Reports

Cancer Therapy: A Possible New Approach

Abstract. *Two substances, one promoting growth (promine) of ascites tumors in mice and the other inhibiting it (retine) have both been found in several tissues, namely, thymus, aorta, muscle, and tendon. In spite of similar solubilities in the solvents used for their extraction, the substances could be roughly separated. The value of the ratio between these substances in the same tissue may be significant.*

About a decade ago the workers in this laboratory became interested in the chemistry of the thymus gland and asked the simple question: Does the thymus contain biologically active substances of low molecular weight which might be designated hormones (*1*)? After exploring several blind alleys, we began to use as a test the influence of thymus extracts on growth. To obtain rapid answers we chose as test material the most rapidly growing tissue, transplanted cancer. The first results were equally fascinating and disconcerting. Some extracts produced a strong inhibition of growth, while others produced a promotion. Even one and the same extract could change, in a couple of days, from an inhibitor into a promoter. These discrepancies were resolved by the demonstration that the extracts contained two antagonistic substances, a promoter, "promine," and a growth-retarding one, "retine." Given simultaneously, the two could completely compensate for each other's action. Retine, being less stable, may decompose on storage. This explains why the activity of an extract could change: the decomposition of retine allowed the promine present to declare itself. The two substances had very similar properties which made their separation difficult. Until this separation was achieved, no statement could be made about the real activity of any extract. This explained our initial failure to demonstrate similar activities in other tissues.

After a crude separation had been achieved we could show that extracts of other tissues, like muscle, big blood vessels, or tendon, showed a similar activity. Retine and promine were thus not specific products of the thymus and were found there only because we looked for them there (*2*). All this supported our earlier conclusion that growth promotion and inhibition, in our experiments, depended on the balance of two active substances (*3*).

The physiological role of these two compounds is a matter of speculation. However, a simple theory can be constructed which answers, also, a hitherto unexplained puzzle. Why do cells which have been dormant for long periods suddenly start to multiply if a wound is made, and why does this growth stop when the wound is healed? Retine contains one or more unstable links, and it is easy to believe that on injury some enzyme is liberated which causes decomposition, leaving the uninhibited promine in command.

Promine makes cancer cells grow faster, while retine tends to stop their growth and can make cancer, already developed, regress. This was equally true for the spontaneous mammary tumor of C3H mice, for the transplanted sarcoma 180, or the solid tumor Krebs 2.

To achieve regression the ratio of the concentration of retine to promine has to be altered significantly. Cancer seems to regress when the ratio is doubled—that is, when amounts of retine are injected daily which correspond roughly to the quantity of retine present in the animal's body.

Mary S. Parshley, at Columbia University, working with tissue cultures, obtained similar results (*4*). She also obtained inhibition of sarcoma 180 in the whole animal by her extracts (*5*). She found also that the division of fibroblasts is inhibited by her inhibitory substance, indicating that these actions are not specific for malignant growth but act on any cell division. Since, in our experiments, retine produces no leucopenia it seems that the blood-cell producing organs are exempt from its actions.

These observations open a wide field for cancer research. Tentatively, a few of the problems may be mentioned which could be approached.

In the one experiment the aortas of old animals contained less retine than those of young ones, which suggested a possible connection between a diminished concentration of retine and the increased incidence of cancer with advancing age. Also the question may be asked whether there is a connection between the ratio of the concentration of retine to promine and the incidence of cancer in various species. In two experiments we found indication of retine in the urine of children. Possibly urine may reflect the ratio of promine and retine in the body and allow the closer study of this ratio in relation to cancer. The variation of the ratio may also alter our concepts of carcinogenesis. Possibly carcinogens, like cosmic radiation, may induce cancer in the young but may lead to disease in older individuals in whom the ratio is less favorable.

Muscle, tendon, and big blood vessels are very rarely seats of cancer. This, in our experience, is not necessarily due to higher retine concentration, since other tissues are equally rich in this substance. However, muscle, tendon, and aorta have greater concentrations of retine than promine so that their extracts, contrary to those from other tissues, are inhibitory even before promine is removed; this is not true for other tissues.

Naturally, the first object of research along this line must be the isolation of the substances in question, their analysis and synthesis. To facilitate work for others we describe, briefly, our experience.

The tumor used for our tests was the Krebs 2 ascites tumor, propagated as such. For the test, 0.25 ml of the cell-containing ascites fluid was injected in mice subcutaneously behind the scapula where solid tumors were formed. Four days later, after a palpable tumor had developed, injections of retine were started and continued daily for 10 days. The retine, dissolved in 0.1 ml of peanut oil, was injected subcutaneously on opposite sides, daily.

In order to obtain reproducible numerical results, the following points were im-

portant: a larger number of animals–15–had to be used, both for the tests and their controls. The animals had to be inbred and of equal age; they had to be young, since the tumor grows faster in younger animals (we used mice 3 to 4 weeks old). At the end, the animals had to be sacrificed, the tumors excised and weighed.

