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The living state and cancer

($\alpha \rightleftharpoons \beta$ period/methylglyoxal as electron acceptor/doping of protein/glyoxalase and entropy/SH catalysis/oncogenic paradox)

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ABSTRACT **Complex living structures developed on our globe after the appearance of light and oxygen. In functions of these structures, solid state phenomena play a major role. The structural proteins were made into radicals by doping, the covalent incorporation of electron acceptors. This lent mobility to their electrons and a subtle reactivity to their molecules. Cancer is unable to go into the radical state.**

We divide the surrounding world into "animate" and "inanimate," alive and not-alive. The division is sharp, unequivocal, which indicates that there has to be some basic difference between the structures of the two. The nature of this difference may be the most important problem of contemporary science. Apart from having this intrinsic interest, the problem also urgently asks for a solution because the understanding, cure, and prevention of a host of degenerative diseases, like cancer, are waiting for our better understanding of the living state. We can control only what we can understand. Shortcuts, in science, mostly turn out to be blind alleys.

The history of life has been divided into two periods by the appearance of light and oxygen. There are reasons to believe that in the first dark and anaerobic period, which I have called the "α period," life could develop but the simplest forms, capable only of the most primitive vegetative functions. Life began to develop and differentiate, build increasingly complex forms capable of increasingly complex and subtle functions, when light and oxygen appeared. The second, aerobic and light, period of development and differentiation I have called the "β period." Accordingly, we have two problems. First, what distinguished the animate from the inanimate in the first, primitive α period? Second, what had to be changed in the basic structure of the living systems to open the way to development and differentiation, the final product of which is us?

The α–β transition

Life originated on a dark and airless globe, covered by dense water vapor. There was no light and no oxygen. Life has left behind very few traces from this first dark and anaerobic period and so we can only philosophize that under the inhospitable conditions of that period life could develop but the simplest forms, capable of performing only the simplest "vegetative" functions. The main product of this period had to be the protein molecule which, by its specific folding, could develop a catalytic activity.

To make life perennial, the living systems, in this period, had to proliferate as fast as conditions permitted. Energy for this proliferation had to be produced by fermentation so that the α period could also be called the fermentative period of unbridled proliferation.

When, owing to cooling, the water vapor condensed and light reached the surface of the earth, life started to develop and differentiate, build increasingly complex forms capable of increasingly complex and subtle functions. To guard the harmony of the whole in the midst of increasing complexity, the unbridled proliferation had to be replaced by regulation.

What life did with the light was to capture its photons and use their energy to separate the elements of water. The hydrogen it fixed by linking it to carbon, producing its own food, the oxygen it released. The invested energy it could recover by reverting the process and oxidizing the hydrogen of food to water again.

The protein molecule developed in the α period was, essentially, a long chain of peptide bonds. There being no oxygen, the atmosphere had to be strongly reducing, dominated by electron donors—that is, molecules tending rather to give off than take up electrons. Under these conditions, the orbitals of the protein molecule had to be occupied by electron pairs, strongly held in their place. The resulting "closed shell molecule" had to have a low reactivity and little tendency to build complex structures, there being no unbalanced forces to hold it together, and no electronic mobility to integrate its function with that of other molecules.

Present biology is a molecular biology. According to it, the main bearers of life are the protein macromolecules with their molecular reactions. One may wonder how such poorly reactive clumsy macromolecules could bring about those subtle biological reactions which characterize life and lend its charm to biology. One may wonder whether these macromolecules are really the main actors of life and whether the main actors are not very much smaller and mobile units, electrons, while the macromolecules themselves are rather the stage than the actors of the drama of life. The problem is whether the electrons of proteins could achieve a greater mobility, lending a subtle reactivity to the protein.

The situation in a closed-shell electronically saturated molecule has often been compared to that in a completely filled garage where there can be no mobility. By taking out a single car, all others are made mobile. Similarly, a closed shell molecule can be transformed into a highly reactive radical with highly mobile electrons by taking out one of its electrons. The electron, taken out, has to leave behind its earlier partner uncoupled, has to leave behind an electron hole and a partially occupied orbital. All this has to upset the electronic balance of the entire molecule. Molecules containing an uncoupled electron are radicals, and radicals are known for their great reactivity.

