and it is this sort of thing which seems to happen in mitochondrial "oxidative phosphorylation" or photo-

[1] Michaelis has shown that in many oxydo-reductions the two electrons can pass from one molecule to the other, one by one, going thus through a charge-transfer stage.

synthesis. Lastly, let us suppose that we transferred an electron to the empty excited level of molecule *A* (Fig. 4*b*), then pulled out, by charge-transfer, one of the two electrons on the highest filled level. Then the electron could drop down from the excited level into the hole thus created, emitting its excess energy in the form of a photon. Something like this may underlie bioluminescence.

I would like to convince you, at this point, that I have not taken you astray into some abstruse field of quantum mechanics but have been talking to you on realities. I will make a few charge-transfer complexes. You will be able to see the transfer of the electron with your own eyes, most charge-transfer complexes being strongly colored. When wanting to make a charge-transfer complex of two arbitrary substances, I am faced with a difficulty. To enable the electron to go from *D* to *A*, I must bring the two molecules into very close proximity, and the attractive forces between

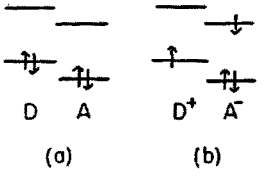


FIG. 4

them may not be strong enough to bring them together. So my first question is, how can I force two molecules into close proximity? There are various tricks I can use. I will show you three. Let us take, first, two water-soluble substances. I will choose two biologically important ones, riboflavin as acceptor and serotonin as donor, dissolved in water. As you see, the acid watery solution of their mixture shows the brilliant yellow color of riboflavin (serotonin being colorless). Now, to push the two together I need only to freeze the solution. Then the water will crystallize out, forcing the two solutes into close proximity. As you see, on freezing, the golden yellow fluid has turned black, like India ink, the free radical of riboflavin being black. On melting, the electron will return to serotonin, and the golden yellow color will return. Now let us take two substances that are soluble in chloroform, like dimethylbenzanthracene and trinitrobenzene. I will simply pour their faintly pink mixture on filter paper and let the chloroform evaporate, leaving the dissolved molecules in close

proximity. As you see, a dark purple color appears. I could also dissolve the two substances in a solvent, like tetrahydrofurane, which mixes with water. So if I add water now, I dilute the solvent, making the solutes insoluble. They have to precipitate together, forming a deeply colored charge-transfer complex.

I hope that these experiments have convinced you that I did not take you far away from reality. Now, to convince you that I did not take you away from biology either, I would like to discuss, briefly, two processes: mitochondrial oxidation and photosynthesis. As you know, mitochondria oxidize our foodstuffs and produce ATP by means of their energy released. "Oxidation" of the foodstuffs means that their electrons become transferred onto O_2. This transfer occurs over a series of intermediary substances, and so it is customary to talk about the "electron flow" in mitochondria. Our question here is: is all this "oxido-reduction," that is, the pairwise transfer of electrons, or is it "charge-transfer," in which the single electrons go it alone?

There are various pathways in mitochondria. Let us take the most representative one, in which the H of the foodstuffs is transferred onto DPN.[2] The reduced DPN then reduces FMN (flavin mononucleotide), FMN reduces a cytochrome, which reduces a second, the second a third, till eventually the electrons are scavenged by O_2, which then binds protons, H^+'s, and is turned into water.

Under specific conditions FMN can be made to take up one electron, being reduced to the FMN free radical which gives a signal in the ESR[3] (Fig. 5, *top*) as shown by B. Commoner. If you observe the hyperfine structure, you find its two sides completely symmetrical, being each other's mirror images. As a next step, we mixed FMN and serotonin, making the same mixture you have seen turn black. The signal was then asymmetrical (Fig. 5, *bottom*). The reason is simple: the fluid then contained two different free radicals, of FMN and serotonin, the former substance having gained and the latter having lost one electron. Then we mixed DPNH and FMN, expecting again an asymmetrical signal. The signal was completely symmetrical, identical with that of the FMN radical. There was no DPN radical. Most probably the DPN radicals "dismutated" to DPN

[2] The oxidized form of DPN is DPN^+, its reduced form DPNH. For convenience I will write the oxidized form as DPN and the fully reduced form $DPNH_2$.

[3] The curves shown are actually the second derivatives of the signal.

and $DPNH_2$, one radical giving an electron to another, both ending, thus, with paired electrons.

