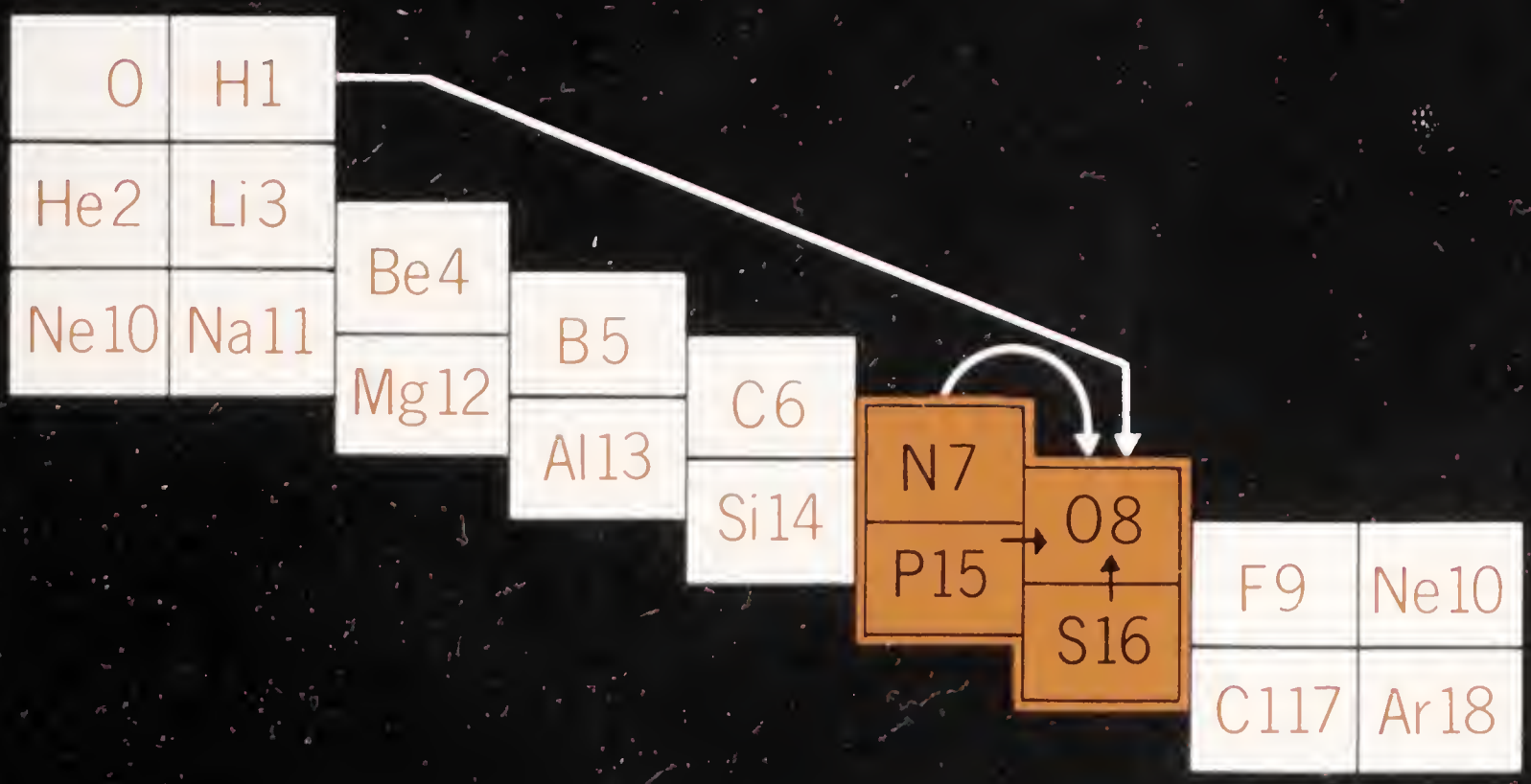
ELECTRONIC BIOLOGY AND CANCER

A New Theory of Cancer



ALBERT SZENT-GYÖRGYI

NOBEL LAUREATE

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A New Theory of Cancer

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Dedicated to Miss Louise Crane
Without Whose Support
This Book Could Not Have Been Written

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My thanks are due to Miss Jane A. McLaughlin, who, for a quarter of a century, shared all my labors and helped to get their results into print.

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ELECTRONIC BIOLOGY AND CANCER

Part I

BASIC CONCEPTS AND REGULATIONS

1

INTRODUCTION

Biology, at present, is a molecular science. Our body is built of molecules, mainly protein macromolecules, and so it seems logical to believe that all biological reactions have to be molecular, making life into a molecular phenomenon. The charm of biology is in the wonderful subtlety of its reactions, and I could never believe that they could be brought about by clumsy macromolecules without the participation of much smaller, mobile and responsive units which could be hardly anything else than delocalized and highly reactive electrons. That there was a major gap in our basic knowledge, that our whole outlook on life may be defective, was suggested to me also by the complete stagnation of research into the nature of cancer and the other degenerative diseases. We may have been "fishing behind the net," and the macromolecules may have been more the stage than the actors of the drama of life. The first step towards the solution of the "cancer problem" had to be the filling of this gap. As Bernal told us, we can control only what we understand.

When I gave expression to these ideas (A. Szent-Györgyi, 1941), which I developed partly in discussions with my young friend and pupil K. Laki, I met general opposition and was told that highly reactive and delocalized electrons would react with photons, and make the proteins colored or opaque, while the

great number of isolated and thoroughly studied proteins was found to be colorless and transparent. This was certainly a powerful argument and little could be said against it; it made, for me, a puzzle out of the problem, and puzzles have to be solved. I found the solution to this one: it is simple and amusing. It is in the fact that, grossly speaking, proteins can be divided into two groups: proteins, present in our body dissolved, in molecular dispersion, and proteins which are linked together to structures which, by definition, are insoluble. The soluble ones perform the simple *vegetative* functions, like fermentation, which need no specially reactive or mobile electrons, and perform molecular reactions. Contrary to this, all the more complex *animal* functions are performed by structures, that is, molecules linked to complex systems, and transform chemical energy into some sort of work, be this work mechanic (muscle), osmotic (secretions), or electric (nervous activity). It is these structures which demand mobile and highly reactive electrons.

The chemist needs crystals; the protein chemist needs protein crystals, and to produce them he needs protein solutions. So what he did was to press out or extract the soluble proteins of biological systems, and called the extracted material *the residue* and sent it down the drain, sending down with it the structures with their highly reactive and mobile electrons.

So when embarking with Jane McLaughlin on this line of research we went in the opposite direction: extracted tissue (liver) and sent the extract down the drain, then we dissolved the "residue" with the help of detergents* to see whether it was

*The organs were blendored in 20 vol distilled water. This treatment hemolyzed the blood and washed out the hemoglobin. The suspension was brought to pH 4.6 and centrifuged. The precipitate was "washed," being resuspended in water and centrifuged. The washed precipitate was suspended in 20% sodium lauryl sulfate.

Figure 1 A suspension of structural proteins of beef liver.

really transparent and colorless. It had the transparency and color of a Swiss Chocolate (Fig. 1). The color of structural protein indicated highly reactive electrons and solid state phenomena.

There can be no doubt that the molecular concept contributed enormously to the clarification of our ideas, building a solid foundation for biology, but what may be a stimulus at one time may become an obstacle later. This may be the situation with our molecular outlook. Blinded by successes of the molecular concept, biology may have lost sight of one of its most basic principles, which is *organization.* "Organization" means that things can be put together in two different ways: at random, and meaningfully. Things put together at random form a "heap" while things put together meaningfully may generate something entirely new. It is the difference between a Greek temple and a heap of stones and bricks out of which it may have been built. This is true for the whole gamut of complexity. If electrons and nuclei are put together meaningfully, an atom is born. If atoms are put together meaningfully, an amino acid may result. If amino acids are linked together meaningfully, a protein molecule may be formed, which

no longer is a group of amino acids but a chain of peptide bonds. A meaningful folding of this peptide chain brings certain atomic groups close to one another, which endows the resulting globule with the catalytic ability to make and break bonds, and in so doing use other molecules as food, rearranging their structure in a way which releases part of their free energy and negative entropy, which the protein molecule can use for its own maintenance. S. W. Fox and K. Dose (1972) showed that large molecules, as complex as those of proteins, and having several of the same biological activities, could form spontaneously.

The process of rearranging the structure of foodstuff molecules is called "fermentation," which is a simple and inefficient process which leaves the greatest part of the energy behind in the products of fermentation. This process, as shown by E. Buchner (1897), can be performed by a system of soluble molecules.

Biology forgot to ask what new qualities are born when single protein molecules are linked together to increasingly complex structures with increasingly complex functions. A "structure" is not a "heap" and by definition "structures" are insoluble. They underlie our individuality and are the instruments of all our "higher" biological functions, like thinking, motion, secretions, reflexes, etc. If I would be put into a hydraulic press which would press out all my juices, then I would be decomposed into two fractions, the "press juice" containing all my soluble molecules, and the "residue" containing all my structures. If it were possible to put the two fractions together again, then the exchange of my juices with those of a young girl would make no difference; but if the two residues were exchanged, then I would become a young girl and she an old man. Individuality is linked to structure.

What I am driving at is that we have to distinguish between two sets of protein macromolecules, the one being the set of soluble molecules, present in molecular dispersion and performing simple "vegetative" functions, like fermentation, the other being that of molecules building structures which perform all the "higher" functions which make us into what we are. So it is legitimate to ask what new qualities are developed when Nature puts molecules together to form structures. What are the connections between the molecules of the structure? What are the instruments of their integration? These must, evidently, be much smaller and more mobile than the clumsy protein macromolecules themselves, which depend for their reactions on high activation energies. It was this last step of organization to which I tried to call attention in my three booklets, *Bioenergetics* (1957), *Introduction to a Submolecular Biology* (1960), and *The Living State* (1972a).

When life originated some three billion years ago, our globe must have been a very unpleasant place, hot and pitch dark, being surrounded by a heavy layer of water vapor. There was no light and no oxygen. We can only philosophize that under those conditions life could have built only the simplest forms, which, to make life continuous, had to proliferate as fast as conditions permitted. The protein molecules formed must have been rather stable, with no loose ends or unbalanced forces. They had to be "closed-shell molecules" with their electrons arranged in pairs. There must have been a strongly reducing atmosphere containing chiefly electron donors, but no electron acceptors. Among the donating groups the strongly reducing SH must have played an important role, involved in the process of proliferation. We can expect that under those conditions the electronic energy bands were saturated, nonconductant, the protein dielectric. This stage

of biological organization I termed the "α state." At this stage the main functions of living systems must have been fermentation and proliferation. Neither of these demands structure or specific electronic qualities. Proliferation had to be favored by the absence of structures and the simplicity of design.

As our globe cooled and the surrounding water vapor condensed, eventually, red light of long wavelength could reach the surface of the earth, whereupon life developed a green dyestuff which could capture the red photons, and still makes our meadows green. With the energy of the captured photons the living systems separated the elements of water, fixing the H to carbon, creating foodstuffs, while releasing the oxygen as O_2 into the atmosphere. Oxygen is an oxidizing agent, an electron acceptor, which could induce profound changes in the nature of the protein by separating its electron pairs, making highly reactive free radicals out of its inert closed-shell molecules. It could desaturate the energy bands, thus making semiconductors out of dielectrics, creating unbalanced forces which could link protein molecules together to increasing complex structures which performed increasingly complex and subtle reactions, leading to differentiation and to a new state of the living systems which I called the "β state" to distinguish it from the α state which preceded the appearance of oxygen.

Before going further on this line I was looking out for an experimental evidence that electron acceptors can actually change the properties of proteins, and also be responsible for the color of the structure proteins. For protein I chose casein while for acceptor I used croton aldehyde, a monovalent electron acceptor, capable of accepting single electrons.

Adding 0.1 M croton aldehyde to my 5% casein solution, adjusted to pH 7.4, and leaving the solution on my bench, next morning I found the pure casein solution unchanged, while the

casein, containing the aldehyde, assumed the brown color of liver, and gave a signal in Dr. H. Kon's electron spin resonance (ESR) spectroscope, was thus a free radical, or a biradical formed by the enal and the protein. Whole electrons had thus to be transferred from the protein on to the enal in the ground state, forming stable free radicals. This simple experiment showed that a monovalent acceptor can make proteins into colored free radicals, inducing profound changes in the nature of protein that may have been responsible for the evolutionary changes following the appearance of light and oxygen.

The unbridled proliferation of the α period was incompatible with the development of complex structures. To maintain the harmony of the whole it had to be arrested and regulated. But even without regulation the semisolid structures must have interferred with cell division, which involves a complete rearrangement of the cellular interior, and demands a more liquid state. To be able to divide, the cell has to dismount its structures to a great extent, dismounting first the most bulky structure, the nucleus, the membrane of which is dissolved and the chromatin condensed into a small number of mobile chromosomes. Also the oxidative mitochondria have to be disassembled, making the cell more dependent on fermentation for energy. All this means that the dividing cell has to dedifferentiate and return, to an extent, to the α state. After completed division the cell has to find its way back to the oxidative-resting β state, building up again its structures and electron transport chains. Should the cell find its road of return to the β state deranged, or should the β state be made unstable by some extraneous factor, then the cell would have to persist in the proliferative α state and tumor would result.

When Nature creates new mechanisms she does not throw the old ones away but builds the new ones on top. So the building

of solid structures in the β period did not mean that the system of soluble molecules was eliminated. It became the basis of metabolism and served as a matrix into which the new insoluble structures were embedded, and continued to perform its simple "vegetative function," fermentation, catering for the embedded structures which released the total energy of food, opening the way to the differentiation and evolution, the end product of which is us. The way to the understanding of these relations leads through the electronic dimension. When going into this dimension of biology I will be traveling on a road, paved by the work of B. and A. Pullman.

2

STABILITY

All physical systems tend toward the maximum of stability. We can also turn this statement around and say that the state of maximum stability is the one which physical systems tend to reach, which is the minimum of free energy and the maximum of entropy. Both matter and energy tend to randomize. In every chemical reaction a definite part of the energy of the system has to go into random heat agitation. The part of the energy, which can avoid this fate and is free to do work, is the "free energy." It is this trend to increase entropy and decrease free energy which drives chemical reactions. Once a system has reached the minimum of free energy and the maximum of entropy, all changes come to a halt. Entropy is randomness. Negative entropy, "negentropy," means pattern.

By establishing a specific pattern, animate (living) systems acquired the ability to increase their free energy and decrease their entropy at the expense of their surroundings. While they decrease the free energy and negentropy of their surroundings, they increase their own. The more free energy or negentrophy they have, the better they work and the better they can increase still further these quantities. The maximum of biological stability is at the maximum of free energy and negentropy. Biological stability is, in a way, the opposite of physical stability. The

further away from the physical stability a living system is, the more alive and stable it is. Death means that biological stability yielded to physical stability.

Physical and biological stability are the opposite of one another. This puts the living systems, so to say, between the devil and the deep blue sea, because life, in itself, does not exist. Nobody has even seen life, as such. What we can see are material systems which have this curious quality of being alive. While the living tends to strengthen its biological stability, the material system to which it is bound tends towards physical stability. Life is linked to a material pattern which always tends to deteriorate. This makes our life dependent on our ability to correct the damage done to our patterns. This ability of ours being limited, the span of life has to be limited too, our ability to correct, being always imperfect.

The accumulating defects would have to make an end to life altogether, should Nature not be able to make intermittently a fresh start, by creating, now and then, a new and perfect individual. Nature does this by fusing two individuals. If the damaged parts of the two do not overlap, and are eliminated, then a perfect new individual is generated. If faults of the two overlap, then an imperfect individual emerges which carries in it the seed of some "degenerative disease."

Creating a new individual by fusion is "proliferation." The trend to proliferate has thus to be an attribute of life, its strongest drive. Each cell has to have it. To preserve the harmony of the whole, this trend of the individual cells has to be suppressed and subjected to regulation, but every cell has to have the trend to break out from under this limitation and proliferate, produce cancer, killing eventually the whole individual. The trend to grow and multiply is one of the most basic qualities of life. So the first problem of cancer research is thus

not why cancer grows, but how the innate trend to proliferate can be kept suppressed. Cancer research has greatly been retarded by our asking why cancer grows, instead of asking what keeps a normal cell from growing. Our ability to correct defects depends on our knowledge of the regulatory system to which this book is devoted. In my next book I hope to be able to show how this knowledge can be used to arrest or prevent cancer.