Single electrons can be taken out from molecules by other molecules that have a low-lying unoccupied orbital in which they can accommodate an additional electron. Such a transfer of an electron from one molecule to another is called "charge transfer," in which two radicals are formed, the "acceptor" having a negative, the "donor," a positive charge. In the α period no such charge transfer could take place in proteins. There being no oxygen, the atmosphere had to be strongly reducing

Abbreviations: MG, methylglyoxal; ESR, electron spin resonance.

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and at the generated high electron pressure there could be no stable strong electron acceptors, with low-lying empty orbitals.

Oxygen as electron acceptor

Oxygen is a strong acceptor. After its appearance the atmosphere was no more dominated solely by electron donors, but was dominated by a new parameter, the D/A quotient, the relation of donors to acceptors.

The appearance of oxygen opened the possibility of taking electrons out of the protein molecules, transforming them into radicals with a high degree of reactivity and electronic mobility. The O_2, as such, could not be used as electron acceptor because, being di- or tetravalent, it tended to take up electrons pairwise. The pairwise transfer of electrons is not charge transfer but burning, oxidation, which does not lead to the production of radicals.

Oxygen can be made into a monovalent acceptor by linking its atoms, by double bonds, to C as in C=O, carbonyl, instead of linking them to one another, as in O=O. The carbonyl group being small, it cannot easily accommodate a whole additional electron, which makes it into a weak acceptor. However, its π electron pool can be extended by placing at its side another double bond, be it in the form of another C=O, or in the form of a C=C. The two double bonds being conjugated, their π systems fuse to a wider π system, which is a strong, though still monovalent, acceptor. Two C=Os make a dicarbonyl, a C=O and a C=C make an enal.

Aldehydic C=O is rather reactive but, being hydrated, is not a strong acceptor. Ketonic C=O is stronger, but less reactive. The smallest ketone-aldehyde which unites the advantages of both ketones and aldehydes is methylglyoxal, MG. MG can rather easily be formed along various metabolic pathways. It can form even spontaneously from triose. 2,4-Dinitrophenylhydrazine precipitates out of a triose solution a hydrazone of MG.

In live tissues there are two streams of electrons. In one the electrons are transferred pairwise to oxygen, O_2, producing energy. In the other, the electrons are transferred one-by-one, the acceptor being the C=O, carbonyl. This stream serves the electronic desaturation of protein.

The glyoxalase

The previous chapter opens the possibility that it might have actually been MG or a closely related ketone-aldehyde that served as acceptor in the electronic desaturation of protein and so might have started up the development and differentiation in the α period. To guard the harmony of the whole in the midst of differentiation, the unbridled proliferation of the α period had to be arrested. It seems logical that Nature should have used the same substance for the arrest of proliferation with which she started the development and differentiation. Együd and I (1) have shown that MG, at a low concentration, can arrest cell division reversibly, without harming the cell, and so could play a central role in cellular regulation.

Regulation always involves two antagonists. Traffic cannot be regulated by red or green light alone, only by red *and* green. The antagonistic influence in the regulation of cell division might have been the production and rapid inactivation of MG.

It was more than 60 years ago that D. H. Dakin, H. V. Dudley, and C. Neuberg discovered simultaneously a most active enzyme capable of transforming the active MG into inactive D-lactic acid. This methylglyoxalase (mostly called "glyoxalase") is one of the most active and widely spread enzymes. Nature does not indulge in luxuries and if there is such a widely spread and highly active enzyme, it must have something very important to do, but nobody could find out what, neither MG nor D-lactic acid lying on a metabolic highway.

The glyoxalase occupied the attention of a great number of leading biochemists whose studies showed that it actually is not an enzyme, but an enzymic system, composed of two enzymes, "glyoxalase I" and "glyoxalase II," which use SH-glutathione as coenzyme. MG and SH-glutathione form spontaneously a hemimercaptal which is the substrate of glyoxalase I, which transforms it into a thioester. The glyoxalase II then splits the thioester into D-lactic acid and free SH-glutathione, recovering the coenzyme.

