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Bioelectronics and Cancer

Albert Szent-Györgyi

Marine Biological Laboratory, Woods Hole, Mass.

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Abstract

The appearance of oxygen on our globe induced profound changes in the nature of living systems which started to differentiate and build complex structures with complex functions. Oxidation was added to fermentation and unbridled proliferation was subjected to regulation. Fermentation demanded no structure, being the result of the action of a series of single molecules. Oxidation, with its electron flow, demanded structure and electronic mobility. To produce meaningful structures and complex functions the action of the single molecules had to be integrated. The question is: how could oxygen bring about these transformations?

These changes are not limited to the distant past because in every division the cell has to revert, to some extent, to the undifferentiated, fermentative, proliferative state of its earlier anaerobic period. After having completed its division, it has to find its way back to its oxidative resting state. If this road of return is deranged the cell has to go on dividing as it does in cancer. By elucidating the details of these processes we can hope to be able to control them. We can control only what we understand (Bernal).

That oxygen can induce profound changes in cell life can be demonstrated even in the acute experiment. L. Pasteur showed that fermentation is inhibited by the admission of oxygen ("Pasteur Reaction"), and H. G. Crabtree demonstrated the opposite effect. The intimate relation of cancer and oxygen was made evident by H. Goldblatt and G. Cameron who provoked malignant transformation in their tissue culture by periodically limiting their oxygen supply.

O. Warburg attributed the changes, induced by O_2, to a wealth of energy it produced. Undoubtedly, without a new and rich source of energy these changes could not have occurred. Energy made them possible, but energy offers no mechanism. The chemical mechanism underlying these transformations will be the main topic of this paper and it will be shown that charge transfer is one of the central biological reactions. A biologist trying to understand life without electronic mobility is comparable to a Martian trying to understand our civilization without knowing about electricity.

This paper will chiefly be concerned with principles. The chemical methods employed will be discussed in a subsequent paper by Dr. L. Egyud.

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Introduction

Biology is dominated by the molecular concept, and the protein molecules are recognized as the main bearers of life. But life is not brought about by the single molecules; it is the result of their integrated function, as a symphony is the product of the concerted action of many separate instruments. This shifts the emphasis from the units to their integration.

To connect the macromolecules to a harmonious whole, smaller and more mobile particles are needed. The smallest units of biological systems are the electrons. To be mobile they need conductors. More than 30 years ago [41] I proposed with my young pupil and friend, K. Laki [28] that proteins may be semiconductors. Our proposition was rejected and at the state of our knowledge, at that time, I was unable to defend it. The knowledge accumulated since allows to rescind the problem.

Proteins are, essentially, chains of peptide links, held together, partly, by hydrogen bonds, that is, by H atoms belonging, simultaneously, to two different peptide links. According to our present knowledge, in such a system the energy levels of the single units can unite to continuous energy bands (M. G. Evans, J. Gergely, J. Ladik and A. Pullman), as the sidewalks before single houses conflue to a street. Whether the electrons, within such a band, are mobile or not, depends on their number. According to the exclusion principle, only two electrons (of opposite spin) can have the same energy in such a system. So, if every unit contributed two electrons to the band, then every allowed place is filled, and the situation becomes similar to that in a box completely filled with marbles: there is no mobility. To create mobility we have to take out some of the pieces.

Many of the dielectrics which we use as electric insulators have such continuous energy bands which, being filled, have their electrons immobilized. To make such systems conductant, we would have to desaturate them, take electrons out of them. The simplest way to do this is to "excite" electrons from the highest filled band to the next higher empty one. If this latter band is so close to the former that even the energy of heat agitation is sufficient for this excitation, then the substance is a "semi-conductor".

The distance between the highest filled and lowest empty band, in proteins, has been calculated and found to be rather great, and there is no energy available in living systems which is high enough to lift the electrons through the "forbidden zone". The energy band thus cannot be desaturated by excitation.

L. Brillouin pointed out that proteins could be made conductant by "acceptor impurities", that is, substances which can take up

electrons, and, if attached to the protein, could desaturate its energy band. Many proteins have been isolated and thoroughly analyzed but no such "impurities" were found, and it was also pointed out that systems, containing mobile electrons, are often colored and opaque, while all proteins studied were found to be colorless and transparent, provided they had no colored prosthetic groups.

I was so convinced of the semiconductor nature of proteins that, for me, the problem was not whether proteins are semiconductors or not. The problem was: where was the solution to the puzzle? How did science miss the solution? The solution is simple. It is this: there are two kinds of proteins, fibrous and globular. The fibrous ones forms threads or rods, while the globular ones form a kind of molecular marbles. There is no essential difference between the inner structure of the two because the globules are but fibers, folded together. However, there is an essential difference in function: only fibers can build structures. Birds build their nests of twigs or lengths of grass, but not pebbles. Nature applies fibers wherever she needs structure, and applies globules where she needs mobility and has to avoid structures.

The basic biological functions all involve transformation of chemical energy into work, be this work mechanical, osmotic or electric. They are all performed by structures. It is these transformations which demand electric conductivity. Being performed by structures (built of fibrous molecules), only the fifibrous, energetically active molecules need to be conductors. But most of the fibrous molecules which build these structures are solidly joined to networks out of which the single units can rarely be extricated without damage, denaturation. Contrary to this, globular proteins can be found in quantity, in molecular dispersion in the mobile body fluids like blood, milk, egg white, or the cell juices. So the chemists needing single molecules for their study, mostly limited themselves to globular proteins and avoided the fibrous ones. As a consequence, the structure forming fibrous proteins which can be expected to be semiconductors were neglected and the question whether proteins are semiconductors has remained untouched.

When embarking on this line the first simple question one had to ask was whether fibrous proteins are really colorless and transparent? With Jane McLaughlin we blendored liver, washed out the blood and dissolved the rest in 10% lauryl sulfate, using 1 ml for 0.2 g tissue. The resulting solution is shown in Fig. 1. It looked like a Swiss chocolate, dark brown and opaque.

The problem is a fundamental one. The question is not merely whether proteins are semiconductors or not. The question is: how far is life a molecular or an electronic phenomenon? And if life is, to a great

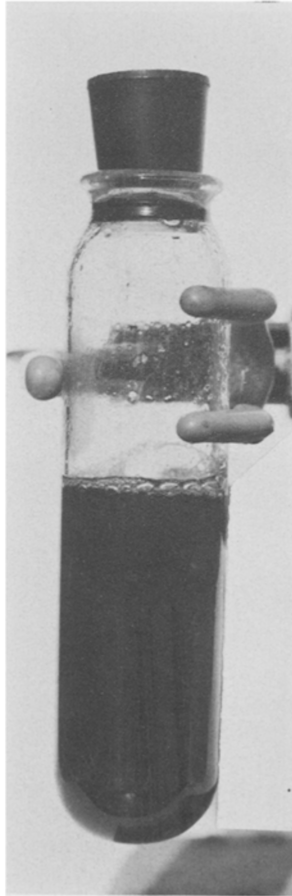


Figure 1. 20% solution of "washed" liver in 10% Laurylsulfate.

extent, an electronic phenomenon, then we may be fishing "behind the net" (as the Dutch say) when looking for the understanding of basic biological problems and cancer at the molecular level. Basic problems demand a wide philosophic outlook for their solution, and one does well to begin at the beginning.

Evolution and Devolution

Life was born on a hot, dark and airless globe, which was covered by dense water vapor. There was no light and no oxygen. No bones or other

fossile remains having been left behind, we can only rely on "educated guesses" about the nature of life in that period. What we know is that life needs energy, and the first primitive living systems probably could derive energy from other molecules only by distorting or splitting them. A very small part of the energy of the food can be released by this method which is now called "fermentation". The greatest part of the energy is left behind in the distorted or dismembered foodstuff molecules.

With such a poor energy supply life could build only the simplest forms, could perform only the simplest function and achieve only a low degree of stability, and to continue, it had to proliferate as fast as conditions permitted. Fermentation demands no structures; it can be performed even in a homogeneous solution, and so cell division, in this period, could have been favored by a semiliquid state.

As our globe cooled the water vapor condensed, and light could get through. Light consists of photons, tiny packages of energy, and life has learned to catch them. With this energy life could decompose water into its elements, hydrogen, H, and oxygen, O, fixing the H to carbon and sending the O into the atmosphere as O_2. To make use of the energy thus stabilized, the process only had to be reversed, oxygen being taken up from the atmosphere, "oxidizing" the H to H_2O again. With the oxygen the cells could also oxidize the products of fermentation and release their energy, which had been lost before.