3

CHEMICAL ENERGY

Our world is built of about 100 different elements. Mendeleef has arranged these in his periodic chart into a coherent system. The top rows of his chart are reproduced in Figure 2. Each horizontal row ends with a "noble gas" which also begins the next one. We call these gases "noble" because they do nothing—do not enter chemical reactions.

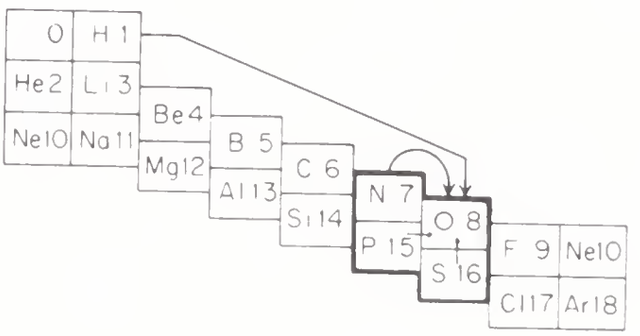

Figure 2 Top row of the periodic chart of elements.

Atoms share the human foible of wanting to belong to the nobility, or, at least, look like it. This they can do by having the same number of electrons as the nearest noble gas. Elements on the left side of the chart have more; those on the right side have less. And so the former tend to give off and the latter tend to take up electrons. If two such atoms interact, the former will pass an electron to the latter. An electron, given off

easily, has a high energy level, while an orbital which tends to take up an electron, has to have a low energy level. The transmitted electron thus goes from a high to a low level, and releases the energy which corresponds to the difference of the two levels. It is this energy which drives life. This drop of energy is symbolized in Figure 2 by a slope. As the figure shows, hydrogen forms a row by itself. The noble gas, corresponding to it, would be the neutron gas (symbolized by O) which has no electron. The H has but one unbalanced electron which it gives off rather easily, which is thus on an especially high energy level. The energy of this electron is the fuel of life. By giving off its electron, the H atom becomes an H^+, a proton, which then merges in the proton-pool of water, the solvent of life.

For a biological electron acceptor we have to look at the right side of the chart in Figure 2. The natural candidate would be fluorine, but this element has various qualities which make it unfit for this role. So the best choice is fluorine's next neighbor, oxygen, which has all the qualities demanded. So the whole energetics of life is turning around the transfer of electrons from H to O, the elements of water, which makes life, energetically, a play of water. The energy released by the transfer of the electron from H to O is symbolized in the figure by the slope of the long arrow.

The closer an element is to the noble gas, the more it tends to take up or give off an electron. So electron transfer can take place also between members of the same horizontal row, e.g., from N or P to O. The energies of these reactions are symbolized in Figure 2 by the small arrows. These energies are too small to support life, but are large enough to play an important role in regulations, or serve as "small change" in energetics. The bigger an atom is, the farther away its outer electrons are from the nucleus, and the less they are attracted by it. So energy can be

gained also by an electron going from the second to the first row, say, from S to O. While the key of energetics is the interaction between H and O, the key to regulation and cancer has to be sought in the interrelation of N, O, and S. The main actors of regulation will thus be the N, S, and P of the protein, acting as donors, and the O acting as acceptor.

4

COLOR AND ELECTRONIC MOBILITY

Color may seem a superficial, accidental quality. It may be so in the case of the color of cheeks and lips of girls, but, more often color, being generated by an interaction of photons with electrons, can give us basic information about the latter. Even a lack of color may give an important lead. The lack of color in soluble proteins, and its presence in insoluble, structural proteins, indicates an important difference between the two, suggesting that the biological reactions produced by the former are molecular while the reactions of the latter are, partly, electronic.

The stronger an electron is attracted by the nuclear forces, the less it can interact with photons. The looser it is held the more it will tend to become delocalized and interact with light. Molecules, having conjugated double bonds are, owing to the mobility of their π electrons, yellow. The more extensive the conjugated system, the darker and deeper the color. B. and A. Pullman have emphasized the biological importance of this mobility, coenzymes being built, to a great extent, of such conjugated systems. Electrons, in well balanced, closed-shell molecules, are arranged in pairs which occupy the same orbital, spinning in opposite direction, and balancing each others magnetic moments. If the pair is separated, a highly unbalanced situation

results. The magnetic forces being very small, it is not their imbalance which causes most of the disturbance, but the partial occupation of the orbital.

The single electrons, occupying now a whole orbital, attract a new partner, are easily excitable, and strongly absorb light. The absorption is usually limited to a narrow range of the spectrum, which makes the resulting peak of absorption very narrow, high, and structureless. To the eye this appears as a strong and pure color. Molecules containing such uncoupled electrons are very reactive and are called *free radicals.* They give an ESR signal.

Mobility, the delocalized nature of electrons, may also have important biological bearings, making the rapid interactions possible. Apart from the mobility of π electrons of conjugated double bonds, there are various possibilities of electronic delocalization. It was more than 30 years ago that I suggested that proteins may be semiconductors.

Another possibility of electronic mobility was pointed out by L. Brillouin (1962, 1966), who showed that in structures, built of a regular array of repeating units, the single energy levels split up to a bundle of levels, forming energy bands separated by *forbidden zones* (the so-called "Brillouin Zones").

The conductivity of such a band depends on the number of electrons it contains. Exclusion principle allows every unit cell to contribute but two electrons to a band. So if the number of underlying units is n, while the number of electrons in the band is 2n, then every available place is taken and there can be no mobility, similar to a fully occupied garage. Such a band can be made conductant by taking electrons out of it. Semiconductors owe their conductivity mostly to the fact that their highest occupied energy band is very close to the lowest empty band above it, so that the energy of heat agitation suffices to excite electrons

from the former to the latter, making both bands conductant. The width of the forbidden zone, separating the two bands in protein, has been measured and calculated (M. G. Evans and J. Gergely, 1949; D. D. Eley et al., 1962; A. Pullman, 1965; and J. Ladik, 1964) and was found to be of the dimensions of several electron volts, and there is no energy available in living systems which could lift electrons through such a wide forbidden zone.

L. Brillouin pointed out another possibility of making a saturated energy band conductant by taking electrons out of it. This could be done by *acceptor impurities*, that is, substances capable of taking up electrons. D. D. Eley, K. M. C. Davis, R. S. Snart, and D. I. Spivey actually showed that the electronic conductivity of protein can be increased by its treatment with electron acceptors, like chloranyl. J. B. Birks and M. A. Slifkin (1963) reported similar observations.

The peptide bond is written, in the chemists shorthand, as in Figure 3, A, but (as shown by L. Pauling, 1959; 1968) is actually a mixture of two mesomeric forms, B and C. In reality it is neither of these but something in between which

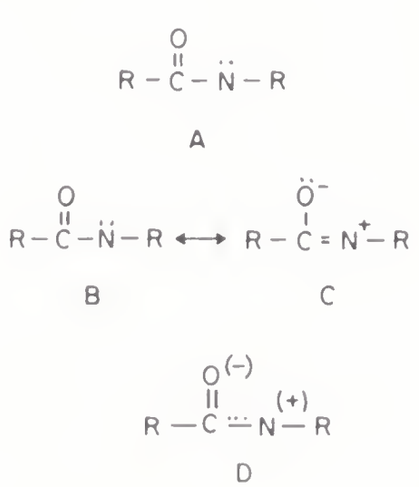

Figure 3 A: classical symbol of the peptide bond. B and C: mesomeric forms. D: combination of B and C.

can be described only by wave equations (which cannot be solved). So, with our chemical shorthand, we could come the closest to reality by writing D, all the bonds being partially double bonds with mobile electrons.

The peptide bonds, in the protein backbone, are connected in two ways by the βC atoms of the constituting amino acids: As shown by A. Pullman (1965), βC atoms of amino acids are saturated and hold their electrons too tightly to allow a conduction. The problem has lately been reconsidered by S. Suhai (1974), and may not be completely closed.

As shown by L. Pauling (1959, 1968), the peptide links, in protein, are connected also by H bonds. The problem of conductivity over the H bonds was studied by M. G. Evans and my associate J. Gergely (1949), who came to the conclusion that a certain degree of conjugation is possible over the p orbitals of the H atoms. Their opinion is more or less generally accepted. Only thin energy bands can be built over the H bonds allowing but a low degree of electronic mobility, and so taking everything together, it can be said that the closed-shell molecules of the α state can be regarded as dielectrics.

5

OXYGEN AND DICARBONYLS

With the energy of light the living systems generated oxygen and the oxygen made them develop and differentiate. The question arises how oxygen can induce these changes. According to O. Warburg (1966, 1967) it was not oxygen which did it, but the energy produced by the oxidative metabolism. I find it difficult to accept this opinion, because energy can drive, but not build, mechanisms. If it was not energy which induced the change, then it had to be oxygen, as such, which did it, and the question is: How?

Oxygen, O_2, owes its central role in biology to its ability to oxidize, that is, take electrons from other molecules. Could it, then, not induce the changes in living systems by taking electrons from proteins, making them into semiconductors and free radicals? However tempting, this assumption is unacceptable for two reasons: First, protein does not yield electrons to oxygen. They are simply built that way. If they would do so, we would burn up. Second, oxygen is a bivalent acceptor, it takes up electrons pairwise, and doing so simply oxidizes off a whole electron pair, makes the molecule smaller, but does not turn it into reactive free radicals. This can be done only by monovalent acceptors which accept but one electron. Michaelis (1935, 1946a,b) has shown that electrons can be transferred to bivalent acceptors

also one-by-one, but this does not change the situation because the final result is a transfer of two electrons.

The oxygen molecule, O_2, consists of two O atoms, linked together by a double O=O, which can open up to $^{-}O-O^{-}$, a bivalent acceptor which can be split into two $O^{=}$s, which too are bivalent.

The situation is different if O is not linked to another O, but is linked to a C, forming C=O, a carbonyl, which is a monovalent acceptor, capable of taking up one single electron only. But the C=O is a weak acceptor. Its electronic system is too small to accommodate easily an additional whole electron. But if two C=Os are linked together, then the two π electronic systems fuse to a wider π system, which is a strong acceptor, but, all the same, is still a monovalent acceptor, which can accept but one electron.

The simplest dicarbonyl is glyoxal (Fig. 4a), the methyl derivative of which is methylglyoxal (Fig. 4b). Dicarbonyls are readily formed in the oxidative metabolism, and as long as there is oxygen there may also be dicarbonyls. They can be formed along different metabolic pathways, but can form also spontaneously from trioses by a loss of one molecule of water. Dinitrophenylhydrazine, added to a solution of triose or phosphotriose, precipitates the hydrazone of methylglyoxal.

The reactivity and energy of the C=O groups depends on the nature of the atoms linked to the two unoccupied valences of C. If one of these is an H, HC=O, an aldehyde results. The

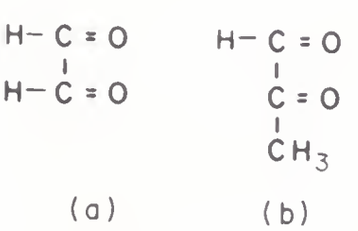

Figure 4 a: glyoxal; b: methylglyoxal.

simplest dialdehyde is glyoxal. If both free valences are occupied by C, then a ketone is formed. If one of the two C=Os is an aldehyde, the other a ketone, then the resulting substance is a ketone-aldehyde. The simplest ketone aldehyde is methylglyoxal. The aldehydes are more reactive than ketones but ketones are richer in energy, the heat of formation of C—H being higher than that of C—C (ΔH of C—H, 98.2 cal/mole; ΔH of C—C, 80.5 cal). The reactivity of the aldehydic group is somewhat tuned down by its hydration.

Dicarbonyls are highly unsaturated compounds which could not have existed in the strongly reducing anaerobic atmosphere of the α state, which makes them into specific features of the β period. In a keto-aldehyde, as methylglyoxal, the aldehydic C=O activates the ketonic C=O. According to the calculations of A. Pullman (personal communication), methylglyoxal has a very low lying empty orbital which makes it into a very good electron acceptor. Though having a very simple structure, dicarbonyls have very specific electronic qualities. While they can enter into bond formation by means of their aldehydic group, they can accommodate a transferred electron on their ketonic C=O, holding the transferred electron far enough from its earlier partner, left behind on the donor, to be uncoupled of it sufficiently to give an ESR signal, the donor and acceptor molecules being separated also by an H bond (Chapter 9).

Summing up, we can say that dicarbonyls possess the qualities needed to act as electron acceptors for protein. By the transfer of electrons to dicarbonyls the closed-shell dielectric proteins of the α period could be transformed into free radicals and semiconductors, opening the way to development and differentiation. However, dicarbonyls, being rather reactive substances, can be present in the cell only in a very limited quantity, while the big mass of protein demands a big mass of an acceptor, and O_2 is

the only acceptor available in unlimited quantity. Nature solved this problem by transforming some of the O_2 into dicarbonyls, which could act as primary acceptors, transmitting then the accepted electrons to O_2, the final electron acceptor of the biosphere.

6

OXIDATION AND CHARGE TRANSFER

The transfer of an electron pair from one molecule to another is *oxidation,* commonly called "burning." It oxidizes off a part of the molecule, makes it smaller, and produces two new well-balanced molecules.

The transfer of *one single electron* from one molecule or atom to another is *Charge Transfer,* which involves the separation of an electron pair.

The dicarbonyls, when accepting a single electron, have to separate an electron pair. This creates an unbalanced situation, a great reactivity. A molecule containing an uncoupled electron is a *free radical.* By the uncoupling the magnetic moments of the two electrons, spinning in opposite direction, become uncompensated, which makes them give an ESR signal. The magnetic forces involved being small, most of the instability comes from the partly unfilled orbital.

Charge transfer and stable free radicals were, hitherto, looked upon by biologists as an oddity, an item of Nature's curiosity shop, the high reactivity and net charge being thought to be incompatible with life. Free radicals were thought to occur only as short-lived transitory members of chain reactions. As will be shown later, charge transfer is one of the most common and frequent biological reactions, leading to the formation of

stable free radicals. Protein structures are built, to a great extent, of free radicals, the unbalanced nature of which explains, to a great extent, their subtle reactivity.

In charge transfer the electron goes from an occupied orbital of the *donor* (D) to an empty orbital of the *acceptor* (A). Such a transfer can take place only if the two orbitals overlap. Orbital overlap demands an intimate contact, a close fit, which can rarely be established in a single collision and may demand specific steric relations, which can make the reaction very specific. The energy of charge transfer is too small to hold A and D together against the forces of heat agitation, and for charge transfer to occur, the two particles mostly have to form a complex, held together by conventional forces. Orbital overlap is then established within the complex. This may take time, even days, which can make charge transfer into a slow reaction.

According to energy relations, the transfer of electrons may have different mechanisms, as symbolized in Figure 5 in which the thick line D, in the middle, stands for the donor. If the accepting orbital (A) has a higher energy than D, extraneous energy is needed for the transfer from D to A. This energy is usually derived from the absorption of a photon which "excites"

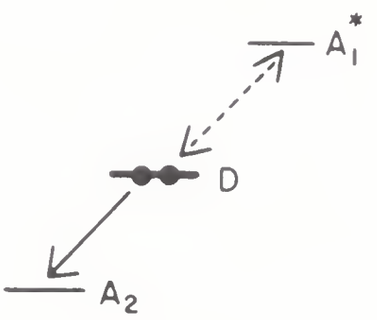

Figure 5 Schematic representation of charge transfer. D: ground state. A_1^*: higher energy level and charge transfer in the excited state. A_2: lower energy level, charge transfer in the ground state.

the electron from D to A*. This is *charge transfer in the excited state.* On the excited orbital A*, the electron is unstable and soon falls back to D, to be excited again. The electron thus oscillates between D and A*, as symbolized by the broken two-headed arrow. The absorption of light can lead to intense colors and declares itself in the spectroscope by a *charge transfer spectrum* in which the energy of the absorbed light corresponds to the energy difference of the two orbitals. Since the electron spends only part of its time on A*, we can also say that only *part of the electron was transferred.* Oscillating between D and A*, the electron remains coupled to its pair. The two spins compensating one another, no ESR signal will be obtained.

If the energy of the accepting orbital is lower than that of the donating one, as symbolized in Figure 5 by A_2, then the electron can go over spontaneously from D to A_2 in the ground state. The transferred electron, then, can stay put on A_2. This is *strong charge transfer in the ground state,* in which *a whole electron* is transferred, and two free radicals are formed. The charge transfer complex may dissociate into radicals A^- and D^+. Free radicals easily absorb photons and if the absorbed light is in the visible, the radical is colored. Spins being uncoupled, an ESR signal is obtained.

