# Cell Division and Cancer

Substances which promote or retard cell growth may provide keys to fundamental problems of cellular biology.

Albert Szent-Györgyi

Life wants to spread and multiply. What sets limits to the proliferation of unicellular organisms are the factors of the environment, such as the quantity of food or energy available. But once cells joined to form more complex multicellular organisms they had to subject their dividing to strict regulations in the interest of their community. They also had to give up their motility and develop a new kind of surface which could link them to their neighbors and mediate the subtle intercellular relations. However, these qualities could not be ingrained irreversibly, for under certain circumstances the cells have to revert, at short notice, to their unicellular way of living. This is the case in regeneration or the healing of wounds. In the epithelium of our undamaged skin, cell is strongly attached to cell, forming the tough structure which we need for our protection. But if we cut ourselves, cells release their neighbors, resume motility, creep into the wound, and multiply till they have filled the gap. Once they have done so and cell touches cell, the wound is healed, and the tissue returns to its initial resting state. The cells thus have retained their capacity to switch back and forth between the two great evolutionary states, the monocellular and multicellular. There must be some sort of a "switch mechanism," a subtle regulation which controls these changes.

What happens in a healing wound can be, so to say, spread out before our eyes in tissue cultures. Abercrombie and his associates (*1*) placed two pieces of tissue on their culture medium not too far from one another. Similarly to the happenings in a wound, cells made themselves free from the surface, crawled away, and multiplied. But where the cells of the two cultures met, their motion and proliferation stopped and the cells returned to their resting state. This is what Abercrombie has called "contact inhibition."

The subtle regulatory mechanism underlying these changes is unknown, but its understanding is tragically urgent, for there seems to be an intimate relation between these regulations and cancer. As shown by Abercrombie *et al.* (*1*), cancer has no contact inhibition. When the two explants were made from cancer tissue, the cells did not stop dividing on contact but grew over one another in a disorderly fashion. They behaved as they do in the sick organism where they invade the surrounding tissue. They seem to have lost the regulatory mechanism which restores and guards the quiescent state. This regulatory mechanism, being a late evolutionary accomplishment, appears to be easily lost, and the question is how it can be analyzed. It cannot; it is too subtle and complex. If we cannot approach a problem the only thing we can do is to wait till the problem approaches us, and I would like to tell, very briefly, how this problem approached me.

## The Thymus, Muscle, and Growth

For a long time my main interest was muscle, and muscle seemed to be connected, somehow, with the thymus, because one of the muscle diseases, myasthenia, is often benefited by the extirpation of this gland. With Jane McLaughlin, we thought (*2*) that it might be the over-production of the hypothetical "thymus hormone" which was responsible, so we started hunting for this hormone. In order to find a biologically active substance, one must have some sort of test by which to show its presence. Our first test animals were myotonic goats. Myotonia is a degenerative disease, the symptoms of which are the reverse of those of myasthenia. So, should an excess of a hormone cause myasthenia, then the lack of it might produce myotonia and the animals would react favorably to injections of the hormone. Unfortunately, this line of work had to be given up, because we worked in Woods Hole, in a marine biological laboratory, and the smell of the goats clearly indicated their nonmarine origin. Led by my theories on energy transduction, I isolated various substances which, eventually, all turned out to be red herrings. Trying to find a new approach, I argued thus: growth involves all normal processes of the cell, and so if the biological balance is upset by any active substance the rate of growth should change. To get rapid answers we looked for rapid growth. Cancer grows rapidly, so we injected various cancer cells (Krebs 2 ascites, Sarcoma 180) under the skin of Swiss albino mice and observed the influence of our injections on the growth rate of the developing solid tumor. In a few cases we also used the spontaneous mammary cancer of C3H mice. A 50-percent increase or decrease in growth rate (as measured by the weight of the excised tumor) we called "one unit of activity." We could thus express the action in numbers, but these numbers showed the most disconcerting inconsistency. In some instances our extracts inhibited growth, in others they promoted it, and even one and the same extract could turn from an inhibitor into a promotor. It took a great deal of the classical mixture of inspiration and perspiration till it dawned on us that we had two antagonistic substances in hand, one which retarded and one which promoted growth. The retarder we called "retine," the promotor "promine."

Later, tissue cultures, introduced in our laboratory by Ruth Johnsson, became, also, an important tool of research. They give much detailed information and have the advantage that they demand very little active material. Johnsson found retine cytotoxic on the cultivated cancer cells (HeLa and KB). While retine killed the cells, promine caused their hypertrophy and increased

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intercellular distances, which suggested that promine may have bearings on the invasiveness of cancer cells. Preliminary histological studies by Johnsson indicate that the changes in size of our tumors, observed in vivo, are due to the same factors that operate in vitro. The reduction in size by retine is due to the loss of cells, while the increase in size by promine is due, at least partly, to a hypertrophy of cells and the increase of intercellular spaces, filled with interstitial fluid.