With this new and rich source of energy, life could differentiate, build complex structures and perform involved and fast reactions. For oxidation a bulky semisolid oxidative machinery had to be built and to maintain complex structures higher cohesive forces had to be developed. To integrate all reactions into harmonious function proteins had to be made into semiconductors which could lend mobility to electrons. Also, a brake had to be put on cell proliferation which was disfavored, anyway, by the cohesive forces of a more solid state and the bulky oxidative machinery. Cell division involves a complete rearrangement of the cellular interior, which is possible only in a semiliquid state. So when dividing, the cell has to dedifferentiate, dismantle part of its oxidative machinery and rely more on fermentation, and dissolve its nucleus, disintegrating its chromatine into single chromosomes. These changes all mean a shift towards the earlier, undifferentiated, anaerobic, proliferative state. Then, after division is completed, the cell has to find its way back to its resting oxidative state again. So the changes which occurred billions of years ago repeat themselves in every cell division, and if deranged, can lead to pathological conditions. This lends an actuality to these transformations and make the clarification of their chemical mechanism into a most urgent biomedical problem.

Oxygen and Charge Transfer

One of the most important unanswered (or even unasked) biological problems is: how could the oxygen induce all these changes which accompanied the transition from the first primitive anaerobic period to the later oxidative state? How could oxygen induce semiconductivity? No doubt, energy was needed for these transformations but energy, as such, cannot induce changes. Energy can drive a car but cannot build it.

Oxygen owes its central role in biology to its oxidative power, to its being able to oxidize. Oxidation means taking electrons from the oxidized material. Oxygen is the general "electron acceptor" of the biosphere. If so, could it not be that it induces conductivity in proteins by taking electrons from it? It could not. O_2 does not desaturate protein energy bands. This prompts the question whether perhaps, some of the products of oxidation do not contain oxygen atoms in a form in which they could still be "acceptors", capable of taking electrons from the proteins, making them into semiconductors? Oxidation of organic substances produces various oxygen-containing atomic groups like COH or COOH which are not "acceptors". There is, however, one, the C = O, the "carbonyl", which is an acceptor. Out of the two bonds which link the O to the C, the one is a σ the other is a π bond, which latter has an empty orbital, on which it can accommodate an additional electron. But C = O is a very small atomic group, too small to be able to take up easily a whole electron. However, its π electronic system can be extended by placing at its side another C = O or C = C in α-β position. Neighboring double bonds are "conjugated", and their π electron systems fuse to a wider π system which, then, is a good acceptor. This leads us to two groups of good electron acceptors, the one containing C = O – C = O, the other containing C = O – C = C. The simplest representative of the first is glyoxal (Fig. 2A), that of the second acrolein (Fig. 2B). The simplest derivative of glyoxal is methylglyoxal (Fig. 2C), that of acrolein croton aldehyde (Fig. 2D). That we did not stray away far from biology is indicated by the fact that, so far as we know, all cells contain an enzymic system for the inactivation of glyoxal derivatives, "the

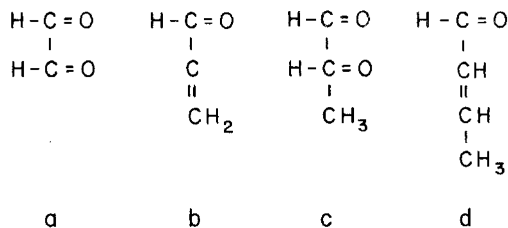


Figure 2. a: glyoxal, b: acrolein, c: methylglyoxal, d: croton aldehyde.

glyoxalase". This system consists of two enzymes glyoxalase I and II, and a coenzyme, SH-glutathione. It transforms glyoxal derivatives into the corresponding hydroxyacids, transforms, for instance, methylglyoxal into lactic acid. The "glyoxalase I" is one of the most active enzymes. Nature does not indulge in luxuries, so this enzyme must have something important to do.

Electrons, in molecules occur, as a rule, in pairs, the two electrons of the pair spinning in opposite directions, compensating each other's magnetic fields. In organic oxido-reductions usually an electron-pair is transferred from one molecule to the other and the two molecules, after having gained or lost a pair of electrons, rearrange their structure and form a new stable and well-balanced molecule. If only *one* electron is transferred an unstable free radical may be formed and the transfer is called *"charge transfer"*. This type of reaction was discovered by E. Weiss 30 years ago, Weiss found that in certain complexes, formed by two molecules, an electron of one, the "donor", could be excited by light to an orbital of the other, the "acceptor". The excited electron then oscillates between the two molecules, spending only part of its time on the acceptor; so we say that only "part of the electron" is transferred. The spins of the transferred electron and that of the electron left behind remain coupled and no electron spin resonance signal can be expected. The absorption of light produces a "charge transfer spectrum". The energy needed for the transfer of the electron is supplied by the absorbed photon, the energy of which corresponds to the energy difference of the ground level of the electron on the donor and its excited level on the acceptor. This is *"charge transfer in the excited state"*.

A "strong donor" is one which has its electron on a high energy level, while a "strong acceptor" is one that has its empty orbital on a low energy level. If a strong donor interacts with a strong acceptor, the electron, going from a high to a low level, may go over spontaneously without absorbing light in the "ground state". It may stay then permanently on the acceptor, a "whole electron" having been transferred. The donor and acceptor may then dissociate into two free radicals. The two electrons of the electron pair having been uncoupled, the radicals give a signal in the electron spin resonance spectroscope. Only such "strong" *"charge transfer in the ground state"* can be expected to desaturate the energy band of a protein and induce conductivity.

Charge transfer can take place in a single collision but, as a rule, the two interacting molecules have to form a complex first, held together by conventional forces, the charge transfer forces being very weak. But even within a complex, charge transfer can take place only if the two orbitals, the donating and the accepting one, overlap. Orbital overlap demands a very close contact, a very precise fit. It takes mostly time for the two

molecules to find this close contact which can make charge transfer into a slow reaction, demanding hours or days for completion. It can also be very specific.

The nitrogen atoms of the peptide bond have "non bonded" electrons which, not taking part in chemical bonding, can be given off relatively easily. M. A. Slifkin *et al.* [33] have shown that the amino N actually can donate electrons to chloranyl, a "strong" acceptor. That the amino N can transfer a whole electron in the ground state, has been shown by myself [42] and by J. E. Kimura and myself [23].

Our problem, then, is: can the discussed dicarbonyls and unsaturated carbonyls act as acceptors for the electrons of amino nitrogen, taking over one whole electron in the ground state? That they can, may be demonstrated in a simple and very pretty experiment which starts with mixing a 1% solution of *p*-phenylenediamine (2HCl) (Fig. 3A) with a 0.2 molar solution of glyoxal. Both solutions are limpid and colorless and remain so after mixing; the acid reaction does not allow them to form a complex. This situation, however, is changed by adding a small amount of sodium bicarbonate or Na_2HPO_2. On neutralization the color of the mixture becomes intensely yellow and soon a yellow precipitate is formed which gradually darkens. The yellow color is, in all probability, due to a charge transfer in the excited state, a precipitate being a charge transfer complex. The gradual darkening is due, as shown by H. Kon and myself [27], to the gradual transfer of a whole electron in the ground state. If the suspension is acidified by strong HCl, instead of discoloring, it turns intensely blue. The precipitate dissolves with a dark blue color which soon disappears, leaving a colorless fluid behind which is identical with out initial mixture. What happened on sudden acidification was that we caught the electron of the amine "red handed" on the glyoxal, both the amine and the glyoxal having formed a free radical. Accordingly, in his electron spin resonance spectroscope, Dr. Kon obtained two signals. The blue color was that of the free radical of the diamine. The experiment was most fascinating because it demonstrated, *ad oculos,* the transfer of an electron both in the excited and the ground state. It should be repeated in every course of chemistry.

The precipitate is not very insoluble. A 0.002 M glyoxal gives no precipitate, only a strong yellow color with a sharp peak of absorption just over 400 mu. The color is still very definite at a 0.0005 M concentration of glyoxal (in presence of 0.1% of diamine). It can be used for the quantitative estimation of dialdehyde. The color is formed slowly, the establishment of orbital overlap being slow, and time has to be given (1 h) for the color to develop. To prevent the autooxidation of the diamine to colored oxides, a small quantity of ascorbic acid can be added. Dithionite, being a strong acceptor, discolors. The formation of the yellow complex is not specific for glyoxal. Also methylglyoxal or croton aldehydes give similar colors, but only glyoxal gives a charge transfer in the ground state and a blue color on acidification.

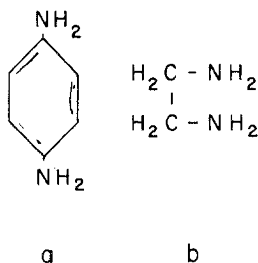


Figure 3. a: *p*-phenylenediamine, B: ethylenediamine.