It is generally assumed that in "strong charge transfer" the two radicals dissociate, going into the ionic state, each with its charge, the one of them positive, the other negative. This notion is probably derived from the fact that the free radicals formed in strong charge transfer give an ESR signal, are thus uncoupled. I am unable to accept this conclusion. I think it believable that the two uncoupled electrons can be located on D and A far enough from each other to have their electron spins uncoupled without dissociation. I see no binding reason for the

two free radicals to dissociate. The forces holding the complex together only become increased by the forces of charge transfer and the mutual attraction of the opposite charges. If the two radicals do not dissociate, they form a *biradical.* A protein molecule may give up several electrons to acceptors and form *multiradicals.* In such polyradicals the charges may neutralize each other's outward actions. It seems likely to me that structural proteins consist, to a great extent, of such multiradicals which play a major role in biology also by influencing the distribution of ions.

The possibility of a third kind of charge transfer reaction can be deduced from the foregoing. If there is only a slight energy difference between the donating and accepting orbital, then the energy of heat agitation may suffice to carry the electron over from the D to the A, making the electron oscillate between the two. It is possible that such weak charge transfer is the most common one and plays an important role in biology. It may be the mechanism underlying a great number of subtle biological or pharmacological reactions. No light being absorbed, no color is produced, and no spins being uncoupled, no ESR signal is obtained and, so, the reaction has no outward signs and may go undetected. A change will be found only in the dipole moment.

What dominates the reaction of D and A is the difference in energy levels, and not their absolute values. As emphasized by the Pullmans, "acceptor" and "donor" are not absolute ideas. A weak donor can act as acceptor towards a stronger donor, and any acceptors may turn into a donor by donating its electron.

What is badly needed in this field is a scale of donor and acceptor strength which would allow one to compare substances belonging to different chemical groups. The scale of k values, proposed by the Pullmans (and discussed more in detail in my *Introduction to a Submolecular Biology,* 1960, pp. 35-45), has rendered good service but is not as universal as desired.

7

CARBONYLS, CELL DIVISION, AND THE GLYOXALASE

In the first anaerobic α period, life must have been dominated by electron donors and the resulting high electron tension, there being no strong electron acceptors. Among reducing groups the strongest donor, the sulfhydryl (SH), must have dominated, and played first fiddle in the process of cell division and protein synthesis.

When oxygen and carbonyls appeared, inducing the aerobic β period, the physical state of the cell became dependent on the relation of acceptors to donors, on a D/A quotient. Proliferation became arrested by the carbonyls which formed complexes with the SHs, putting them out of commission. As shown by M. P. Schubert (1935, 1936), methylglyoxal forms stable hemimercaptales with SHs. The dicarbonyls counteracted proliferation also by making the proteins less soluble and structures more stable. When cell division was called for, the cell had to shift towards the α state by inactivating the dicarbonyls and setting free the SH groups, and loosening up the protein structures.

L. Egyud and I (1966a,b) have shown that methylglyoxal (and the same holds for glyoxal) suppresses cell division at a low concentration, and does so reversibly, without harming the cell, as could be expected from a physiological regulator. Proliferation was arrested in all systems tested, bacteria, flagellates, fertilized

sea urchin or frog eggs, germinating plant seeds, or viruses (M. Baylor and L. Egyud, 1967). Inhibition of nucleic acid was poor, that of protein synthesis was strong, indicating that the inhibition was mainly on the ribosomal level.

This inhibition had an unusual feature: if the methylglyoxal was applied in a low concentration, after a while the cells resumed proliferation at its initial rate. This meant that the cells could inactivate the carbonyls; the inhibition caused by them was reversible.

This resumption of cell division seemed rather fascinating, because if it is the ketoaldehydes which keep the cell in interphase, then the cells must have also a chemical mechanism for their inactivation if cell division is called for.

It was more than 60 years ago that H. D. Dakin and H. W. Dudley (1913) and C. Neuberg (1913) discovered an enzyme the "glyoxalase," which can transform methylglyoxal into the biologically inactive D-lactic acid, transforming thus the ketone aldehyde into the corresponding hydroxyacid. Owing to the studies of F. G. Hopkins and J. Morgan (1948), K. Lohman (1932), M. Jowett and J. H. Quastel (1933), S. Yamazoye (1936), E. Crook and K. Law (1952), B. Mannervik and colleagues (1972, 1973, 1974), and last but not least, E. Racker (1951, 1952), we know today that "glyoxalase" is not a single enzyme but a system of two enzymes, "glyoxalase I and II" which use SH-glutathione (SHG) as coenzyme. When methylglyoxal and SHG meet, they form a hemimercaptale which is transformed by glyoxalase I into a thioester by shifting two of its H atoms. Glyoxalase II then splits this thioester into lactic acid and free SHG, recovering the coenzyme (Fig. 6).

As far as we know, glyoxalase is present in all living cells. Glyoxalase I is one of the most powerful enzymes, with a transfer number of 40,000, Glyoxalase II is about one-fourth as active,

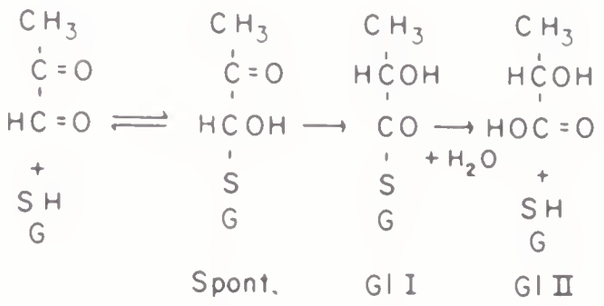

Figure 6 Transformation of methylglyoxal by the glyoxalase. G = glutathione; GI = glyoxalase.

indicating that what Nature wants to do in haste is the inactivation of the aldoketone; the recovery of the coenzyme, SHG, she does more leisurely. Nature does not indulge in luxuries, and if there is such a widely spread and most active enzymic system, it must have something very important to do, but until present its function was a mystery, the regulatory function of dicarbonyls being unknown, and methylglyoxal or D-lactic acid not lying on any major metabolic pathway. The formation of SHG-hemimercaptale of methylglyoxal is reversible, and the compound is dissociated to some extent into its components; only by its transformation into the irreversible thioester does methylglyoxal lose its biological activity. The glyoxalase attacks not only methylglyoxal, but also other ketoaldehydes, as phenylglyoxal and glyoxal. It decides not only the activity of the aldoketones, but decides also what SHG should do. In its presence SHG inactivates the dicarbonyls while, in its absence, SHG can catalyze electron transfer, as will be shown later (Chapter 8).

The distribution of glyoxalase I in various tissues of the mouse is shown in Table 1, elaborated by J. McLaughlin. The numbers give the micromoles of methylglyoxal decomposed by the extract of 1 g of various tissues in 30 min at 37°C. In these experiments the glyoxalase was estimated by the method of N. M. Alexander and J. L. Boyer (1971) which measures the

Table 1 Distribution of glyoxalase I in mouse tissues

Tissue	μmoles
Liver	640
Spleen	100
Intestine	60
Heart	60
Kidney	46
Leg muscle	40
Lung	38
Brain	26
Ehrlich carcinoma	24
Sarcoma 180	15

quantity of the undecomposed methylglyoxal spectroscopically as semicarbazide.

Acting as electron acceptor for protein, and the inactivation of SH, is not the sole biological activity of ketone aldehydes. They also seem to contribute to the stability of the resting β state and its structures. This function can be demonstrated by treating gelatine with glyoxal. The gel of gelatine, treated with 0.1 M glyoxal, no longer melts on heating. That methylglyoxal makes protein molecules stick together, once they come in close contact, can be shown by precipitating casein, in presence of salt and methylglyoxal, by acetone. The precipitate formed is almost insoluble, while casein precipitated in absence of methylglyoxal dissolves easily. This stickiness of molecules, induced by carbonyls, may also have a hand in "contact inhibition." I expect methylglyoxal to induce contact inhibition in ~~cancer~~ NORMAL cells, and expect glyoxalase to abolish contact inhibition in ~~normal~~ CANCER ones. I only can regret lacking the means for testing these assumptions.

There is no reason to suppose that the action of carbonyls is limited to the discussed instances. There are various electron donors in the cell with which dicarbonyls could react, besides SH. B. and A. Pullman (1963) have shown guanidine to be a good electron donor. R. Shapiro and J. Hachmann (1966), as well as M. Litt and V. Hancock (1967), have shown carbonyls to react also with nucleotides, in the first place guanidine. Carbonyls may thus interact also with nucleic acid, in the first place single-stranded RNA. Shapiro and Hachmann isolated and identified complexes of methylglyoxal and guanidine.

If ketone-aldehydes stabilize structures and the interphase, then their inactivation by the glyoxalase must promote cell division. The question arises how a cell can be in interphase at all in presence of glyoxalase. As will be shown later, in the resting cell dicarbonyls and glyoxalase are, in all probability, separated from one another (Chapter 14), the enzyme being kept in a nondiffusible state, making, possibly, the diffusibility of glyoxalase one of the main factors of the regulation of cell division.

8

DISTRIBUTION AND REACTIVITY OF SULFHYDRYL

Since F. Hammett (1929) called SH the "hormone of cell division," and L. Rapkine (1929, 1930, 1931) found a definite relation between SH and the mytotic cycle, the close connection between sulfhydrils and proliferation was common knowledge, even if this relation was not understood. So before entering upon the discussion of the regulation of cell division, I will briefly consider the distribution and reactivity of SH.

The SH can influence the electronic atmosphere of a cell, and influence cell division in two ways: by giving off electrons and thus increasing the electronic tension ($p\epsilon$), and by binding the dicarbonyls. The relation of SH and dicarbonyls dominates the D/A quotient, the relation of donors and acceptors, one of the main cellular parameters.

The SH fulfills various functions and accordingly, there are different kinds of SHs. On first approximation one can divide the SHs into two groups: "soluble" and "fixed." "Fixed" means bound to protein. The "soluble" SH can be identified, for practical purposes, with the SH of glutathione. The two kinds of SH can be separated by trichloroacetic acid which precipitates the protein with its "fixed SH," leaving the SHG in solution.

The "fixed SH" can again be divided into two groups: "free," that is, SH which reacts with conventional SH reagent also while the protein is in the native state, and "masked," which reacts only after the protein has been denatured.

Jane McLaughlin estimated the three different kinds of SHs in various organs of the rat. The results are summed up in Table 2. The numbers mean millimolar concentration of SH, 1 g of the tissue having been equated with 1 ml. They represent the averaged result of five experiments. (In the case of livers only four measurements were made.) The single measurements were fairly concordant and were in agreement with the earliest measurements made by H. E. Tunnicliffe (1925).

The SH was estimated by means of Ellmans reagent. The organs were blendored in water, 2 ml being used per gram tissue. Then the proteins were precipitated with trichloroacetic acid, and separated by centrifugation. The SH of the supernate was regarded as the SH of glutathione. The SH of the precipitate was the sum of the free (F) and masked (M) fixed SH. To find the

Table 2 Sulfhydryls in various organs of rats

Organ	SH Glutathione (mM)	Fixed free SH (mM)	Fixed masked SH (mM)
Leg muscle	1.64	1.00	7.90
Kidney	2.64	1.53	11.44
Heart	1.28	2.27	5.83
Lung	1.01	4.90	6.86
Brain	1.41	2.27	5.70
Intestine	4.22	1.60	5.79
Liver	7.99	3.47	13.49
Regenerating liver	8.80	1.97	10.54
Liver tumor	2.32	0.23	5.33
Sarcoma 180 solid tissue	1.16	0.01	5.10
Ascites	1.39	1.37	6.25

concentration of "masked" SH, the tissues, blendored in water, were treated with an excess of Ellmans reagent, allowing time for the free SHs to interact with this reagent. Then the proteins were precipitated with trichloroacetic acid, the precipitate separated, and its SH content estimated. This SH corresponded to the "masked fixed SH." Subtracting M from F + M, the value of F was found.

As Table 2 shows, the glutathione concentration of various organs was found to be somewhat over 0.001 M, with the exception of liver, intestine, and kidney, whose values were higher. The greatest part of the SH of the tissues is "masked" and "fixed," that is, linked to protein, reacting with reagents only after denaturations of the protein. The mechanism of "masking" has not yet been established with any finality.

Sulfhydryls are weak acids. As strong acids expel weak acids from their salts, so can more reactive SHs replace less reactive SH in their compounds.

Unfortunately, the main instrument of ordering reactivities, the electrode, fails, the SH giving no well-defined potentials. We have tried to obtain some information about the reactivity of SH and its affinity to glyoxal and methylglyoxal, by observing how long it takes for the SH to reduce a very easily reducible dye in presence of carbonyls, expecting that the greater its reactivity the stronger it will be linked to the dicarbonyls, and the longer it will take to reduce the dye. The results are summed up in Table 3. Higher numbers mean greater affinity to dicarbonyls.

The 0.05 M solution of various sulfhydrils was mixed with an equal volume of an isomolar glyoxal or methylglyoxal solution, buffered by phosphate at pH 7. Ten minutes later an equal volume of 0.05% solution of 2,6-dichloroindophenol was added. Then the time, needed for complete discoloration, was

Table 3 Interaction of sulfhydryls with dicarbonyls

Sulfhydryls	Water (sec)	Dicarbonyls	
		Glyoxal (sec)	Methyl-glyoxal (sec)
SH-glutathione	0	15	25
Cysteine	0	2400	1200
Cysteamine	0	1200	30
Ethanethiol	300	1800	2400
Mercaptoethanol	0	300	50
1-Thioglycerol	0	1200	45
Mercaptoacetic acid	30	75	150
Thiolacetic acid	105	105	90
Thiolactic acid	10	40	90
3-Thiopropionic acid	10	50	90
Ethylenedithiol	0	7000*	60
1.3-Dithiopropane	0	75	15
2.3-Dimercaptopropanol	0	120	30
1.4-Dithiobutane	0	17	18
1.2-Dithiobutane	0	4000*	270
Benzenethiol	0	0	0
Mercaptoacetanilide	0	0	0
N-acetyl-L-Cysteine	15	60	90
N-acetyl-*dl*-homocysteinamide	0	45	60
Homocysteinamide	0	45	60

Zero indicates discoloration in less than 5 sec.
Asterisks mean incomplete reduction.

measured. Numbers mean seconds, 0 means discoloration in less than 5 sec. Asterisks mean incomplete discoloration.

As Table 3 indicates, the reactivities of different SHs are very different, the interaction of SH with dicarbonyls being modified by other groups present in the molecule. The great affinity

of cysteine to glyoxal and methylglyoxal is, evidently, due to its amino groups, which enables it to form six-membered rings with both glyoxal and methylglyoxal. Cysteamine seems to be able to make such rings only with glyoxal, not with methylglyoxal, the methyl group interfering with the reaction. This difference in the reactivity of cysteine and cysteamine may allow one to distinguish between glyoxal and methylglyoxal. The strong toxicity of cysteamine suggests that glyoxal plays an important role in cell life.

As the table shows, the reactivity of the SH of glutathione is lower than that of most other SH compounds. The probable reason for this low reactivity was given by M. Calvin (1954), who pointed out that the SH of glutathione can form an H bond with the CO of the peptide link between cysteine and glutamic acid, which cuts down the reactivity. Calvin's explanation is supported by the low reactivity of the SH in N-acetyl-L-cysteine, N-acetyl-*dl*-homocysteinamide, and homocysteinamide, in which the SH can interact under formation of a six-membered ring with the C=O of the acetyl in the first, and the NH_2 of the two latter substances.

These reactivities agree with the observations made in the biological experiment. Egyud and I (1966a,b) found that the inhibition of bacterial growth, arrested by methylglyoxal, could readily be released by cysteine, but not by glutathione. Evidently, the SH of glutathione has a lower affinity to methylglyoxal than the SHs of the ribosomes have. Cysteine can compete with these SHs for the carbonyl, while glutathione cannot. Nature seems to have tuned the affinities of the SH of glutathione most carefully. With its low affinity, glutathione will not prevent the interaction of carbonyls with the more active SH of ribosomes. At the same time it will protect other SHs of lower reactivity from the carbonyls.

