

BIOELECTRONICS

A STUDY IN CELLULAR REGULATIONS, DEFENSE, AND CANCER

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DEDICATED TO THE MEMORY OF MY BELOVED TEACHER

SIR FREDRICK GOWLAND HOPKINS

PREFACE

The greatest stride in biology, in our century, was its shift to the molecular dimension. The next will be its shift toward the submolecular, electronic dimension. A bold beginning in this direction was made by the Pullmans, as witnessed by the classic "Quantum Biochemistry" (1963). Wave mechanics opens the way not only to the submolecular but also to the supermolecular, allowing the linkage of single macromolecules to higher meaningful structures.

In my two earlier booklets, "Bioenergetics" and "Introduction to a Submolecular Biology," I advocated the extension of biology into the sub- and supramolecular dimension. The present writing completes my trilogy. It is a report on my latest attempts to understand biological phenomena. This experience I owe to grant GM10383 from the National Institutes of Health, Bethesda, Maryland, and the generous hospitality of the Marine Biological Laboratory, Woods Hole, Massachusetts, which has been my scientific home for more than twenty years.

My deeply felt gratitude is due also to my associates Miss Jane A. McLaughlin and Dr. Laszlo G. Együd, who, for many years, shared my work with all its joys and disappointments.

I. INTRODUCTION

THE PROBLEM IS STATED

If you would ask a chemist to find out for you what a dynamo is, the first thing he would do is to dissolve it in hydrochloric acid.* A molecular biochemist would, probably, take the dynamo to pieces, describing carefully the helices of wire. Should you timidly suggest to him that what is driving the machine may be, perhaps, an invisible fluid, electricity, flowing through it, he will scold you as a "vitalist."

No doubt, molecular biochemistry has harvested the greatest successes and has given a solid foundation to biology. However, there are indications that it has overlooked major problems, if not a whole dimension, for some of the most exciting questions remained unanswered, if not unasked. It failed to explain the wonderful subtlety of cellular regulations. Neither did it explain the mechanism of energy transduction, the transduction of chemical energy into mechanical, electric, or osmotic work. These transformations are closely connected to the very nature of life. I do not know what life is, but I can tell life from death, and know when my dog is dead: when he moves no more, has no reflexes, and leaves my carpet dry, that is, performs no more energy transductions. These failures, hiding in the shadow of success, warrant a fresh approach.

The cell is a machine driven by energy. It can thus be approached by studying matter, or by studying energy. The study of matter, or structure, leads to molecular biochemistry and what D. D. Eley calls the steric factor approach which

* This quip is not original but I was unable to trace its origin. It may have come from a Victorian physicist, possibly Tyndall.

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dominates present biochemistry. I will approach from the energy side. Needless to say, a final understanding can be achieved only by the synthesis of the two lines which are but the two sides of the same coin.

Molecular biochemistry left no room either for one of the most fundamental rules of life, that of nonadditivity. One particle, plus one particle, put together at random, are two particles, $1+1=2$; the system is additive. But if two particles are put together in a meaningful way then something new is born which is more than their sum: $1+1 > 2$. This is the most basic equation of biology. It can also be called *organization*. This equation holds true for the whole gamut of complexity. If an electron and a nucleus are put together in a meaningful way, a hydrogen atom is born which is more than an electron and a nucleus. If atoms are built into a molecule, again something new is born which can no longer be described in terms of atoms. The same holds true when small molecules are built into macromolecules, macromolecules into organelles, organelles into cells, cells into organs, organs into an individual, and individuals into a society, etc. If living nature has qualities which are very different from those of the inanimate this is not because it is subject to different laws, but because life drives this “putting together” much farther than the inanimate world does.

This book is devoted to the question of whether or not there is a closer analogy between the dynamo and the living system. This latter, too, may be permeated by an “invisible fluid,” the particles of which, the electrons, are more mobile than molecules and carry energy, charge, and information, and act as the fuel of life. These electrons may help to connect molecules to meaningful structures and may also be responsible for the charming subtlety of biological reactions.

THE IONIZATION POTENTIAL AND ELECTRON AFFINITY

Approaching biology from the energy side the first question is: What is energy? What does it mean that an aggregate of atoms or molecules, like the cell, has energy? To simplify this problem let us consider one atom only, the simplest of all, hydrogen. What does it mean that hydrogen has energy?

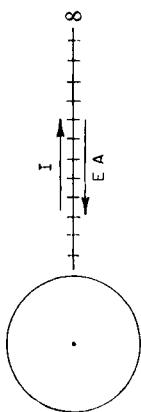


FIG. 1. A hydrogen atom.
For explanation see text.

In Fig. 1 is represented an H atom. The circle stands for the electron of H moving rapidly around the nucleus, the dot in the middle, the proton. The nucleus, as such, has no energy, nor has the electron. So, if H has energy, it can reside only in the relation of the two. The electron would, evidently, fly off if it were not held by forces pulling it

toward the nucleus. We can measure these forces by measuring the energy which is needed to tear off the electron from the atom, and take it to infinity, that is, to a distance at which it interacts with the nucleus no longer. This tearing off is symbolized in Fig. 1 by the straight line leading from the atom to infinity; the energy needed for doing this against the attraction of the nucleus is what is called the *ionization potential* (I), which is usually measured in electron volts (eV).

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The ionization potential of H is about 13 eV. This means that I would have to invest 13 eV worth of energy to tear the electron off. I could get my invested energy back by dropping the electron from infinity to its now empty orbital. This amount of energy released by the dropping electron is the *electron affinity*.

If, somehow, I would cause the electron to be attracted by the nucleus only half as strongly, then the ionization potential would be only 6.5 eV. I would need, now, only 6.5 eV to take the electron off, but would get only 6.5 eV by dropping it back. The electron behaves now as if I had taken it halfway toward infinity, between notches 6 and 7 in Fig. 1.

If, in this new situation, the electron comes off earlier, this also means that it will interact easier with other atoms or molecules. The ionization potential is thus a measure of both the electron's energy and reactivity. The *lower* the ionization potential, the *greater* the electron's energy and reactivity, while the greater electron affinity indicates a greater willingness of the orbital to accommodate the electron.

One of the main reactions discussed in this book will be the transfer of an electron from the occupied orbital of one molecule or atom to an empty orbital of another, the so-called *charge transfer*. The particle giving the electron is called the *donor* (D) while the one receiving the electron is called the *acceptor* (A). The transfer could also be called a *DA interaction*.

In theory, we could find out how much energy we use or release in transferring an electron from D to A, by transferring the electron in two steps, first by taking it away from D, removing it to infinity, then dropping it from infinity onto the empty orbital of A, as symbolized in Fig. 2. In this figure the thick lines stand for occupied orbitals, the thin lines for empty ones. The electron in such interactions is usually

transferred from the highest filled orbital of the donor to the lowest empty orbital of the acceptor. In this procedure we would have to invest in the first step an amount of energy which corresponds to the ionization potential of D. In the second step we would gain energy which is equal to the electron affinity of A. The energy gained or used in this whole process would be equal to the difference in the length

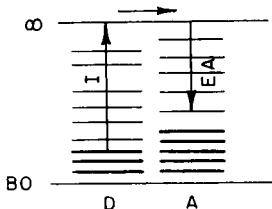


FIG. 2. Transfer of an electron from donor (D) to acceptor (A).

of the two arrows, that is, the difference between the ionization potential of the donor and the electron affinity of the acceptor,

$$E = I - EA + \Delta \quad (1)$$

where Δ stands for the correction one has to take for the perturbations caused in A by the dropping electron. If I is greater than EA then we would have to invest energy to help the electron over. If EA is greater than I then we would gain energy in the process and could expect the electron to move over spontaneously.

THE BIOPOTENTIALS

The ionization potential is one of the most useful and simple of concepts. All the same, it is somewhat confusing

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and disturbing for the biochemist. What is confusing is that the energy and reactivity of an electron, its potential to react, is inversely proportional to its ionization potential. The *smaller* the ionization potential, the *greater* the energy and

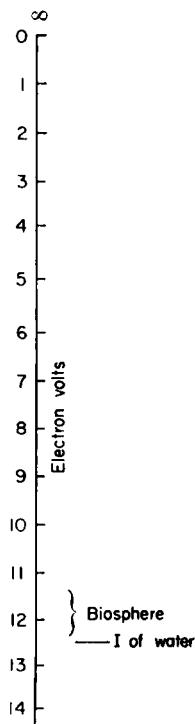


FIG. 3. Ionization potentials.

reactivity, and *vice versa*. This is confusing. Moreover, our reference point is infinity, and infinity is far away. Life is, energetically, a very poor and modest phenomenon. Its actual energy changes are very small, below 1.5 eV. All the

energy we have is the energy of the photons absorbed by the plants. Measuring these small energy changes, with infinity as reference point, is like finding out the distance from my corner to the next block by measuring their respective distances to the moon. The question is: Can we find no closer reference point, a reference point which, at the same time, would allow us to straighten out the disturbing inverse relationship between potential and reactivity?

The lack of proportion in the scale of ionization potentials is borne out by Fig. 3. Zero ionization potential (infinity) is at the top of the scale. As will be seen biological reactions are crowded into a very narrow range, far from infinity, the reference point.

The electrons, in biological systems, reach the minimum of their energy by being *oxidized*, that is, being coupled to oxygen, forming water. We excrete these electrons, deprived of their energy, in the form of water. From the energy point of view water is thus the bottom of nature. It has the lowest energy and highest ionization potential among all biological substances, 12.56 eV. But if water is the bottom of nature, why not take it also as the bottom of our energy scale, call it zero and count from there? Then all values will be positive, and will be in direct relation to energy content and reactivity. Water is, in any case, the most central substance of living nature. It is the cradle of life, the mother of life, and its medium. It is our *mater* and *matrix*.

To be able to crowd the biological data onto the electron volt scale, we must stretch out the latter. This has been done in Fig. 4 where the whole scale corresponds roughly to the narrow region marked in Fig. 3. The ionization potential of water is taken here as zero. This scale will be called the scale of biopotentials *B*.

The advantage of this scale is that all values are positive,

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the energy and reactivity of the single substances increase with their potential, and the single potentials have biological meaning of their own, giving the energy which can be gained by oxidizing the substance in question to water. The energy values can be expressed also in calories, 1 eV being equal to 23 calories.

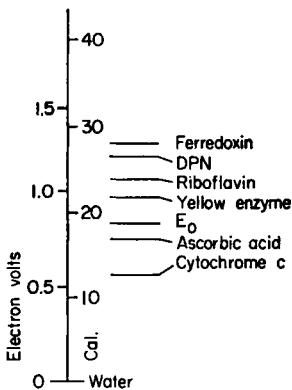


FIG. 4. Biopotentials.

Redox potentials, as measured against the H_2 electrode, can also be plotted on this scale. If we shunt an O_2 and a H_2 electrode against one another water will be formed. So the potential of the O_2 electrode can be used for zero. Since the difference between O_2 and H_2 electrode is 820 mV, the potential of the H_2 electrode on the *B* scale will come to lie at 820 mV (0.82 eV). If ferredoxin were 430 mV more negative than the H_2 electrode, then its potential would lie on the *B* scale at $820 + 430 = 1250$ mV (1.25 eV).

The energy of orbitals is identical with the energy of electrons occupying them, and so can be plotted as the latter's ionization potential. If the ionization potential of

water were 12.56 eV, and an electron has an ionization potential, say of 10, then the biopotential of this electron (or its orbital) would be 2.56 eV (cf. Fig. 3). What is usually meant by the ionization potential of a given substance is the ionization potential of its electron which has the lowest potential.

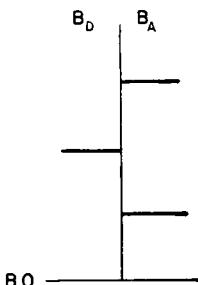


FIG. 5. Biopotentials of donating and accepting orbitals.

An empty orbital is just as much a physical reality as an occupied one. Its energy is equal to the energy of the electron which would occupy it. If the ionization potential of this electron would be say 10 eV, then the electron affinity of this orbital would be 10 eV, and this much energy would be gained by dropping an electron from infinity onto this orbital.

The *B* scale makes it easy to find the energy gained or lost in the transfer of an electron from donor D to an acceptor A. The biopotential of a hypothetical donor is plotted on the left side of Fig. 5, while the *B* of two hypothetical acceptors is plotted on the right side. The energy change will be simply equal to the distance of the D and A levels, and a corresponding amount of energy will be gained or lost by transferring the electron to the lower or the higher one of the two empty orbitals.