But how could MG act as regulator in the presence of a highly active enzyme that destroys it? Evidently, in the resting cell it is stored in an inactive state. Physiology has a great number of examples of keeping a substance in an inactive state. Enzymes can be kept in an inactive state, for instance, by being stored as proenzymes or by being enclosed in impermeable vesicles. They can also be anchored to structures.

Whatever the case may be, the inactivation of a potent enzyme within the narrow boundaries of the cell demands a high degree of order. Any disturbance of this order (say by a carcinogen) would then lead to the release or activation of the glyoxalase, the destruction of MG, and the consecutive induction of proliferation. If it was MG that brought the cell from the α to the β state, then the destruction of MG has to have the opposite effect, and bring the cell from the resting β to the proliferative α state. This could explain why my cells begin to proliferate if I cut myself; the cut is a disturbance which activates the glyoxalase; the glyoxalase destroys MG and brings about proliferation which eventually fills the discontinuity, restores order, and inactivates the glyoxalase.

The oncogenic paradox

The malignant transformation of tissues involves a paradox which, to my knowledge, has not been pointed out before.

This transformation is a very specific process which must involve very specific changes in a very specific chemical machinery. Accordingly, one would expect that such transformation can be brought about only by a very specific process, as locks can be opened only by their own keys. Contrary to this, a malignant transformation can be brought about by an infinite number of unspecific influences, such as pieces of asbestos, high-energy radiation, irritation, chemicals, viruses, etc. It is getting more and more difficult to find something that is not carcinogenic. That a very specific process should be elicited in such an unspecific way is very unexpected.

The paradox can be solved by supposing that the process in question takes place in two steps, an unspecific and a specific one. The unspecific action may be making disorder. Disorder in an ordered system can be produced by practically any influence. Disorder, in cells, may lead to the activation of glyoxalase, the destruction of MG, and the release of proliferation from its inhibition, leading the cell into the proliferative α state. Proliferation will then go on as long as the carcinogen is present and disorder persists.

It is also believable that the destruction of MG pushes the cell into some vicious circle out of which it cannot emerge without outside help. Without MG the cell may be unable to find its way back to its β state. Such a vicious circle is known to most of us: without our spectacles we cannot find our mislaid glasses.†

† If these considerations are correct, then cancer should be produced also by substances that inactivate MG, as 2-naphthylamine or dimethylhydrazine (2) does.

Structure versus solute

In the α period the main components of living systems had to be the soluble protein molecules catalyzing various reactions which did not involve solid-state phenomena. In the β period extensive systems of structures were built. If Nature develops a new system she does not throw the old one out, but builds the new one on top. So she built the system of structures into the solution of dissolved proteins which continued to perform their primitive functions, catering also for the structures. It was the function of the structures which demanded free radicals and electron mobility.

Pohl, Gascoyne, and I (3) separated the soluble proteins and the structures (which, by definition, have to be insoluble), by blender-treating tissues in ice-cold half-saturated ammonium sulfate, then centrifuging out the insoluble proteins. These gave a strong electron spin resonance (ESR) signal, consisting to a great extent of radicals, while the soluble proteins (separated after full saturation with ammonium sulfate) gave practically no signal at all.

This duality in the nature of proteins has, till now, not been fully appreciated. When I proposed, more than 30 years ago (4), that proteins may be semiconductors, the main and apparently decisive argument against my proposition was that none of the great number of proteins isolated in crystals showed any signs of semiconductivity. It was overlooked that crystalline proteins have to belong to the soluble group and so cannot be expected to be semiconductors. It is the structures that are semiconductors, which cannot be crystallized.

The doping of protein

Two molecules can be transformed into radicals by transferring an electron from the one to the other. If the two radicals dissociate, the donor carries with it a positive, the acceptor, a negative charge. Radicals are highly reactive, especially if they carry a charge. Many biologists doubt the possibility of the existence of such charged stable radicals, thinking their high reactivity incompatible with life.