These relations explain a peculiarity of methylglyoxal, noted also by C. T. Gregg (1968) and J. F. Scaife (1969): the dicarbonyls have no toxicity below a certain concentration, but are very toxic above it. The 0.25 ml of a 0.1 M glyoxal solution is supported without trouble by a mouse of 25 g, while the same amount of 0.15 M solution may kill the animal. The 0.25 ml of a 0.1 M solution of methylglyoxal establishes in a mouse of 25 g an average concentration of 0.001 M, which is also the average concentration of glutathione present (Table 2). These relations suggest that the carbonyls are harmless as long as their concentration does not exceed that of glutathione, but are very toxic if they exceed this limit and the glutathione can no longer protect SH groups of lower reactivity. A lethal dose of glyoxal is supported by a mouse, if injected with an equivalent amount of SHG. These relations also explain the high concentration of glutathione found in liver and intestine (Table 2). These are the organs which have to meet the carbonyls ingested with food, which holds true, to a lesser extent, also for the kidney which has to excrete the carbonyls. These relations also explain why an otherwise harmless dose of methylglyoxal may kill a mouse when injected with dimethylsulfoxide which speeds up its resorption, allowing the concentration of the resorbed carbonyl to exceed momentarily the concentration of SHG. Similarly, the toxicity of a given amount of carbonyl was found to be the higher the greater the concentration was in which it was injected.

The importance of the SH made it desirable to build an alternative method for the estimation of its reactivity. Such a method was based on the interaction of dopamine and glyoxal. It was found that dopamine and glyoxal interact under formation of highly colored free radicals (see Chapter 12). This reaction is autocatalytic, being the result of a radical chain reaction. It starts slowly (Fig. 7) and becomes gradually faster, reaching,

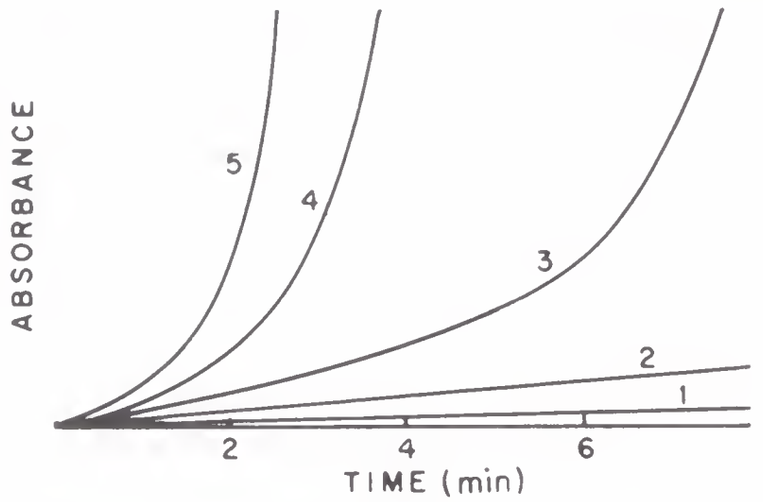

Figure 7 Inhibition of the complex formation of glyoxal and dopamine by SH-compounds.

eventually very high rates. The reaction can be followed in the spectroscope by measuring the absorption at 388 nm. Sulfhydryls can inhibit the reaction by combining with the catalytic glyoxal. The more reactive the SH, the more it will inhibit the reaction. As the curve 3 in Figure 7 shows, glutathione inhibits the reaction moderately, as compared to the control (curve 4). N-acetylcysteine inhibits stronger (curve 2) while cysteamine arrests the reaction almost completely (curve 1). N-acetylhomocysteine, thiourea, thioglycerol had no inhibitory C action (curve 5). The slight promotion may have been accidental.

9

AMINES AND CARBONYLS

Our central problem is whether proteins can give off single electrons to carbonyls from their peptide bonds and form free radicals. Such charge transfer would also desaturate the π electron band, and increase conductance.

As shown by R. E. Miller and W. F. K. Wynne-Jones (1959), as well as J. B. Birks and M. A. Slifkin (1963), the electron which is most likely to be given off by the protein is one of the nonbonded pairs of nitrogens. Since these electrons are taken up in the π pool of the peptide bond, it might be more exact to say that the electron given off most easily by the protein is one of the π pool of the peptide bond, which has the strongest relations with the N. So we can approach the interaction of protein and dicarbonyls by studying the interaction of amino N and CO. Proteins being very complex, I approached the problem by studying the interaction of dicarbonyls with simpler amines, my main problem being whether it leads to the formations of free radicals.

The simplest nonvolatile amine is ethylamine, $CH_3CH_2NH_2$. If its dilute watery solution is mixed with that of a dicarbonyl, the gradual development of a yellow color indicates a reaction in which a "Schiff base" is formed under loss of water:

$$RNH_2 + \underset{\underset{R}{|}}{\overset{\overset{H}{|}}{C{=}O}} \rightleftharpoons RN{=}\underset{\underset{R}{|}}{\overset{\overset{H}{|}}{C}} + H_2O$$

In the spectroscope the yellow solution yielded a simple line with the absorption rising smoothly towards the short wavelength (Fig. 8). There was no indication of the formation of free radicals. Either none were formed, or else they deteriorated too

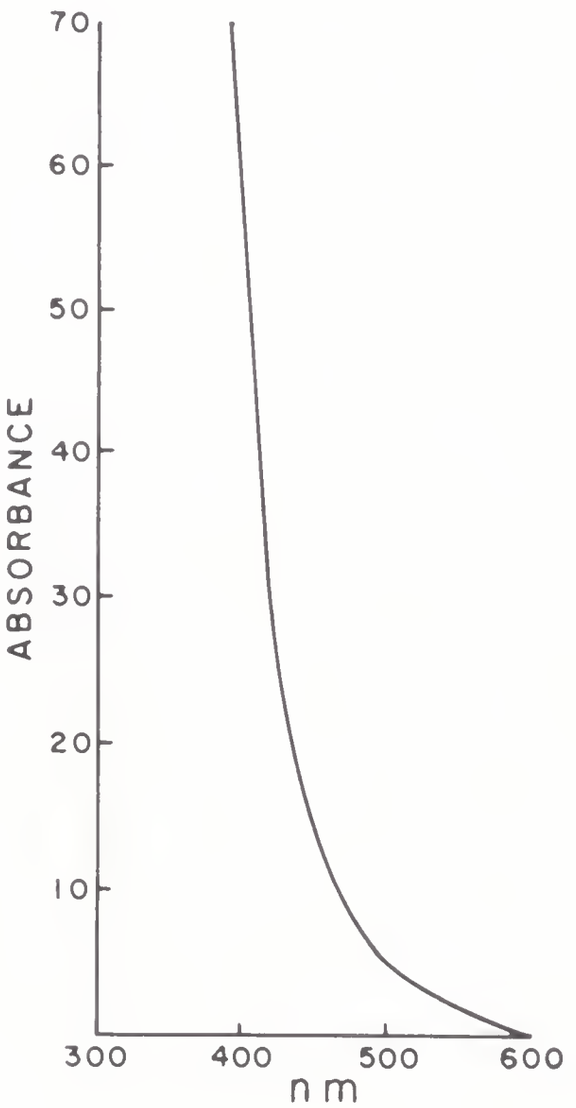

Figure 8 Absorption spectrum of the complex formed by ethylamine and methylglyoxal at room temperature.

fast to become visible. To become visible they must accumulate, and be formed faster than they deteriorate. Conditions had to be found which favored their formation and stability. Acid reaction and decreased temperature could be expected to favor stability, but at an acid reaction the amine and dicarbonyl do not interact. So these had to be mixed at the alkalinity of the amine at a lowered temperature and acidified after they interacted.

A sample of 40% commercial methylglyoxal was cooled to 0°C and mixed with an equal volume of 25% ethanol solution of equally cold ethylamine. The mixture was kept in melting ice. It rapidly darkened, its coloration reaching maximum in about 5 hr. Then it was diluted with a 0.1 M solution of citric acid to pH 5.6. The resulting solution had an intense mahogany-red color. In the spectroscope its absorption gave the curve A of Figure 9, which indicated two reactions. The rise of the absorption towards the shorter wavelength indicated the formation of the Schiff base which gave the curve in Figure 8, while a superimposed hump indicated a second reaction. The absorption of the substance, responsible for this hump, could be obtained by differential spectroscopy, that is, by placing the yellow solution of Figure 8 into the reference beam while placing the red one into the primary beam. The differential spectrum, thus obtained, is shown by curve B in Figure 9. It is a narrow, structureless peak as is mostly given by free radicals. That the underlying substance was actually a free radical was shown by Dr. H. Kon in whose ESR spectroscope the solution gave a signal. This conclusion could be corroborated also by adding a free-radical-scavenger, dithionite, whereupon the hump disappeared (Fig. 9C), leaving an abosrption similar to that shown in Figure 8. The interaction of ethylamine and methylglyoxal involved thus charge transfer in which a whole electron was transferred in the ground state from the amine to the carbonyl, leading to the formation of stable free

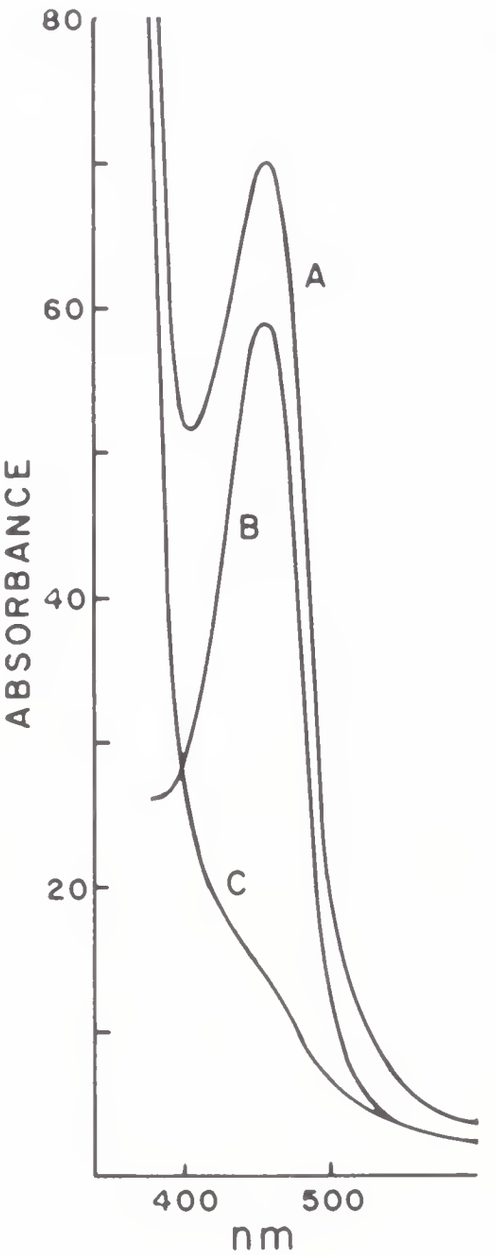

Figure 9 Curve A: same as Figure 8, obtained at lower temperature (see text). B: differential spectrum of curve A. C: solution of curve A after addition of dithionite.

radicals. On storage the hump gradually disappeared, the free radicals having deteriorated.

This simple experiment leads to the conclusion that in the interaction of amines and dicarbonyls free radicals are formed by charge transfer, a whole electron being transferred in the ground state. The dicarbonyl owes its electron acceptor ability to its low

lying empty orbital. By interacting with an amine a biradical is produced. The charge transfer between amine and dicarbonyl enables the dicarbonyl to exchange its low lying orbital for being a free radical. The Schiff base usually formed in the interaction of amine and carbonyl has to be a secondary stabilization product of the free radical.

Figure 10, B was obtained by adding 0.2 ml of a 40% methylglyoxal solution and 0.2 ml of a 5.5 M alcohol solution of

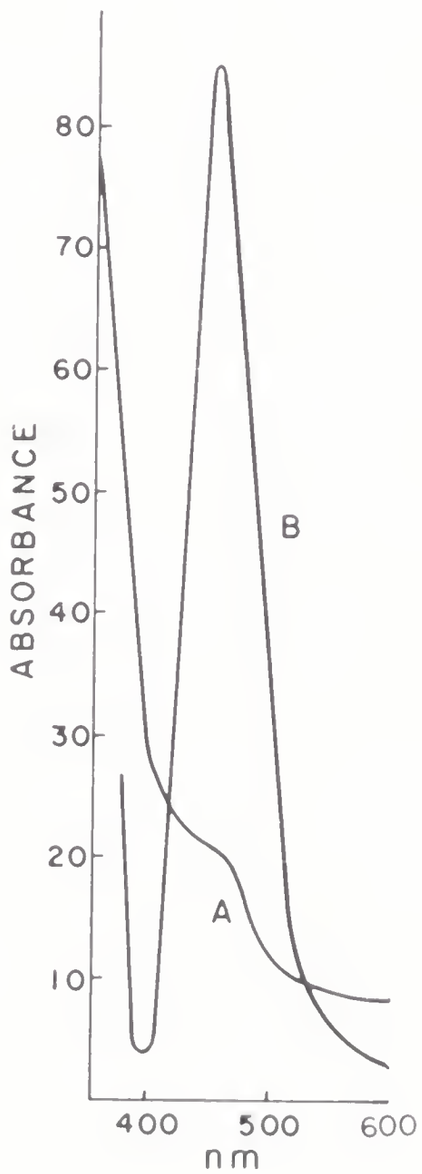

Figure 10 Curve B: similar to curve A of Figure 9, in dimethylsulfoxide. Curve A: same in water.

ethylamine to 2 ml of dimethylsulfoxide, and diluting the mixture, 10 min later, 100-fold with 0.1 M citric acid. There is practically no Schiff base; the whole curve is dominated by the absorption of the free radicals present. On storage the red color faded out yielding its place to the yellow color of a Schiff base, into which the free radical deteriorated.

The lower curve A in Figure 10 was obtained with the same reagents in an aqueous medium in which only a trace of free radicals was formed, as indicated by the small hump.

As a rule, charge transfer has to be preceded by the formation of a complex held together by conventional forces. The primary link between the amine and the carbonyl can hardly be anything else than an H bond, across which the charge is transferred. If the transferred charge is taken from the π pool of a peptide link, and is eventually located on the ketonic CO of the dicarbonyl, then the electron-hole produced on the peptide bond becomes separated from the transferred electron by four atoms, which include the H of the H bond. This distance is big enough to have the two electrons of the separated pair uncoupled, and allows them to give an ESR signal. It is difficult to see why the complex of the two radicals should dissociate. The forces holding the complex together only become increased by the charge transfer energy and the mutual attraction of the two charges. Such complexes were usually supposed to dissociate into the ionic state because of their ESR signal. The specific structure of methylglyoxal makes the uncoupling possible without dissociation; the probable product of the charge transfer are not two free radicals, but a biradical, that is, a complex formed by the two oppositely charged radicals.

In order to be able to study the formation of the free radicals in more detail, a solvent had to be used which stabilizes them. Such a solvent is, in my experience, dimethylsulfoxide,

DMSO, which not only facilitates charge transfer, but also stabilizes the radicals formed.

The free radical having a strong absorption at 475 nm, the rate of its formation can be followed in the spectroscope. The curve marked "air" in Figure 11 shows the line obtained. It indicates an autocatalytic reaction. However, the reaction is autocatalytic only in presence of O_2. In absence of air, in N_2, or *in vacuo*, a straight line was obtained (Fig. 11, N_2). The presence of oxygen made the reaction autocatalytic. "Autocatalysis" means that the

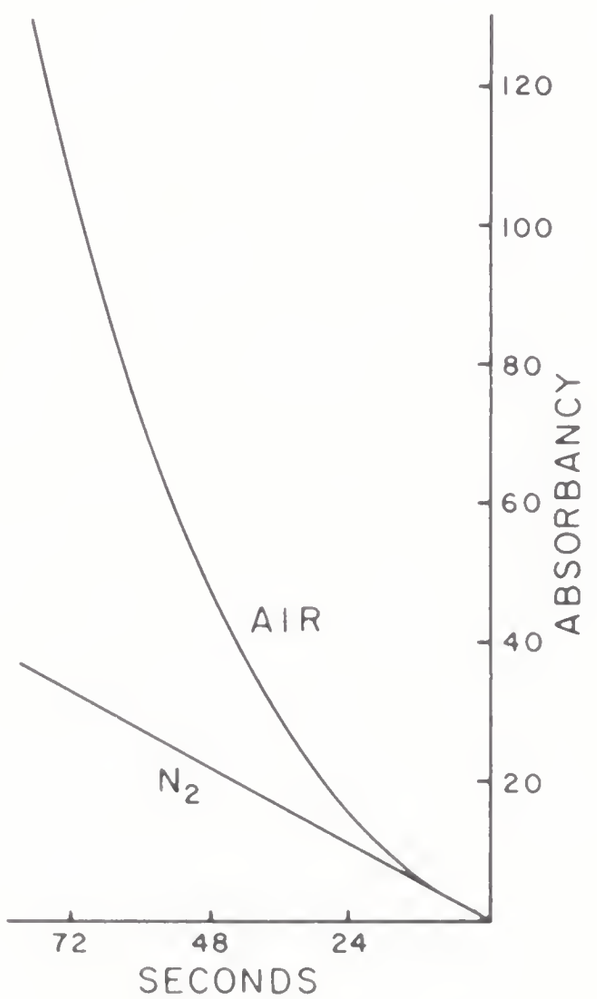

Figure 11 Formation of ethylamine-methylglyoxal in presence and absence of O_2. (2 ml dimethyl sulfoxide, 0.1 ml 2.75 M methylglyoxal, 0.05 ml 25% ethylamine.)

product of the reaction facilitates the formation of more product. The formation of the amine-dicarbonyl biradical is made autocatalytic only by the oxygen present. This makes it reasonable to suppose that the biradical formed a complex with the molecular oxygen. O_2 being a fairly inert molecule, which needs activation to react at ambient temperature, the biradical and O_2 seem to have mutually activated one another. In Chapter 12 another example will be given of such an activation of O_2 by a biradical. The paramagnetic oxygen is activated by paramagnetic biradicals, or paramagnetic atoms, like the ferromagnetic Fe of cytochrome A_3. In the electron transport system, connecting protein and oxygen, the radicals and O_2 mutually activate one another.