The SH Catalysis

Aromatic amines, like the *p*-phenylenediamine, used in the above experiment, are rather reactive. In order to find out whether acyclic amines also can donate whole electrons, Jane McLaughlin and I [43] added 0.5 M phosphate to ethylene diamine to give a solution of pH 7.4. We chose diamine to represent aliphatic amines, because it tends to give colored products observable by the naked eye. To its solution we added a 0.5 M methylglyoxal solution (Fig. 2C). Soon a yellow color indicated a reaction. On storage it gradually turned brown.

Though positive the result was disappointing because the reaction was slow, gave no indication of free radicals, and the spectrum of the product was dull (Fig. 4B). To have any biological meaning it had to be catalyzed in some way by a cell constituent.

One of the most widely spread cell constituents is glutathione, a tripeptide with an SH group, the function of which is poorly understood. So we repeated the reaction in presence of biological concentrations of SH-glutathione (0.1%). Adding methylglyoxal to the diamine, in presence of SH-glutathione, a purple color appeared which rapidly deepened, and then disappeared. This was most striking. The spectrum of the purple solution resembled the earlier dull spectrum, but had a hump (Fig. 4A). Evidently, superimposed on the earlier reaction there was a second interaction, responsible for the hump and the purple color. It was possible to obtain the spectrum of this latter alone by compensating for the first. This could be done by placing the glutathione-free solution into the reference beam of the spectroscope, while placing the SH-glutathione containing solution into the primary beam. Doing this, a single structureless peak was obtained (Fig. 4C), characteristic for charge transfer. Evidently, two independent reactions took place, the dull and spontaneous one which, probably, was the formation of a Schiff base, and a second, catalyzed by the glutathione. That it was the SH group which was the catalyst could be shown by blocking it by

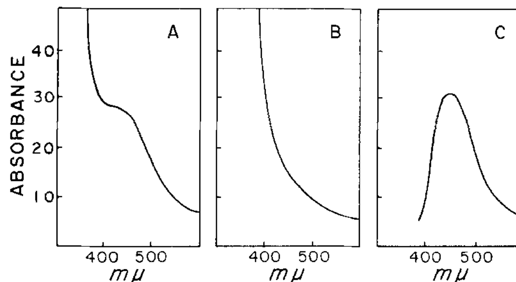


Figure 4. Absorption spectrum of the products of interaction of ethylenediamine and glyoxal. A: in presence of SH-glutathione. B: in absence of SH-glutathione. C: differential spectrum of A and B.

mercurysalicylate (mersalyl), in presence of which there was no catalytic activity. That the SH was the functional group was indicated also by the fact that other sulfhydrils showed a similar though considerably weaker catalytic activity. Glutathione, for instance, was three times more active than the equivalent amount of cysteine, indicating that this catalysis is not merely an item of nature's curiosity shop but has its physiological meaning. This was indicated also by the fact that the catalysis had its pH optimum at 7.4, the physiological pH. What was of prime import for our problem was the question whether the peak produced in the interaction of amine and carbonyl was a "charge transfer spectrum" or was the absorption of a free radical formed by the transfer of a whole electron in the ground state which could desaturate energy bands, transforming the protein into a semiconductor. This question was answered by Dr. H. Kon of the National Institutes of Health (1973) by showing that the purple solution gave a strong and wide spin resonance signal which appeared and disappeared with the color, leaving no doubt that the peak was the spectrum of a free radical, that a whole electron was transferred in the ground state, the transfer being catalyzed by the SH of glutathione. If methylglyoxal, the acceptor, was replaced in the above experiment by glyoxal, the results were similar. In a way they were even more striking because in absence of SH there was no visible reaction at all, not even if incubated. The color developed in presence of SH-glutathione was initially yellow, then turned brown. It pH optimum was again at 7.4. Owing to the binding of amino groups during the experiment the pH decreased and the solution took on a blackish tint which indicated delocalized electrons.

The contents of this section could thus be summed up by saying that SH-glutathione catalyses the transfer of electrons between amines glyoxal derivatives. SH-glutathione is one of the most widely spread tissue constituents.

Primary and Secondary Reactions

C. Neuberg and M. Kobel noted in 1927 that methylglyoxal and related substances readily interacted with amino acids and guanidine. The first change was in optical rotation, followed by deepgoing reactions, accompanied by the liberation of CO_2, ammonia, and the formation of aldehydes. Methylglyoxal, or similar compounds can occur in the animal cell only in minimal concentration and so it seem unlikely that reactions should produce such deep going changes, as were observed by Neuberg and Kobel. It is only the primary reaction products or their immediate derivatives which are of interest for our problem.

The observations, presented in the foregoing section, could be interpreted by supposing that the primary reaction between diamine and methylglyoxal was the formation of the purple free radical which, being unstable, went over spontaneously into the poorly colored brown Schiff base. The color of our mixture depended on the relative rate of the formation and disappearance of the free radical. If its formation was fast and its disappearance slow, then its purple color dominated; if its formation was slow and its disappearance fast, no purple color could appear at all. What the SH-glutathione did was to catalyze the formation of the free radical which thus reached a concentration in which its color became visible.

These assumptions were borne out by interaction of methylglyoxal and ethylamine (used instead of the diamine). At acid pH, where the radical is more stable, there was no reaction, while at alkaline reaction the radical was too unstable and so only brown Schiff bases could be obtained even in presence of glutathione. In order to make the radical visible, it had to be stabilized, which could be done by lowering the temperature. To be able to work below 0° C, alcoholic solutions had to be used. The molar solution of methylglyoxal and ethylamine in 95% ethanol were cooled to –25° C, mixed and kept at this temperature overnight, then diluted with an 0.01 M aqueous citric acid solution. The resulting fluid had a vivid mahogany-red color. In the spectroscope it gave a curve with a high hump (Fig. 5A). A similar solution prepared at room temperature had only a brown color and gave a flat curve (Fig. 5B). The differential of the two curves gave in the spectroscope a sharp peak (Fig. 5C) which had all the earmarks of a charge transfer reaction. That this peak was the spectrum of a free radical was shown by Dr. Kon who found a strong spin resonance signal. This behavior indicated that the primary reaction between amine and carbonyl is a charge transfer in which a whole electron is transferred in the ground state.

Using croton aldehyde as acceptor, similar results were obtained, as shown by Fig. 6 which shows the spectroscope behavior of the products

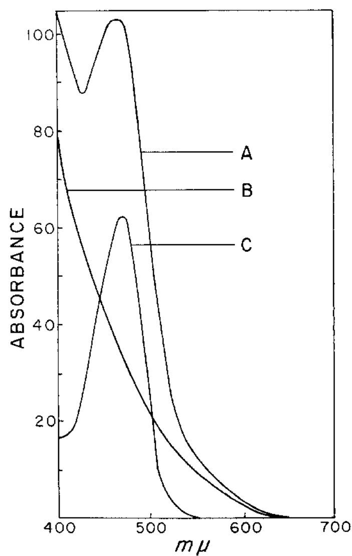


Figure 5. Absorption spectrum of the products of interaction of ethylamine and methylglyoxal. A: at –25°C. B: at room temperature. C: differential spectrum of A and B.

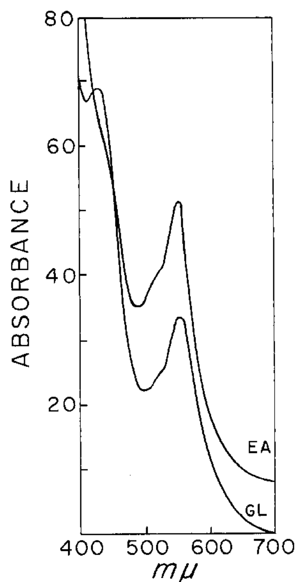


Figure 6. Absorption spectrum of products of interaction of croton aldehyde and glycine, croton aldehyde and ethylamine.

of the reaction of this aldehyde with ethylamine and glycine. In this case the mixtures were warmed in the water bath at pH 8.5.

On the whole, glyoxal and methylglyoxal reacts readily with amines, but react poorly with amino acids. The opposite tendency is observable with croton aldehyde. This difference in the reactivity might be taken as a suggestion that the two groups of acceptors serve different functions, the first being involved in fast changes connected with cell division, while the latter may be connected with more permanent changes, as changes in conductivity. The participation of glyoxals in rapid changes is also suggested by the existence of glyoxalase which is one of the most active known enzymes.

