Keto-Aldehydes and Cell Division

Glyoxal derivatives may be regulators of cell division and open a new approach to cancer.

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Living matter has the inherent tendency to multiply. In monocellular organisms it is the quantity of food which limits growth. However, when cells form multicellular organisms the unlimited growth has to be checked, in the interest of the community: a brake has to be put on. The cell is then comparable to a car parked on a downgrade: all that is needed to set it going is to release the brake. The cellular brake has to be released in wound healing or regeneration, where multiplication has to be resumed at short notice.

When a cell divides, its whole physical state changes. It becomes tender, pliable, semifluid. When it returns to its resting state it freezes up again. This involves many changes. One may thus ask: Are all these changes governed by different regulatory mechanisms, working in concert, or else are they all induced by one substance, a common chemical signal? The first assumption involves unsurmountable difficulties. The second demands that all parts of the cell—most of its macromolecular systems—should have a receptor for the signal, an atomic group common to all, which has a major influence on their physical state. There is one such atomic group, the sulfhydryl (SH). The recent advances in protein chemistry have brought out the importance of this group for conformation, while W. Gordy's extensive studies on electron spin showed the specific relation of SH groups to electronic distribution. Huggins, Tapley, and Jensen (*1*) demonstrated, long ago, the queer catalytic interplay of SH groups. The indispensability of free SH for cell division has been known for 30 years (*2*).

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It was found, many years ago in this laboratory, that certain tissue extracts inhibited growth while others promoted it (*3*), and it was natural to ask whether the retarder (for convenience called "retine") was not the brake and the promotor (called "promine") its release, the behavior of the cell depending on the balance of the two. Though we were neither the first nor the last to observe such activities, the chemical structure of the underlying substances was never ascertained.

In order to find out the structure of a biological substance one has to "isolate" it. In order to isolate it one needs a test. We tried to isolate "retine," using, as a test, the growth rate of implanted cancer in mice. This turned out to be a waste of time. The great variability of the growth rate of cancer frustrated our attempts. Having no test, and being thus unable to isolate to find out what the substance is, we turned the story around—tried to guess, first, what the substance is and then isolate. Infrared spectra of partially purified preparations suggested the presence of a ketone aldehyde, a derivative of glyoxal: R–CO–CHO (*4*). This scent seemed worth following, since all cells, be they bacterial, vegetable, or animal, contain a very active enzymic system, "glyoxalase," for the conversion of the chemically reactive α-keto-aldehydes into an inert hydroxy-acid, R–CHOH–COOH. Such a widely spread enzymic system must have a basic biological significance. This is unknown. Great biochemists, like Hopkins, Lohman, Dakin, and Racker, who worked on this enzyme, were unable to find its substrate, and what's the use of an enzyme without a substrate? If "retine" should be a glyoxal derivative it could be the substrate, and the glyoxalase could act as "promine."

Glyoxal Derivatives and SH

As an introductory study, the homologous series of methylglyoxal ($C_3H_4O_2$) was synthetized up to $C_{13}H_{24}O_2$, with a few related compounds in addition. These were tested for their action on bacterial growth. They all inhibited proliferation—in a small concentration, reversibly—without damaging the cells (*5, 6*). They strongly inhibited protein synthesis (*7, 8*). The activity increased with the number of carbon atoms up to six. Further lengthening of the chain caused a gradual decrease of inhibitory activity, which, in the case of C_{12}, turned into promotion (*6*). Only a moderate effect on DNA and RNA synthesis was observed.

The inhibition could be released instantaneously by the addition of equimolar cysteine (*5*), which indicated a great affinity of aldoketones to SH. The ready interaction of cysteine—respectively, its SH and NH_2 groups—with methylglyoxal had been shown earlier by Schubert (*9*). The closely related cysteamine, though containing SH and NH_2, was relatively inactive, showing that the activity involved the whole molecular structure. Glutathione was very slightly active. Molecules containing two sulfhydryls were active only if these two were neighbors. With one carbon in between, they were less active, with two they were inactive. Thus, both the activity of sulfhydryls and the activity of carbonyls depends on neighboring atoms, on the whole molecular structure, which could make reactions very specific, opening new alleys for chemotherapy, as indicated also by the encouraging experience of F. E. Knock (*10*). All this suggested that the common chemical signal for the arrest of cell multiplication may be a glyoxal derivative, while promine may be any substance which can inactivate aldoketones, such as those containing SH groups, or enzymes, like glyoxalase.