10

ETHYLENEDIAMINE, METHYLGLYOXAL, AND SULFHYDRYL-GLUTATHIONE

To be one step closer to proteins, Jane McLaughlin and I (A. Szent-Györgyi and J. A. McLaughlin, 1972) repeated the experiments, described in the previous chapter, using ethylenediamine instead of ethylamine. The diamine, $H_2NCH_2CH_2NH_2$ is more akin to protein than ethylamine, having in its backbone the same atomic sequence, NCCN, as protein has. Similarly to ethylamine, at room temperature, the diamine formed a yellow complex with the carbonyl which gave an absorption (Fig. 12, A). There was no vivid color and no hump to indicate the formation of free radicals.

As far as we know, all living cells contain SHG, which, as shown by M. P. Schubert (1935, 1936), readily forms a hemimercaptale with methylglyoxal,

$$R{-}SH + \begin{matrix} H \\ | \\ C{=}O \\ | \\ R \end{matrix} \rightleftharpoons R{-}S\begin{matrix} H \\ | \\ C{-}O{-}H \\ | \\ R \end{matrix}$$

the dissociation constant of which is, according to E. E. Cliffe and S. G. Waley (1961), 2.10^{-3}. So, if methylglyoxal enters a cell, it has to form a complex with its SHG. What we had to

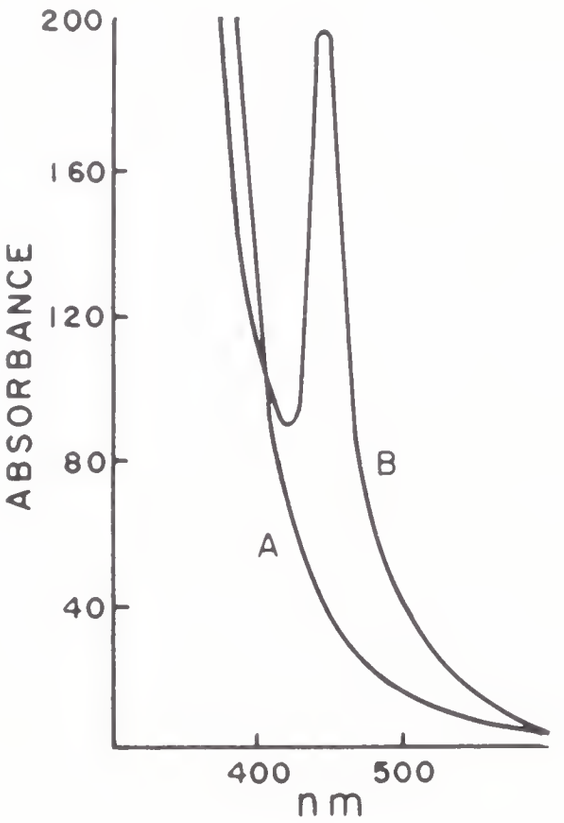

Figure 12 Absorption curve of the product of the interaction of ethylenediamine and methylglyoxal. A: in absence of SH-glutathione. B: in presence of SHG.

look into was thus a possible interaction between ethylenediamine and the hemimercaptale of methylglyoxal formed with SHG.

A 0.1 M solution of methylglyoxal was mixed with 1.2 vol of a neutralized 0.1 M solution of SHG. A few minutes were given for the formation of the hemimercaptale, and then a double volume of a 0.1 M ethylenediamine solution of pH 7.4 was added. Soon a pink color appeared which rapidly deepened to an intense purple. In the spectroscope the diluted solutions gave the absorption reproduced in Figure 12, B, which was analogous to that in Figure 9, A. It was the expression of two reactions, complex formation and charge transfer, the first giving the line gradually rising towards the shorter wavelength, and the second giving the hump. The absorption of the substance, underlying the hump,

could be obtained again by compensation. It was a structureless peak with a maximum at 450 nm. The narrowness of the peak suggested a high degree of electronic delocalization. Dr. H. Kon found a strong ESR signal accompanying the appearance of the purple color which indicated a charge transfer in the ground state. The purple color was thus that of a relatively stable free radical. On addition of HCl the purple color changed into brilliant blue, the maximum of absorption having shifted to 575 nm.

Glyoxal, used instead of methylglyoxal, gave only a yellow complex.

That the purple color and the absorption peak were due to the presence of a free radical could also be shown by adding a free-radical-scavenger, sodium dithionite, which made the color and peak disappear.

The central role of the SH group could be shown by blocking it with mercury (mersalyl), which prevented the appearance of the purple color. It was also brought out by the fact that the SHG could be replaced by other sulfhydryls. The purple color developed the fastest if the SHG was stored for a few minutes with the methylglyoxal before the diamine was added, which indicated that the SHG interacted with the carbonyl, and the amine reacted with the SHG-methylglyoxal complex. Excess of SH inhibited the reaction.

The intensity of color given by various sulfhydryls is shown, qualitatively, in Table 4. What is noteworthy in this table is the poor activity of cysteine. Free cysteine forms a very stable complex with methylglyoxal, in reacting both with its SH and NH_2. The 1,2 dithiols, which form very stable compounds with dicarbonyls, also gave a poor reaction. Separation of the two SH groups by one C made the reaction stronger. Separation by two Cs led to full activity, as shown by comparing

Table 4 Intensity of color given by various sulfhydryls

Sulfhydryls	Intensity
Cysteine	+
Cysteamine	(++)
SH-glutathione	++++
Acetylcysteine	+++
Mercaptoacetic acid	+++
Thioglycerol	++
3-Thiopropanol	+
3-Thiopropionic acid	+++
Benzenethiol	+++
Ethanedithiol	+
1,3-Dithiopropane	++
2,3-Dimercapto-1-propanol	+
1,4-Dithiobutane	+++
1,2-Dithiobutane	+
Acetylhomocysteine	+++
Homocysteinamide	+++

1,2- and 1,4-dithiobutane. The formation of a very stable complex between SH and aldoketone interferes with the formation of free radicals. The lack of specificity of the reaction opens the possibility that the SH of protein may react similarly.

The reaction of SHG, methylglyoxal, and diamine deserves a thorough study. It is not impossible that the SHG-methylglyoxal complex acts as donor towards protein, a possibility now being studied in association with Doctors J. Ladik and K. Laki. Should this complex donate electrons to the lowest empty orbital of protein, this would open the possibility of conduction along the backbone of the protein over the lowest empty σ band.

What transpires in the experiments is that charge transfer and the formation of free radicals is involved in the interaction of carbonyls and amines.

11

DICARBONYLS AND BIOGENIC AMINES

As described in Chapter 1, casein stored in the presence of croton aldehyde assumed the brown color of the liver, and it was assumed that this change was due to transfer of electrons from the protein to the enal. The structure of croton aldehyde is very similar to that of dicarbonyls: both have conjugated double links, the dicarbonyls two C=Os, the enals a C=O and a C=C, which makes both substances into monovalent acceptors of similar reactivity. Accordingly I expected that casein, stored in presence of glyoxal or methylglyoxal would show a change in color, similar to that induced by the croton aldehyde, but no colors appeared. There seemed to be no reaction at all. Since the acceptor ability of both substances was similar, the difference had to be in the ability to bind to the protein. Dicarbonyls seemed to be unable to bind to it.

Jane McLaughlin and I (1975) noticed earlier that dicarbonyls form spontaneously highly colored complexes with biogenic amines, which complexes had very unusual qualities. What struck the eye most was the strong yellow color due to a high absorption peak around 400 nm. The narrowness of the band suggested electronic delocalization. The complexes gave, in the hands of Dr. H. Kon, strong spin resonance signals, indicating that they were stable free radicals formed by the transfer of a whole

electron in the ground state. That these reactions had a biological meaning was suggested by the fact that they were given only by the biologically active amines, though the active dopamine and serotonin (Fig. 13) differ from the inactive tyramine and tryptamine only by the presence of an OH, while the ethylamine side chain, which has to react with the dicarbonyls, is equally present in all. Some of the properties of the complexes are summed up in Table 5.

If a 0.1 M solution of dopamine was mixed with 0.1 M solution of glyoxal at pH 7.4, soon a yellow color appeared which rapidly deepened to orange-red, and then a precipitate was formed, consisting of dark floccules, which, after drying, gave an elementary analysis suggesting a 1:1 complex of amine and carbonyl. Methylglyoxal behaved similarly to glyoxal, though acting a bit more sluggishly. Other biogenic amines (adrenalin and noradrenalin) behaved likewise. Though the molar absorptivities were somewhat varied, they were all high and their maximum was always around 400 nm. The serotonin-glyoxal complex had a weak absorption also at 590 which gave it a greenish tint. Histamine, though having a similar ethylamine group, gave no such reactions.

Dr. Daniel I. Arnon had the great kindness to test the dopamine-glyoxal complex for its ability to catalyze photophosphorylation. The dried complex he found active only in

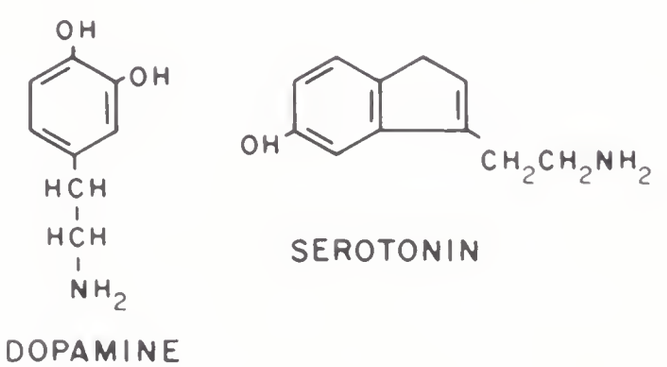

Figure 13 Dopamine and serotonin.

Table 5 Properties of complexes formed by dicarbonyls and biogenic amines

Properties	Adrenalin		Noradrenalin		Serotonin		Dopamine	
	G	MG	G	MG	G	MG	G	MG
Wavelength of maximum of absorption (nm)	400	392	400	395	366	366	393	397
Time needed (min) to reach maximum at 60°C	52	40	10	10	30	40	6	12
Molar absorbency calculated	4100	1500	5800	4900	2100	2100	21,000	5600

G = glyoxal; MG = methylglyoxal. The calculated molar absorbency involves the improbable supposition that the whole quantity of carbonyl and amine was present simultaneously as a biradical.

pseudocyclic photophosphorylation, while freshly formed complex (produced in a gently heated solution of glyoxal and dopamine without drying) was active as a catalyst both in pseudocyclic and cyclic photophosphorylation. His results are reproduced in Tables 6 and 7. In pseudocyclic photophosphorylation the catalyst accepts electrons from illuminated chloroplasts and donates them to oxygen. In photophosphorylation the catalyst transfers electrons from the illuminated chloroplasts to a membrane-bound component of the photosynthetic apparatus.

The experience could be summed up by saying that biogenic amines form spontaneously charged transfer complexes with the dicarbonyls glyoxal and methylglyoxal, which are stable free biradicals formed by the positively charged amine and the negatively charged dicarbonyl. The properties of these complexes

Table 6 Dopamine glyoxal complex (DG) as a catalyst of photophosphorylation by spinach chloroplasts

Cyclic photophosphorylation	Gas phase, N_2 (Q)[a]
Control, no cofactor	6
DG, 100 μM	17
DG + DCMU[b] (10^{-6} M)	26
PMS,[c] 100 μM	170
PMS + DCMU	208
Pseudocyclic photophosphorylation	**Gas phase, air (Q)[a]**
Control, no cofactor	15
DG, 100 μM	111
DG + DCMU (10^{-6} M)	17
MV,[d] 100 μM	149
MV + DCMU	18

[a] μmoles ATP formed/mg chlorophyll/hr.
[b] 3-(3,4-dichlorophenyl)-1,3-dimethylurea.
[c] Phenazine methosulfate.
[d] Methyl viologen.

suggest a wider biological role. Since tissues contain both dicarbonyls and biogenic amines, there is a possibility for their formation.

By writing Ba for biogenic amine and Dc for dicarbonyl we could symbolize the situation by:

$$Ba + Dc \rightleftharpoons Ba^{+}Dc^{-}$$

$Ba^{+}Dc^{-}$, having a strong and specific absorption, its formation could be followed in the spectroscope. In the case of

Table 7 Methyl glyoxal dopamine (MGD[a]) complex as a catalyst of photophosphorylation

Cyclic photophosphorylation	Gas phase, N_2 (Q)[b]
Control, no cofactor	7
Phenazine methosulfate, 100 μM	179
MGD, 0.1 ml	54
Pseudocyclic photophosphorylation	**Gas phase, air (Q)[b]**
Control, no cofactor	12
MGD, 0.05 ml	212
MGD, 0.1 ml	155
MGD (0.1 ml) + DCMU[c] (10^{-6} M)	23
Methyl viologen, 100 μM	309
Methyl viologen + DCMU	26

[a] Dopamine-HCl (50 mg) dissolved in 10 ml of 0.05 M MES [2-(N-morpholino) ethane-sulfonic acid] buffer, pH 6.0. To 2 ml of this solution, added 0.2 ml of glyoxal and immersed test tube in boiling water for 3 min. The colored complex formed became turbid on cooling. Clarified by adding DMSO (to 20%) and used in test.

[b] ATP formed, μmoles/mg chlorophyll/hr.

[c] 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

dopamine and glyoxal the curve obtained was very similar to the curve "air" in Figure 11. It indicated an autocatalytic reaction. In absence of air, *in vacuo,* the curve obtained was similar to Figure 11, N_2. Evidently, the catalytic action of oxygen was similar in both cases. I will return to it in Chapter 13. Other amines behaved similarly to dopamine. Methylglyoxal, used instead of glyoxal, also gave a catalytic curve, though the autocatalytic effect was considerably weaker.

I have limited myself to the discussion of the complexes of dicarbonyls formed with biogenic amines. The possibility is left open that other amines act similarly. The identification of the amine which mediates the supposed electron transport between protein and dicarbonyl as well as the final identification of the dicarbonyl itself are urgently needed.

12

THE ELECTRON TRANSFER CHAIN

The cell contains biogenic amines and dicarbonyls in a very low concentration while it contains a great quantity of protein. It follows that the biogenic-amine dicarbonyl complexes could not act as final electron acceptors for protein. They could act as acceptors only if they could transfer the accepted electrons to another electron acceptor present in greater quantity. There is only one acceptor which is present in tissues in unlimited quantity, and this is oxygen. So the question is whether $Ba^{+}Dc^{-}$ could act as electron transmitter between protein and oxygen. To be able to do so it would have to enter charge transfer complexes both with oxygen and protein, forming charge transfer complexes with them.