The ESR signal given by FMN (obtained by Dr. Isenberg) can convince you that a charge transfer has taken place; one electron was transferred without DPN forming a radical. This molecule seems to be built so that it should be able to give off one electron without forming a free radical itself. If it would form a free radical, then the positive charge of this radical would attract the electron given to FMN and would not let it go on its way toward oxygen. From FMN the electron goes through a series of cytochromes, which are capable of a one-electron change only, so the whole series seems to be a one-electron business.

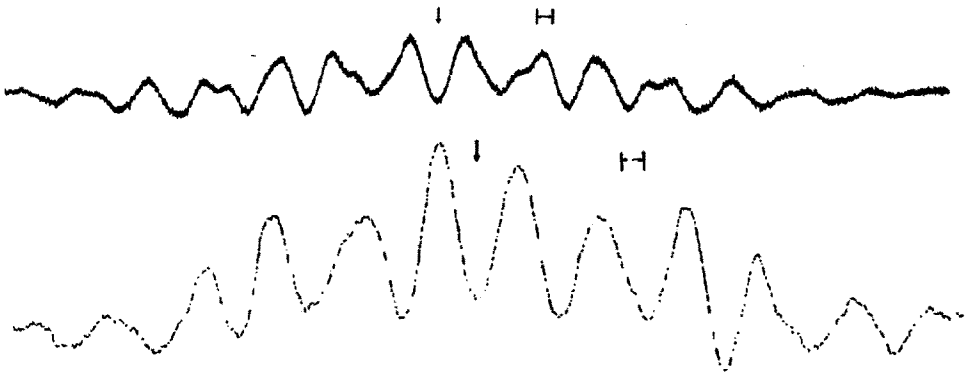


FIG. 5

In photosynthesis something similar happens, the difference being that the electron is raised to the excited level by the energy of the photon absorbed. The electron then cascades down to its ground level, producing ATP and $DPNH_2$ on its way by its energy released. These two substances can thus be looked upon as stabilization forms of the very unstable excitation energy. These substances cannot be stored in quantity; thus, a further stabilization occurs, as is shown by D. Arnon, and the energy of ATP and $DPNH_2$ is converted into the energy of "foodstuffs"—carbohydrates and fats. We feed on these carbohydrates, then reconvert their energy into $DPNH_2$ and ATP. This takes me to the central theory which is dominating my research at present. I strongly believe that when ATP and $DPNH_2$ drive the living machine, their energy is reconverted into electronic energy; that is, they liberate electrons of a high energy, and it, the energy of these electrons which is driving us, is driving the whole living world.

We have been unable to understand the simple signs of life because we have overlooked this electronic dimension.

If it is this energy of electrons, released in charge transfer, which is driving the living machine, then it becomes desirable to approach these changes in a more quantitative, numeric fashion. This can be done by imagining that we transfer the electron from *D* to *A* in two distinct steps. In the first, we take the electron off from *D* altogether, into infinity, then drop it from infinity onto *A*. The first step consumes, the second liberates energy. The energy consumed in the first step I have symbolized in Figure 6 by the upward arrow, while the energy liberated in the second step is symbolized by the downward arrow. The energy gain, or loss, in the total process is represented by the difference in length of the two arrows. Both arrows correspond to well-defined physical concepts, the upward arrow to what is called "ionization potential," *IP*, the downward arrow to what

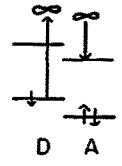


FIG. 6

is called "electron affinity," *EA*. Both can be expressed in electron volts or calories. If we cannot express the energy change simply by *IP–EA*, this is because dropping the electron onto *A* involves various secondary reactions which make *EA* less definite. So, we can build, only, on the *IP*, which is well defined. We can dodge this difficulty by always using the same acceptor while working with different donors. Then *EA* will remain constant and the only variable will be the *IP*. We biologists can apply this trick because the final electron acceptor in biology *is* always the same substance, O_2. It follows, then, that we can express the energy of an electron unequivocally by its *IP*, which can be measured. It can also be calculated, and such calculations have been done for a host of biological substances by the Pullmans.[4]

According to the theory of Mulliken, if *D* and *A* get together, part of the electron goes over spontaneously while part of it may need the energy of a photon for its transfer. The electron will thus absorb a photon. Ab-