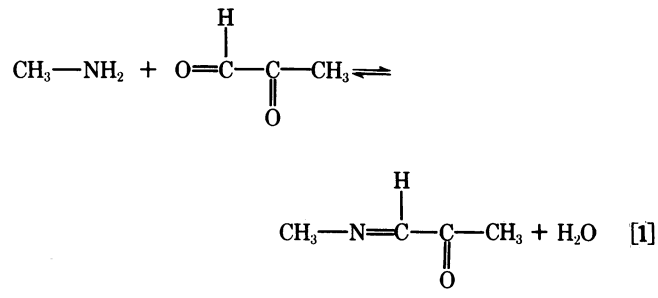
What would be the situation if a protein macromolecule transferred one of its electrons onto an acceptor of small size, but would, before transferring it, incorporate this acceptor into itself? In this case again, two charged radicals would be formed, transforming the protein macromolecule into a biradical, but this biradical would have no net charge, would thus not make trouble. This process of incorporating a small acceptor would be very closely analogous to the doping used in the semiconductor industry. This seems to be the way in which Nature transformed protein macromolecules in the β period into highly reactive radicals without endangering life. She discovered doping 800 million years before man did.

If the protein macromolecule donates an electron to a ketoaldehyde, such as MG, then the transferred electron can be expected to be one of the nonbonded electrons of an amino nitrogen and can be expected to become located mainly on the ketonic C=O, which Abdulnur showed to have a very low-lying triplet level (5).

Proteins being very complex, I started studying this transfer reaction on a model, composed of MG and a simple amine. The simplest aliphatic amine is methylamine.

If dilute (0.1 M) solutions of methylamine and MG are mixed, the gradual appearance of a yellow color indicates the formation of a Schiff base (reaction 1).

The yellow color of the Schiff base is due to the chromogenic C=N group. It is easy to show, by using butylamine instead of methylamine, that the reaction takes place in two steps, a colorless intermediary product being formed first.



Both methylamine and MG are freely soluble in acetone, but their complex is not. If 0.5 M acetone solutions of methylamine and MG are mixed, a heavy colorless precipitate is formed, which rapidly becomes a dark purple, showing a strong absorption at 475 nm. As shown by H. Pohl (unpublished), this purple substance is a tetramer. It can readily be separated on the centrifuge. It gives a strong ESR signal with a rich hyperfine structure, which leaves no doubt that electrons have been transferred from the amine to the ketoaldehyde. The ketonic C=O being connected with the amino N by a bridge of conjugated double bonds, the electron can move over freely, leading to the formation of two covalently bound radicals.

The question is: do proteins give similar reactions? As material for these experiments I chose casein.

If dry casein powder was suspended in methanol containing 10% (wt/vol) neutralized commercial (40%) methyglyoxal and was incubated overnight, next morning the casein was found to have turned brown, having taken on the color of liver. The color could not be detached from the protein by repeated precipitation, dialysis, or "washing" with water or alcohol. The dried brown casein gave a strong ESR signal (3), very similar to that given by methylamine–MG complex. The number of spins tended towards one per casein molecule, which suggested that it was a special amino group that was responsible for the reaction, possibly the terminal amino group of the protein. Similar results were obtained with other proteins, such as serum albumin. Pethig and I (6) found the brown casein to have a greatly increased electronic conductivity.

All this indicates that proteins give reactions with MG very similar to those of methylamine, and protein can be converted into a biradical by incorporating MG into it.

All this suggests that the brown color of the liver is due to the formation of analogous charge transfer complexes. If a mouse liver is blender-treated in an ice-cold half-saturated ammonium sulfate solution and the structural proteins (which have the brown color) are separated by centrifugation, they are found to give a strong ESR signal similar to that given by the MG-treated casein (3). The underlying Schiff base could be split from the liver protein by treating it with 2,4-dinitrophenylhydrazine in 1 M HCl at an elevated temperature. The dinitrophenylhydrazone of the substance split off from the protein and behaved chromatographically similarly to the dinitrophenylhydrazone of MG. Its final identification is not yet established.

On SH

There being no oxygen in the α period, there could have been no sulfates, and the sulfur of the atmosphere had to be present to a great extent as SH, sulfhydryl, participating in the biological processes, becoming involved in their mechanism.