To decide whether $Ba^{-}Dc^{+}$ complex could form charge transfer complexes with protein, a 5% casein solution was mixed with 1/2 vol of 0.1 M glyoxal and dopamine in presence of a pH 7.4 phosphate buffer, and incubated in absence of air at 38°C for half an hour. Then the casein was precipitated by acetic acid, separated on the centrifuge, redissolved in 0.25 M Na_2HPO_4, re-precipitated with acid, then centrifuged out again. The precipitated casein was grey. The $Ba^{+}Dc^{-}$ attached itself to the protein, forming with it a charge transfer complex, turning the protein into a black free radical (grey is diluted black). Writing P for protein:

$$Ba^{+}Dc^{-} + P \rightarrow P^{+}BaDc^{-}$$

In order to see whether the $Ba^{+}Dc^{-}$ complex could interact with O_2, a 0.1 M solution of dopamine and glyoxal were mixed with a pH 7.4, phosphate buffer in absence of air. The mixture soon developed a deep red-orange color. On shaking the mixture through with air it assumed a blackish tint, which indicated an interaction with oxygen. On storage the black tint disappeared to return on renewed shaking with air. After repeated shakings a very dark, blackish precipitate was formed, evidently consisting of the complex of $Ba^{+}Dc^{-}$ formed with O_2. It is a reasonable guess that the O_2 joined the complex as a peroxide, taking over the electron of Dc^{-}. The reaction thus could be symbolized by:

$$Ba^{+}Dc^{-} + O_2 \rightarrow Ba^{+}DcO{-}O^{-}$$

Combining the two last reactions:

$$P + Ba^{+}Dc^{-} + O_2 \rightarrow P^{+}BaDcO{-}O^{-}$$

the net result being the transfer of an electron from protein to oxygen.

The last equation was transformed into an experiment by shaking gently a 5% casein solution with an equal volume of 0.1 M glyoxal and dopamine in presence of a pH 7.4 phosphate buffer in an open beaker for 30 min at 38°C. The solution turned black. The casein was precipitated with acetic acid, redissolved, reprecipitated. It yielded a black protein which could be reprecipitated without losing its color, yielding a colorless supernate. The result was similar if serotonin or noradrenaline were used. Adrenaline gave a less deeply colored protein. Figure 14 shows the result

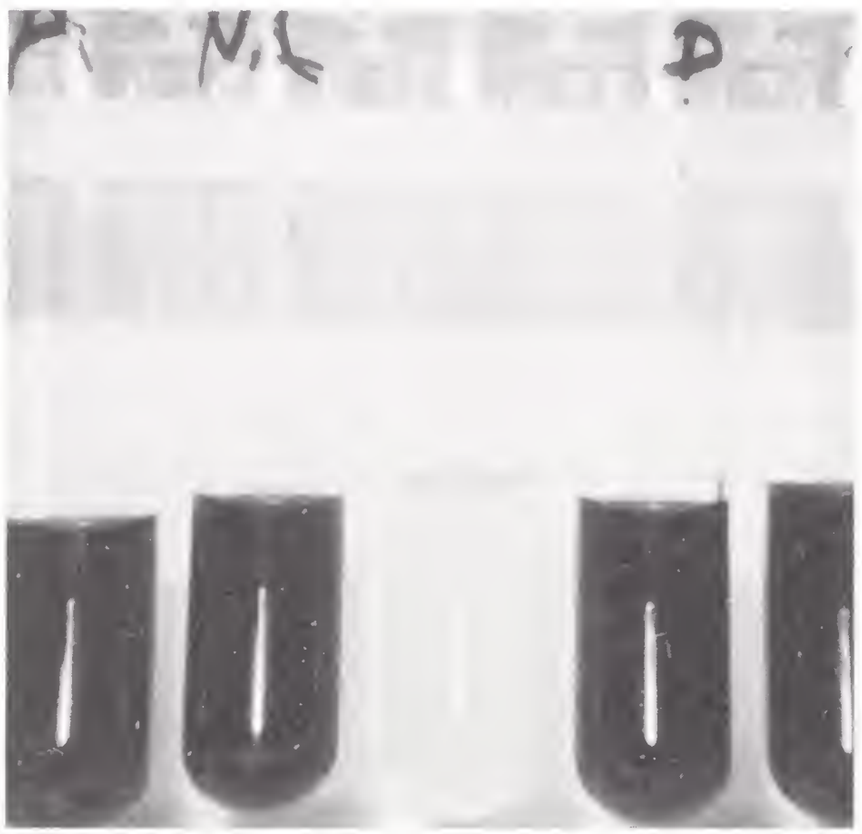

Figure 14 Middle tube: 5% casein solution. Left tube: same incubated with adrenaline, glyoxal, and peroxidase. Second tube from left: same, noradrenaline instead of adrenaline. Fourth tube: same with dopamine. Fifth tube: same with serotonin.

of the experiment. The tube on the left was the one containing adrenaline as amine, the next one noradrenalin. The tube in the middle contained casein without additions. The next one to the right had dopamine and the last one serotonin. The very dark color indicated the transfer of electrons to oxygen. It is likely that the blackened protein was a multiradical of casein with the amine radicals attached to it. Such a complex must have very unusual properties, being both free radicals and conductors. The electric charge of such a complex would cause no perturbations in its surroundings, the charges compensating one another inside the complex.

That the final product of the charge transfer was actually a substance containing a peroxide, could be shown by repeating the experiment with dopamine in presence of peroxidase. In presence of this enzyme the solution blackened much faster and its casein was less soluble than the casein in absence of peroxidase. The peroxidase, evidently, activated the peroxide and enabled it to accept a second electron from the protein. Accepting a second electron, the peroxide had to be detached as $^{-}O—O^{-}$ which, by capturing two protons, had to become hydrogenperoxide. The hydrogenperoxide in the cell would have to be decomposed by the catalase. The complete set of reactions involves a peroxidase *and* a catalase. D. Keilin and E. F. Hartree (1945, 1951, 1955) showed that "catalase" is a bifunctional enzyme, capable not only of decomposing hydrogen peroxide, but capable also of acting as peroxidase, fulfilling both required functions.

The experiments indicate that the color of the structure proteins of the liver, described in Chapter 1, are the visible expressions of the presence and function of an electron transfer system which transfers electrons of protein over biogenic amine and dicarbonyl to molecular oxygen, the universal final electron acceptor of the biosphere.

What has been shown in this chapter is that biradical complexes, formed by biogenic amines and dicarbonyls, can transfer electrons from protein to O_2. There is no evidence available yet that it is actually these amine-dicarbonyl complexes which transport the electrons in the living cell. The identification of the substances which perform this hypothetic activity still belongs to the unfulfilled tasks of research.

The described electron transport chain strongly suggests that catalase, with both of its functions, is involved in the electron transport chain. This sheds a new light on the meaning of J. Greenstein's (1954) catalase inhibitor, produced by cancer cells.

By inhibiting the catalase and peroxidase activity, this factor tends to inactivate the electron transport system and push the cell toward the α state. It may be one of the regulators of the α-β transition.

13

THE BIOLOGICAL FUNCTION OF OXYGEN

Oxygen is the universal electron acceptor of the biosphere. It produces the energy which drives life, and produces the negative entropy which preserves its pattern. It is tacitly assumed that this release of energy and negentropy is *the* function of oxygen which is only indirectly involved in biological processes through energy and entropy.

My experiments suggest two more important and direct roles for oxygen: transformation of the protein into free radicals and semiconductors, and promotion of electronic reactions, making charge transfer and the formation of free radicals autocatalytic.

In the transformation of protein, oxygen is involved both as monovalent electron acceptor, with its atoms linked to carbon in dicarbonyls, and as a divalent electron acceptor, with its atoms linked together to O_2.

In humans the most frequent immediate cause of death is the lack of oxygen, and it was generally assumed that lack of oxygen kills through the lack of energy. The contents of the previous chapters suggest the possibility of death being due to the dropping out of the catalytic function of oxygen. If the O_2 supply to the central nervous system (CNS) is cut, consciousness ceases instantaneously. This is difficult to explain with the lack of energy, because the brain has its reserve energy in the form of

high energy phosphates and can produce energy by fermentation, which should enable it to function, at least for a while, without oxidative energy. In absence of O_2, the biradicals lose this autocatalytic character and their formation must drop to a low level. In this case the readmission of O_2 alone cannot restore function; the biradicals would have to be introduced as well to speed up their autocatalytic formation.

The substances involved in the radical chain reactions are not identified yet. They all have to be able to form a stable free radical.

It seems a pertinent question whether ascorbic acid, which seems to be an indispensable cellular constituent, does not owe its vital importance to the participation in these radical chain reactions. As far as the writer is aware, no free radical of ascorbic acid has been isolated yet. This acid was looked upon as a bivalent redox agent. To make free radicals visible, these must be accumulated, being formed faster than destroyed. As shown earlier, free radicals can be stabilized by DMSO and their formation can be accelerated by an alkaline reaction.

A 0.5 M solution of ascorbic acid was prepared in DMSO and poured into a bigger (2 liter) bottle in which the air was replaced by O_2. Dry ammonia gas was introduced until the fluid showed an alkaline reaction. Then the bottle was stoppered and connected to an O_2 cylinder which maintained the O_2 pressure. The fluid was stirred and its reaction was intermittently tested. If necessary ammonia was introduced to maintain the alkaline reaction. Soon after mixing, a faint purple color appeared and then deepened with increasing speed to an intense purple. In the spectroscope the fluid showed two absorption peaks, one at 500 nm, and the other about 2½ times higher at 375 nm. In the undiluted fluid the specific absorption was 200 at nm 500. By measuring the absorbance at 500 nm, the formation of color could

be followed. It showed an autocatalytic character giving curves similar to the one marked "air" in Figure 11. After evaporation of the DMSO, the residue gave a strong ESR signal which indicated that the purple substance was a free radical. This conclusion could be supported by diluting the solution with water and adding a small amount of a free-radical-scavenger (dithionite), which caused immediate and complete discoloration. The optical absorption of our fluid, diluted with water, was identical between pH 4 and 9, and disappeared above and below this pH. The color is the most stable at pH 6 and only somewhat less stable at pH 7. The color persisted for days. It, evidently, was the color of the stable free radical of ascorbic acid, the production of which could greatly be promoted by manganese.

14

DEFENSE AND REGULATION

If I cut myself my life is in danger as long as the wound stays open. The cut makes my cells proliferate, fill the gap, and heal the wound. My cells form a system which is activated by the cut, and corrects the damage. This reflects the basic principle of defense. The damage is made to correct itself by activating a dormant mechanism.

We find this principle reiterated in the various defense mechanisms. If I am wounded, and bleed, the injury, which caused the wound, releases the trombokinase. The trombokinase activates thrombin, thrombin produces fibrin, and the fibrin plugs my bleeding blood vessels. If I go out into the sunshine naked, I get a sunburn which is a damage to my skin. The damage activates the tyrosinase, the tyrosinase produces pigments, and the pigments protect me against the sunshine. These are most ingenious mechanisms. They are not limited to the animal world. If I drop my banana, apple, or pear, it becomes damaged. The damage breaks the cell walls and opens the gate to bacterial invasion, but at the same time it also activates a phenoloxidase. The phenoloxidase oxidizes a polyphenol to a quinone, and the quinone kills the invading microorganism. At the same time, the quinones complex with protein, forming a protective sheet over the open wound and making the protein unfit for bacterial food. All this I can see happening with my naked eye, because

the quinone-protein complexes are brown or black and make the damaged place of my banana or apple brown or black. What happens, when I cut myself, and make my cells proliferate, is similar: the cut releases the glyoxalase, the glyoxalase inactivates the glyoxal derivatives which have kept my cells at rest. So my cells proliferate and heal the wound.

The question is: How was this system kept in an inactive state before it was activated by the cut, which was the damage which had to be corrected? The simplest assumption is that its single parts were kept separated, enzymes being separated from their substrates, or proenzymes being separated from their activator. The glyoxalase had to be kept separated from the glyoxal derivatives, as the phenoloxidase is separated in intact plants from the polyphenols, tyrosinease from tyrosine, and prothrombin from thrombin.

The next question is: How can two substances be kept separated within the narrow confines of the cells? One way would be to enclose one of the reactants (the colloidal enzyme) in small vesicles. Activation, then, would consist of breaking the vesicles open, or making their wall permeable. This would be analogous to cathepsin which is kept by the resting cell in small vesicles, "lysosomes." A grave damage done to the cell breaks the lysosomes open, releases the cathepsin, which then digests the badly damaged cell and clears it out. This method of activation is not limited to correcting damage, but is used also for physiological regulations. The material of the hypertrophic pregnant uterus, for instance, is preserved by being kept separated from proteolytic enzymes, as shown by F. R. Goodall (1965, 1966). These latter are enclosed in lysosomes, stabilized by progesterone. On termination of pregnancy the production of progesterone drops out, the lysosomes become unstable and release the proteolytic enzymes which reduce the

uterus to its normal size by digesting its excess proteins. Nervous excitation in muscle makes the vesicles, holding acetylcholine, break up. More subtle regulations may be achieved by regulating the permeability of the wall of the vesicles. The classical example of such a regulation is given by the regenerating rat liver. If two of its three lobes are cut out, the remaining lobe grows, in 8 days, to the size of the whole normal liver. It is made to grow, evidently, by messengers which inform it of the increased functional demand.

It is also believable, that an enzymic system may be kept inactive by being fixed to points of the structure, which makes it nondiffusible. The activation, in this case, would consist of releasing the substance from its bondage.

We do not know yet how, in the resting cell, the glyoxalase is kept separated from its substrate, the glyoxal derivative. It is possible that it is kept enclosed in vesicles, or kept indiffusible, being linked to structure. The hypothetic vesicles, I will call (in analogy to lysosomes), "promosomes," since glyoxalase has to promote cell division. If a cut induces cell division, it may do so by breaking up the promosomes, or by releasing the glyoxalase of its binding. Jane McLaughlin and I tested a series of substances for their ability to release glyoxalase from its bondage. Lobes of mouse livers were kept suspended in Ringer's solution under gentle agitation overnight at 1°-2°C, after which the Ringer and the liver lobes were analyzed for glyoxalase. Only very small amounts of the enzyme leaked out in pure Ringer, and leaking was not increased by ATP, ADP, AMP, histamine, adrenaline, noradrenaline, dopamine, ascorbic acid, glutathione, or chloroform. Three substances were found to increase it considerably: thiourea, cyclic AMP, and cysteamine. These actions seemed most intriguing.

Two facts lend fascination to the action of thiourea. The one is that this substance is known to promote cell division, and was used medicinally to make torpid wounds heal. The other is that M. Rachmilewitz, A. Rosin, and L. Doljanski (1950) discovered that thiourea, administered in high doses to rats *per os,* elicits an outburst of cell division in the liver. These relations suggest that substances which increase the diffusibility of glyoxalase, produce cell division by leading to the inactivation of the carbonyls by the glyoxalase made diffusible.

What makes the action of cAMP intriguing is the fact that this substance plays an increasingly important role in physiology, mediating different pharmacological or hormonal reactions. This makes it seem possible that a cut, or a functional demand, activates proliferation by promoting the production of cAMP, which, then, makes the promosome permeable. The action of growth hormone may also be mediated by cAMP acting as a "second messenger."

The third substance which increased leaking out of glyoxalase was cysteamine. Cysteamine has a very high affinity to glyoxal and so inactivates the glyoxal and makes the promosomes, stabilized by it, more permeable.

Our observations can be put together into a tentative hypothesis of how the resting β state is stabilized against the innate tendency of the cell to shift into the proliferative α state. In this stabilization of the β state dicarbonyls play an important role. They decrease the D/A quotient and $p\epsilon$ which dominate the α-β balance and the physical state of the cell (see Table 8). They inactivate the SH groups which increase the D/A and $p\epsilon$, and tend to bring the cell into the proliferative α state. The SHs are indispensable for cell division and protein synthesis. The dicarbonyls inactivate them by forming inactive hemimercaptales with them. Moreover, the dicarbonyls play a central role in the chain of reactions which desaturates the energy bands of the structural proteins, which they

transform into free radicals, making the proteins less soluble and stabilizing their structures.

It is tempting to suppose that dicarbonyls are produced all the time in the oxidative metabolism, bathing, so to say, the cell in a constant stream of glyoxal derivatives, the excess of which is decomposed by the indiffusible glyoxalase. If cell division is called for, the glyoxalase is made diffusible and inactivates also the glyoxal derivatives which have stabilized the β state, giving free reins to the innate tendency of the cell to go into the proliferative α state.

The C=O groups seem to be Nature's universal tool to suppress cell proliferation. The animal body has to suppress the proliferation of its own cells, and do this reversibly, without causing damage. This can be done by the use of the moderately active aliphatic dicarbonyls. Vegetable cells have to suppress the proliferation of invading microorganisms, and do this irreversibly. They have to kill. This they can do by the highly active aromatic dicarbonyls, the diquinones of catechol derivatives. Having no blood circulation, the vegetable cell has to suppress bacterial proliferation locally, for which it can use such toxic substances as *o*-diquinones, there being no blood circulation to spread these poisons. The animal, with its blood circulation, does not need such local defense, being able to invoke the immunological defense mechanism of its whole body. It could not use toxic substances either which would be spread by the circulating blood.