I found the reaction of croton aldehyde and glycine catalyzed by molecular oxygen and mentioned this observation in my paper at the annual meeting 1973 of the National Academy of Sciences. Later I found a serious methodical error in these experiments and so want this observation to be disregarded.

Our real problem was whether proteins enter charge transfer reactions with carbonyls, and are capable of transferring whole electrons in the ground state. To decide this question 5% caseine was dissolved in phosphate buffer of pH 8. Then 0.1 M croton aldehyde was added and the solution placed in the boiling water bath for 3 min. It turned red brown (Fig. 7A). The electron spin resonance spectroscope of Dr. Kon again registered signals. At a more alkaline reaction (pH 11) only a flat brown color was obtained (Fig. 7B). The differential spectrum of the two gave a single sharp peak (Fig. 6C) due, evidently, to the free radical of caseine.

If the caseine solution was incubated with croton aldehyde at 37°C and pH 7.4, it assumed the brown color of the liver, supporting the assumption that the color of this organ is due to the formation of charge transfer complexes with carbonyls. Caseine incubated with glyoxal also showed similar color changes which were speeded up by SH-glutathione.

M. L. Tanzer reported lately an interaction of endogenous aldehydes in collagen, forming a variety of covalent cross links. If this interaction would involve charge transfer between aldehydic and amino groups, it could desaturate the energy bands and make the protein into a semiconductor without extraneous "acceptor impurities".

It seems possible that the described charge transfer interactions between carbonyls and amino groups represent a specific type of charge transfer in which the spins of the electrons are uncoupled without a dissociation into free radicals. For such a reaction the two electrons of the pair would have to be kept sufficiently far apart to be uncoupled. Possibly, the carbonyls discussed, with their conjugated double links, are specifically built so as to be able to do this. Such a charge transfer may demand smaller difference in the energy levels of the donating and

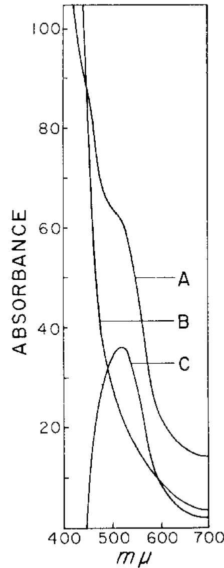


Figure 7. A: Absorption spectrum of caseine treated with croton aldehyde pH 8. B: same at pH 11. C: differential spectrum of A and B.

accepting orbitals as is the case in "strong" charge transfer which leads to free radicals. The complex thus formed would have no net electric charge and would not cause a perturbation of the electric balance of the cell.

Chemical Evolution and Cancer

The observations and considerations presented in the foregoing sections can be linked up to a simple theory. When oxygen appeared on our globe and living systems learned how to use it, the oxidation of metabolites produced carbonyls which acted as electron acceptors for protein, desaturating their energy bands and transforming them into *p*-type semiconductors. This made electronic mobility and the integration of the function of bigger molecular systems possible. The electric charges thus induced increased cohesions which made the building of complex structures possible and, at the same time suppressed proliferation which was inhibited also by the bulky machinery of biological oxidation.

Electronic delocalization leads to an increased light absorption. This gives a tentative explanation of the brown color of the organs (liver and kidney) involved in energy transformations.

If all this is correct one has to expect that rapidly growing tissues have less color than resting ones. To test this point two lobes, 2/3 of the liver

of a number of rats was cut out, and four days later the color of the regenerating part was compared with that of the resting liver, the two lobes which were cut out. As is generally known, rats can regenerate 2/3 of their liver in eight days. It was found, on inspection, that the regenerating liver was considerably lighter colored than the resting one.

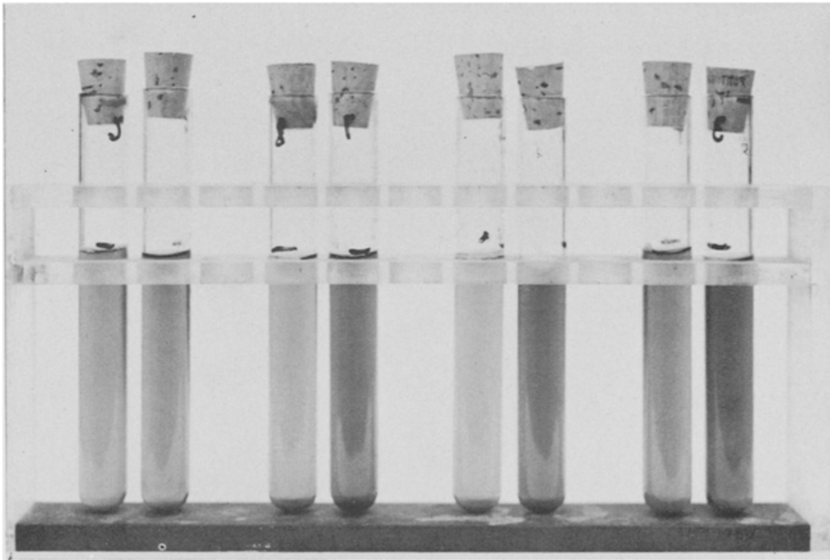


Figure 8. Left tube contains "washed" resting liver. Right tubes: analogous suspension of regenerating liver.

To exclude a role of blood the livers were blendored in an excess of distilled water, then the structure proteins were precipitated isoelectrically at pH 4.5, spun out and dissolved in 10% laurylsulfate (5 ml being used for 1 g of liver). In Fig. 8 the left side tubes contain the suspensions of the resting, the right hand tubes the suspensions of the regenerating livers. The latter are definitely lighter. The peak of regenerative activity is at the end of the first day after the operation (N. L. R. Bucher, Th. R. Schrock and F. L. Molten). Probably, at that time the color difference would have been still stronger.

Similar considerations hold for the rapidly growing parenchymal liver carcinomas. Professor George Weber of the University of Indiana, Indianapolis, kindly supplied a rapidly growing Morris Hepatoma 3924-A. The tumor was excised when reaching about 1″ in diameter. At this size the tumors are not necrotic and consist mostly of rapidly

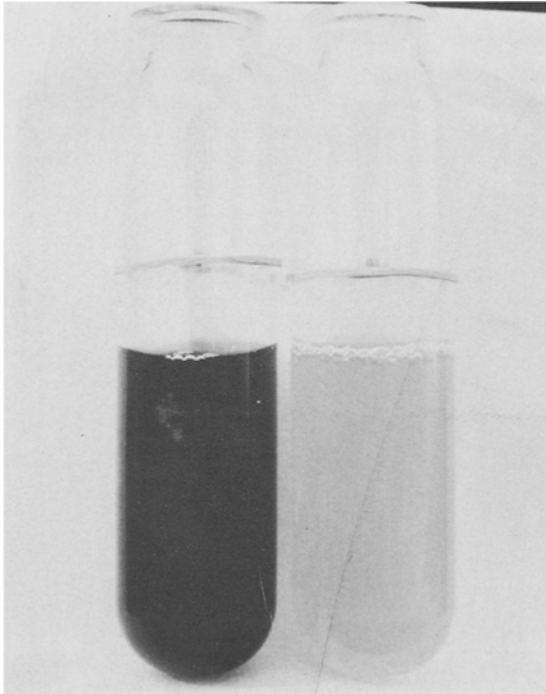


Figure 9. Right: "washed" suspension of rat liver. Left: washed suspension of parenchymal liver tumor of the same rat.

growing tissue. They were "washed" and then dissolved in laurylsulfate. The livers of the same animals were also excised and treated likewise. Figure 9 shows the resulting solution, the tube on the left containing the suspension of normal liver, the tube on the right that of the cancers.

The Brain

What has suggested semiconduction in proteins to the writer 30 years ago was the speed and subtlety of biological reactions which is the most striking in the brain. So if our assumptions were correct the brain had to contain a relatively great amount of a carbonyl acceptor. This made the brain into a "test case".