MG has a strong affinity to SH and readily forms a hemimercaptal with it. There can be little doubt that the immediate arrest of cell division by MG is due to a great extent to this interaction.

SH not being electroactive, one has to use devious methods to get information about the reactivity of various SHs. In my experience, one can divide, very roughly, the SH groups of tissues into three classes: highly, moderately, and poorly active. The SHs partaking in the mechanism of cell division belong to the first, the SH of glutathione belongs to the second, and the SHs of metabolic enzymes belong to the last group. However crude and qualitative, this division is a useful one. The MG introduced into the cell will react first with the most reactive SHs involved in the mechanism of cell division. The less active glutathione will not interfere with this reaction. An excess MG will then react with the glutathione, which will protect the less active SHs of metabolic enzymes from the action of MG. Until free SH-glutathione is present the MG is not toxic, but it becomes very toxic as soon as its concentration exceeds that of glutathione, which is present in the mouse in an average concentration of 1 mM.

Extending these studies to diamines, I mixed a 0.1 M neutral ethylenediamine and MG solution. The slow development of a yellow color indicated the formation of a Schiff base. If SH-glutathione was added, the color rapidly turned dark purple. The color was accompanied by the appearance of an ESR signal. Evidently, the SH catalyzed the charge transfer. The change was strongest if MG and SH were about equivalent. Excess SH inhibited.

With this reaction in hand, I went over the various charge-transfer reactions of MG and found that all of them were strongly accelerated by SH. This led to the unexpected conclusion that SH is a general catalyst of the charge-transfer reactions of MG and amines. All these charge-transfer reactions, if taking place in the presence of SH-glutathione, are not the reactions of the MG as such, but reactions of its hemimercaptal. SH has a general catalytic effect on charge-transfer reactions. The mechanism of the action of SH is not understood. J. Ladik (personal communication) made the unexpected discovery that SH can act not only as electron donor but also as electron acceptor, which may have far-reaching biological consequences.

Summing up, we can say that the SH is a catalyst of charge transfer between MG and amines and has to play a central role in the electronic desaturation of proteins.

Theory, therapy, and prevention

The foregoing suggests that cancer essentially is increased entropy, randomness, disorder. It suggests that the cancer cell is unable to arrest growth because it is unable to inactivate its glyoxalase, which destroys the ketone-aldehyde that keeps the cell at rest and in the radical state. The essential difference between a cancer cell and a normal cell is in the degree of order. The question is whether this difference can be equalized or utilized in some way to inhibit the growth of the cancer cell.

To achieve a therapy the normal ketone-aldehyde would have to be restored to the cell. Until the exact chemical nature of the ketone-aldehyde is known, methylglyoxal, which is available commercially, can be used.

The difficulty is that ingested MG is rapidly destroyed. The expired air and urine of mice injected with radioactively labeled MG contains radioactivity soon after injection, the MG having been destroyed by the glyoxalase. This loss cannot be compensated by injecting large quantities of MG because the MG, while harmless at a low concentration, becomes toxic when its concentration exceeds that of the SH-glutathione present.

This leaves but one way open to maintain a low MG concentration, and this is by continuous infusion. P. Conroy of the Biochemical Department of Brunel University is studying the effect of such intravenous infusion in mice, the technique of which is cumbrous and difficult. Whether the maintenance of a low MG concentration will arrest growth of cancer has to be shown. MG, being a physiological substance, does not kill cells. It is possible that a cancer cell may be killed simply by arresting its proliferation, but it is also possible that it may be killed by the lowering of the pH, due to the production of D-lactic acid. The vascularization of cancer being poor, the acid may accumulate to a toxic concentration. If a tissue, such as liver, is treated in a blender in Ringer's solution, and is incubated with MG, within minutes its proteins are denatured and precipitated isoelectrically by the acid formed, the glyoxalase having been activated by the blender treatment. It seems possible that the cancer cell can be made to commit suicide by means of its own active glyoxalase, if a low concentration of MG is maintained. All this may open also very interesting possibilities for the local treatment of cancer. It seems not impossible that ways to the prevention of cancer may also be found by means of decreasing the electron donor/acceptor quotient.

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