About half of the plants contain no phenoloxidase and no polyphenol. Most of these plants are characterized by the presence of a very active peroxidase, and are called, accordingly, "peroxidase plants," (A. Szent-Györgyi, 1928). They produce no dark coloration on damage. Many of these plants are rich –some of them very rich –in ascorbic acid, at the side of which I (1931) found an "ascorbic acid oxidase," a copper-containing enzyme, which oxidizes ascorbic acid to dehydroascorbic acid, a

dicarbonyl. The chemical structure of this substance satisfies the requirement of an inhibitor of cell division.

The oxygen uptake of the cabbage leaf I found commensurate with the oxygen uptake of the ascorbic acid oxidase it contains. This made it probable that the oxidase is involved in respiration, and is, with its Cu atom, responsible for oxygen activation, Cu being a transition metal, similar to Fe.

In the intact plant the ascorbic acid is re-reduced by the dehydrogenases. If the plant is damaged the dehydrogenases cease to work and the dehydroascorbic acid is reduced no more and remains in its oxidized form, playing the same role in peroxidase plants as the quinones do in the phenoloxidase plants, arresting the proliferation of microorganisms.

Possibly, the free radical described in Chapter 13 is the primary oxidation product of the oxidation of ascorbic acid. The bactericidal action of this stable radical has not yet been studied. It can be expected to be high, and it seems possible that it is actually this radical which protects the plant against bacterial invasion when its cells are damaged.

15

SYNOPSIS

The history of life can be divided into two periods: the periods preceding and following the appearance of light and oxygen. In the anaerobic electron donating atmosphere of the first, the proteins must have consisted of soluble, closed-shell dielectric molecules, with paired electrons and saturated energy bands. The protein, at this stage, had to have two main functions: fermentation and proliferation. This state of the living systems was termed the "α state," in which reactions had to be molecular, molecules and ions being the smallest particles capable of independent motion.

This situation was changed by the appearance of oxygen, a strong electron acceptor which could separate electron pairs, transforming molecules into free radicals and desaturating energy bands, making them conductant, lending a high reactivity and mobility to electrons. The unbalanced forces allowed to link molecules together and integrate their actions, creating increasingly complex structures with increasingly complex and subtle functions. This made electrons the actors in the drama of life, using molecular structures as their stage. This made life, to a great extent, into an electronic phenomenon. This new state was termed the β state.

Cells, containing extensive semisolid structures, cannot divide. To be able to do so they have to dedifferentiate, dissolve

part of their structures, and return to an extent to the proliferative α state. After completed division they have to find their way back to the β state again, rebuilding their structures. This makes the α-β transformation reversible. It is supervised by a complex regulatory system. Cell division is, in a way, a repetition of the evolutionary development. Dividing cells being in the α state, they have to share the properties of this state: the lack of color, free radicals, fetal proteins, and low ESR signals, as found by B. Commoner, J. Townsend, and E. C. Page (1954) in cancer and embryonic tissue. The properties of the two states α and β form a package deal and are summed up in Table 8.

The discussions presented in the previous chapters were based chiefly on the observation of the color of the liver, and it could be objected, that apart from the kidney and liver, no other organs have a similar color, and so no general conclusion can be drawn from our observations. Needless to say, different tissues have different functions, which do not have to depend equally on electronic reactions. The greatest interest may be attached to the function of the CNS which produces electric work. Its main function is performed by the "grey matter" which, as the name shows is grey, and grey is diluted black. Evidently, the black color of the nerve cells is diluted by other uncolored, poorly transparent material, as the glia, which has a static function, which does not involve electronic interactions. Some of the centers of the brain also can be recognized by their color, as the "red" nucleus (nucleus ruber). The studies of G. C. Cotzias et al. (1974) have shown the importance of dopamine for the normal function of the CNS. Dopamine, as has been shown, can act as a member of an electron transport free radical chain.

Electronic reactions can be expected to have little meaning for tissues which have static functions, as most connective

Table 8 Properties of resting (β) and proliferative (α) states in cells

α State	$\rightleftharpoons$	β State
High D/A, low pϵ		Low D/A, high pϵ
Unbridled proliferation		Regualtion of cell division
Undifferentiated, molecular dispersion, lack of structures		Differentiated, insoluble structures
Soluble proteins		Insoluble proteins
No contact inhibition		Contact inhibition
Fermentation		Oxidation
No carbonyls		Carbonyls and carbonyl-biogen amine charge transfer complexes
Free SH		Complexed SH
Anaerobic dark reactions		Aerobic reactions, photosynthesis
Dielectric		Semiconductor
Closed-shell molecules, molecular reactions		Free radicals, electronic reactions
Structured water		Random water
Lack of color		Colored
Fetal proteins		No fetal proteins

tissues have, and electronic reactions may play in other organs less prominent roles than in the liver, and so their concentration may not be sufficient to lead to visible color changes. Also, the absorption maxima of the produced free radical may lie outside of the visible range of the spectrum.

The intense purple color of the ascorbic acid free radical suggests the possibility that the pinkish tint of healthy human bodies is not solely due to the blood they contain, but may also be due partly to free radical state of the ascorbic acid present. The deterioration of this radical, after death or in ill health, may be responsible partly for the pale color.

PART II

ON CANCER

16

INTRODUCTORY REMARKS

Cancer, like diabetes, is one of those fascinating faults of Nature which allow us a deeper insight into the mechanisms of life. What retarded its research was that the suffering it causes made us put the cart before the horse, searching for a cure before an understanding. Looking at it through the concepts developed in Part I of this book, cancer appears as a cell, stuck in the α state, or somewhere between the two basic states, α and β. As a consequence, it has to share the properties of other proliferating cells. What makes it into such a threat to life is not the rate of proliferation. There are various cellular systems in our body proliferating faster than cancer does. What makes it into such a threat is that the cancer cell cannot stop dividing when no proliferation is needed. It has lost what Walter B. Cannon called the "wisdom of the body." Something has gone wrong in its regulations.

Cell division and its regulation demands a complex mechanism while cancer can be caused by an almost inexhaustible variety of physical, chemical, or biological factors. It is not believable that all these factors could create the mechanisms involved in cell division. These mechanisms had to be there, and were only deranged by the oncogenic factor. The cancer cell is comparable to a car parked on a slope. If it starts moving one does not ask, "What drives it"?, but asks, "What has gone wrong

with the brake"? The ability to grow is an attribute of life. As W. S. Bullough (1962) put it: "It is the nature of the cell to prepare and undergo mytosis wherever not prevented. Opportunity, and not stimulus is all that is needed for cell division." The basic question is thus not what makes cancer grow but what has kept its cells at rest before, in spite of their innate tendency to multiply: What is this mechanism? Where and why has it gone wrong? What was the brake? How was it released? How can it be put on again? Before these questions are answered there is little chance to control cancer.

As has been shown earlier, oxygen and its electron-transport chain are intimately connected with the development of the β state. The regulatory mechanism, in the case of the liver, declared itself to the naked eye by the color of the structure proteins. This suggests a comparison of the color of structure proteins of normal and cancerous tissue. It speaks for itself that such a comparison can have a value only if the cancer is compared with the homologous normal tissue, if, for instance, liver cancer is compared with normal liver. Such material is difficult to come by for the biochemist working at a marine biological laboratory. Owing to the kindness of Dr. G. Weber, the author is in possession of a rapidly growing parenchymatous rat liver tumor (Morris Hepatoma 3924 A) which, if inoculated subcutaneously into rats, grows in solid lumps which have a relatively low tendency to necrotize.

Figure 15 shows the suspension of the structural proteins of such a tumor, side-by-side with an analogous suspension of the proteins of the normal liver of the same animal. As the figure shows, the proteins of the normal liver are brown, (left tube), while the proteins of the tumor are colorless (right tube), which indicates that in the tumor the whole electron transfer chain was missing, the connections between structure and oxygen were cut,

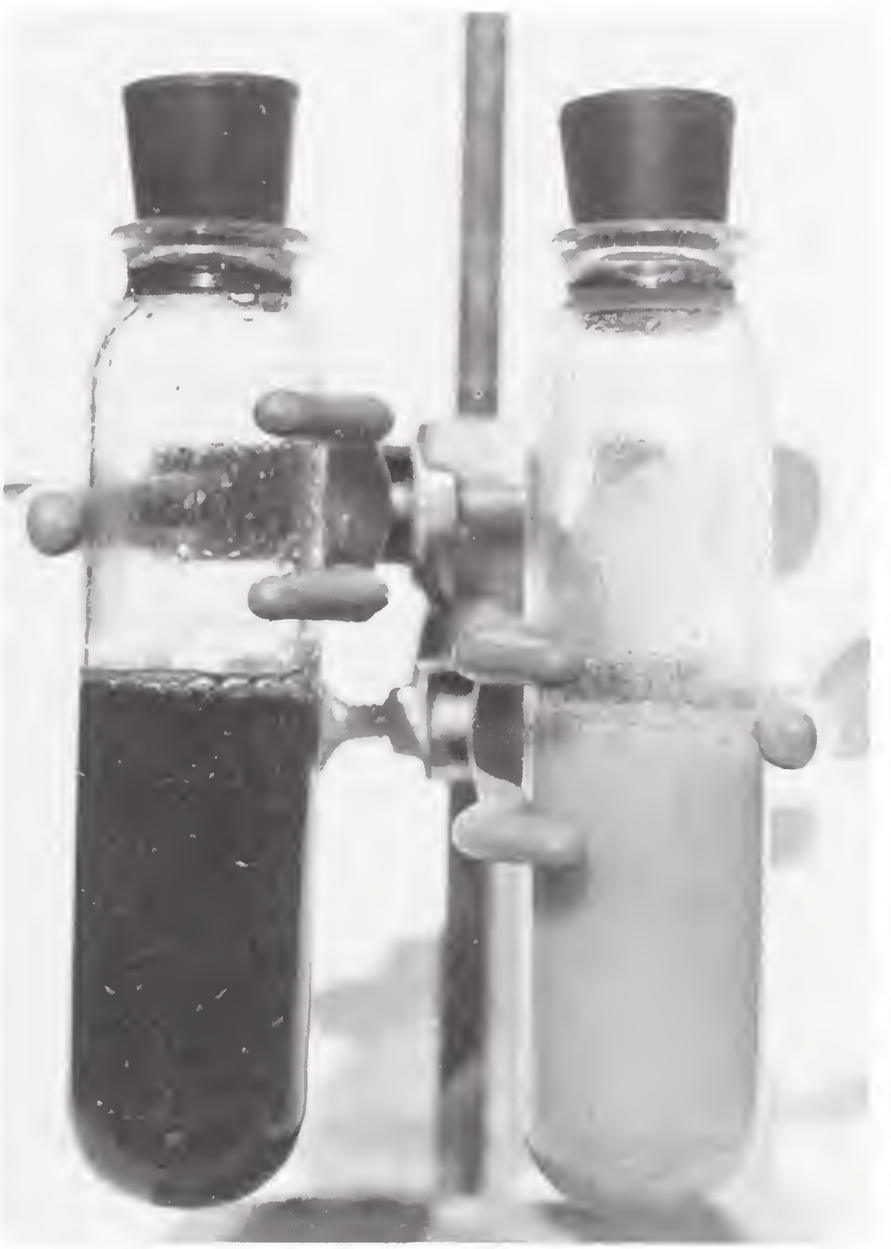

Figure 15 Suspension of structural proteins. Left tube: rat liver. Right tube: parenchymatous rat liver tumor.

making the cells unable to utilize oxygen and build up its β state. (For colors see Fig. 9 in Szent-Györgyi, 1973.)

That this difference in color was due to a failure of the electron transport system and not to change in the nature of the proteins could be shown by treating the proteins of the cancer with an electron acceptor, croton aldehyde, whereupon they assumed the color of the proteins of the normal liver.

The next and more complex question is: Why is the cancer cell unable to build up its electron transport chain? What complicates this problem is the nature of a chain. A chain is an indivisible unit. However long it may be, and however different its single links may be, they are equivalent because if any of them breaks, the whole chain is broken and becomes inoperative. This makes the qualities enumerated in Table 8 into a "package deal" creating a "domino" relation, the change in one factor inducing a change in all the others.

There may be changes which, in themselves, could make the building of an electron transfer chain impossible. If, for instance, some mutation on DNA would deprive the cell of its ability to immobilize the glyoxalase, then the cell could not maintain its dicarbonyl level and would have to go on dividing. Such a disturbance could be corrected only by correcting the primary change. But there are also factors which, in themselves, could not cause cancer, but, owing to the interdependence of the parts of the regulatory system, could push the cell into a vicious circle out of which the cell could not emerge without outside help, even if the original disturbance has been corrected. This case may be illustrated with the observation of H. Goldblatt and G. Cameron (1953), according to which a temporary partial lack of oxygen can induce a malignant transformation in tissue cultures. It is easy to believe that a lack of O_2, which induces changes in other factors, will eventually take the cells back from the oxidative β state to the fermentative α state. One may be tempted to paraphrase Ben Franklin's poem about the lost horseshoe nail:

For the lack of oxygen oxidation was lost,
For the lack of oxidation carbonyls were lost,
For the lack of carbonyls regulation was lost,
And all this for the lack of oxygen.

A disturbance, like the lack of oxygen, may push the cell into a vicious circle which the cell is unable to break and which may land it in the proliferative α state. Without oxygen no dicarbonyls can be produced and without dicarbonyls no oxidation system can be built. Such vicious circles may play a major role in oncogenesis. The introduction of dicarbonyls or their radical complexes may break them. The disturbance of the regulatory mechanism, at any point, may break the electron transport chain and lead to senseless proliferation. A mutation on DNA may leave the cell unable to make the glyoxalase non-diffusible, letting it destroy the dicarbonyls, or an excess of Greensteins catalase inhibitor may start proliferation by inactivating the catalase and arresting charge transfer to O_2. The possibilities are very numerous. The work of T. T. Puck, K. R. Porter, and their associates suggests that many changes are reversible. I expect that dicarbonyls will induce contact inhibition in cancer cells.

These relations open the way to a new philosophy of therapy and prevention. Until now cancer was looked upon as a hostile intruder which had to be eliminated. It might be looked upon also as a cell in trouble, which needs help to return to normal. Naturally, changes may become constitutive, irreversible. Whether there is a "point of no return" is unknown.

17

ON CAUSE, CURE, AND PREVENTION

The research of my laboratory was led by the conviction that we have to understand before we can cure. Accordingly, our work aimed at an understanding and no serious attempts were made to develop a cure, and here I will limit myself to a few general remarks on this line.

It is believed by some that cancer is not one disease, but many, as many as there are factors which can cause it. Cancer is but one disease, the disturbance of the regulatory mechanisms. This mechanism, however, is a complex one, and consists of many parts which can be deranged. So cancer is many diseases, as many as there are parts in its regulatory mechanism which can go wrong. This is an important point because a deranged machinery can be repaired only where it has gone wrong. Accordingly, a therapeutic agent which is active in one case may be entirely useless in another, supporting F. E. Knock's contention that chemotherapeutic agents should possibly be tested on the cancer which has to be treated. Dr. G. Klein of the Karolinska Institute, Stockholm, was kind enough to test, at my request, glyoxal for its carcinostatic action on three of his cancer strains. The experiment consisted of exchanging the drinking water of the mice with an 0.05 M solution of glyoxal. The cancer ELD (an Ehrlich ascites tumor) was completely arrested, while TA 3

and MDAY (a sarcoma originally produced by methylcholanthrene) remained totally unaffected. This makes it evident that the elucidation of the regulatory system is one of the most urgent tasks of research. It is rather disturbing that the regulators are not yet idientified definitely. The efforts of my laboratory, on this line, were cut short for lack of financial support.

To achieve a cancer therapy, the regulatory system would have to be known, and methods would have to be worked out for the identification of the locus of the disturbance. A kind of "cancer science" would have to be developed.

Cure and prevention of cancer are two different propositions and may demand different means. Prevention is not necessarily a preventive therapy. Cars, out of order, have to be repaired at the disordered point, but disorder can be prevented by good servicing. Greasing may prevent, but not repair it.