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ELECTRONIC ENERGY AND BOND ENERGY

The electrons, driving life, are brought to their high biopotential in plants by the absorption of photons. From this high energy level they drop back to their lowest water level stepwise, like a ball rolling down a staircase. The energy given up in the single steps is driving life.

Since there is no light at night, and the cells need energy all the time, and since electronic energy cannot be stored, the energy given up by the electrons in their single steps is converted into bond energy—the *high-energy* phosphate bonds of ATP, symbolized by $\sim P$. This conversion of energy occurs in *oxidative phosphorylation*. The gradual “rolling down” of the electrons is what is called the *electron flow*.

Owing to its osmotic pressure ATP cannot be stored in quantity, therefore, its energy is converted into a form able to be stored in quantity; it is used to build foodstuffs—fats and carbohydrates. These foodstuffs, being insoluble, can be accumulated. If the energy of these foodstuffs has to be utilized it is reconverted into ATP. This reconversion, oxidative phosphorylation, is essentially the repetition of photosynthetic phosphorylation. In this process the electrons of the foodstuffs are taken off one by one. They still contain the energy which has been invested into them in photosynthesis. They once more “roll down the staircase” in an *electron flow*, their energy being given up in the single steps being invested again in the building up of $\sim P$.

This distinction between *bond energy* and *electronic energy* demands an explanation. What is meant here by electronic energy is the high biopotential of a single electron, while the bond energy is the collective energy of the elec-

trons forming that bond. So if we symbolize the energy of a single electron by a line, as is done on the right side of Fig. 6, then we can symbolize the bond energy by a bundle of lines. There is a great difference between the two. Owing to its high biopotential, the single electron can go to other atoms or molecules, to other orbitals of lower energy, which happens in the electron flow; thus the electron has a certain

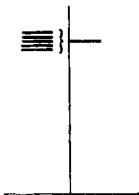


FIG. 6. Biopotentials of bond energy (left) and electronic energy (right).

mobility. With its negative charge it will generate an electric field around itself. The \sim creates no field about itself and is immobile, being linked to molecules. This difference in the qualities of electronic energy and bond energy warrants the use of special symbols. If we denote a high energy bond by \sim , then we can denote the electronic energy by $\{$. Oxidative and photosynthetic phosphorylation could then be written:

$$\{ \rightarrow \sim \quad (2)$$

As has been pointed out, the most characteristic features of life are the energy transformations in which the chemical energy is transduced into mechanic, electric, or osmotic work, as in the work of muscle, nerve, and secretions. The energy for these functions is supplied by ATP. This brings us to one of the most major problems of biology: How is the \sim transduced into the various forms of work? This is not

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only one of the most major, but also one of the most obscure problems. It may have remained unsolved because a possibility has been overlooked, the reversion of $\sim \rightarrow \sim$. It seems likely to me that when the energy of $\sim P$ has to be utilized it is retransformed into \sim ,



the reaction being reversible:



Summing up, we could distinguish between three types of energy transformations: (1) transformation of electronic energy to bond energy, (2) *group transfer reactions* in which one high energy bond is exchanged for other endergonic bonds, and (3) production of work. In this last process the bond energy would be exchanged for electronic energy which, then, induces the physical changes in the system by which the work is produced, (3) being the reverse of (1).

Dr. Koloman Laki has kindly drawn my attention to the fact that the elucidation of the mechanism of Eq. (4) is not only a major problem of biology but is also the limiting factor of space travel beyond the moon. The clarification of this equation would allow the transformation of radiation energy into foodstuffs to provide for astronauts on longer flights.

Equation (4), as the foundation of production of work, is not without support. T. Hill showed that small changes in electric field may induce cooperative phase transitions. It is believable that the electric field, generated by electrons of high B , induces the changes which declare themselves by contraction in muscle and by superprecipitation in actomyosin.

Oxidative phosphorylation can be uncoupled by certain

substances, like 2,4-dinitrophenol or chloro- and iodophenols. It was Orgel who noticed that uncouplers are quenchers of fluorescence and phosphorescence. The nitrophenol, being a good electron acceptor, picks up the excited electrons and dissipates their energy before they can emit light or induce phosphorylation. Thyroxine, with its electronegative iodine, may act likewise.

2,4-Dinitrophenol increases the dephosphorylation of ATP by myosin. In muscle, splitting and contraction are obligatorily coupled, and so this action of the dinitrophenol can also be looked upon, in a way, as uncoupling of muscle contraction, the dissipation of its energy without production of work.

The basic problem of muscle contraction is how ATP produces contraction, how its energy is transduced into motion, mechanical work. This problem has been pushed into the background by the theory of sliding filaments. This theory does not answer the main problem. It clears only the mechanics on the microscopic level, but does so for cross striated muscle only.

THE PULLMAN K

The Pullmans proposed another measure for the characterization of the energy and reactivity of electrons or unoccupied orbitals: k , in the case of occupied orbitals, and $-k$ in the case of unoccupied orbitals. The methods of wave mechanics allow to calculate k . It has been described by the Pullmans in their volume "Quantum Biochemistry" and has been discussed also more recently by B. Pullman (1965). It

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has also been treated in my book "Introduction to a Sub-molecular Biology" (1960), so I will not discuss it here. Suffice it to say that the lower the k the greater the reactivity. A value of +0.25 means a high reactivity and a low ionization potential, a strong "donor"; $k = +0.5$ means a moderate donor; +1.0 a weak one. Similarly, -0.25 means a high electron affinity and a very good acceptor property; -0.5 is moderate; -1.0 is weak. However, the $+k$ of a donor can go below zero, become negative, meaning a very strong donor. The electron in this case has an antibonding character which tends to be given off spontaneously, making the substance auto-oxidizable.

The k is a very useful index. Extensive use was made of it by the writer who was never disappointed by it.

The k is a linear function of the ionization potential, the $-k$ a linear function of the electronegativity. If one plots the k of a series of substances against their ionization potential a straight line is obtained, but the slope of the line is somewhat different in different substance groups.* In the group of substances, studied by myself, and described previously (1960), a change of 0.1 in the k corresponded to change of 0.6 eV in the ionization potential. The linearity of the relations seemed to break down at low values of k .

* The energy of an orbital is $E = \alpha + \beta k$, α being the *Coulomb integral* and β the *exchange integral*. The value of α and β is somewhat different in different substance groups. The difference in slope is due to this difference in the value of β .

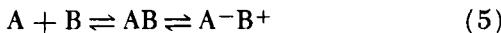
II. ELECTRONIC MOBILITY

THREE CASES OF DELOCALIZATION

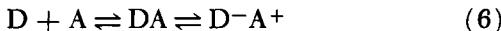
According to a Chinese saying, a long journey of a thousand leagues also begins with the first step. So, in discussing electronic mobility the first question is: How can an electron move from one molecule to another? The passing of one electron to another molecule is called *charge transfer*.

This was discovered by J. Weiss in 1942. In the author's opinion this was one of the most momentous discoveries, the biological importance of which has not yet been recognized. It means that molecules or atoms are not as closed and isolated structures as was supposed, that the electron clouds of two molecules can fuse, an electron of the one using the orbital of the other. The quantum mechanics of charge transfer was worked out and systematized by R. S. Mulliken.

Weiss, working with complexes of aromatic hydrocarbons and quinones or nitrophenols, noticed that these had a strong dipole moment. He recognized that what happened was that an electron passed from the donor molecule to the acceptor,



or



Weiss worked with flat aromatic molecules, the flatness and the extensive π electron pool of which makes a close approach easy. He used strong donors and strong acceptors in which a whole electron is transferred spontaneously. Later, interest centered around the interaction of weaker donors or acceptors, in which the energy of light is needed to transfer the electron. This transfer is connected with the absorption of a photon and, thus, the development of color.

Since we have no pairs of strong flat aromatic donors and

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acceptors in our body, the latter's presence being incompatible with life, and since we have no light in our body to transfer electrons, the biological importance of DA interactions was not recognized, and charge transfer assumed, more or less, the reputation of a chemical curiosity.

It will be shown later that charge transfer can occur readily between moderate donors and acceptors, and DA interactions belong to the most frequent and fundamental biological reactions. It seems possible that many hormonal or pharmacological reactions have charge transfer at their base. I suggested earlier (1960) that chlorpromazine owes its tranquilizing action to its strong donor character, and that lysergic acid owes its hallucinogenic action to electrons transferred in a *local* charge transfer from C-3 of the indole ring, a conclusion corroborated by Snyder and Merril.

Energy band structure is linked to the name of L. Brillouin. The basic idea is this: If, within a molecule, there are regular repeat units, and these units are coupled electronically, they disturb one another's energy levels which split up. Each level splits in two so that there will eventually be twice as many levels as there are units. If there are many units the band will contain many levels. If these are so close to one another that the energy of heat agitation suffices to lift an electron from one into the other, then they can be looked upon as one continuous system of electrons. The situation may be illustrated by Fig. 7 which was constructed based on a similar figure of Brillouin's. In this figure the lines represent the energy levels of the single repeat units, while the sinusoid line represents the energy barriers between these units. The lower energy levels are separated by wide energy barriers, are not coupled, and do not split up. Where the barrier is narrower they are coupled weakly by tunneling and show a weak splitting. The higher levels are separated

by narrow barriers, and splitting becomes pronounced. The highest levels may not be separated at all and form wide energy bands. Such a wide energy band can be looked upon as an electron gas pervading the whole molecule.

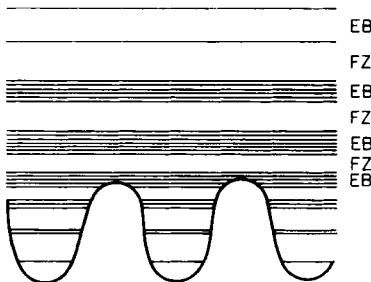


FIG. 7. Energy bands. EB, energy bands; FZ, forbidden zones. For explanation see text.

The single energy levels and bands of the electrons are separated by "forbidden zones." Whether an energy band will conduct electricity depends on the number of electrons occupying that band and the width of the forbidden zone.

As is generally known, only two electrons (of opposite spin) are allowed on the same energy level within an atom. Accordingly, the maximum number of electrons allowed on an energy band is $2n$ if n is the number of contributing repeat units. The meaning of $2n$ is that the band is saturated and the electrons have no mobility, and therefore the substance is not a conductor. The situation is similar to a crowded cocktail party where there is no place to move. If you would push an electron in one way, another would have to move in the opposite direction and there would be no net displacement. Metallic conduction, such as that of copper, is due to the fact that each atom contributes but one elec-

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tron to the common level and so the band is only half filled; the electrons have plenty of room to move.

On top of the occupied bands there are empty ones which cannot conduct electricity having nothing to conduct with. Conduction, in this case, will depend on the possibility of raising electrons from the highest filled band to the lowest empty one, through the forbidden zone separating the two. Such a transfer would make both bands conductant. What could raise an electron into the empty band is heat agitation with its energy kT , which is 0.03 eV. Conductivity will thus depend on the width of the forbidden zone. If this is too wide, say 3 eV, then heat agitation will have no chance to raise electrons through it, from one band to the other, and the substance will be an insulator. If paraffins are dielectrics this is not because they have no energy bands, but because the bands are saturated and are separated by wide forbidden zones. If the gap is narrow and heat agitation can raise electrons into the empty band then we have a *semiconductor*, the conductivity of which depends on temperature.

As was pointed out by Brillouin, there is also another way in which saturated energy bands could be rendered conductant: by *impurities*. If we attached to the system molecules which have a high electron affinity then these could draw electrons from saturated bands and render them unsaturated, conductant. Theoretically, electron donors should equally be able to render a dielectric conductant, donating electrons to an empty band.

Proteins, according to the extensive work of D. D. Eley and his associates, behave as semiconductors. They are not dependent on such extraneous electron donors or acceptors having, in every peptide, a CO and an NH built into their structure. As will be shown later, CO can take up and NH

can give off electrons. Proteins also have strongly linked to them a great amount of electron donors (see Chapter 4). According to Rosenberg they behave as electronic conductors.

While charge transfer could thus explain intermolecular motion of electrons, energy bands might explain motion inside macromolecules. However, energy bands could extend

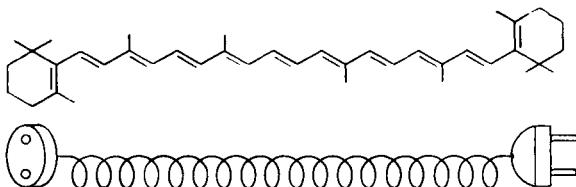


FIG. 8. β -Carotene and an electric extension cord.

over wider systems of macromolecules, like membranes, if these macromolecules stood in close contact in a regular array.