[4] The final result, in this case, was not expressed in terms of *IP*, but in terms of *K* or *P*, which are linear functions of the *IP*.

sorption of photons in the visible range means color, so when you observe these charge-transfer colors, you actually observe the energy of the electron transfer. The energy of light, as well as its color, depends on its frequency, so there has to be some direct relation between the *IP* (or *P*) of the donor—that is, the energy needed for the transfer—and the color of the complex. We can, in fact, directly read the energy change from the color. Let me demonstrate. I have dried on this paper from right to left, a solution of indole, chrysene, benzanthracene, 9,10 dimethylbenzanthracene, and phenothiazin. The *IP* of these substances decreases in the given order. Now, if I pour over this a solution of trinitrobenzene, which acts as an acceptor, you see strong colors appearing. Indole gives a yellow color, indicating that blue light was absorbed, the high energy of this light, about 80 calories, being needed for the transfer. Chrysene is somewhat orange, benzanthracene is deep orange—light of longer and longer wavelength—that is, less and less energy being needed for the transfer. The red color of the complex of the dimethylbenzanthracene indicates a very low ionization potential, while the blue color of the phenothiazin complex indicates that even less energy, about 50 calories, was enough to induce transfer.

Trinitrobenzene, which I used as acceptor, is a rather bulky molecule which acts with its π orbitals as an acceptor. We might ask, what would happen if we used a more compact little acceptor, such as the iodine molecule, I_2? As you see, the result is different. Indole colors dark, chrysene and benzanthracene give no reaction, while dimethylbenzanthracene also gives a black color. Why, then, did indole give a strong reaction with I_2 when it gave a poor reaction with trinitrobenzene? This takes us to a fascinating field. In a highly conjugated molecule, as is that of indole, the electrons belong to the whole system but are not equally distributed. The C atom in position 3 is exceedingly rich in electrons; it has a high electron density. If we take away this high electron density by converting indole into carbazole, the substance does not react with I_2 any more, though carbazole has a lower *IP* than indole. Evidently, indole reacted with I_2 at this C atom in a "local" charge-transfer, and it was not solely the *IP* which decided the issue, but also the "formal" charge on C_3. This is most fascinating because it brings into the picture the individuality of the single atoms within the molecule, and the whole reactivity begins to assume that subtlety which we find in biological systems.

This reaction has fascinated me also for another reason. The Pullmans

have shown that aromatic hydrocarbons, which induce cancer, have two C atoms in their "K-region," which C atoms have a very high charge density. So, if our reasoning is correct, these substances should also give a black complex with I_2, and if this has something to do with cancer, then other, closely related, substances, which have no carcinogenic activity, should not give this reaction. This expectation was borne out before when I showed you that the non-carcinogenic benzanthracene gave no black complex while its strongly carcinogenic dimethyl derivative did. The expectation was borne out by a number of other examples. So, for instance, the non-carcinogenic 1,2 benzpyrene gave no reaction, the carcinogenic 3,4 benzpyrene a very strong one. Further experiments showed that carcinogens belonging to quite different groups, like diazocompounds, behaved likewise, the carcinogens reacting strongly, the non-carcinogens not reacting, or reacting poorly. This suggests that production of cancer is, in some way, connected with a "local" charge-transfer, which may someday bring us closer to the understanding of this disease.

With Isenberg and Karreman, we also attempted to find out whether some pharmacological reactions might be connected with charge transfer. We reasoned as follows. Let us take a drug with a unique action. If this action has something to do with charge-transfer, the substance should also have unique electronic qualities as donor or acceptor. We chose chlorpromazine, which has such a favorable influence on the symptoms of schizophrenia. We found it to have unique electronic qualities: it is the substance with the lowest *IP* known among stable compounds. Serotonin we found to be an especially good donor. This drug, too, has a unique activity.

So I hope to leave you with the impression that a new dimension is opening up for biochemistry—that of the electrons, a dimension in which a molecule assumes a new importance as a quantum mechanical framework. Every single atom with its electronic profile acquires a new and subtle personality. Ideas on which we built, till the present, such as the mass law action, lose much of their importance, while the whole system, with its great subtlety, begins, if I may say so, to smell of life. I also hope to have convinced you that the distance between those abstruse quantum mechanical calculations and the patient's bed is very small, and that further work on these lines may not only deepen our understanding but may also help to complete the armory of medicine with which we try to eliminate human suffering.