The human brain is thought to contain 30 billion nerve cells. If each of these is connected only with one centimeter of a nervous path this makes 30 billion centimeters which is the distance to the moon. In these terms the different centers of the brain are miles apart, and most

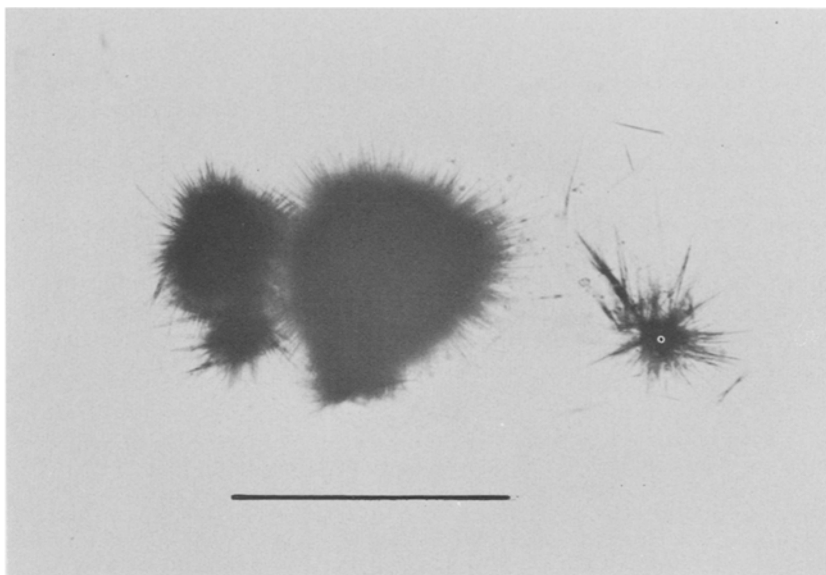
functions of the brain involve the simultaneous interactions of various centers. Such integration demands an exceedingly complex wiring diagram. The brain and its cells do contain a wonderful net of very thin fibers, neurofilaments or tubules, built of protein. Science ascribed to them only the role of "mechanical support". Should these fibrous structures be semiconductors, then they might be comparable to electric wires and could represent the wiring of this most wonderful of computers.

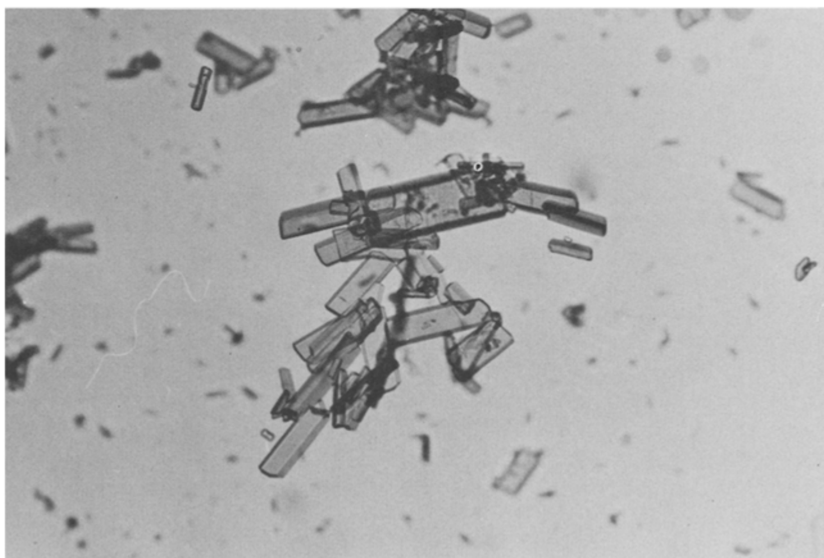
Blendored brain forms a white mass. If an acid solution of the yellow 2-4 dinitrophenylhydrazine is mixed in, the pulp becomes yellowish green. In an hour or so the color changes to canary yellow indicating the formation of a yellow hydrazone. Next morning the suspension is found brick red, due to the admixture of something intensely red. So far as I could see, this reaction is specific for the brain.

Calve's brains were blendored in two volumes of water, then 10 vol. % of a solution was added which consisted of 1 part concentrated HCl, 1 part methanol and 1 part of 10% of 2,4-dinitrophenylhydrazine, dissolved in dimethyl sulfoxide. This fluid was added in a thin jet to the brain homogenate under strong stirring. The suspension was stirred overnight, then an equal volume of ethylacetate was added and stirring continued for 1 h. The homogenate was centrifuged, the ethylacetate separated, shaken out with an aqueous $NaHCO_3$ solution which extracts most of the hemin present. The ethylacetate was distilled off under reduced pressure, the residue dissolved in methanol, left overnight at $0^\circ C$. The great mass of cholesterol crystallizing out was separated by filtration. The methanol was distilled off, the residue extracted with benzene. The insoluble part was dissolved in a small quantity of methanol and dichloromethane was added. On cooling the red dinitrophenylhydrazone crystallized mostly in the form of needles. It could be purified by chromatography on a silica gel column with benzene/pyridine (100 : 7 vol/vol). On thin layer chromatography it moved, under similar conditions, with an RF 30.

The carbonyl, forming the red hydrazone, is a substance of low molecular weight. The elementary analysis of the crystals indicates 6 C and 8 H atoms, one double bond and a carbonyl. Its hydrazone could be crystallized and recrystallized from chlorinated paraffins ($CHCl_3$ and CH_2Cl_2), mostly in the form of needles (Fig. 10 top). Sometimes it crystallizes in the form of exceedingly fine, apparently endless needles, fibers (Fig. 10 bottom). From phenol it crystallized in the form of brickshaped plates (Fig. 10 middle). Its crystallization in the form of very thin fibers may be a reflection of its possible biological function: association with the neurofilaments and tubules which thereby might be made conductant. Dissolved in ethylacetate, the dinitrophenylhydrazone gives in the visible a single sharp structureless peak with a maximum at 493 mu.

The carbonyl compound and its charge transfer with the brain proteins may underlie learning and memory, bringing the analogy





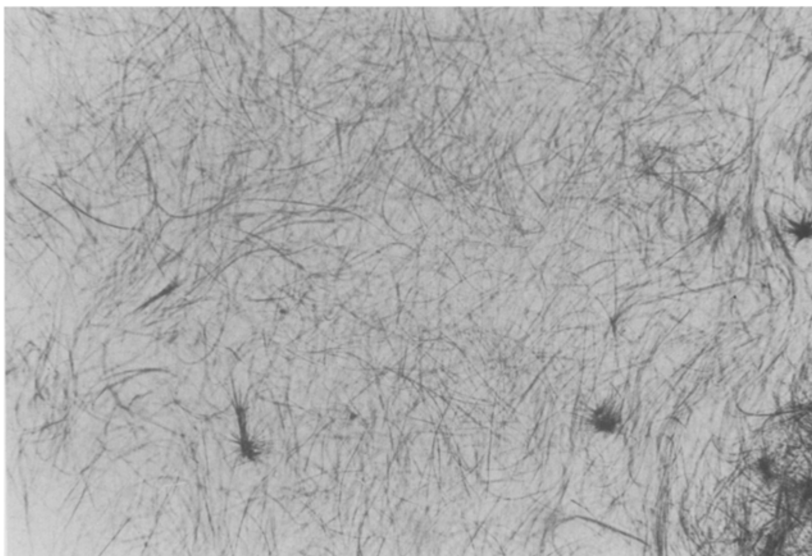


Figure 10. Crystals of 2,4-dinitrophenylhydrazone of carbonyl isolated from liver.

between the brain and a two digit computer still closer. Learning and memory may be due to the gradual development of orbital overlap, while the elementary process, underlying these processes may be the transfer of an electron.

It may be objected that according to our previous observations the brain should be colored owing to its delocalized electrons. The brain is actually colored, at least its "gray matter", gray being in fact the color which corresponds to an absorption in the whole visible range. The "Nucleus Ruber" may contain a reddish charge transfer complex.

Retine and Promine

Many years ago Jane McLaughlin and I happened to find that extracts of the thymus gland inhibited the growth of inoculated cancer. Thinking this action to be due to the unknown "thymus hormone" we set out to isolate the inhibitor. Later we found that this action was not specific, and was shown by liver extracts much stronger. This activity was described earlier by P. E. Herbut and W. H. Kremer [22]. We have spent a decade in futile efforts to isolate the inhibitor. Though being a low-molecular substance, it had properties which made isolation exceedingly difficult. It had no "chemical handle", no reactive group,

but all the same readily adhered to any precipitate. Some of our thymus extracts *promoted* growth and we called the underlying agent *promine,* in contrast to *retine* which *retained* growth.

Our extracts were prepared in the following way: beef livers were frozen as soon as possible after the death of the animal, then transported and kept in this frozen state. The livers were reduced to a snow which dropped into 3,5 volumes of methanol. Then 2.5 vols of water were added, containing 3.5 ml glacial acetic acid pro Kg liver. After a few hours storage the pulp was separated on a drum centrifuge and the fluid clarified on a Sharples supercentrifuge. Then the extract was concentrated under reduced pressure to 1 ml for every 10 g of liver. Further purification could be achieved by adding 2 vols of methanol, which precipitated inactive matter.

The existence of retine and promine can be deduced without experiment from everyday experience. If one cuts oneself, the cells on the side of the cut begin to proliferate and go on doing so till the gap is filled, and the wound is healed. Then growth stops as suddenly as it started. There must be thus in the tissue an "on" and an "off" switch for growth. Such switches mean, for the chemist, substances which have to be isolated and identified.