A third possibility of electronic mobility is offered by *conjugation* (Fig. 8). If, in a chain of C atoms, every second link were a double bond, then the π electrons would form a continuous electronic system, lending metallic conductivity to the substance. As emphasized repeatedly by the Pullmans, most biological catalysts, such as coenzymes, contain chains of conjugated double bonds. However, no macromolecules are known to contain extensive conjugated systems, and so this mode of electric conductivity can be only a short-range one.

The three modes of conductivity have their limited possibilities. None of them, in itself, may satisfy the needs of nature. However, there is no need for nature to limit herself to any of them. As pointed out, earlier, by putting two

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things together in a meaningful way new and unexpected qualities may be generated. This is organization on which life is built. Similarly, by putting different qualities or processes together in a meaningful way, entirely new and unexpected possibilities may arise. So while charge transfer in itself can mediate only the transfer of an electron from one molecule to another, it may turn extensive systems of insulators into conductors by transferring electrons to empty bands or subtracting electrons from saturated ones. Conversely, a band system may mediate the transfer of electrons (and energy) between two orbitals lying wide apart. Similarly, relatively short systems of conjugated bonds may lead to extensive conductivity if this system connects different structures, such as membranes, each of which has its own conductivity. Figure 8 may illustrate this point.

CHARGE TRANSFER

For an electron to pass from one molecule to another the orbitals, the donating and the accepting one, must touch, overlap. If there is no overlap, that is, if there is a zone between them in which the probability of finding an electron is zero, then the electron is unable to move over. Orbital overlap means a very intimate contact and, as a rule, D and A must form a complex before charge transfer can take place. For this to happen the molecules must be brought and held together by conventional forces, like van der Waals attractions, charges, or dipole attractions. But even if held together, the molecules may need time to find the exact steric relations which allow orbital overlap. This makes charge transfer reaction often very slow. Once the overlap

is established it will tend to bring the molecules into a relative position in which the overlap is maximal, and hold them there. Flat aromatics may establish the demanded proximity easily, while specific structural relations may be required in other instances, making the interaction very specific.

Charge transfer can also be achieved, occasionally, in one single encounter. This is called *contact charge transfer*, to distinguish it from the complex-forming type.

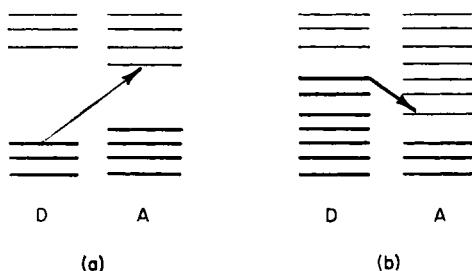


FIG. 9. Schematic representation of charge transfer. Thick lines stand for occupied orbitals; thin lines for empty ones. (a) Light-induced transfer; (b) spontaneous (strong) charge transfer.

Charge transfer is symbolized in Fig. 9 in which the thick lines stand for occupied and the thin ones stand for empty orbitals; the arrow connects the two interacting orbitals.

Once the two molecules are brought together, have formed a complex, and orbital overlap is achieved, then further happenings depend on energy relations. Schematically, we can consider three possibilities: (1) the biopotential of the accepting orbital is higher than that of the donating one, (2) the two potentials are about equal, and (3) the biopotential of the accepting orbital is lower than that of the donating one.

Possibility (1) is represented in Fig. 9a. In this case, extraneous energy is needed to move the electron over. This

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energy can be supplied by light of the corresponding wavelength, that is, by light quanta, the energy of which is equal to the energy difference of the two levels. This light being absorbed, a *charge transfer spectrum* is developed, and if the absorption is in the visible range a color appears. Many of such charge transfer complexes are highly colored. The charge transfer absorption is at a longer wavelength than the donor's own singlet absorption. This is natural because the excited electron of D, evidently, prefers the empty orbital of A to its own because less energy is needed to reach it. The absorption peak is structureless. The electron will not stay on the accepting orbital but will drop back very soon, in 10^{-9} sec or so, to its ground state on D. If the light is continuous the electron will oscillate between the two levels, spending only a very small part of its time on the acceptor, only *a very small part of the electron being transferred.*

Two factors are involved in pulling the electron back to its original ground state on the donor. One is the general tendency of physical systems to occupy the lowest level of free energy. The other factor will be the electrostatic attraction between the negatively charged electron and the positive charge it has left behind on the donor.

The energy of the absorbed light is equal to the energy needed for the transfer, and the energy of light is proportional to its wave number (300 wave numbers being equal to 1 calorie). So if we transfer an electron from a series of different donors to the same acceptor, the wave number of the absorbed light will be found to be in direct relation to the ionization potentials (or k values) of the single donors. Similar, though less strict relations hold for the transfer from one donor to various acceptors. In this case the wave number will be in direct relation to the electron affinities

of the acceptors employed [McConnell *et al.*, as well as Hastings *et al.*].

This type of charge transfer in which the energy of the accepting orbital is higher than that of the donating one, and in which the electron is moved over by light, is called *weak* charge transfer. Since the electron spends only little time on A (or only a small part of the electron is transferred), no dipole moment will be found. Since the transferred electron is still coupled to its ground state the electron will give no spin resonance signal either.

Possibility (3), in which the biopotential of the donating orbital is higher than that of the accepting one, $B^A \ll B^D$, is represented also in Fig. 9b. If the energy difference, the energy gained by the transfer, is great enough to compensate for the electrostatic attraction between the electron and its positive "hole," then the electron may move over permanently, a whole electron being transferred. The complex will show a dipole moment and if the dielectric constant of the solvent is high enough to depolarize electrostatic attractions, the two molecules D and A may dissociate into free radicals which will give an electron spin signal. This case has also been termed *strong* charge transfer, to distinguish it from the *weak* transfer mediated by light. The complexes formed is often strongly colored because free radicals are often strongly colored, delocalized electrons being able to absorb light of different wavelengths. The complexes, on which J. Weiss made his discovery, were of this type. Since both weak and strong charge transfer are associated with development of color, charge transfer was, in general, associated with color.

For reasons stated earlier, neither of these two cases can have general biological importance. The weak charge transfer is eliminated by the fact that, except for the skin and

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the eye, there is no light in our body, while strong charge transfer is eliminated by the fact that the existence of strong acceptors is incompatible with life. For the biologist the most important possibility is (2), in which the energies of the donor and acceptor are of medium range and roughly of the same magnitude, $B^D \approx B^A$. What could make the electron still move over from D to A is the delocalization energy it gains by doing so. However, this charge transfer, having no outward sign, will be difficult to detect. No free radical being formed or no light being absorbed, no color will be developed, nor will there be a spin resonance signal or dipole moment. The small increase in binding energy would be difficult to detect. So it is no wonder that this most important type of charge transfer should have been overlooked.

One of the most important differences between the ways the physicist and living nature set up experiments is this: the physicist tries to isolate the system he studies, to be able to observe it undisturbed. Nature seldom sets up such isolated experiments, most biological reactions being chain reactions, the single reactions being but steps in a series. So in many cases the transfer of an electron is but a single step of an *electron flow* in which a stream of electrons moves in one direction. This means that if the donor transfers an electron to the acceptor, the place of the transferred electron on D may be taken by an electron, coming from an outside molecule, say D_1 , the next number of the electron transfer chain. By giving off an electron D becomes an acceptor, having an empty place on a relatively low-lying orbital. If the vacated place of the transferred electron is taken by an electron coming from an outside source, the whole situation changes. The transferred electron will be no more attracted by its "hole," this being filled by the out-

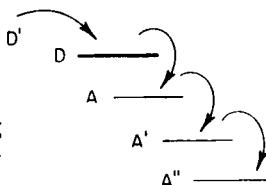


FIG. 10. Electron flow involving excited states. See text for explanation.

side electron. The transferred electron could not even return now to its original ground state, its place having become occupied. So the transferred electron would be free to generate a free radical or to move on. If this process were repetitive then a permanent electron flow would result, as is found in photosynthetic and oxidative phosphorylation. Such situations are symbolized in Figs. 10 and 11. In Fig. 10 the flow is supposed to be started up by electrons being transferred from the ground state of D to the empty excited orbital of A, the place of the electron on D being taken by an electron coming from D_1 . In Fig. 11 the electron flow is supposed to be started up by an electron being taken off from a ground state of D by oxygen, its place being filled by an electron coming from the ground state of another molecule D_1 , etc.

Following Mulliken one can classify charge transfer reactions also according to the character of the electron transferred. If it is a σ electron then we talk about a σ electron donor or acceptor. If the electron is donated by a lone pair

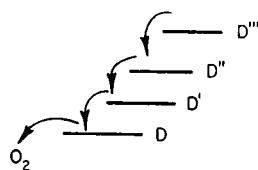


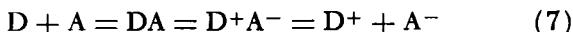
FIG. 11. Electron flow involving ground states. See text for explanation.

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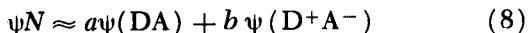
of an O or N atom, then we talk about n electrons, while a vacant orbital is a v acceptor. Charge transfer takes place most easily between flat aromatics with their extensive π electron system and their flat surface which allows close proximity, but, even in the presence of such an extensive system of π electrons, charge transfer can take place from one single atom with especially high electron density, as C-3 of the indole ring (local charge transfer), even if this C atom draws then on the π -pool (Szent-Györgyi *et al.*, 1961).

Electron transfer can take place not only between different molecules but also between the different parts of the same molecule. Also, one and the same molecule can act, alternately, or simultaneously, as donor *and* acceptor, containing both an occupied orbital of relatively high energy and a relatively low-lying empty orbital. As emphasized by Mulliken *acceptor* and *donor* do not denote two different types of molecules, only different *modes of action*, though a high-filled or low-empty orbital may dominate the reactions of a molecule making it into a good donor or acceptor.

Simpler charge transfer reactions can be described in the shorthand of chemistry. So, for instance, the formation of a charge transfer complex and subsequent dissociation can be described as



All the same, the language of classical chemistry is inadequate and misleading when describing electronic phenomena. The adequate language is that of wave mechanics, and the correct notation for the formation of a charge transfer complex would be



where N means ground state, ψ is the wave function, and the coefficients a and b indicate the fraction of the electron taking part in the reaction. In the excited state (weak charge transfer):

$$\psi_E \approx b^* \psi(DA) - a^* \psi(D^+ A^-) \quad (9)$$

These wave equations need not frighten the biochemist. They cannot be solved anyway. All the same, they offer an easy shorthand, allow us to express and systematize the reactions, and emphasize the fact that these are, essentially, electronic reactions which cannot be covered by equations of classical chemistry.

Oxidoreductions also involve a transfer of electrons, so a word may be said about the difference between this group of reactions and charge transfer. Oxidoreduction between organic molecules, usually, involves two closed shell molecules and transfer of *two electrons*, whereupon both molecules rearrange and assume a stable configuration again. In charge transfer *one electron* is transferred only to an empty orbital and there is, in principle, no major rearrangement of electronic structure, though rearrangements often follow such transfer. This difference is not eliminated by the fact that, as shown by Michaelis, in biological oxidoreductions the electrons may be transferred one-by-one and that the intermediary radical can be stabilized by excessive pH. Charge transfer is, in principle, a monovalent reaction.

The two electrons, given off by the reductant in an oxidoreduction, can just as well be given off to a metallic electrode. Accordingly, most oxidoreductions lead to a well-defined redox potential. This is not the case in charge transfer. Charge transfer is a very personal affair between an acceptor and donor which involves great intimacy, an orbital

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overlap. Such orbital overlap is difficult to establish between the donor and a piece of metal. The lack of well-defined redox potentials may also be one of the reasons why the biological importance of charge transfer has not been fully recognized.

It is more difficult to draw a line between donors and acceptors and what Brönsted and Lewis called *acids* and *bases*. For the biologist acid means H ions, a base means OH ions. Brönsted and Lewis gave an entirely different definition to these substances. In their definition an acid is a substance which can accept electrons, a base is a substance which can donate them. Charge transfer reactions are thus, in a way, but specific cases of the more general idea of the Lewis acid-base balance. Donors are a special case of Lewis' bases, and acceptors are a special case of Lewis' acids (see Luder and Zuffanti).