The thymus is a relatively rich source of retine and promine, but is a less advantageous material than aorta, tendon, or muscle, because of its high content of extractible matter. Tendon is difficult to handle and collect. We worked chiefly with aortas of calves. This material was frozen soon after the death of the animal and transported to the laboratory in frozen condition; it was reduced here to a snow which was dropped directly into methanol —100 liters of alcohol for 36.4 kg (80 lb) of tissue. Then the mixture was brought to the boiling point of methanol and cooled; the methanol was separated from solid matter on a basket centrifuge and clarified on the Sharples centrifuge.

The methanol extract was evaporated at low temperature to 3 liters, adjusted to *p*H 4 with HCl and shaken out, repeatedly with chloroform. The methanol extract was then adjusted to *p*H 1 and again shaken out with the same solvent. The united chloroform extracts, which contained both retine and promine, were shaken out with water of *p*H 1 which eliminated promine. The chloroform was evaporated, and the residue was dissolved in butanol, saturated with 1*N* HCl. The solution was subjected to chromatography on a cellulose-column wetted with 1*N* HCl saturated with butanol. Butanol saturated with 1*N* HCl was used as the moving phase. The first colored eluates were rejected, and the column was extracted with acid methanol. The methanol was evaporated under reduced pressure, and the residue was dissolved in chloroform. The retine was then shaken out with alkaline water (*p*H 9), and the watery extract was acidified (*p*H 1) and then shaken out with benzene which extracted fatty acids. After this the water was shaken out with chloroform which extracted the retine. After evaporation of the chloroform the residue was dissolved in peanut oil. The oily solutions were stored at −20°C. Precipitates forming were eliminated by centrifugation.

Our yields approximated about 50 percent. The final material injected had a dry weight of 2.2 mg/kg (1 mg/lb) of aorta extracted. We called a "unit" the quantity of retine which slowed down the growth of cancer to one-half. This corresponded to about 200 μg of dry weight. The aorta contains about 33 units per kilogram.

Our limited experience suggests that the promine and retine are of small molecular weight and have a high potency. Retine seems to contain one or more unstable links since it decomposes at room temperature in a week. It is more sensitive to alkali than to acid. It also seems to contain a group which dissociates at an alkaline reaction, though its solubility in alkali may have been due also to the accompanying substances, mainly fatty acids. Promine seems to have an alkaline group which makes the molecule more soluble on dissociation in acid water.

We found no harmful side effects either with retine or with promine. In this respect, these substances seem to differ from all the antimetabolites used in cancer therapy which are not specific in their action and interfere with some fundamental process or substance, common to all cells, so that even if cancer cells may be more sensitive, side effects do result. Retine and promine, being natural substances produced by nature, might perhaps specifically influence cell division; one might have here substances which will stop cancer growth and even produce regression without toxicity. Possibly even the growth-promoting substance may acquire a medical application, in analogy to weed killers which kill by promoting growth. One could, perhaps, introduce in the body some antimetabolite and then make the cancer grow fast and kill itself. The growth promoter seems not to induce malignancy by itself. It might also find application in accelerating the healing of wounds (*6*).

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Recombination Events in the Bacterial Genus Nocardia

Abstract. *Genetic recombination was demonstrated in the bacterial genus* Nocardia. *The compatibility mechanism governing recombination differs from that of* Escherichia coli *and the streptomycetes. Mutants of* N. erythropolis *or* N. canicruria, *of homologous origin, were incompatible, but mutants of heterologous origin were compatible. Such a compatibility system is suggestive of classic heterothallism which up to now has not been known among the bacteria.*

Members of the genus *Nocardia* are morphologically intermediary between less complex organisms, the Mycobacteriaceae, and more complex forms, the Streptomycetaceae (*1*). If one is allowed to speculate on phylogeny and evolution of bacteria, the nocardiae could be considered to be either the point from which divergence of the primitive and complex bacteria occurs or the endpoint of convergence (*2*). The inviting question concerning hereditary mechanisms of such an intermediary group is: Would studies of the nocardiae reveal genetic mechanisms similar to those found in the Eubacteriales, exemplified by *Escherichia coli* (*3*), or to those found in the streptomycetes (*4*), or would the genetic mechanisms of nocardiae have characteristics of both groups? In order to find an answer to this question, genetic studies have been undertaken with *Nocardia erythropolis* and *Nocardia canicruria*, members of the genus which morphologically typify the phylogenetically intermediate category (*1*).

These two taxonomically designated species grow relatively rapidly and form colonies in 5 to 7 days on a medium containing only mineral salts and glucose as well as on more complex but convenient laboratory media. They exhibited little colonial morphologic variation on the several media upon which they were tested. It seemed perfectly feasible to use bacterial genetic techniques which had been developed previously and to test for genetic interactions between complementary pairs of nutritionally characterized mutants of homologous or heterologous origin.

The velveteen method of replication (*5*), whereby identical plates of colonies are made from a master plate, was used for the indirect selection of auxotrophic mutants which were induced by an ultraviolet irradiation dose which killed more than 99.9 percent of the starting population. As a result of ultraviolet induction many replicated colonies capable of growth on the medium of mineral salts and glucose supplemented with amino acids,