The behavior of cells in a wound brings out also another important point: the ability to proliferate is an attribute of life which can be activated wherever far going differentiation does not interfere with it. This proliferative ability must be controlled under normal conditions, by the "off" switch. This is important because cancer research has often been derailed by asking: "What makes the cancer cell multiply?" There is no point in asking this question because the ability to grow is innate, and the problem is not what makes cancer grow, but what has kept the cell at rest before, what was the brake which went wrong? Cancer is comparable to a car parked on a slope. If it starts to move one does not ask: "What is moving it", but asks, "What's wrong with the brake?".

During our futile efforts to isolate retine, Dr. Egyud found methylglyoxal in our retine preparations [15]. Later he also found glyoxal (unpubl.) and identified, with B. Andresen mesityloxide (unpubl.) to which I will come back later. So our attention turned to carbonyls. We found methylglyoxal to inhibit cell division in very low (0.001 M) concentration in the most varied material, like bacteria, fertilized sea urchin or frog cells, flagellates, germinating plants seed, normal and cancerous cells [16] and viruses [5]. Methylglyoxal inhibited protein synthesis [17] on the ribosome level [33]. The ketone aldehyde acted stronger on cancer than on normal cells and Shun-ichi Hata showed that its action was stronger than could be explained by its electron affinity. All these inhibitions were reversible, could be effected without causing damage.

Extracts of 1 g of liver, injected into tumor bearing mice twice daily,

caused a 50% reduction of tumor growth without causing any untoward side effects.

Our experiments consisted of inoculating a number of animals with a known number of ascites cells (Sarcoma 180 or Krebs 2). Mostly we implanted 10^7 under the skin behind the scapula on one side. Eight days later the animals were sacrificed, the tumors excised and weighed. The weight of the animals was checked daily. We assessed the activity by the usual T/C x 100 value (weight of tumor in treated/control x 100). The single sets consisted of 10 animals of 25 g weight. The substances studied were injected twice daily intraperitoneally in a volume of 0.25 ml.

It could be argued that if retine, present in the liver could inhibit growth in the whole animal, then it could certainly inhibit growth in the liver itself and be the brake responsible for keeping the cells at rest, be the "off" switch, while the "on" switch could be some system responsible for the inactivation of the retine. The "glyoxalase" could be such an "on switch", changing the reactive glyoxale derivatives into the corresponding inactive hydroxy acids, like lactate. Glyoxalase attacks also glyoxal.

Vegetable Defense Systems. Principles of Defense

Plants and animals are both but leaves of the same age-old tree of life and there is no basic difference between the two. However, there are considerable differences in their organization. The plants have no blood circulation and are protected by a cellulose cell wall. While this wall is undamaged, the cells are protected against bacterial invasion. Mechanical damage to the membrane throws the cell open to bacteria. Having no blood circulation, the plant must fight local infection locally, while the animal, with its blood circulation, can throw in the immune defense system of its whole body

The plant cell protects itself by harboring an inactive enzymic system which becomes activated by the damage. The activated enzymic system then produces carbonyls which, acting as electron acceptors arrest bacterial proliferation. I have hitherto studied three such defense systems [44]. The first was the phenoloxidase complex. Plants containing this system, like apples, bananas or potatoes, can easily be recognized by the dark coloration which marks the damaged part. This coloration is due to the formation of quinones, aromatic carbonyls, which form dark charge transfer complexes with proteins, kill the bacteria and produce a protective sheet over the wound. My second study on this line was concerned with the "peroxidase plants", characterized by the presence of an active perioxidase. The isolation of ascorbic acid was the byproduct of this study. Mechanical damage in these plants, destroys

hydrogen activation which then, no longer reduces the dehydroascorbic acid, produced by the ascorbic acid oxidase. The final result is that the damaged part is left with unreduced dehydroascorbic acid, a dicarbonyl, a diketone. The third such oxidative system was discovered by I. Banga and myself: the system of the dihydroxymaleic acid oxidase, studied more in detail later by I. Banga and E. Philippot, and L. Marsart in my laboratory in Liège, Belgium, and I. Robezieks, at Szeged. This leaves the damaged plant with diketosuccinic acid.

If the animal body is damaged mechanically and a wound is made, cell division is activated, fills, and heals the wound. Cell division in this case also acts as a defense mechanism, activated by the damage. Bacterial infection is taken care of by the immunological system. What the cells have to suppress is not the proliferation of bacteria but their own proliferation when no proliferation is needed. This suppression is again done by carbonyls. Proliferation is released by inactivation of these carbonyls. In the resting state the enzymes responsible for this inactivation have to be kept separated from the carbonyls. Damage brings them together and starts cell division by having the carbonyls destroyed.

The general principle of defense, which transpires from these observations, is this: cells contain enzymic systems, the function of which is such that it protects the cell against the damage or its consequences. In the resting cell these enzymic systems are kept in an inactive state and are activated by the damage against which they have to protect. Sunshine, for instance, damages our skin, causes sunburn. The damage activates the tyrosinase system which produces pigments. The pigments, then, protect us against the sunshine. If we suffer a wound and bleed, the damage activates an enzymic system which produces fibrine. The fibrine then plugs the leaky blood vessels. In phenoloxydase plants the invading bacteria activate a phenoloxydase which produces quinones which then kill the bacteria.

How these enzymic systems are kept in an inactive state in the undamaged cell is not fully known. The simplest assumption is that the enzymes and thier substrate are kept separated. In some systems the enzyme is kept in inactive state and is activated by a third substance. In this case the enzyme and its activator have to be kept separated and it is the activator which is released by the damage. In any case, inactivity means separation, be it the enzyme and its substrate, or proenzyme and its activator which are kept separated. The separation may be achieved by binding the two substances to different loci, or else by enclosing them in fragile vesicles which are broken by the damage. Whatever the case may be, separation of two substances within the very narrow limits of the cell demanands a very high degree of order which can easily be disturbed. Damage, essentially, means disorder, increase in entropy.

If "retine" is glyoxal or a glyoxal derivative, then the enzyme which

starts proliferation could be the glyoxalase which transforms the carbonyls into inactive hydroxyacids. As shown by L. Egyud [15], animal cells do contain glyoxal and methylglyoxal. They contain also glyoxalase so when no proliferation is needed, these two, the glyoxalase and the glyoxal derivatives must be kept separated, and damage could induce proliferation by creating disorder and letting them meet. This could explain the biological role of glyoxalase which has been a mystery. These assumptions are in agreement with the work of N. M. Alexander and J. L. Boyer [1], who found an increased glyoxalase activity in the regenerating rat liver (quoted from manuscript). Our countless failures to produce tissue extracts capable of arresting cell division, may have been due, partly, to a release of glyoxalase, which then destroyed the glyoxals.

Regulations

The glyoxalase system is not the only mechanism by which carbonyls can be inactivated. The reactive aldehydic C = O can be transformed into the relatively inactive ketonic C = O, which gives no charge transfer with amino nitrogen, by methylation. As shown by G. L. Cantoni [11], the main methyl donor in the animal cell is a complex of mathionine and adenylic acid. J. B. Lombardi, A. W. Caulter and P. Talalay [29] have shown that substances which inhibit the formation of this methionine complex have a carcinostatic action. This suggests strongly that methylation is also involved in the inactivation of inhibitors of cell division. Pteridines may also play a role in methylation, explaining the cancerostatic action of folic acid antimetabolites. E. Tyihak and E. Patty [46] also suggest a role for trimethyllysine and dimethylarginine. Methylation is widely used by nature for the inactivation of cell constituents. Many years ago I found methylation to be used in citrus fruits for the inactivation of the highly reactive flavone Eryodictiol, which is kept inactive in the unripe fruit methylated to hesperidine.

The failure of different parts of the regulatory system may promote proliferation and be involved in carcinogenesis, and may demand different means for their correction, giving support to the contention of F. E. Knox [24] that the adequate inhibitor should be found where chemotherapy is resorted to.

Proliferation may be called in at short notice, which may be reflected in the enormous activity of the glyoxalase. Methylating enzymes work slower, and may influence carbonyls involved in slower changes, as the production of conductivity. Conductivity plays an important role in the physical state and function of the whole system and so also has an influence on cell division.