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MIDDLE-RANGE CHARGE TRANSFER

Charge transfer reactions between specific substances under specific conditions have been demonstrated in various quarters, some of them in my own laboratory. These have been reviewed by A. and B. Pullman (1966). However, what is of prime interest is not whether specific substances can give DA interactions under specific conditions. What is of prime importance is whether charge transfer has a general biological importance, whether metabolites of average reactivity can give charge transfer.

As pointed out before, a middle-range transfer would have no measurable outward sign, and can, thus, not be detected. To approach this problem we can argue thus: If it could be demonstrated that these metabolites can give a whole electron to a strong acceptor, then one could suppose that they could give part of an electron to an acceptor of average reactivity. The donation of a whole electron is easy to detect, owing to the color and spin resonance signal of the generated free radical.

As a strong acceptor, phenazine (Fig. 12) can be used. It has a strongly colored free radical which can be identified by its spin resonance spectrum. However this radical is too unstable in water to be detected. So a solvent is needed in

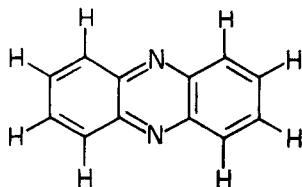


FIG. 12. Phenazine.

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which it is stable. This solvent must also be a weak donor, and a small molecule which can reach all reaction sides. Last, but not least, it should be a good solvent, equally capable of dissolving water- and fat-soluble substances.

Dimethylsulfoxide (DMSO) satisfies these requirements. It is a small molecule which has the shape of a trigonal pyramid, with the S in the apex and the CH₃ and O at the corners (Thomas *et al.*, 1966). All its reactive groups are thus in an exposed position. Its dielectric constant is 45.* Its charge transfer spectrum with iodine suggests a weak donor of a somewhat higher ionization potential than that of methanol. DMSO is also a good solvent both for hydrophilic and hydrophobic substances.

The molecules of DMSO are strongly associated, as witnessed by its high viscosity and boiling point. This suggests hydrogen bonding between the hydrogens of the methyls and the oxygens. This strong tendency for association may facilitate transfer and stabilize free radicals. Isenberg and the author have shown earlier, that charge transfer reactions can be provoked by freezing, which induces an association of the solvent molecules.†

With a strong acceptor and solvent in hand we can choose freely our metabolite of average reactivity. Dihydroxyacetone (Fig. 13), a triose with which I was well acquainted from earlier work, was chosen. Phenazine and dihydroxyacetone were dissolved in DMSO‡ and mixed in

* Merck Index, Merck and Co. 1960.

† When an aqueous 10% ATP disodium salt solution is diluted with 6–8 volumes of DMSO, the solution, on short storage, sets to a gel which will not run out from the inverted test tube.

‡ Phenazine is photosensitive. It is advisable to use a fresh solution. Its solution can be preserved in the deep-freeze.

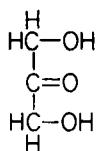


FIG. 13. Dihydroxyacetone.

equimolar quantity. On addition of alkali a deep red color developed which had its maximum absorption at 500 m μ (Szent-Györgyi, 1967).

That this color was due to the transfer of a whole electron from the triose and the formation of the free radical of phenazine was indicated by a strong electron spin resonance signal (Fig. 14). That this signal was due to the phenazine free radical was shown by the hyperfine structure (Fig. 15) which, with its splitting, allowed its identification with that of the free radical of phenazine produced by Carrington and Santos-Veiga (1962). No similar reaction was obtained in water, and the color disappeared on diluting the DMSO with water. In DMSO the radical is stable for at least 1 hr. The reduction does not go beyond the one electron step.

This reaction may have a special biological interest because the adrenal steroids, like cortisone, corticosterone, Reichstein's compound S, prednisolone, deoxycorticosterone, and cortisol, contain a keto-alcohol configuration in their side chains, similar to that found in dihydroxyacetone (Fig. 16). Treated in a similar fashion, they all developed an identical color, and gave an identical electron spin resonance signal (see Fig. 14).

All this shows that under favorable conditions metabolites and hormones can enter DA interactions, giving off a whole electron, acting thus as powerful electron donors in a strong charge transfer. These observations also suggest that

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the corticosteroids actually owe their vital function to the transmission of electrons from their ketol side chain, while the specificity of action is due to the hydroaromatic structure which leads to specific binding.

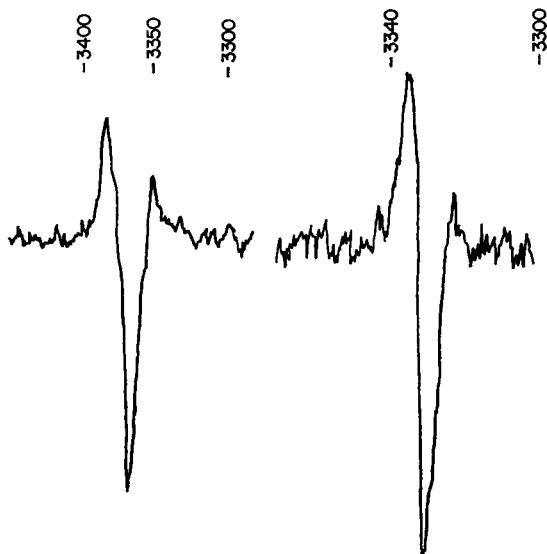


FIG. 14. Electron spin resonance signal given by phenazine-free radical produced by dihydroxyacetone (left) and cortisone (right).

Other steroids which have no such ketol side chain will not give similar reactions (diethylstilbestrol, estriol, estrone, estradiol-17 β , progesterone, testosterone, dihydrosterone, dehydroepiandrosterone).* In a different position CO groups, not activated by a neighboring vicinal OH did not act as donors under these conditions.

* The author is greatly obliged to Professor Charles B. Huggins for samples of all these steroids.

While corticosteroids seem to be electron transmitters with a prevailing donor activity, other steroids or related substances might fulfill an analogous function acting as acceptors. If stilbestrol or estradiol- 18β were added to the solution of the phenazine free radical in equivalent quan-

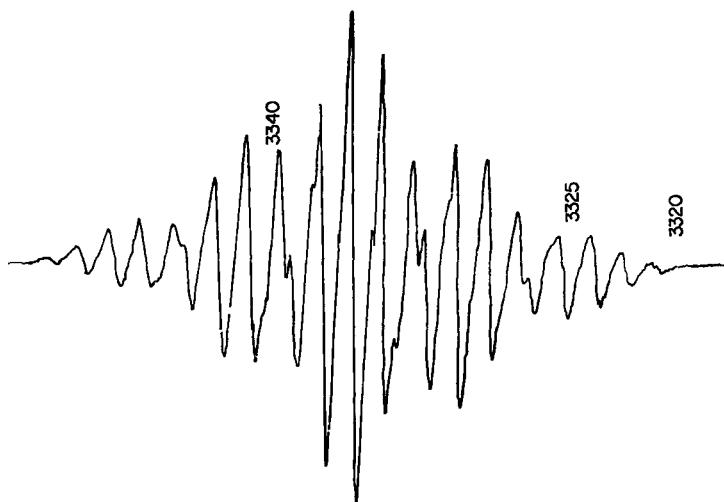


FIG. 15. Hyperfine structure of electron spin resonance signal of phenazine-free radical produced by dihydroxyacetone.

tity, the color and signal disappeared, indicating that these sexual hormones took the free electron over, supporting the contention of G. Williams-Ashman and P. Thalalay that estrogens are catalytic electron transmitters. In these experiments phenazine mediated the reaction between cortisone and estrogen acting as the catalytic electron transmitter, taking the electron from the cortisone and passing it on to the estrogen. One would expect, on thermodynamic grounds, that such an electron transfer from cortisone (or

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dihydroxyacetone) to estrogen would also take place directly, without the intervention of phenazine. This was not the case. While both cortisone and estrogen could react with the flat heterocyclic phenazine, they could not react directly with one another, giving a new example of the specificity of charge transfer reactions.

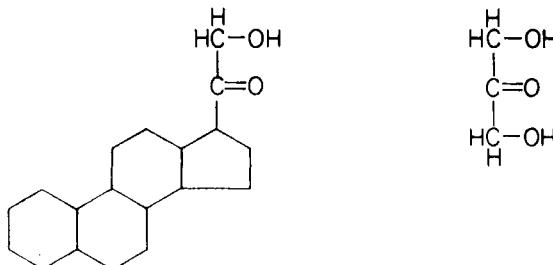


FIG. 16. Adrenal steroid (left) and dihydroxyacetone (right).

The 2,4- and 2,5-dinitrophenols are also good monovalent electron acceptors and act like phenazine. In DMSO they are reduced by dihydroxyacetone or a corticosteroid to a black substance, in all probability, a free radical.

ELECTRON DONORS OF TISSUES. THE HOPKINS POOL

Since charge transfer between a pair of molecules, say D and A, may be promoted by the presence of an extraneous weak donor, which can fill the place of a transferred electron, it seemed interesting to know whether tissues contain weak donors, and contain them in quantity.

Jane A. McLaughlin tried to answer this question. To do so she needed a good solvent, and a good acceptor which could also serve as an indicator. DMSO was used as solvent but as an acceptor she preferred methylphenazonium (phenazine methosulfate) "PMS," shown in Fig. 17. This

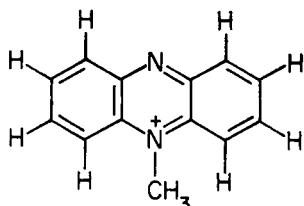


FIG. 17. Methylphenazonium.

substance can also be reduced monovalently into a purple colored substance which has been shown by J. Kimura (unpublished) to be a free radical which could be identified as that of the methylphenazonium by its hyperfine structure. The radical can be reduced bivalently to its colorless leucoform, and seems to have a special affinity for biological systems. It is not a newcomer in biochemistry, fitting snugly into various biological systems. Geller and Gregory found that it increased the photophosphorylation of chloroplasts, and now it is widely used in research on photosynthesis. Singer and Kearney found that it could reactivate soluble succinoxidase which otherwise showed no activity. While phenazine needs a definitely alkaline reaction for its reduction, PMS reacts at the physiological pH 7.4, its reactivity rising rapidly above pH 7.

McLaughlin (1968) blended pieces of various animal tissues in water and then added DMSO in excess, phosphate buffer pH 7.4, and PMS. An intense red color developed with an absorption maximum at $540 \text{ m}\mu$. Even if the

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homogenate was diluted 500- to 1000-fold, it still showed an absorbency of about 50% indicating the presence of a donor in high concentration. The various tissues, like heart and body muscle, kidney, lung, brain, spleen, intestine, and transplanted sarcoma and cancer, all gave about identical values, containing a donor whose concentration in the tissues had to approach 0.1 M , and was thus about ten times higher than that of glutathione. The donor thus detected was a typical monovalent donor, reducing the dye monovalently. It was bound in the tissue to the protein and released from the protein by trichloroacetic acid, after which it readily passed the dialyzing membrane. No attempt was made to determine its chemical nature and homogeneity. Different amines may have been involved in its formation, since amino or acid amide nitrogen readily reduces PMS under the given conditions. Only glutamine was identified as one of its constituents.

All this may solve an old and more or less forgotten puzzle. In 1925, F. G. Hopkins estimated the quantity of *fixed SH* in washed muscle. He treated the muscle with a solution of S—S glutathione which became reduced by the fixed SH to SH-glutathione. He found that if he allowed this SH-glutathione to be reoxidized by O_2 , ten times more O_2 was used up than corresponded to the fixed SH present in the muscle. He was puzzled but found no solution.

The observation of Hopkins indicates that there is, in muscle, an unknown reducing agent in quantities ten times greater than glutathione; this reducing agent is strongly bound to protein.

The observations of Hopkins indicate that tissues contain a rich store of electrons of high biopotential. These electrons are not electrons of SH groups but are in equilibrium with the SH pool and can be translated into SH, the actual

SH concentration being an indicator of the level of the electron pool. The reducing agents demonstrated by McLaughlin may be part of the picture.

The finding that amino and acid amide N can also transfer a whole electron to methylphenazonium, reducing it monovalently, was unexpected. Nitrogen has its lone pair of electrons, but that these should have a potential high enough for such charge transfer was a surprise.



FIG. 18. Isomeric forms of thiourea.