### Extension of the Research

Extending our studies, with A. Hegyeli (*3*), to other tissues, we found similar actions. What we were dealing with was thus not a thymus hormone, but a general tissue constituent. Later, following the suggestion of our friend Charles B. Huggins, we tested human urine and found there the same activities, which made urine a propitious material for big-scale work. Urine is not only available in quantity, but is in many ways also superior to tissue extracts, having passed a very specific filter, the kidney.

M. Rosarii Schmeer (*4*), working at Woods Hole, wondered whether molluscs have retine. She was impressed by the fact that no malignant growth had yet been reported in them. Clam extracts gave a strong inhibition of cancer growth, and there seemed to be little promine present, which simplified matters a great deal. We corroborated her results and extended our own work to mushrooms, in which we found a similar inhibitory action, accompanied by relatively little promine. All this indicated that retine may represent a universal cell constituent, and that retine and promine may actually have been the regulators of cell division, developed in the evolutionary step from monocellular to multicellular organization.

What made the going slow and difficult was the great similarity between retine and promine. Judged by their physical and chemical qualities they almost had to be one and the same substance. Since they could completely antagonize each other's action, it was impossible to state their concentration in an extract till they were separated, but their similarity made the separation very difficult. While this similarity complicated matters, it also had its fascination, because it suggested that retine and promine are chemically closely related and that nature might have produced a switch mechanism by creating a substance which could be transformed from an inhibitor into a promotor by some minor change.

These studies represent the most difficult research I have ever undertaken. The similarity of the two antagonists, the slowness and clumsiness of our test, and the many variables slowed progress. One variable in a set of experiments is manageable, two make life difficult. We had half a dozen. One day the substances behaved as a colloidal macromolecule, the other day as a crystalloid of very small molecular size, which readily distilled away even with low-boiling solvents, like chloroform, indicating that the molecule could contain but a very small number of carbon atoms. Some extracts were stable, others deteriorated rapidly, and so forth.

All this seems simple now. The studies of Hegyeli show that retine and promine have very small molecules but are bound, in vivo, to a hydrophilic colloid from which they can, more or less easily, be released. While bound to its carrier, retine is stable, nondialyzable, nonvolatile, and strictly water soluble, whereas free retine is unstable, dialyzes, and can be extracted from its watery solution at a low *p*H by chloroform. M. Parshley's (*5*) interesting cancer-inhibitory tissue extracts probably contained retine in this form.

### Turning to Cancer Research

Though primarily interested in cancer only as a test object, willy-nilly we found ourselves in cancer research, and this is not a happy hunting ground. Because of the untold suffering cancer causes, the researcher who enters this field and sees the faintest hope of progress loses his freedom and leisurely ways, feeling obliged to go on relentlessly and follow the narrow path which might give the most hope to patients. While we depend on natural resources for retine, it can have no therapeutic importance. To become useful it would have to be produced by the ton, which presupposes its isolation, analysis, and synthesis. So one has to put on shutters right and left, shy away from all the fascinating functional problems this field abounds in, and concentrate on the intellectually less satisfying chemical isolation.

Hegyeli achieved partial isolation and stabilization of the active substance by forming its reineckate. He also showed that free retine has a fairly low boiling point, distilling with acetic acid (bp, 118°C). When treated with mineral acid, retine underwent a change which declared itself in the further lowering of the boiling point, distilling now even with chloroform (bp, 61° to 62°C). Concomitantly, the absorption at 273 millimicrons was greatly increased and the product became very toxic, though maintaining its retarding activity on tumors. This change was probably due to an isomerization and the splitting-off of some group from the molecule, which group might have contained nitrogen, since the intact retine forms a reineckate, while the "split retine" does not. The split retine is also less soluble in water. S. Layne of the Worcester Institute for Experimental Biology and Medicine, who kindly studied Hegyeli's preparation, found in the infrared an absorption which indicated a ketone.

### Chemistry

L. Együd, who, meanwhile, had joined in, took advantage of the low boiling point of retine and started his preparatory work with steam distillation at a high *p*H. The infrared spectrum of his purified preparations indicated the presence of a ketone *and* an aldehyde, while the low boiling point indicated a very small molecule.