Cancer may be prevented by shifting the basic parameters, as the D/A relation in a sense which favors the β state and disfavors proliferation. I have, myself, made but one tentative experiment on this line. It consisted of setting up two groups of "retired breeders" of C3H mice, of the Jackson Laboratory (Bar Harbor, Maine), each consisting of 50 animals. Both groups received standard food. One of them received, for drink, pure water, while the other received a 0.05 M glyoxal solution. During the first 16 weeks of the experiment no cancer was observed in the group receiving the carbonyl, while 11 tumors appeared in the other, a difference too great to be accidental. This indicates that cancer can be prevented simply by decreasing D/A by dicarbonyls, which can be done without major inconvenience *per os* medication. After the 16th week the difference between the two groups faded out, suggesting that we might have to distinguish between cancer of senescence, and the cancer which hits at an earlier age, which may be called "untimely cancer." Cancer, in a

way, is a natural phenomenon. If we would live long enough we would, probably, all die of cancer, brought on by the final collapse of the living machinery, which cannot be prevented by carbonyls. In any case, this experiment is worth repeating on a bigger scale and is encouraging. It suggests that while cancer of old age might not be preventable by a shift of D/A, "untimely cancer" can. It is probable that more effective electron acceptors will be found than glyoxal.

Since the balance between the α and the β state depend on many factors, it seems possible that a therapy can be achieved only by influencing simultaneously several of them. It seems also possible that the shift in any one factor, favoring the β state, can be used to support other therapies, as therapy by high energy radiation, which can be supported by dicarbonyls, sensitizing the tumor, as has been shown by Ashwood-Smith et al. (1967).

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REMARKS ON DICARBONYLS

The experimental material in Part I suggests that dicarbonyls arrest cell division by interacting with SH groups, essential for proliferation. They also stabilize the resting β state by increasing the cohesive forces, and building semisolid structures which interfere with division.

As far as our experience goes, glyoxal and methylglyoxal are harmless as long as their concentration does not exceed that of SHG present. Beyond this point they become toxic. My mice supported the intraperitoneal injection of 0.25 ml of 0.1 M solution of these dicarbonyls without trouble, but died when this dose was exceeded. A 0.25 ml dose of a 0.1 M solution in a mouse of 25 g establishes an average concentration of 0.001 M, which is the average concentration of SHG. Two such injections *per diem* mostly inhibited the growth of cancer by 50%.

The injected dicarbonyls are eliminated rapidly. Dr. L. Egyud (1965) found, soon after the injection of radioactively labeled methylglyoxal, radioactivity in the urine and the expired air. The glyoxalase also has to contribute to the inactivation of the dicarbonyl. This rapid elimination cannot be compensated for by injecting more dicarbonyl because of the narrowness of the limits set by the concentration of SHG. In order to prevent cancer rejuvenating itself by a cell division, the concentration of

the dicarbonyls would have to be maintained, which is very difficult in small experimental animals like mice, but meets no difficulty in man. This makes testing of the therapeutic activity of dicarbonyls in mice very difficult. In my laboratory 50% inhibition of the growth of inoculated cancer could be achieved by two daily intraperitoneal injections of glyoxal or methylglyoxal. A 50% inhibition is much too low for a therapy, but if the injected dicarbonyl were eliminated in, say, 3 hr, then two daily injections left the animal unprotected to 16 hr of the day, leaving the cancer cell free to multiply. If the inhibition lasted only 3 hr, it may have been a 100% inhibition for this short period and would have meant a cure in a continuous application.

Being physiological regulatory substances, dicarbonyls, applied in a nontoxic dose, cannot be expected to kill the cancer cell. They can kill it only if applied in a concentration which exceeds that of the SHG. In this high concentration the dicarbonyls can be applied but locally. M. A. Apple and D. M. Greenberg (1967, 1968) as well as L. Egyud and myself (1965, 1966a,b, 1968) have shown that methylglyoxal can cure ascites cancer if applied locally by intraperitoneal injection.

Various attempts have been made to make the increase in the quantity of injected dicarbonyls permissible. I found that the quantity of injected methylglyoxal could be increased beyond the allowed measure by injecting it with an isomolar SHG. What limited possibilities on this line was the high molecular weight and high price of SHG, the first of which made the quantities bulky, the second the expense prohibitive. N-acetyl cysteine had similar effects, has a smaller molecule, and is cheaper. Homocysteineamide and *d*-acetylhomocysteinamide had no protecting action, their affinity to glyoxal being too low. Contrary to this,

the affinity of cysteamine was too high, rendering the glyoxal completely inactive.

Attempts were made also to protect the aldehydic group of dicarbonyls by covering it with some substituent which could be split off in the body and would protect the dicarbonyl against the glyoxalase, make it less toxic, and make the concentration of free carbonyl more uniform.

It seems difficult to predict a therapeutic action in man from experience gained on mice with inoculated cancer, in which the changes may have become irreversible during the countless passages that such cancer has possibly undergone.

The oncostatic action observed, in the case of dicarbonyls, depends also on the size of the inoculum. With 10^7 inoculated cells I could not obtain inhibition higher than 50%, while with 10^2 inoculated cells I could arrest cancer completely. What lends interest to this point is that cancer or metastasis starts with a small number of cells. As to the mechanism of this dependence of growth inhibition, the possibilities are numerous. One of them is that cancer cells defend one another by producing glyoxalase or J. Greenstein's (1954) catalase inhibitor.

In order to be able to maintain a steady low local concentration of free glyoxal, I synthesized a compound of this substance with ethylaminoethanol, which has no taste, and no smell, and produces a low concentration of free glyoxal by its gradual dissociation. This substance is being tried in the treatment of basal cell skin cancer with very encouraging results.

In conclusion I would like to mention a line opened up by E. Schauenstein and his associates (1964a,b, 1967a,b,c, 1968), who found that hydroxy enals are formed in autooxidation of highly unsaturated fatty acids, and have a strong carcinostatic action. These substances are closely related to dicarbonyls.

Instead of two neighboring C=Os, they have a C=O and a C=C. Croton aldehyde belongs to this group. Enals would have the advantage over dicarbonyls that they are not inactivated by the glyoxalase. This made me try croton aldehyde for its carcinostatic activity. I found it rather toxic, which I attributed to additions on the double link. I tried to prevent such addition by a methyl substitution in position 3. Methyl-croton aldehyde (seneceic acid) was kindly synthesized for me by Mr. Skip Nelson in Dr. Max Tishler's laboratory at the Wesleyan University, Middletown, Connecticut, and by Dr. L. Bursics of the Isotope Laboratory of the Hungarian Academy of Science. Dr. Bursics also synthesized the corresponding methoxy derivative. My profound gratitude is due to both researchers and to the Hungarian Academy of Science. Both substances were found to be toxic and offered no advantage over dicarbonyls.

It has been objected to the therapeutic use of dicarbonyls, that if these substances would arrest cell division they would have to kill the host before killing the cancer, by inactivating the hematopoietic apparatus. This objection may hold for unphysiological substances which have a general action on mytosis, but does not hold for dicarbonyls which are physiological regulatory substances, tuned to a definite regulatory mechanism. The regulation of the hemoatopoietic apparatus is different from that of other body cells, and so dicarbonyls need not stop their activity. During the long years of work with dicarbonyls I have never seen any sign of the failure of the production of blood corpuscles, which is regulated by different means. The production of red blood corpuscles can be speeded up by low barometric pressure, which has no such action on other cells. Similarly, the production of white blood corpuscles can be elicited by ingestion of antigens. So it seems possible to arrest the growth of cancer cells without interferring with hematopoiesis.

19

SCATTERED OBSERVATIONS

According to the theory presented, factors which favor the action of glyoxalase and disfavor that of carbonyls, should favor carcinogenesis, while factors which disfavor the action of glyoxalase and favor that of carbonyls, should be carcinostatic. In this chapter I want to review a few scattered observations of others, to see how far they bear out these expectations.

I have touched upon thiourea before (Chapter 14), which is known as a weak carcinogen which, as shown by M. P. Schubert (1935), complexes (in its "pseudoform") with methylglyoxal. As shown in Chapter 14, it also makes the promosomes permeable to the glyoxalase, should thus be oncogenic, and promote cell division. As shown earlier, they do.

Hydrazines form strong complexes with carbonyls, should thus produce cancer, but, until lately, were not known to be carcinogens. They may have been too poisonous, killing the animals before they could produce cancer. It was discovered but lately by A. E. Rogers and M. Newberne (1973) that dimethylhydrazine, given to rats *per os* in small doses, produces cancer of the colon. Evidently, its quantity administered was too small to kill the animal, but its local concentration high enough to produce cancer.

Naphthylamine is a classical carcinogen. It produces cancer of the bladder in workers of aniline industry. Being secreted by the kidney and stored with the urine in the bladder, it has a chance to interact with the bladder epithelium. Naphthylamine is a puzzle because only 2-naphthylamine is oncogenic while 1-naphthylamine is not, though the two isomers are very similar. As shown by B. and A. Pullman (1952) almost all electronic indices of the two are identical.

Aromatic amines react readily with dicarbonyls, forming complexes with them. The methylglyoxal complexes of the two napthylamines are different. The α isomer forms a vivid green complex, the color of which is, probably, due to "charge transfer in the excited state." The complex formed by the oncogenic β form is grey. The darker color indicates absorption in a wider spectral region and might be due to charge transfer in the ground state.

Attention has turned lately towards chemically indifferent carcinogens, like plastics and asbestos. Asbestos is a strong carcinogen. Its mechanism of action seemed a mystery. It was discovered lately by P. Davies et al. (1974), that it induces a rapid, massive, and selective release of lysosomal enzymes. If it damages lysosomes it can be expected to do the same to the more unstable promosomes. The same may apply to various plastics which are known to produce cancer. If implanted in tissues, they may upset the subtle balances of the resting state, produce disorder.

Keeping enzymes and substrates apart within the very narrow limit of the cell demands a very high degree of order. Any factor which creates disorder and interferes with the separation of carbonyls and glyoxalase should be carcinogenic. Chronic irritation is such a factor and is known to be carcinogenic. Disorder is a very unspecific state and can be induced by an endless

number of factors which may explain why cancer can be produced in such a variety of ways.

The disorder of protein structure has to entail the disorder of water structures. According to R. Damadian (1971, 1974), the disordered water is one of the characteristics of cancer, characteristic enough to be used as diagnostic means as measured by nuclear magnetic resonance. Disorder has to interfere with the good working order of promosomes.

It may be well to try to clarify here the relation of the presented material to the virus theory of cancer. As a biologist I can discuss only the biological end of the story. What is clear to me is that cell division is brought about by a very specific, involved, and subtle biological mechanism. This mechanism has been built by Nature through millions, if not billions of years. Viruses cannot produce such a mechanism. What they can do is only to disturb the regulations of this mechanism, set the machine going, not create it. They can set it in motion by creating disorder. So the virus theory and theory presented in this book are not contradictory, but complementary.

The most common carcinogen is age. If we would live long enough all of us would die of cancer. Any mechanism tends to deteriorate in time, become disordered. So age may predispose for cancer by the disorder it entails, but there may be more to it. Disorder is not the most prominent symptom of age. The greying of hair and feathers is a more striking and common symptom, which is due to the failure of the oxidative machinery which produces pigments. This may be an expression of a general tendency of oxidative processes to slow down, a tendency towards an increased D/A quotient. As I pointed out earlier, the newer a process evolutionarily, the more easily it is discarded. So the decrease in oxidative processes may be the expression of a tendency to shift back in senescence from the newer β state

towards the more archaic α state, when, in old age, the whole edifice of life begins to crumble, become disordered. As I mentioned before, the cancer of old age may be different, in its genesis, from cancer of an earlier age, in which cancer may be called "untimely." While the cancer of old age may be an expression of the general collapse, the latter may be due to a failure of the regulatory machinery at a specific point, which might be accessible to repair or compensation.

20

NOTES ON HISTORY

The research presented in this book had its origin in the observation that tissue extracts contain factors capable of promoting or retarding the growth of inoculated cancer. The *pro*moting factor I called "*pro*mine," the *ret*arding one "*ret*ine." I was neither the first nor the last to make such observations. The existence of two such antagonistic factors could have been predicted even without any experimental observation. Regulation always involves two antagonistic factors. Traffic cannot be regulated by a red or green light alone, only by a red *and* a green light. This made it seem possible that "retine" and "promine" were connected with regulations, and I wanted to know more about them. The experiments performed with Dr. L. Egyud (L. Egyud and A. Szent-Györgyi, 1965, 1966a,b, 1968) tended to indicate that "retine" may be a dicarbonyl. This led to electron acceptors and donors, and, eventually, to the wider natural philosophical outlook presented in this book.

The oncostatic action of carbonyls has a long history. The first to observe the growth inhibitory action of an aldehyde, heptylaldehyde, were L. C. Strong and L. F. Whitney (1938). In the sixties, public attention was directed to the carbonyls by W. F. Koch (1961), who claimed a therapeutic action for quinones. Unfortunately, the data given by him were not detailed enough

to allow repetition. I found quinones devoid of therapeutic activity. They inhibit cell division but are very toxic.

After G. E. Underwood (G. E. Underwood, 1956; G. E. Underwood and S. D. Weed, 1956) had shown a ketoaldehyde to be highly active against Newcastle disease virus, B. I. Tiffany and his associates (1957) extended this experience to other carbonyls. In 1958 F. A. French and B. L. Freedlander started their series of papers on the "Carcinostatic Action of Polycarbonyl Compounds and their Derivatives," reporting on the action of "Kethoxal," β-ethoxy-α keto butylaldehyde, which is still on the market as cancerostatic agent. Subsequently, various hydrazones of glyoxal and methylglyoxal were found to have oncostatic activity. Glyoxal-bis-guanyl hydrazone and glyoxal-bis-thiosemicarbazone (B. L. Freedlander and F. A. French, 1958), methylglyoxal-bis-thiosemicarbazone (V. C. Barry, M. L. Conalty, and J. F. O'Sullivan, 1966), methylglyoxal-bis(N^4-methylthiosemicarbazone) (E. Mihich and C. A. Nichol, 1965), hydroxymethyl-bis-guanylhydrazone (L. Li et al., 1963), methylglyoxal-bis-guanylhydrazone (E. Freireich, E. Frei, and M. Karon, 1962), glyoxal-bis-thiosemicarbazone (F. A. French and B. L. Freedlander, 1958), were found to be active, suggesting that cancer cells can split the CN link. That these compounds have not achieved a major therapeutic importance is probably due to the toxicity of hydrazine moiety.

C. P. Kenny and R. G. Sparks (1968, 1969) found in their tissue cultures a bacteriostatic ketone aldehyde (α keto-4-hydroxy butylaldehyde).

A most important contribution to the field was made by E. Schauenstein and his associates (1964a,b, 1967a,b,c, 1968) who showed that in autooxydation of highly unsaturated fatty acid, unsaturated aldehydes, hydroxyenals were formed which had a strong oncostatic action. They have shown lately (G. Nohammer, E. Schauenstein, and P. Weber, 1973) that 4-hydroxypentanal

can bind to the thiols of structural proteins of Ehrlich ascites tumor cells. Should enals be involved in regulation, then the question would come up how they are inactivated. They may be deactivated by methylation which transforms the aldehydic group into a less reactive ketone. In this case factors inhibiting methylation should have a carcinostatic action. It has been shown by J. B. Lombardini, A. W. Coulter, and P. Talalay (1970) that substances which interfere with the formation of the methionine-adenyl complex, the main methyl donor, inhibit the growth of inoculated cancer. Possibly, the mesityloxide found by Dr. L. Egyud and B. Andresen (personal communication), in brain extracts was but a 2-methylcrotonaldehyde inactivated by methylation. The oncostatic action of carbonyl is thus a well-established field. What was not known before is that they are involved in normal regulatory processes, are thus normal cell constituents which played a major role in evolution.

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"Cancer research has been greatly retarded by our asking: 'why does cancer grow' instead of asking: 'what keeps a normal cell from growing'."

–from *Electronic Biology and Cancer: A New Theory of Cancer*

To be able to control cancer, we must have a detailed knowledge of the principles and mechanisms underlying cell division and its regulation. To understand cancer, we must extract it from the narrow confines of medicine and look upon it not as a disease but as a natural phenomenon. We must utilize the wide outlook of natural philosophy to search for the solution to the problem on the electronic level.

The extension of biology from the molecular to the electronic dimension is a necessary adjunct to twentieth century civilization's transition from mechanical to electric and electronic technology. Dr. Albert Szent-Györgyi—renowned for his discovery of ascorbic acid (Vitamin C) and his pioneering research in metabolism, muscle chemistry, and quantum biology — views the uncontrolled cell growth that is cancer as a derailment of normal processes.

"Every cell has to have the [capacity] to proliferate . . . the first problem of cancer research is how the innate [drive] to proliferate can be suppressed. . . . Our ability to correct defects depends on our knowledge of this regulatory system." Rendered in lay language, imbued with a spirit of humanistic concern for human health and well-being, Nobel Laureate Albert Szent-Györgyi's *Electronic Biology and Cancer: A New Theory of Cancer* is an excursion to the frontiers of scientific thought.

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