Protein, in every peptide, has a CO and NH group. That the lone pair of electrons of N and O can be translated into SH (reducing SS or CS), in the presence of a suitable catalyst, has been demonstrated earlier (Szent-Györgyi, Együd and McLaughlin, 1967). As is generally known, nitroprusside (in the presence of ammonium sulfate and ammonia) gives an intense purple color with SH. It is the classical reagent for SH. So if to an aqueous tissue extract nitroprusside is added (in the presence of ammonium sulfate and ammonia) a purple color appears, which is due to the reduction of the nitroprusside by the SH of the glutathione present. This color very soon fades out. Thiourea gives only a very faint color with nitroprusside, evidently due to the equilibrium of its two resonating forms shown in Fig. 18, of which the keto form is strongly favored, SH being present in traces only. If, however, thiourea is added to an aqueous tissue extract (in the presence of ammonium sulfate, ammonia, and nitroprusside), then, on incubation,

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a very deep purple color is developed, indicating the gradual formation of a high concentration of SH. Evidently, the CS of the thiourea was reduced to CSH or CS^- , which then passed on its electron to the nitroprusside. This indicates that the reducing power of other donors present can readily be translated into SH if a suitable catalytic electron transmitter is present. In the presence of such a substance there may thus be an equilibrium between SH and other reducing groups of the cell.



FIG. 19. Two resonating forms of the peptide bond.

Dihydroxyacetone or acid amides also strongly reduce nitroprusside under the above described circumstances, evidently reducing the CS of thiourea to SH by means of their lone electrons.

Our outlook on the nature of proteins, dominated by steric considerations, is ripe for a revision. We look upon the peptide link as a segment of the backbone with static function.

Every peptide link has two electroactive donor groups, CO and NH, which will be discussed in the next chapter. The link itself is present in two isomeric forms shown in Fig. 19. The acceptor-donor properties of the two forms have not been considered yet. The two forms can be expected to have different acceptor and donor properties, and so the presence of donors and acceptors must have a major influence on the stability of the two forms. Con-

versely, the two forms must have a major influence on the acceptor-donor properties. As has been discussed before, the sulfur atom can act not only as an electron acceptor or donor, but can act also as a catalyst between the lone pairs of N and O and the system of SH and S—S links. The S atom of cysteine, in proteins, is separated from the peptide chain by a saturated C atom, so that the S atom seems to be isolated electronically. However, as pointed out by the Pullmans (1963), the SH group can fold back

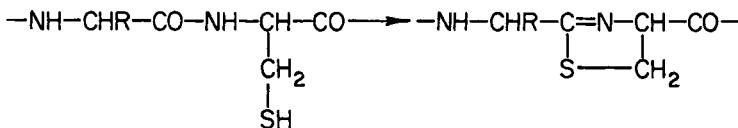


FIG. 20. Folding back of the SH group of cysteine in protein.

on the peptide chain to form a thiazole ring, whereby its electronic structure merges with that of the peptide chain (Fig. 20). The Pullmans also have pointed out that a free electron donated to the protein chain would preferably be localized on the α -carbon atom and would greatly extend conjugation along the backbone, in addition to the conjugation across the H bridges.

There is one point about proteins which I found neglected throughout my research career, and this is the difference between structural and globular proteins. Energy transductions are linked to the structural proteins so that one has to conclude that structure must be intimately connected to the electronic changes involved in energy transduction and the production of external work.

The nonstructure-forming proteins are globular and have no field of unsatisfied forces surrounding them, factors

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which are essential for mobility. They have, more or less, a passive career role, and mobility is their main physical feature. Contrary to this, the structural proteins have axial asymmetry and have unsatisfied forces to connect them. Structures can be built of elongated particles only, not from spherical ones. Accordingly, globular proteins, like serum or milk proteins and extractable tissue proteins, are more easily accessible and so it was mostly globular proteins which served as material for studies on protein. *Protein chemistry* is, to its greatest extent, the chemistry of globular proteins. Structural proteins are very difficult to secure since most structures cannot be disentangled without damaging their particles. Only in muscle are the particles arranged coaxially in bundles which easily fall apart.

FUNCTIONAL GROUPS AND REGULATIONS

If electron donors and acceptors dominate the activity and the physical state of the cell, the question arises: How can such donor or acceptor groups be generated? The basic elements of the cellular edifice are C, N, H, O, and S, and so, evidently, the functional groups must be built of these. The way in which they can be put together is limited. As Birks and Slifkin have shown, the NH₂ of amino acids can donate electrons from the lone pair of their N to chloranil. Slifkin showed them to do the same to oxygen (1962) or riboflavin (1963). McLaughlin showed them to reduce methylphenazonium. Primary amines are better donors than secondary amines, ethylamine being a better donor than dimethylamine, and dimethylamine better than trimethyl-

amine. α -Amino acids or acid amides are better donors than ethylamine, suggesting that the proximity of oxygen increases the donating ability.

The oxygen has its lone pair of electrons, too, and can thus act as a donor. As the reaction of phenazine and dihydroxyacetone showed, it is a good donor. As the action of thiourea showed, the electron donor ability of oxygen can be greatly increased by the CS group which can take over one of the lone electrons and form CSH, acting thus as a catalytic electron transmitter for the electrons of oxygen.

The S atom also has its lone pairs of electrons, and can thus be expected to be a good donor, even in S—S links. The donor ability is favored by the "soft" nature, the deformability of this atom (Niedzielski, Drago, and Midgaugh). Taking everything together, we can say that there is, in the cell, an ample source of transferable electrons. To this we have to add the electrons activated in metabolism, since most metabolites are oxidized by means of *H-activation*. H-activation, essentially, is an activation of electrons and a transfer of the activated electrons accompanied by a proton. The wealth of tissue in active electrons is demonstrated also by the ready reduction of methylene blue, which demands electrons of high potential. Summing up: The cell abounds in active electrons.

The situation is different with acceptors. The cell is poor in these. The main electron acceptor of the cell is the carbonyl group, C=O, be it the carbonyl group of ketones or aldehydes. CO can act as an *n* electron donor with the lone pair of O, especially if supported by a catalytic CS like that of thiourea. But, at the same time, the CO can act with its π electron system also as a *ketoid* π electron acceptor (Mulliken). The aldehydic CO is a poorer ac-

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ceptor than the ketonic one; in an aqueous solution the aldehyde R—HCO is present, to a great extent, as a hydrate (Fig. 21). Contrary to this, the aldehyde has a greater

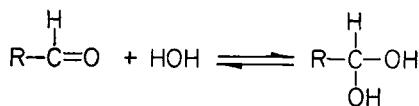


FIG. 21. Aldehyde and aldehyde hydrate.

chemical reactivity which enables it to add SH compounds, such as glutathione, as a thiohemiacetal (hemimercaptal, Fig. 22). This reaction can be interpreted as a charge transfer reaction between the aldehydic CO and SH, with a subsequent intramolecular rearrangement. A similar mechanism may underlie also the formation of Schiff bases from aldehydes and amines, amines being good donors.

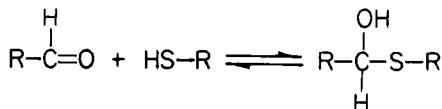


FIG. 22. Formation of hemimercaptal from aldehyde and SH.

A single CO is not a very active acceptor. Being a π acceptor its acceptor strength can be increased by extending its π pool. This can be done by adding a second CO in α position. The same can be achieved by a double link in $\alpha\beta$ position (Fig. 23). A wider system of conjugated bonds,

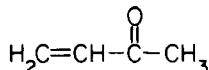


FIG. 23. Methylvinylketone.

as that of aromatics, very greatly increases the acceptor strength. Aromatic ketones, that is, quinones, are strong oxidizing agents, *o*-diquinones stronger than the *p*-diquinones. These are powerful acceptors which can be used as chemical claws or fangs in self-defense against invading hostile life.

Ketones or aldehydes do not oxidize iodide nor do dialdehydes (glyoxal). An α -ketoaldehyde, with its two CO's, does so at high concentration and acidity, and so do ketones with an α - β double link (Fig. 23). Quinones readily oxidize iodide even in dilute solution.

The CO group being ambivalent, capable of acting both as π acceptor and n donor, may play a prominent role in shaping the cells' DA balance. The same is true of the CS group. As acceptor it can go over into CS^- which, by binding a proton, can turn into CSH . The biopotential of various SH groups seems to be very different, depending on the neighboring groups. There seems to be a whole gamut of SH biopotentials. The SH's involved in cell division seem to have a rather high reactivity and so will be the first to interact with acceptors, reacting even with weak acceptors. This difference in reactivity of the various SH groups opens a possibility of influencing various cell functions in a specific way.

The CSSC bridges play an important role in the proteins' conformation. The bridge formation makes the structure more solid, the breakdown makes the structure more liquid. Huneeus-Cox, Fernandez, and Smith (1965) liquefy the axoplasm in their neurons by cysteine. SH, as donor, is more active than O or N, and so if the cell shifts electrons from its oxygens and nitrogens to its sulfurs, breaking up S—S, this has to have a major influence on the cell's physical state and activity. The S—S bridges are the link between the physical

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state of the cell and its DA balance. The shift of this balance in favor of D, that is, the accumulation of SH, will declare itself in an increased SH-glutathione concentration, which acts as the indicator of the accumulating electrons of high biopotential. Being an indicator only, we cannot expect that the addition of a corresponding amount of SH-glutathione should shift the cell's physical state and activity.

As shown by Gordy, Ard, and Shields, free electrons in protein tend to get localized on S which is also said to have low-lying *d* orbitals.

Since the cell is rich in donors but poor in acceptors, we can expect no striking changes in physical state and activity by introducing a small amount of additional donors. The opposite being true for acceptors, these may have a regulatory activity.

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DEFENSE MECHANISMS

This chapter has sentimental overtones. One of my first biochemical papers was on the action of polyphenoloxidases (1925). It was this little piece of work which brought me into contact with my beloved teacher Sir Frederick Gowland Hopkins, who made the continuation of my research career possible. I have learned from him what science is, while Cambridge has become my spiritual home-country.

As is commonly known, about half of the plants or fruits turn black or brown if damaged. Bananas, pears, apples, potatoes, and mushrooms may be the best-known examples. They discolor whenever we hit or drop them. The chemical mechanism underlying this discoloration is one of the most important and fascinating defense mechanisms in nature of a high survival value.

The intact plant contains a polyphenol and polyphenoloxidase side by side, without the two interacting with one another. In all probability the two are separated. The mechanism responsible for this separation is a most sensitive, subtle one and any damage will disturb it, whereupon the enzyme and its substrate get together and the phenol becomes oxidized to a quinone. The quinone interacts with the protein present forming highly colored, dark complexes. Figure 24 shows a banana which has been dipped for a minute halfway into chloroform.

At the time of my studies various complex theories were in circulation about the chemical mechanism of this oxidation. They involved peroxides and peroxidases. I could show that what happened simply was that the oxidase oxidized the phenol to quinone (Fig. 25). The potatoes, on which I

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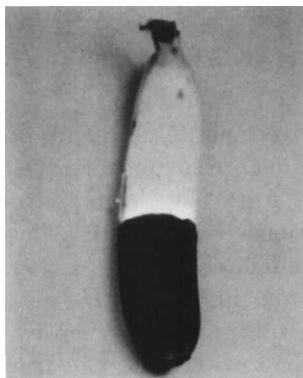


FIG. 24. Banana, a day after having been dipped for a minute halfway into chloroform. (Photo by Felker.)

worked, contain a derivative of such an *o*-dihydroxyphenol, catechol, which becomes oxidized to an *o*-diquinone.

o-Quinones are powerful electron acceptors. They are powerful bacteriostatic agents, too; so if it is microorganisms which have damaged the plant, the quinone thus formed will kill them. Nature, cunningly, has set a trap for them and makes them commit suicide.

The interaction of the quinone with the plant's own protein also has its meaning. Nature often kills several birds

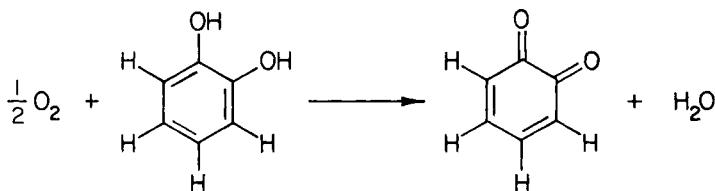


FIG. 25. Oxidation of catechol (left) to *o*-diquinone (right).

with one stone. By its interaction with the protein the quinone becomes bound and will not penetrate into the healthy tissue and cause damage. The quinone will precipitate, tan the protein, closing the gate to further damage or bacterial invasion, forming a semisolid protective film. The dark color observed (see Fig. 24) is actually the color of the protein-quinone complex. Quinones by themselves are poorly colored.