The simplest ketone-aldehyde is methylglyoxal ($CH_3COCHO$), which readily forms a crystalline hydrazone with 2,4-dinitrophenyl hydrazine, with a well-defined melting point. A hydrazone was prepared from Együd's extracts with practically the same melting point. Methylglyoxal has a characteristic absorption peak in the ultraviolet at 272 to 275 millimicrons, which shows a shift in acid and alkaline reaction, typical of a keto-enol transformation shared also by Együd's extracts. Like the active extracts, methylglyoxal greatly increases its absorption at 272 to 275 millimicrons on boiling with strong acid or alkali. All this, taken together, left little doubt that in retine we were actually dealing with a methylglyoxal derivative.

The carcinostatic action of methylglyoxal derivatives is known. It was observed first by Bahner and his associates (*6*). Furst, French, and Free-

lander (*7*) synthesized and studied a greater number of these derivatives. Several, such as $\beta$-ethoxy-$\alpha$-ketobutyraldehyde (Kethoxal), are on the market. The derivatives bis(guanidylhydrazone) and bis(thiosemicarbazone) have lately attracted much attention. Freelander and French found one of them, the hydroxymethylglyoxal bis(guanidylhydrazone), in certain concentrations to be an inhibitor, in others a promoter. If the same substance can act, according to conditions, once as inhibitor, another time as promotor, then it seems even more possible that two closely related substances can be antagonists, as we supposed for retine and promine.

If retine is a regulator of cell division in general, then it should be able to act not only on cancer but also on other cells, and if the ketone-aldehyde is its active group, then other ketone-aldehydes should also show some such reaction. For this reason J. Roslansky tried the action of methylglyoxal on fertilized sea-urchin eggs (*Strongylocentrotus drobochiensis*). It completely inhibited the division of all cells at $10^{-4}M$ concentration. The starfish (*Asterias*) eggs were somewhat less sensitive. In both cases methylglyoxal left the spindle intact.

Johnsson found that, like retine, methylglyoxal was also very toxic to KB cells in the tissue cultures, though there were also differences between the action of the two. The KB cells were killed by methylglyoxal in 24 hours by concentrations of $10^{-3}M$ to $10^{-4}M$.

These cytotoxic effects may explain why clams and mushrooms contain retine in a relatively high concentration, unbalanced by promine. Clams live in mud, mushrooms in decaying matter. Both must be exposed to the attack of parasites. Possibly, they use retine for protection, preventing the development of parasites.

The fact that retine is, in all probability, a methylglyoxal derivative not only cleared up the nature of the active atomic group but also gave us an excuse for all our stumbling. The ketone-aldehyde formation is a most reactive one; it easily undergoes various isomerizations or polymerizations. It can also make the molecule rather unstable. But as far as our experience goes, retine is not identical with methylglyoxal or any of its derivatives hitherto studied. It is more active. Accordingly, the next major aim of the work on this line will have to be the isolation and identification of "retine," the natural glyoxalate derivative.

## Biochemical Relations

The participation of a methylglyoxal derivative in the regulation of cell division seems also to solve an old puzzle of biochemistry. Cells contain a powerful enzymic system for the transformation of methylglyoxal into lactic acid, a reaction in which glutathione is involved as a cofactor (*8*). This enzyme system consists of two catalytic proteins, glyoxalase I and II, isolated by Racker (*9*) and Crook and Law (*10*). Glyoxalase I links up methylglyoxal with glutathione to a complex postulated by Jowett and Quastel (*11*), while glyoxalase II splits the complex into glutathione and lactic acid. This widely spread and active enzymic system, which can also act on other methylglyoxal derivatives, occupied the greatest biochemists, like Hopkins and Dakin (*12*), without, however, anybody being able to fit it into the framework of metabolism. The lactic acid of tissues is not formed over methylglyoxal. The involvement of methylglyoxal in the regulation of cell division may answer the question. Perhaps the function is analogous to that of acetylcholine esterase. These observations may also establish a link between cell division and the system of SH groups. Aldo-ketones being good chelating agents, methylglyoxal also may establish more intimate connections between chelation, calcium, cell division, and malignancy, a link postulated by D. R. Coman (*13*).

## Promine

Hitherto, we have concentrated, in our work, on retine and have neglected promine, partly because of the possible medical use for retine, but partly, also, because it will be easier to identify promine once we have retine in hand. Then, we will be able first to suppress growth with retine and then to release it from this inhibition by promine, which will be easier to do than to promote growth directly. Our temporary neglect of promine, however, does not mean that this substance, as such, may not have a biological and medical importance. We observed that tumor grows much faster in the young than in the old animal. One can eliminate this difference, slowing growth with retine in the young and speeding it up with promine in the old. This suggests that these substances may have something to do with that wonderful condition called "youth" and the less attractive "senescence." Studying these relations one will have to consider two factors. The one is the relative concentration of retine and promine, the "$R/P$ quotient," the other, the absolute concentration of the two. The animal experiment gives information about the first. The extracts we obtained from various quiescent tissues mostly showed some excess of retine, just sufficient to keep the tissue at rest. For good regulation, a high concentration of both is probably needed. It is not impossible that in senescence and in tissue cultures the concentration of both drops, predisposing to uncontrolled growth. However this may be, promine is likely to acquire some practical importance in speeding up regeneration and making wounds heal faster.