Experiments on tissue cultures and bacteria showed that the inhibitory action of an aldoketone depended less on its concentration than on the relation of its quantity to the number of cells acted upon. Ten times more cells demanded ten times more of the aldoketone to achieve the same inhibition. This suggested that the ketone aldehyde reacted in a stoichiometric fashion with certain sulfhydryls of the cell. In our experiments, 15,000 cells of our

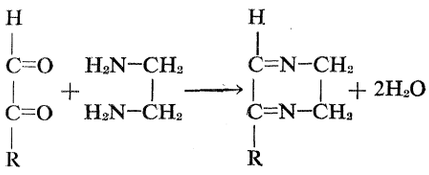

Fig. 1. (See text.)

tissue cultures needed $10^{-3}M$ aldoketone to arrest their division. Since solid tissue contains a hundred times more cells, such tissues needed about $0.1M$ for the same action. But could there be a substance in such a high concentration which nobody could find? This could be only if its detection and isolation were attended by major and unexpected difficulties. The crucial question was thus: Do tissues contain glyoxal derivatives in a concentration of approximately $0.1M$ (which, for a biological substance, is a very high concentration)?

Tests

Keto-aldehydes are precipitated by 2,4-dinitrophenylhydrazine at room temperature. The dark red color indicates that each of the two carbonyl groups has bound a hydrazine molecule.

Aldoketones give a yellow compound with ethylene diamine. The amino groups interact with the carbonyls with loss of water (Fig. 1). The color is proportional to the concentration of the keto-aldehyde. This reaction is carried out in 50 percent alcohol in the boiling water bath.

Another fascinating reaction can be based on the observation of Doljanski, Eshkol, Givol, Kaufman, and Margoliash (*11*) with thiourea, which also sheds some light on the possible biological role of glyoxal derivatives. These authors found that thiourea, if given to rats by mouth, provoked an outburst of cell division in the liver. Thiourea is known to promote cell proliferation in healing wounds (*12*). This seemed exciting because, if cells are kept in the resting state by retine, then the thiourea could promote proliferation by reacting with it; and if retine is a keto-aldehyde, then thiourea should react with keto-aldehydes also, in vitro. Thiourea, in solution, is a mixture of two isomeric forms (Fig. 2), the equilibrium being shifted much to the left.

If a thiourea solution is mixed, in the presence of ammonium sulfate, with a solution of keto-aldehyde, no visible change takes place, but a reaction can be made visible by the presence of nitroprusside, which, in an alkaline reaction, develops a red color. Most SH-containing substances give a red color in an alkaline reaction with nitroprusside, but this color rapidly fades out, while the red color generated by aldoketones in the presence of thiourea develops but gradually. As to the mechanism of this reaction, it seems likely that the ketone aldehyde reacts with the NH, stabilizing, thus, the SH, which then gives the red color with nitroprusside (see Fig. 2).

Material

Once a test is available, the next problem is the choice of material. It is common knowledge that the more a cell is differentiated, the less it tends to divide and the less it needs a growth inhibitor, and vice versa. Accordingly, when hunting for an inhibitor, we have to use a material which is poorly differentiated. Such a tissue is the liver, which can grow very fast, as is shown by the rapidly regenerating rat liver. The liver of younger animals regenerates faster, so we chose, as our material, the liver of "baby beef."

Extraction

We extracted the liver in various ways but could find no reaction with dinitrophenylhydrazine. An aldoketone, if present in the expected concentration, should have given a heavy precipitate. Eventually, a weak reaction was obtained when the liver was "blendored" in 50 percent methanol and incubated for a short period at 60°C. We expected the methanol to have a double role: to inactivate the glyoxalase and promote dissociation of complexes. While the reaction with hydrazine was weak, the extracts gave a strong reaction with ethylenediamine, which was increased to the expected level by the presence of As_2O_3. So our extracts did contain a glyoxal derivative in the expected concentration, of the order of $10^{-1}M$, as calculated for the tissue, but it could not react with hydrazine, being present in a bound form with its carbonyls blocked. The action of As_2O_3 indicated that these were blocked by SH. Why they could not react with hydrazine but could do so with the diamine is easy to see. The first of the two reactions has to be

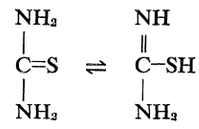

Fig. 2. (See text.)

carried out at room temperature (to avoid the involvement of carbohydrates), while the second has to be carried out at 100°C in the presence of alcohol. Under these latter conditions the complex dissociates and the dissociating aldoketone is caught up and fixed by the diamine, while the SH is blocked by the arsenic.

Sephadex column chromatography indicated that the substance to which the keto-aldehyde was bound is a semicolloid of molecular weight of about 1000 grams. This colloid is soluble in methanol but is co-precipitated with other colloids, and usually sent down the sink as "residue." Earlier workers, looking for glyoxal derivatives, may thus have lost it at the first step. But even had they dissolved the complex they could not have obtained reactions for an aldoketone, the carbonyl groups being blocked. Summing up, we can thus say that liver does contain the expected high concentration of a ketone aldehyde which can be extracted, linked to a semicolloidal material by SH groups. It can be purified, to some extent, and then precipitated as part of this colloid. Then it can be detached from its carrier and purified further in its free form.