The separation of interacting substances is one of the most widely spread regulatory principles of nature. It is widely used not only for normal regulation, but also for the repair of mechanical damage. In this case, as in the potato, the system is built in such a way that the damage activates an enzymic mechanism which, eventually, leads to the correction of the damage. The best known example may be blood coagulation. If the tissue and its blood and blood vessels are mechanically damaged, two factors, thrombokinase and prothrombin, interact. In the intact system they were kept separated. The result is the activation of prothrombin to thrombin which, eventually, leads to coagulation which plugs up the broken vessel. Another example may be the catheptic enzymes. They are contained in the intact tissue in a state in which they cannot act and digest protein. However, if the cells are severely damaged, then it is of vital interest to the organism that they be eliminated. So severe damage sets the catheptic enzymes free which digest the cells in question. UV light damages our skin, the damage sets the tyrosinase free, the tyrosinase produces pigments, and the pigments protect us against the light.

At the time of my experiments, I could not yet see that the potato oxidase involved two basic principles. One of them was the principle of self-correction, just discussed. The

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other was the exchange of a donor group, the phenolic OH, for an acceptor group, CO. The acceptor nature of this group is strongly boosted up by the neighboring CO and the extensive conjugated system on which the CO's are superimposed. Nature uses such vicinal CO's where it wants to stop proliferation, proliferation of bacteria in the present case. Where nature wants not only to inhibit but to kill she builds these CO's on top of an extensive system of conjugated double bonds. Living nature is based on a relatively small number of basic principles which are cleverly adapted to the most varied ends.

PHYSICAL STATE AND CELL DIVISION

Life wants to grow and multiply. As Bullough puts it, it is only the opportunity and not the stimulus it waits for, to do so. In monocellular organisms it is mostly the quantity and quality of food available which limits growth; not so in multicellular organisms. When cells organized to build more complex organisms, a brake had to be put on and growth had to be subjected to regulation in interest of the whole. However, this brake had to be put on loosely, reversibly, to allow turning the cells loose on short notice should growth be needed.

My skin is tough. With a little tanning it would make good boots. This toughness allows it to fulfill its most important role, to hold body together and protect it from mechanical or chemical injury. "Tough" means that in my quiescent skin the cells are semisolid, are strongly attached to one another and their surroundings.

This situation changes with a stroke when I cut myself. The cells, on the cut surface, make themselves free, assume a semiliquid consistency, creep with ameboid motion into the wound and divide. The newly formed cell mass is soft, mushy. This goes on till the gap is filled, cell touches cell on all sides. Then everything stops, the cells become quiescent again, attach themselves to their neighbors, and return to their original semisolid resting state.

This change of physical state involves all parts of the cell down to the single macromolecules. It involves also the nucleus, the chromatin of which breaks up into the chromosomes.

How can such a change be achieved, which involves all the countless parts of the cell? Do we have to suppose a special regulating mechanism for each of these, working in concert? This would lead to unacceptable complexity. Such a synchronous change in so many parts can be brought about only by a change in some basic parameter which dominates the physical state of the entire cell, in both its intra- and intercellular relations. pH might be such a parameter, but no change in pH which could be held responsible has been observed as yet in cell division.

The abruptness of the described transitions suggests that the cell knows two states only, that of rest and that of activity. So our first approach may consist of trying to correlate these two states to physical or chemical parameters.

The *semisolid state*, which characterizes the resting cell, means high intracellular cohesion. A strong attachment to the surrounding means high intercellular cohesion. If the latter would remain high the cell could not make itself free, while with high intracellular cohesion no ameboid motility could be developed, nor could the mitotic aster be pulled

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through the whole cell interior. All this demands a semi-liquid state of low cohesion. The fact that this change in cohesion engulfs the whole system suggests that it is a cooperative phenomenon which tends to be an all-or-none reaction.

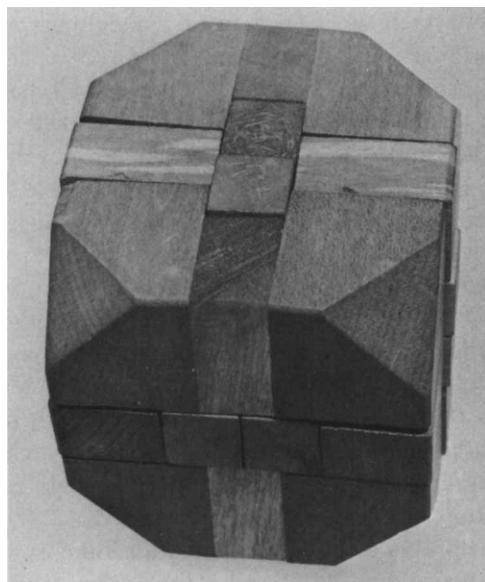


FIG. 26. Chinese puzzle, collected.

Examples of cooperative action are known from everyday life. The Chinese puzzle shown in Figs. 26 and 27 is one. The collected puzzle is a solid structure. It may symbolize a resting cell. It could be dropped without going to pieces. If, however, one piece is removed, the whole system collapses and eventually forms a loose heap of particles, as shown in Fig. 27, which may symbolize a cell in an ameboid

motion. The strength of the whole intact structure of Fig. 26 is not reflected in the forces between any two particles. The whole phenomenon is cooperative. Such a cooperative structure is also the "zip." If it lets loose on one point the perturbation will spread spontaneously till the whole system opens up.

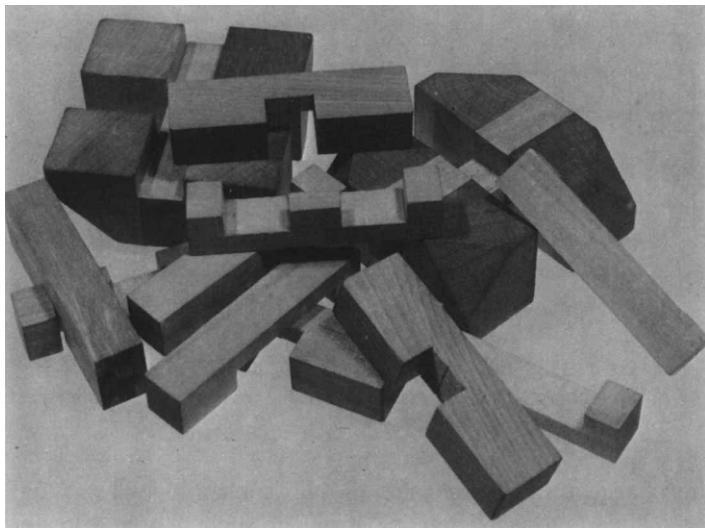


FIG. 27. Same as Fig. 26, disjointed.

Cohesion means the mutual neutralization of the forces surrounding the single particles. The conventional forces responsible for van der Waals attraction are attraction of charges or dipoles supported by the London dispersion forces. A force which has been overlooked till now is that of charge transfer. It will not pull particles together, but once they are brought together and orbital overlap has been established, it may help to hold the particles together

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and even bring them into positions in which the overlap is maximal (Mulliken). In a way, the "snaps" on ladies' dresses are analogous to charge transfer complexes: They have to be pushed together to hold. If Fig. 28a symbolizes a macromolecule, with a donor group on one side and an acceptor group on the other (as symbolized by the protuberance and indentation), then Fig. 28b may be taken as

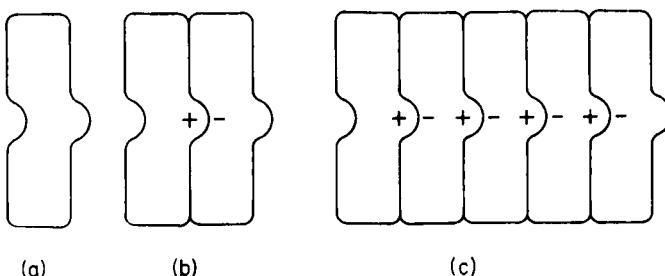


FIG. 28. (a) Symbol of macromolecule with donor and acceptor group; (b) charge transfer complex formed by two such molecules; (c) row of similar complexes.

the charge transfer complex of two such molecules. What Fig. 28c suggests is that in a row of such charge transfer complexes (as may be the case in a membrane), the dipole developed in a charge transfer may promote the DA interaction with the next molecule, making charge transfer cooperative. The structures thus generated may be two- or three-dimensional. The surface of a protein molecule consists of the outer occupied orbitals, surrounded by the empty ones. So if two such molecules touch, their orbitals touch. Where energy relations are favorable, and an occupied orbital of high biopotential touches an empty orbital of equal or lower biopotential, charge transfer must take place.

So there may be a great number of DA interactions between neighboring molecules on all sides, contributing significantly to cohesion, even though the energy of the single links, thus formed, may be low. The weakness of these bonds would make them cooperative. If the system begins to break up at one point the neighboring links may not be strong enough to hold and so the breaking up may spread.

There may be no direct evidence, yet, available for these DA interactions. What can be said with certainty is that the peptide chain contains donor and acceptor groups in equal number, the CO of the single peptide bond being an acceptor, the NH a donor. This 1:1 relation creates favorable conditions for charge transfer. Accordingly, it seems possible, if not likely, that a great number of DA interactions are established between adjoining cells. There is a qualitative difference between weak and strong bonds. If two surfaces are held together, say, by ten weak bonds of 2 calorie energy, the same force will be needed for their separation as for the separation of two surfaces held together by one strong bond of 20 calories. This, however, is true only until we pull at a right angle to the surfaces. If we start separating from the side only a very weak force (corresponding to 2 calories) will be needed to separate the surfaces held together by the weak bonds. Accordingly, these surfaces will preserve a certain deformability, while the one strong bond will make the system rigid. The weak bonds are cooperative. The qualities of living tissues rather suggest a great number of weak bonds than a few strong ones, and these weak bonds may be due to DA interactions.

According to the functional state of the cell, cohesion varies between wide limits. The conventional forces usually made responsible for cohesion, like charge and dipole moment, cannot be expected to show such variation. Con-

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trary to this, the DA interactions could easily be disturbed by the introduction of excess electrons which upset the resting DA balance and break up the DA complexes by competing for their A's. The cells have an unlimited source of electrons available in their metabolism, but are poor in acceptors. If the resting cell is at the maximum of its cohesion then accumulation of electrons will have to loosen up its structure; loosening up will greatly be promoted by the breaking up of the SS bridges which are reduced to SH's. As shown by Hopkins, the glutathione of the cell juice is in balance with the fixed SS-SH system, and so the shift of this system toward SH will be accompanied, also, by an increase of the concentration of SH-glutathione.

The SH groups are also involved in function. The SH groups participating in various functions have a varied biopotential. Those, involved in cell proliferation and protein synthesis, seem to have an especially high biopotential, being easily inactivated by acceptors, even by acceptors of low biopotential. So introduction of acceptors may have a profound influence on the cell's activity.

Though Hammett's contention, that SH is the hormone of cell division, may not have been borne out by later work, there can be little doubt that there is an intimate relation between SH and cell division. Rapkine's finding, an increase in SH-glutathione during cell division, was confirmed by H. Stern who also reviewed the subject. More recently, the problem was ably reviewed by J. S. Harrington (1967). If the concentration of SH-glutathione is only an indicator of the filling of the electron pool, then it is easy to understand why the introduction of a small amount of excess glutathione had no effect on physical state and function. The opposite can be expected to be true for acceptors introduced into the cell or generated by them.

METHYLGlyOXAL AND THE REGULATION OF CELL DIVISION

The accumulation of SH during cell division indicates that the balance between donors and acceptors is disturbed in favor of the former. This disturbance can be compensated for by the introduction of acceptors. Single CO groups are poor acceptors. As shown by Együd (1967) they produce in bacteria but a transient weak inhibition of cell division. Only formaldehyde and acetaldehyde stop cell division. They kill the bacteria owing to their chemical interaction with proteins.

The CO group is a π electron acceptor and so its acceptor strength can be increased by extending the π pool. As mentioned earlier, this can be done by introducing a double link in α - β position, as is the case in methylvinylketone (Fig. 23). As shown by Együd (1967), this substance completely suppresses cell division in $10^{-3} M$ concentration.* Since no substance of this structure is known to play a major biological role I will not discuss it further.