It seems worthwhile to pause for an instant at mesityloxide found in our retine preparations by L. Egyud [15] and identified by B. Andresen

and L. Egyud (unpubl.). Mesityloxide (Fig. 11) is a croton aldehyde methylated at C1 and 3. The methylation at C_1 may serve for inactivation, while the methylation at C_3 may serve to eliminate the strong tendency of this C atom for additions. This C_3 being activated by the C = O group, easily enters into additions, and addition can make molecules toxic. S. Nelson, at the laboratory of Professor Max Tishler, at Wesleyan University, Middletown, Connecticut, kindly synthetized for me 3-methyl croton aldehyde. It shared most reactions with croton aldehyde but was considerably less toxic.

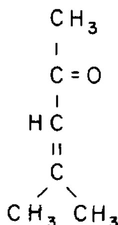


Figure 11. 3-methylcrotonaldehyde.

Damage is not the only factor which can elicit rapid cell proliferation, as shown by the regenerating rat liver. In this case it cannot be the damage, the cut, which elicits proliferation, since it is the cells in the undamaged lobe which proliferate. What elicits proliferation in this case must be the disturbance caused by a suddenly increased functional demand on the liver, 2/3 of which have been removed. There are thus various factors which can elicit proliferation in one and the same organ. It speaks by itself that the proliferation, in different organs, which have different functions, may be elicited by entirely different influences. The production of red blood corpuscles can, for instance, be speeded up by a decrease in oxygen pressure by a high altitude, while the production of white blood corpuscles may be elicited by foreign proteins. It is rather fortunate that different systems depend on different impulses, otherwise it would be impossible to arrest the proliferation of cancer cells without killing the host by suppressing its hemopoietic apparatus.

If damage, essentially, means disorder, and it is disorder which elicits proliferation, then permanent damage must elicit permanent proliferation, that is, produce cancer.

Disorder is very unspecific and can be induced by an infinite variety of means (including viruses), which has led to the erroneous conclusion that cancer is not one disease, but many, as many as there are ways to produce it. Certainly, there are many roads to Rome but they all may have to pass through the same gate.

Masking

If the cell is kept at rest by carbonyls, then tissue extracts have to contain carbonyls, and carbonyls, as a rule, give an immediate precipitate with 2,4-dinitrophenylhydrazine at a strongly acid reaction. As found by Dr. Egyud and the writer, tissue extract do not give such a precipitate. They give it only after a few hours' incubation, after time was given to the acid to hydrolyse off a substance which has covered up the aldehydic group. This is important from a preparative point of view but may be important also biologically. The latentiating group may fulfill different functions, as protecting glyoxal derivatives against the glyoxalase. It may also lend specific affinities to the aldehyde. It brings also new enzymes into the regulatory system. It is possible that the masked substance acts as such, but it is more likely that the masking group has to be split off, the CO liberated where it can react with the protein. This would bring into the picture a hydrolaze. It is equally possible that the carbonyl is transferred by a "transferase" from its masking group to the protein, without being hydrolyzed first. In any case, this masking gives new problems for research. The nature of the masking group has to be established, and the enzymes involved in its production and handling have to be defined. The masking of CO can also explain much of the difficulties of the isolation of retine

The great reactivity of carbonyls, which have extended π electronic systems, exclude the possibility to their being stored by the cell in free condition. With a masked CO they can be stored. The liver is especially rich in carbonyls, which suggests that they may be produced by the liver.

These relations also add new factors to the possible mechanism of cancerogenesis. The lack of the enzymes which produce the masked carbonyl, liberate the carbonyl, or transfer and attach it to the protein, all may cause continued proliferation.

It seems possible that, in a way, carcinogenesis is autocatalytic. A temporary lack of oxygen, for instance, will arrest oxidation and if there is no oxidation no carbonyls will be formed. The lack of carbonyls on its turn may cut down on semiconductivity, and the failure of semiconduction may further cut down on oxidation and the production of carbonyls, driving the cell towards the fermentative-proliferative state which, eventually, may become stabilized, constitutive. Similarly, a permanent inactivation of carbonyls, be it by enzymes as the glyoxalase, or by substances, like α-naphthol (see later), may interfere with the resting oxidative state and drive the cell into the fermentative-proliferative state. Fortunately, changes may be autocatalytic both ways, and an increase in the concentration of carbonyls, introduced from the outside, may start up changes in the opposite direction and help the cell return to normal.

Cancer, Cure and Prevention

The contents of the previous sections suggest that cancer, essentially, is disorder. This disorder declares itself already in the microscopic dimension by the heteromorphous nature of the cancer cell. Microscopic disorder is the result of molecular disorder The spin echo studies of Damadiam indicate that even the water of the cancer cell is disordered, being bound less by the protein. It is easy to find connection between disorder and proliferation. It is believable, for instance, that as the protein cannot bind water, so it cannot fix the glyoxalase, which then inactivates the carbonyls responsible for keeping the cells at rest. Should the glyoxalase turn out to be the culprit, then the cancerostatic activity of octylglutathione [47] would demand a thorough study. It interferes with the activity of glutathione as coenzyme of the glyoxalase.

Where the ultimate source of this disorder is, has to be established. Since cancer cell generates cancer cell, it seems likely that the root of cellular disorder is in nucleic acid, in a deletion nucleotides or the disturbance of sequences. This, then, may produce disorder in cell body, a deletion of enzymes, or a deletion and disorder of amino acids. To use Pauling's nomenclature, the cell, to be normal, has to be "orthomolecular". To be "orthoelectronic" it has to be orthomolecular. Whatever the case may be, to be able to control cell division and control cancer we need a detailed knowledge of the whole regulatory mechanism.

We cannot replace enzymes, but can replace their products. We cannot make the diabetic pancreas produce insulin, but can put things straight by introducing insulin. Similarly, a failure to produce carbonyls could be corrected by introducing them. Naturally, to be able to do this, we would need a detailed knowledge of these carbonyls, and have them in sufficient quantity at our disposal – which is not out of reach.

This suggests a new philosophy for cancer therapy. The cancer cell hitherto, was looked upon as a hostile cell which had to be destroyed. To destroy it we used unspecific noxious agents as high energy radiation, or poisons of basic biological processes making it a gamble which will suffer most, the cancer or the patient. The cancer cell is not a hostile cell. It is a sick cell which has to be helped to return to normal, or, at least, allowed to wither away. To do this we can use normal cell constituents which do no harm to the patient.

Having no command yet over the natural growth regulators, but having some indication about their chemical nature, we can, at present, synthetize compounds which share some of the properties with the natural inhibitors. Our first attempts on this line were rather encouraging. Dr. L. Egyud synthetized carbonyls with masked carbonyl groups and subjected them to the National Screening Program which found some of them active as carcinostatic agents. I, myself, synthetized

an ethylamin derivative of methylglyoxal and tested it for its ability to prevent spontaneous cancer in C3H mice, obtained from the Jackson Laboraatory, at Bar Harbor. A group of 100 "retired breeders" was divided into two groups of 50, the one of which received pure water for a drink, while the other received a freshly prepared 1/30 molar solution of the synthetic compound. The results are shown in Fig. 12 in which crosses mean spontaneous cancers. As the table shows, no cancer was observed during the first 16 weeks of the experiment in the treated mice, while 11 cancers were developed in the control groups. After this time, results became mixed and the experiment collapsed owing to the senile deterioration of the animals. Since application *per os* does not cause major inconvenience, this experiment indicates that it may be possible to prevent cancer and eliminate it altogether. No doubt, these results can greatly be improved. The ethylamine complex, in its free radical form, is too unstable to be useful.

Weeks	1-4	4-8	8-12	12-16	16-20
Treated	0	0	0	0	xx
Untreated	xxxx	xx	xx	xxx	x

Figure 12. Top: incidence of spontaneous tumor in mice treated with glyoxal. Bottom: incidence of spontaneous cancer in untreated mice. See text.

I have found that the inhibition of inoculated cancer depends on the size of the inoculum. The results, extrapolated, indicate that it should be possible to inhibit one cell, or a few, by 100%. This may be important because cancer or a metastasis starts with one cell, or a few only and so it should be possible to prevent its development. Bigger inocula or more developed cancer can be cured by methylglyoxal but only if the drug is injected into the tumor [2, 18] which excludes a wider medical application.

In many ways conditions for therapy are more favorable in man than in mice. One factor which has to be kept in mind is time relations. The substances in question have a small molecular size and can be excreted rapidly. If the drug injected is excreted within three hours and two injections are given daily, the mouse will be protected for 6 h and left unprotected for 18 h, during which time the cancer can rejuvenate itself by dividing. A constant concentration in the blood is too difficult to maintain in a great number of small experimental animals. There is no difficulty on this account in the human.