Crystallization

If the precipitated semicolloid, with the attached aldoketone, was dissolved in water and incubated in the presence of As_2O_3, the reaction with nitrophenylhydrazine remained poor or negative, indicating that the aldoketone had not become liberated. However, if methanol or acetic acid was used as solvent, the aldoketone dissociated in 24 hours at 40°C. Then it could be precipitated by dinitrophenylhydrazine in a semicrystalline form and could be recrystallized from ethyl acetate in the form of needles or long hairlike threads, united into bundles. These had no melting point and decomposed around 250°C. Measurement of the bound hydrazine indicated a small molecule, while the first combustion analysis (*13*) suggested a close relation to carbohydrates. Through the loss of the keto-

aldehyde and the blocking of its SH groups, the colloidal carrier became unstable and turned into a dark brown substance which could readily be precipitated by alcohol, while the free ketone aldehyde remained in solution even in more hydrophobic solvents.

Discussion

How could the physical state of the cell be dominated by a glyoxal derivative if a very active glyoxalase is present in the same cell? This is possible only if one of them is kept in an inactive state, or else if the two are separated. Both tricks are often used by nature for regulation.

We have not studied the distribution of the keto-aldehyde yet in detail, but we have found it, thus far, in all tissues tested, except one: cancer. A lack of the aldoketone may thus be involved in the senseless proliferation of this tissue. As suggested, also, by F. E. Knock (*10*) and by unpublished results of M. Watson, cancer cells seem to be more sensitive to aldoketones than normal cells, indicating that, if aldoketones are involved in oncogenesis, it is rather the lack of the inhibitor than of its receptor which is responsible for the misbehavior. In any case, a detailed study of the distribution of keto-aldehydes is indicated, under various conditions (for example, different age). Urine contains keto-aldehydes, and it can be hoped that its study may give information about the equilibria in the body and their possible relation to cancer.

Suspensions of disintegrated liver cells are precipitated by concentrations of glyoxal derivatives which inhibit cell division, showing that aldoketones can interact with various macromolecular systems, altering their physical state.

The weak cancerostatic action of aldehydes was noted long ago (*14*) and has been studied by several authors. Aldehydes, also, react with sulfhydryl compounds (*15*), and it can be expected that their reactivity is greatly increased by a neighboring ketone group, as shown by Együd (*6*). Reports on cancerostatic action of glyoxal derivatives, especially semicarbazones, appear in the literature with increasing frequency, and one member of this group, Kethoxal, is even on the market.

Conclusion

Many problems are left open in this article. Its publication may be excused by the suffering cancer causes, which urges the researcher to publish as soon as he thinks he may have found a new trail, which also may be taken by others. What emerges clearly is that SH groups, with their specific reactivities, offer a hopeful target in the search for cancerostatic substances, among which the natural repressor of cell division may hold out the most promise. The glyoxal derivatives also have antiviral properties (*7, 16*) and may be in the center of a hitherto unknown system of equilibria which deserves a thorough study. The low molecular weight of the glyoxal derivative reported justifies the hope of an early clarification of its structure, as well as its synthesis (*17*).

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18. This research was supported by National Institutes of Health grant GM10383.

NEWS AND COMMENT

Pitt Picks Chancellor: Agrees that Modesty Is the Best Policy

Pittsburgh. The University of Pittsburgh seems to have crawled out of the pit of financial trauma and academic worry. The most recent indication of Pitt's movement toward recovery was the announcement on 13 January that the university had found a new chancellor—Wesley W. Posvar, a 41-year-old career Air Force officer with an impressive collection of academic credentials. His selection ended a 1½-year search to replace the controversial Edward H. Litchfield.

The decade of dynamic thrust which the university had enjoyed under Litchfield hit a roadblock in the summer of 1965 when it became apparent that the university had accumulated debts of more than $20 million and had run out of operating funds. (Pitt's difficulties were examined in a three-part series published in *Science* on 4, 11, and 18, February 1966). Litchfield, after suffering a mild heart attack in June of 1965, left the university in July of that year. Litchfield now divides his time between his corporate directorships and his service as chairman of the board of the Governmental Affairs Institute in Washington, D.C.

Although many at Pitt still praise Litchfield for the pioneering work he did in helping bring Pittsburgh into the ranks of nationally known universities, some also add that he often tended to be arrogant in his dealings as chancellor. Trustees sometimes felt ignored, the faculty occasionally felt slighted, and neighboring educational institutions felt little inclination to cooperate with Pitt's aggressive leader. As a result, the powers at Pitt were ready for a less Olympian chancellor.

They found him in an unexpected place—at the Air Force Academy where he was chairman of the division of social sciences. In contrast to the usual civilian stereotype of the military officer, the soft-spoken Colonel Posvar gives the impression of being a modest and self-effacing man. At his first press conference at Pitt, on 18 January, Posvar said he had no plans for sweeping