The π electron system of the CO group can be extended also by introducing a second CO on the neighboring C atom. This will increase the acceptor strength. If this CO is aldehydic, it will lend a greater chemical reactivity to the

* Owing to its activation by the CO link the β -carbon atom of the double link is highly reactive toward substitution, e.g., substitution by R—SH, R—NH₂, or even HOH. Accordingly, the inhibition of cell division induced by methylvinylketone is readily relieved by equivalent amounts of cysteine. It is worth noting that the mesityloxide, which contains the same configuration, was found to be biologically inactive. This is probably due to the steric hindrance, introduced by the second methyl group which prevents the reactions of the carbon atom.

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molecule, aldehydes being more reactive than ketones. To test the biological activity of such a formation, a greater number of cyclic and aliphatic α -ketoaldehydes were synthesized, including the whole homolog series of methylglyoxal up to C₁₃. All members were found to inhibit cell division at a low concentration (Együd, 1967). The strongest inhibition was found in the lowest members of the aliphatic series, in methyl-, ethyl-, and propylglyoxal (Fig. 29), which inhibited cell division completely in 10⁻³ M concentration.

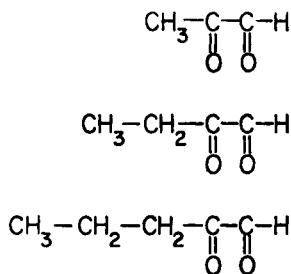


FIG. 29. Methyl- (top), ethyl- (middle), and propylglyoxal (bottom).

Methylglyoxal differs from trioses only by one H₂O, and trioses are among the most common metabolites. Accordingly, methylglyoxal was found, by various researchers, to be formed by bacteria, yeast, animal cells, and tissues under various circumstances. Evidently, it is easily formed, and the cell would have no difficulty in making it should it want to use it for its regulations. So, if ketoaldehydes play a role in regulations, then it is, in all probability, methylglyoxal which fulfills this role.

What makes these possibilities exciting is the fact that, as far as we know, all living cells contain a most active enzymic system *glyoxalase* for the transformation of α -ketoaldehydes

into the corresponding inert α -hydroxy acid, e.g., the transformation of methylglyoxal into lactic acid (Fig. 30). Glyoxalase is not a single enzyme but an enzymic system, consisting of two enzymes, glyoxalase I and II which use

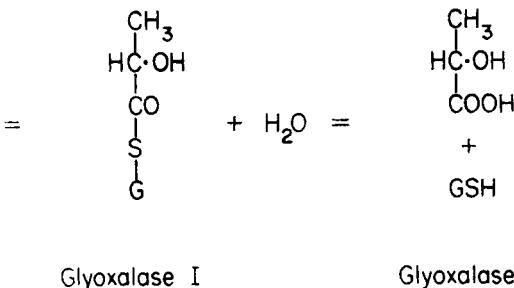
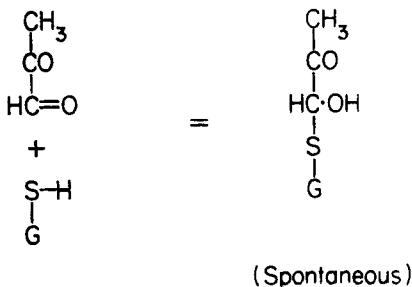


FIG. 30. Action of glyoxalase on methylglyoxal, in presence of glutathione. (GSH, glutathione.)

glutathione as coenzyme. When methylglyoxal and glutathione are brought together they link up spontaneously to a hemimercaptal (Fig. 30). Then glyoxalase I shifts two H's from carbon 1 to carbon 2, forming lactoylglutathione. Finally, glyoxalase II splits the compound into lactic acid and glutathione.

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In the first half of this century glyoxalase occupied many of the leading biochemists, like Neuberg, Hopkins, Lohman, Dakin, and Racker. The interest gradually faded out because no glyoxal derivative was found in the tissue and no major enzymic pathway seemed to lead over this substance—and what's the use of an enzyme without a substrate?

Needless to say that such a ubiquitous and active enzymic system must have its major biological significance. Nature does not indulge in luxuries. If methylglyoxal were its substrate then it would be easy to see why this could not be isolated: It readily interacts with SH and amines, and can thus be bound by the various cell constituents, if not decomposed by glyoxalase. With its high biological activity it can be present in the normal cell only in trace amounts.

All this brings the idea close that it may actually be methylglyoxal which inhibits cell division and keeps the cells in the resting state (Szent-Györgyi *et al.*, 1967). This assumption depends on the demonstration that methylglyoxal can actually inhibit cell division in a low concentration in a more or less specific manner. Dr. L. G. Együd tested the action of methylglyoxal on the proliferation of *E. coli* and found that cell division was completely suppressed by 10^{-3} M in a reversible and specific way (Együd and Szent-Györgyi, 1966a). When the methylglyoxal was decomposed by the glyoxalase, or inactivated chemically by the addition of cysteine, the growth resumed; the inhibition was thus reversible. Other functions, such as respiration and mobility (flagellates), demanded considerably higher concentrations for their inhibition. Muscle (glycerinated rabbit muscle) demanded very high concentration, though SH is known to be involved in contraction. The action was thus specific, which opens the possibility of arresting growth without interfering with life.

The main results were tested on various materials, like germinating seeds, flagellates, fertilized sea urchin eggs, cells of tissue culture or viruses. On all materials the same result was obtained: a cessation of growth at low concentrations.

The action of ketoaldehydes had a number of unexpected features. The inhibitory action depended not only on the concentration of the drug but also on the number of cells or bacteria on which it acted. A greater number of these demanded correspondingly higher concentration of the drug, indicating that the latter reacted stoichiometrically with its target, there being a high affinity between the two. The quantity of the ketoaldehyde necessary to inhibit or kill an ascites cancer cell could be roughly calculated; 5×10^{-12} moles of methylglyoxal was needed to inhibit growth and 1×10^{-11} moles to kill a cell. For bacteria the values were found to be similar. Cancer cells, on the whole, seemed to be somewhat more sensitive to these drugs than normal ones.

The question arises: What is the nature of the atomic group which reacts with methylglyoxal in such a stoichiometric fashion? In other words: Which group, inactivated by methylglyoxal, arrests proliferation?

Schubert has shown that methylglyoxal readily interacts with SH groups. This made it seem likely that the target of methylglyoxal was actually SH. Accordingly, cysteine, added to the cell suspension, in quantities equimolar to the methylglyoxal present, completely abolished inhibition. Együd (1968) has compared various SH compounds for their ability to revert methylglyoxal inhibition. Among monothiols, cysteine seemed especially active, owing to the participation of both its NH₂ and SH groups in the reaction with the aldehydic CO with which they form, under elimi-

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nation of water, a substituted thiazolidine-4-carboxylic acid (Fig. 31). Dithiols acted most strongly if the two SH's were in the vicinal position. A methylene group inserted between them strongly reduced activity. The protective action of dithiols toward ionizing radiation showed similar relations (Doherty, Burnett, and Shapiro, 1957).*

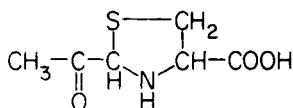


FIG. 31. 2-Methylketothiazolidine-4-carboxylic acid.

This affinity of the CO group for SH suggests that glyoxal derivatives exert their inhibitory action on cell division by interacting with SH. The specificity of the action of methylglyoxal on cell division indicates that the SH's involved in proliferation are especially active and have a greater affinity for CO than the other SH's involved in other cellular processes.

Együd found glutathione fairly inactive in releasing this inhibition. Since glutathione also forms hemimercaptals with ketoaldehydes, this means that the glutathione complex still inhibits cell division. The SH involved in cell division, evidently, has a greater affinity for the CO than the SH of glutathione. Possibly, the cell uses glutathione to tune down the acceptor ability of CO to the level of the SH's involved in division. Since glutathione readily forms hemimercaptals the cell can contain no free methylglyoxal while glutathione is present. Conversely, it seems possible

* In dithiols, also, it can be assumed that the two SH's react with the aldehydic CO. The higher homologs of dithiols are probably less active owing to the instability of the ring formed.

that part of the glutathione is present as a hemimercaptal, and the increase in glutathione concentration, observed by Rapkine during division, is due to its decomposition. This hemimercaptal will be detectable only in the absence of glyoxalase.

Cell division involves many partial processes, and it is legitimate to ask which of these is involved in the action of methylglyoxal. Experiments along this line, with radio-labels, showed that this ketoaldehyde cuts out protein synthesis (Együd and Szent-Györgyi, 1966b; Otsuka and Együd, 1968). The synthesis of DNA was inhibited only partially, by 27–50%, RNA synthesis by 20–25%. Also, these inhibitions were found to be reversible, being released instantaneously by cysteine. Since the DNA synthesis proceeds at a reduced rate, DNA was found to accumulate during inhibition of protein synthesis, as has actually been shown in phage-infected bacteria (Baylor and Együd, 1967). Also the formation of the virus protein was inhibited. The protein synthesis was inhibited on the ribosome level (Otsuka and Együd, 1968).

That the inhibition of cell division by various methylglyoxal derivatives was an expression of their acceptor nature was indicated by the parallelism between this inhibitory function and the oxidation of the iodide ion to metallic iodine by the various α -ketoaldehydes. Glyoxal derivatives, being monovalent electron acceptors, can oxidize monovalent iodide. Simple ketones or aldehydes have no similar inhibitory or oxidizing power.

The assumption that methylglyoxal is actually involved as acceptor in regulation of cell division, supported also experiments of Rachmilewitz, Rosin, and Doljanski, performed thirty years ago. They found that thiourea, ingested to rats *per os*, elicited an outburst of cell division in the liver.

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If methylglyoxal is responsible for keeping the cells at rest, then thiourea could do this only by eliminating this inhibition. It is easy to show that thiourea can actually turn the acceptor methyl- or ethylglyoxal into a good donor by activating its lone pair of electrons. In the presence of thiourea these glyoxals readily reduce nitroprusside, while in the absence of thiourea they oxidize it. Thiourea has been used for a long time for promoting cell proliferation in torpid wounds.

As is generally known, SH protects against radiation damage. If methylglyoxal interacts with the SH groups, which play a role in our natural resistance, then methylglyoxal should sensitize for high energy radiation. Ashwood-Smith, Robinson, Barnes, and Bridges have shown that both cells of tissue culture and bacteria can be sensitized to radiation by methylglyoxal.

As far as experience goes, all observations are in harmony with the assumption that an α -ketoaldehyde is involved in cellular regulations and that it is actually the methylglyoxal which keeps the cell in the resting state. Once this assumption is made we also have to assume that the glyoxalase is kept, in the resting cell, separated from the methylglyoxal. It is released and allowed to act on the glyoxal only if cell division is needed, as is the case when a wound is present. In this respect, glyoxalase falls in line with the system of the vegetable polyphenoloxidase and analogous enzymic systems discussed previously (see p. 55), which are kept under normal conditions in an inactive state and become activated only by the damage which their function has to correct.

I have described, earlier, the dramatic cellular changes which take place in my skin when a wound is suffered. These can now be put together, tentatively, into a logical

sequence and correlated to methylglyoxal and glyoxalase. When cut, the exposure on the sides of the wound disturbs equilibria and activates the glyoxalase, freeing it from its bondage. The glyoxalase, thus released, inactivates the methylglyoxal. The cell, thus released from its inhibition, goes into the active state and divides. It does so till the wound is filled, cell is surrounded by cell again, whereupon the resting equilibria are restored and the glyoxalase is brought back to its inactive state.

ON CANCER

If cancer research, hitherto, was not more successful it may have been for a dual reason: Not only did we put the cart before the horse but we put the cart before the wrong end of the horse. Not only were we too hasty in trying to cure cancer before understanding it, but we also may have asked the wrong question: Why do cancer cells divide? Living matter has the inherent tendency to proliferate. The problem is not what makes cells proliferate, but what stops them from doing so in multicellular organisms, when proliferation is not needed. What is the brake? The cell is comparable to a car parked on a slope. All that has to be done to set it going is to release the brake, so the first question is: What is the brake? The difference between cancer and normal cells is not that cancer grows. The difference is that normal cells stop growing when growth is needed no more, while the cancer cell multiplies senselessly. Many normal cells proliferate faster than cancer cells. If a neoplasm grows in 3 months from 10 to 100 grams it will be said to grow fast, be very malignant. A growth from 10

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to 100 means somewhat more than three cell divisions, and one cell division per month is a low rate indeed.*

A cancer cell is, in many ways, similar to a dividing normal cell, say an epidermal cell in a healing wound. As shown by R. Virchow 100 years ago, cancer cells even show ameboid motion. As shown by Warburg, their metabolism is shifted toward fermentation, as is the case with any fast growing tissue. Since fermentation is a more archaic process than oxidation, one may be inclined to think that cancer is simply a reversal to an earlier evolutionary state of monocellular existence, the latest and least deeply rooted regulatory mechanism being lost. This damage may be introduced by the most varied agents, and hence the great diversity of factors causing cancer, while the result is the same. Many roads lead to Rome, but there is only one Rome. The cancer cell does not know how to stop, its newly acquired superstructure, its brake have become disordered.