The carcinostatic action of keto-aldehydes has been known for a long time [20]. A ketoaldehyde (Kethoxal of Upjohn & Company) is on the market as a cancerostatic agent. Unsaturated aldehydes with strong cancerostatic action have been produced also by the autooxidation of highly unsaturated fatty acids by E. Schauenstein and his associates.

What was not known is that these substances inhibit cancer because they reflect reactions involved in the normal regulations of cell division. The possible importance of carbonyls in regulation and carcinostatis was emphasized by W. F. Koch. A narrow relation between cancer and electronic changes was clearly suggested by the discovery of the K region of aromatic carcinogens. It is believable that these carcinogens cause cancer by interfering with electronic conductivity being bound to semiconductors and saturating their electron deficient energy bands with the electron density of their K region. The involvement of electronic changes and glyoxals in carcinogenesis was also suggested by the carcinogenic property of α naphthol, which gives highly colored, yellow-green charge transfer complexes with glyoxal and methylglyoxal, while the inactive β naphthol fails to do so. α naphthol may cause cancer by complexing with "retine" and inactivating it.

Conclusion

The cancer problem is finite and solvable. What is needed for its solution is imagination, the high spirit of inquiry, and the wonderful new tools of research. It is not solvable by the combined arrogance and ignorance of politicians now trying to command science, nor is it solvable by bureaucratic dullness.

In the present paper a wide alley is opened for an attack on cancer. It is made possible to pinpoint and correct the disturbance. The road is marked out: it leads over extensive basic studies, a thorough study of all the components of the regulatory system. There are shortcuts only to failure.

The progress of science is marked by the penetration into increasingly small dimensions. From macroscopic anatomy biology moved to microscopy, from light microscopy to electron microscopy. At present it is dominated by the molecular concept. The next natural step will be a penetration into the electronic dimension. Cancer, partly, is a disturbance in the electronic dimension.

Progress in the art of healing has always been induced by progress in basic knowledge. The present regrettable emphasis on applied research, at the expense of basic research, and the search for a cure before an understanding, can only delay the final solution. Our inability to cope with diseases like cancer indicates major gaps in our basic knowledge which have to be filled before progress can be made.

The carbonyls with extended π systems may be the key not only to cancer but also to other "degenerative diseases", like schizophrenia, muscular dystrophy and various pathological conditions of the heart muscle. These substances represent a specific field of research which demands, for its exploration, specific experience. The substances in question cannot be isolated and identified without use of the most

modern analytical methods, and the idea must be accepted that the days of "string and sealing wax" are past also in biochemistry. It is for society, or rather its government, to decide whether it wants to meet the expense of having these diseases eliminated. The writer is paralysed by the lack of means, his last three applications having been rejected by the main granting agency in biomedical research, the National Health Institutes. However expensive research on these lines may be its cost is negligible, as compared to the sums spent on instruments of killing and destruction. The present research could be brought to fruition by the means spent on the training of the hundreds of S. Vietnamese pilots, trained by this country in contempt of all human values.

References

1. N. M. Alexander and J. L. Boyer, *Analytical Biochemistry*, 1971.
2. M. A. Apple and D. M. Greenberg, *Cancer Therapy Rep.* 51 (1967) 455, and 52 (1968) 687.
3. I. Banga and A. Szent-Györgyi. *Hoppe Seylers Z.f Physiol. Chem.*, 255 (1938) 57.
4. I. Banga and E. Philippot, *Hoppe Seylers Z.f. Physiol. Chem.*, 258 (1939) 147.
5. M. Baylor and L. Egyud, *Virology*, 31 (1967) 380.
6. J. B. Birks and M. A. Slifkin, *Nature*, 197 (1963) 42.
7. J. B. Birks and M. A. Slifkin, *Ibid.*, 197 (1963) 42.
8. I. Bobeznieks, *Hoppe Seylers Z.f. Physiol. Chem.* 255 (1938).
9. L. Brillouin, *Giant Molecules and Semiconductors.* In: *Horizons of Biochemistry*, M. Kasha and B. Pullman (eds), 1962, Academic Press, New York.
10. N. L. R. Bucher, Th. R. Schrock, F. L. Moolten, *Johns Hopkins Journal of Medicine*, 125 (1969) 250.
11. G. L. Cantoni, *J. Biol. Chem.*, 189 (1951), 203 and 745; 204 (1953) 403; G. L. Cantoni and J. Durell, *J. Biol. Chem.*, 225 (1956) 1033.
12. H. G. Crabtree, *Biochem. J.*, 23 (1929) 536.
13. H. D. Dakin and H. V. Dudley, *J. Biol. Chem.*, 14 (1913) 155.
14. R. Damadian, *Science*, 171 (1971) 1151.
15. L. Egyud, *Biochem. J.*, 96 (1965) 19c-20c.
16. L. Egyud and A. Szent-Györgyi, *Proc. Natl. Acad. Sci.*, 55 (1966a) 388.
17. L. Egyud and A. Szent-Györgyi, *Proc. Natl. Acad. Sci.*, 56 (1966b) 203.
18. L. Egyud and A. Szent-Györgyi, *Science*, 160 (1968) 1140.
19. M. G. Evans and J. Gergely, *Biochem. Biophys. Acta*, 3, (1949) 188.
20. F. A. French and B. L. Freelander, *Cancer Research*, 18 (1958) 172.
21. Shun-ichi Hata, *Bioenergetics*, 1 (1970) 325.
22. P. E. Herbut and W. H. Kremer, *Am. J. Pathol.*, 36 (1960) 105.
23. J. E. Kimura and A. Szent-Györgyi, *Proc. Natl. Acad. Sci.*, 62 (1969) 286.
24. F. E. Knox, *Anticancer Agents*, Ch. C. Thomas, 1967, Springfield.
25. F. E. Knox, R. M. Galt, Y. T. Oester and R. Sylvester, *Oncology*, 26 (1972) 515.
26. W. F. Koch, *The Survival Factor in Neoplastic and Viral Disease*, 1958.
27. H. Kon and A. Szent-Györgyi, *Proc. Natl. Acad. Sci.*, 70 (1973) 1030.
27a J. Ladik, *Nature*, 202 (1964) 1208.
28. K. Laki, *Studies. Inst. Med. Chem. Univ. Szeged*, 2 (1942) 43.

29. J. B. Lombardini, A. W. Caulter and P. Talalay, *Molecular Pharmacology*, 6 (1970) 481.
30. J. B. Lombardini and P. Talalay, *Advances in Enzyme Regulations*, 9 (1971) 349.
31. L. Massart, *Hoppe Seylers Z.f. Physiol. Chem.*, **258** (1939) 190.
32. C. Neuberg, *Biochem. Z.*, **49** (1913) 502.
32a. C. Neuberg and M. Kobel, *Biochem. Z.*, **185** (1927) 477, and **188** (1927) 197.
33. H. Otsuka and L. Egyud, *Currents in Mod. Biol.*, 2 (1968) 106.
34. L. Pasteur, *Compt. Rend. Acad. Sci.*, 52 (1861) 1260; **80** (1875) 452.
35. L. Pauling, *Science*, **160** (1968) 265.
35a. A. Pullman, *Hydrogen Bonding and Energy Bands in Proteins, Modern Quantumchemistry*, Part 3, 283, Academic Press, New York (1965).
36. B. Pullman and A. Pullman, *Quantum Biochemistry*, Interscience Publishers (1963).
37. E. Schauenstein, W. Wohl and I. Kramer, *Z. Naturforsch.*, 23-**b** (1968) 530.
38. E. Schauenstein, B. Wunschmann and H. Esterbaur, *Z. Krebsforsch.*, **71** (1968) 21, and 72 (1969) 325.
39. M. A. Slifkin, *Nature*, **195** (1962) 693.
40. A. Szent-Györgyi, *Oxidation, Fermentation, Vitamins, Health and Disease*, Williams and Wilkins Co., New York (1939).
41. A. Szent-Györgyi, *Nature*, **148** (1941) 157.
42. A. Szent-Györgyi, *Proc. Natl. Acad. Sci.*, **58** (1967) 2012.
43. A. Szent-Györgyi and J. McLaughlin, *Proc. Natl. Acad. Sci.*, **69** (1972) 3510.
44. A. Szent-Györgyi, *The Living State*, Academic Press, New York (1972).
45. M. L. Tanzer, *Science*, **180** (1973) 561.
46. E. Tyihák and A. Patthy. Quoted from their manuscript.
47. R. Vince and W. B. Wadd, *Biochem. Biophys. Res. Comms.*, 35 (1969) 593.
48. O. Warburg, *The Prime Cause and Prevention of Cancer*, Lecture at the meeting of Nobel Laureates, June 30, 1966, at Lindau, K. Tiltsch, Würzburg (English by Dean Burk).

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