The colloid to which retine and promine are bound will also demand a more thorough study. I have always been convinced that a substance which has as low a boiling point (and as high a vapor pressure) as retine and promine can have no biological importance because we would lose it with the air we expire. This loss is, evidently, prevented by its binding to a colloid. This colloid seems also to be involved, to some extent, in the biological activity. In the whole animal, free retine and the colloid-bound retine have a similar action. In our early experiments with clam extract, free retine was found to be 25 times less active in tissue cultures than colloid-bound retine. If this observation is correct, it suggests that the colloid has a hand in activity and that the injected retine finds plenty of it in the animal but not in the tissue culture.

## Retine, Promine, and Cancer

A host of most intriguing questions come to mind. The first are, perhaps, these: Have retine and promine anything to do with cancer? Can retine retard and cure it? These questions will be answered when these substances are available in sufficient quantity and purity. What can be said at present, with fair confidence, is that *if* retine is instrumental in suppressing normal growth and *if* it is involved in contact inhibition, then the loss of the ability to produce it should lead to unbridled growth. In this case it would also seem possible that various carcinogenic agents act by injuring the retine production. In our experience the various cancers

tested all reacted readily to retine. In Johnsson's early experiments they reacted even more strongly than the L929 fibroblasts. So if retine is involved in oncogenesis, it must be the ability to produce retine, rather than the ability to react with it, which is lost. Its excessive enzymic destruction by glyoxalase may also be considered. Since retine acts equally by mouth and by injection, and does so in very small quantities, and seems to be, in therapeutic doses, devoid of untoward side effects, some of these possibilities can be tested on patients without causing inconvenience, once the substance is available.

Closer study of the $R/P$ quotient and of the absolute concentrations in various age groups, animal species, and various organs which have a different cancer incidence may help to clear problems of oncogenesis, but will have to wait for the development of a reliable micromethod for the estimation of these regulators. With the knowledge of the active group and the relations to the SH groups, such a method may be actually in sight.

The mechanism of action of retine and promine is unknown. It may be connected with the SH system, or nucleic acids, or something else. But in spite of all these incertitudes it seems likely that better knowledge of these substances will open a new alley for an attack on cancer and some of the fundamental problems of cellular biology.

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# Some Current Problems of Government Science Policy

## What should be the balance between expenditures on pure and on applied science, and who should set it?


Harold Orlans


In the fall of 1963, much concern was evident in the scientific community about the course that several congressional committees would take in their inquiries into federal research and development programs; and the concern of interested parties is always evident at the time of the President's budget message and subsequent appropriations hearings in Congress. Now that the Select Committee on Government Research has completed its work and the House Subcommittee on Science, Research, and Development has finished its round of hearings and reports, I believe it would be generally conceded that the committee members and their staffs did an excellent and constructive job. Both committees—particularly the select committee chaired by Representative Carl Elliott of Alabama—broke new ground. It is not necessary to agree with every one of their recommendations to acknowledge that, under severe time pressure, they asked trenchant questions and gathered and published fresh and insightful information about the nation's gargantuan research-and-development enterprise. However, the fact that this special congressional effort was required to bring to light current and comprehensive statistics on such matters as the geographical distribution of federal R&D funds and the amount received by leading universities and companies suggests that the executive agencies responsible for informing the public about these expenditures had not been doing their job adequately. Let us hope that in the future these agencies maintain the standards of fuller and more timely reporting which have now been set with the assistance of Congress; for we can hardly expect to have either good current policies or adequate consideration of desirable new policies without comprehensive, timely, *and public* information about existing R&D programs.

As the rate of increase of federal R&D expenditures has been declining and as the volume of expenditures in major agencies like the Department of Defense, the National Aeronautics and Space Administration, and the Atomic Energy Commission has leveled off or declined, a major issue of public policy—and of public and private conflict within many agencies and their constituencies—has been posed: how much of the pie should go to basic research? Or, to put the matter another way, how much should go for research at universities, and how much for research and development in industry?

### The Doctrine of the Sparrow

The answer of academic scientists is not entirely surprising: more should go to them. With a monotony that bespeaks a unison more than an originality of thought, they and their spokesmen in Washington argue that


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