We can approach cancer by considering a few of its well-established features. One of these was revealed by the work of D. R. Coman, who found that cancer cells are more easily detached from their neighbors than normal cells. In this respect, they resemble regenerating cells which can even make themselves entirely free to creep into the wound. Since cells are held together by cohesive forces we can formulate Coman's observation by saying that the cohesive forces between cancer cells and their neighbors are diminished.

Another feature, well-established by Abercrombie and his associates, was that clones of normal cells, when growing toward one another in the tissue culture, stop growing when they meet, and cell touches cell. They stick together.

* Cells of the intestinal epithelium divide every 2 days.

This he called *contact inhibition*. P. Weiss called it *organization*. Cancer cells show no contact inhibition, don't organize. Both these phenomena, that of Coman and Abercrombie, have close relations to the invasiveness of cancer, one of its most dreaded properties.

"Sticking together" means cohesion, and if there is no sticking together this means that cohesive forces are diminished, which brings Coman's and Abercrombie's observations to the same denominator. The fact that cancer cells divide and show ameboid motion indicates that cohesion is diminished not only between the cells but also inside the cell. We can sum up by saying that low cohesive forces characterize the cancer cell. As discussed earlier, low cohesion is characteristic for the active state, the state of proliferation, as compared to the high cohesion of the resting state.

As far as observation goes, the cancer cell behaves as a proliferating normal cell. The difference between the two is that the cancer cell fails to return to the resting state.

According to the theory developed on the previous pages, the resting cell is kept at rest by methylglyoxal and the proliferating state is induced by the liberation of glyoxalase which releases the brake, inactivating the methylglyoxal. The return to the resting state involves the binding of glyoxalase. This theory may be right or wrong, but once we made it we may ask: What would happen, according to this theory, if a cell would lose the ability to bind its glyoxalase? Since the binding of the glyoxalase is a very subtle process, it is easy to believe that this may happen. Evidently, such a cell would be unable to return to the resting state and would continue proliferating even when proliferation is no longer needed; it would behave like a cancer cell. This leads to an exceedingly simple theory of

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oncogenesis which can explain why the most varied of noxious influences can lead to the same result: cancer. The theory simply says that a cancer cell is a cell which has lost its ability to bind and inactivate its glyoxalase.

Two factors favor this theory. First, it can be tested, can be proved, or disproved. Second, it suggests various ways for chemotherapy.

One could assume that under certain conditions a therapeutic effect could be achieved by replacing the methylglyoxal decomposed by the glyoxalase. It has been shown by Apple and Greenberg as well as by Együd and Szent-Györgyi (1968) that ascites cancers can actually be cured in mice by local application of methylglyoxal.

With solid tumors no cure was achieved. Very little keto-aldehyde is needed to inactivate the SH groups, essential for proliferation, since these SH groups are very reactive. However, when the corresponding small quantity of glyoxal derivative is introduced which is just sufficient to inactivate these groups, it is soon eliminated, attacked also by the glyoxalase. If, to compensate for this, we introduce bigger quantities of the glyoxal derivative, then its action will spread also to SH's involved in other functions. Hence the toxicity of methylglyoxal puts narrow limits on the quantities which can be applied. The LD for an average Swiss albino mouse in a single dose is around 10 mg. This suggests various approaches to chemotherapy. One of these could be the building of substances which have the essential features and the activity of glyoxal derivatives but are not attacked by glyoxalase. A ketonic CO with an α - β positioned double bond may belong to these features. Semicarbazones, thiosemicarbazones, guanylhydrazones share these features and are reported to have cancerostatic activity. Various quinones or semiquinones could also take over the function

of methylglyoxal. Their biopotential, that is, acceptor ability should be tuned to that of the SH groups they have to inactivate.

Another approach would consist of maintaining a permanent low concentration of methylglyoxal in the animal body. This could be done by introducing a nontoxic compound in quantity, which could be gradually split, liberating an active glyoxal derivative. These possibilities are being explored by L. G. Együd.

A further approach could consist of having an electron acceptor gradually produced in the animal's body by enzymic action. This approach is taken at present by the writer. One could also consider maintaining a low methylglyoxal concentration by continuous infusion or at least by frequently repeated small doses.

Finally, an approach may consist of developing an inhibitor of glyoxalase, an anticoenzyme, which competes with glutathione. Such an inhibitor is ophthalmic acid (Waley, and Cliffe and Waley). This approach is also being explored by the writer.

The inhibition of cell division by glyoxal derivatives is reversible, and so it is possible that no permanent cure can be achieved with glyoxal derivatives. To achieve this, some other noxious action might have to be combined with the action of glyoxalase. Such an action may be that of high energy radiation. A hopeful alley may have been opened up by Ashwood-Smith and his associates who showed that methylglyoxal sensitizes cells for X rays. This could lend a double edge to the action of glyoxal derivatives.

All these approaches center around SH. The idea of attacking cancer through its SH is not a new one. F. E. Knock is the outstanding pioneer of this idea. She inactivates SH by arsenicals.

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Another merit of the cancer theory presented may be that it converts a mystery to a biological process analogous to the phenoloxidase action. In both the glyoxalase and phenoloxidase systems, the binding of the enzyme is the process which most likely goes awry. Senescence has a general debilitating influence, and if a banana is allowed to get old, small black dots appear on its skin indicating that in certain areas the separation of the oxidase and its substrate failed. As time goes by, these black spots grow, and eventually the whole banana turns black. Similarly, in man, in senescence, cells begin to proliferate senselessly forming cancer cells, and if permitted they would eventually engulf the whole organism.*

* Cancer cells affect their surroundings in various ways. They induce proliferation in the surrounding tissue, have an angiotactic effect, cause loss of weight in the host organism, and induce wasting (cachexia). It seems possible that all these effects are due to the glyoxalase I diffusing out of the cancer cell. If so, the serum of cachectic patients, or perhaps of all cancer patients, should contain glyoxalase. A simple method has been developed in my laboratory for the estimation of serum glyoxalase; a description will be supplied on request.

V. CONCLUSION

My final conclusion could be summed up by saying that the living cell is essentially an electrical device. The macromolecular structure is its framework, as the visible parts of a dynamo are a framework, in which the transduction of electrical energy into mechanical work takes place.

The fuel of life is the electron, or, more exactly, the energy it takes over from photons in photosynthesis; this energy the electron gives up gradually while flowing through the cellular machinery.

The amazing subtlety of biological reactions finds its explanation in the quantum mechanical dimension, in the mobility of electrons. Single molecules are not necessarily sharply isolated and closed units. There is more promiscuity among them, as is generally believed. The merging of orbitals provides the possibility of sharing electrons without covalent bonding. The balance of electron acceptors and donors of varied biopotential is one of the basic parameters of life and is used in the regulation of function and physical state. The sources of donated electrons are the activated electrons of metabolism and the lone pairs of N, O, and S, while the main acceptor group is the carbonyl, the activity of which can be increased by extending its π pool. These considerations offer a new approach to many physiological and pathological problems.

While genetics, the conservation and transmission of the genetic code, is dominated by strict steric relations, the understanding of vital functions and the underlying energy transformation demand a more dynamic outlook on the electronic level. The object of this book is to call attention to this fascinating, promising, and almost virgin field of inquiry.

The contents of this book may give the impression that

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the conclusion that methylglyoxal is involved in cell regulation was arrived at by straight logic. This was not the case; the real story is different.

Many years ago, J. McLaughlin and I were led by odd associations to study the effects of thymus extracts on growth of spontaneous cancer. Some of the extracts retarded, others inhibited. We called the retarder "retine," the promoter "promine." The idea that growth should be regulated by two antagonists seemed too neat to be abandoned.* This was my first round with cancer which I won. The second round was won by cancer, which took what was dearest to me, and knocked me out for several years. Meanwhile, attempts to isolate retine were continued and indications were obtained that it may be a ketoaldehyde (Egyid, 1965). Returning to work, I wasted a couple of years in vain efforts to reproduce the strong inhibitions of growth obtained in transplanted cancer in my absence. Eventually, I was led to believe that these inhibitions may have been due to a methodical error. I also have become convinced that one had to look deeper into the nature of the cellular regulations. This has led to my third round, described in this book, which supports the idea of two antagonists, the inhibitor being methylglyoxal, the promoter glyoxalase, and, possibly, an additional catalyst acting similarly to thiourea, shifting the DA balance by activating the lone pairs of electrons of O and N. Looking back on this long exasperating struggle with its backbreaking work and heartbreaking disappointments, I am reminded of a saying of T. Avery, quoted lately by E. Racker: "It does not matter if you fall if, while getting up, you pick up something from the ground." The contents of this book are what I have picked up.

* This idea is fully borne out by vegetable systems.

VI. EPILOGUE

It was shown in Chapter IV that the nitrogen atom, be it amino or amide N, is a good donor capable of giving off one whole electron. The evidence was based on the color obtained with methylphenazonium which, in analogy to the color given by phenazine, was supposed to have been given by the free radical of the dye.

Color, in itself, is tenuous evidence. My electron spin resonance laboratory being inoperative at that time, I was unable to apply the more powerful ESR technique to the problem. This gap was since filled by J. Kimura (J. Kimura and A. Szent-Györgyi, *Proc. Natl. Acad. Sci. U.S.*, in press) who showed the transfer of a whole electron by means of ESR spectroscopy.

The poverty of my evidence made me rather timid in discussing the possible consequences of intra- and intermolecular charge transfer in proteins, though I was much impressed by the fact that each peptide link contains a donor and an acceptor group, an N and a CO. This opens the possibility of charge transfer between different peptide chains, or different parts of the same molecule. Such bonds, with the underlying orbital overlap, would mean strong electronic coupling between peptide chains. The peptide link itself being conjugated could explain the electronic conductivity of proteins, as demonstrated by B. Rosenberg.

Charge transfer bonds could thus be involved in various features of proteins. They could be involved in stability. Most proteins are denatured around 80°C. Heating from 20° to 80°C increases kT from 0.7 calories only to 1 calorie. This suggests weak bonds. The charge transfer bonds are very weak, much weaker than hydrogen bonds. The fact that the denatured protein does not return spontaneously to the native state suggests that the bonds which are broken

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demand a very specific orientation for their formation. Charge transfer interactions mostly demand such relations.

DA interactions could also contribute to the specificity of proteins. Accordingly, electron acceptors, combining with free donor groups, should decrease specificity and improve the chances of a "take" in grafts. Such improvement has actually been observed in mice by V. Brunn (personal communication) after injection of methylglyoxal.

What impressed me most in the course of this work was the deportment of the cell which, in division, behaved as one single entity when going over from the resting to the active state. This transition involved a major drop in cohesion, an activation of protein synthesis, and an increase in SH concentration. All these changes may be induced by a shift in the balance of electron donors and acceptors, that is the relation of highest filled and lowest empty orbitals, i.e., in a relation of ionization potentials and electron affinities. A shift in this relation in favor of the donors actually means an increase in the concentration of highly reactive electrons, a sort of *electron pressure*. Such an increase would have to open up S—S links, with the consecutive shift toward the liquid state and the generation of the SH groups instrumental in protein synthesis. The breaking of charge transfer links would also contribute to the decrease in cohesion. The electronic coupling, brought about by the orbital overlap of charge transfer links could lend mobility to the active electrons. These electrons would also increase the bonding of water, that is the hydration of protein.

All this, taken together, leads to a picture of cell life which is vastly different from that offered by a one-sided molecular outlook which allows but one motion, the motion of molecules pushed about by the random heat agitation.

The research presented started out more than a decade

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ago with the assumption that cell division was regulated by two antagonistic substances, a promotor, "promine," and an inhibitor, "retine." Though the arduous efforts to isolate retine failed, the experiments allowed us to identify this substance, tentatively with a ketoaldehyde, probably methylglyoxal, while the glyoxalase could act as promine, inactivating the inhibitor.

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