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CELL DIVISION

EX AEDIBVS ACADEMICIS IN CIVITATE VATICANA

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CELL DIVISION

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SVMMARIVM — Auctor de nunnullis investigationibus primariis disserit, quae non solum efficere possunt ut melius tota materia de tumoribus intelligatur, sed etiam ut tumoribus medeatur vel ut prius quoque eis occurratur.

After fifty years of research the greatest mystery, to me, is the cell, and the most interesting cellular process is division. This process acquired a tragic importance by the fact that sometimes a cell is unable to return, after division, to its resting state and goes on dividing until it kills its host. All of us present here have a thirty percent chance to die of cancer. But cancer is, at the same time, also a most fascinating phenomenon. It is one of those magnificent experiments nature performs for us. It is similar, in a way, to diabetes which revealed the existence of insulin. The study of cancer can also be expected to reveal important secrets of nature.

If cancer research did not make the progress it could have made, this may be due to two factors. The one was

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that we were too anxious to relieve suffering and cure before understanding. To try to cure, that is repair such a complex mechanism as a cell, without understanding it, is a shortcut to failure. The other reason may have been that we have asked the wrong question: why do cancer cells divide? As I will show presently this is the opposite of what we should have asked.

There is a simple experiment which can put these problems into the proper light. The experiment is this. We take a rat, open its abdominal cavity and cut out two-thirds of its liver, then we sew up the wound and open the animal eight days later again. To one's amazement one finds a complete liver, as if nothing had happened; the cut has elicited an explosive growth which seemed to stop when the liver reached its original size. This is amazing, because a cut cannot create a new mechanism. It can create but one thing and this is disorder. The ability to proliferate must have been there and our cut could only release a suppressed ability. The ability of unlimited proliferation is an attribute of life. The problem of cancer is then, not why a cell grows. The problem is what has kept a cell at rest before? If a car, parked on a slope, begins to run, you do not ask what makes it run. You ask what has gone wrong with the brake? We are thus faced with the failure of a complex regulatory mechanism. There are two ways to approach any problem: the deductive and the inductive. "Deductive" means to figure out things. "Inductive" means to find out how nature does it.

Deductive Approach.

The simplest way to stop a cell division is to cut its fuel, its energy supply. There is no function without energy. The only fuel of the biosphere is the hydrogen, or more exactly the electron of the hydrogen atom. We can thus arrest a cell by

introducing an "electron acceptor" which will bind these electrons. "Electron acceptor" is a substance which can take up an electron. It has to be a negative atom or atomic group. The most negative atom is fluorine, but there is too little fluorine in nature. The next most negative element is oxygen. There is plenty of it which makes it into the general electron acceptor of the biosphere.

As you all know, electrons, in atoms, are located in specific regions called "orbitals". In order to be able to accept an electron the atom must have an empty orbital on which to accommodate the accepted electron. Most oxygen complexes have no empty orbital on which they could accommodate an electron, but oxygen can develop such an orbital by making a double link either with another oxygen atom (O=O) or with a carbon atom (C=O). The one of the two bonds is a δ bond which does not interest us further, but the other bond is a π bond which has an unoccupied (antibonding) orbital. However, an additional electron would upset electronneutrality in such a small system too much and nature tends to guard electronneutrality. This makes the C=O into a poor acceptor. We can improve its acceptor ability by extending its electronic system. This can be done by placing a double bonded oxygen atom on the next carbon. The π electrons of the two neighboring (conjugated) double bonds conflue into a wider π system which is a good electron acceptor.

If the C=O has an H attached to it as in H-C=O, then this makes an aldehyde. If there is no H attached to the C=O, then the resulting substance is a ketone. Aldehydes are reactive but are thermodynamically poor. Ketones are thermodinamically good but are less reactive, and we need both qualities. By coupling an aldehydic C=O with a ketonic one we form a ketone aldehyde, which has both good qualities. The simplest keone aldehyde is methylglyoxal H_3C-CO-HCO which can be derived, at least on paper, from glyoxal HCO-HCO.

If a biochemist hears of glyoxal derivatives he must

become excited because — as far as we know — all cells contain a powerful enzymic system which can transform glyoxal derivatives into the corresponding inactive hydroxyacid, converting, for instance, methylglyoxal into lactic acid. This enzymic system is called the "glyoxalase". Nature does not indulge in luxuries and if there is such a very widely spread and active system, it must have something important to do. Discovered in 1913, it attracted a number of leading biochemists but nobody could find a substrate for it and what's the use of an enzyme without a substrate? So the interest gradually faded away.

Dr. L. Egyud synthesized in my laboratory, a greater number of glyoxal derivatives and these all inhibited cell division in a low concentration without hurting the cell. They inhibited also protein synthesis, which demands much energy. Glyoxal derivatives could cut off energy supply in two ways, by binding the active electrons, or by forming complexes (hemimercaptals) with the SH groups which are instrumental in transmitting these electrons.

Methylglyoxal, or other glyoxal derivatives, injected into the cancer bearing animal could not inhibit the growth of cancer, being decomposed by the glyoxalase of the blood before reaching the tumor. However, methylglyoxal can be protected against the enzyme by covering up its aldehydic C=O. In this case the blood glyoxalse cannot attack it and it can reach the tumor. Such derivatives of methylglyoxal have been synthesized earlier by French and Freelander and others, and were found to have a definite cancerostatic action. The compounds tested, various hydrazin derivatives, were rather toxic, so that they could not acquire a medical importance. We couplet methylglyoxal with various non-toxic nitrogenous substances and arrived at compounds which had a high cancerostatic action without being toxic, and we hope that some of them will give medicine new weapons in its hands to fight cancer.

Inductive Approach.

Trying to find out how nature suppresses growth, I made extracts of tissues and injected them into cancer bearing animals and found that they inhibited the growth of the inoculated tumors. Livers yielded an astonishingly active extract. The extract of one gram of liver, injected twice daily, had a strong inhibitory action. If the extract of one gram of liver could inhibit cell division in the whole animal of 25 grams, then the inhibition must have been certainly strong enough to arrest cell division in the liver cells themselves, and was evidently responsible for the suppression of proliferation in the intact liver itself. We had thus in hand a regulatory substance which I called "*retine*", since it *retarded* proliferation.

Could retine alone be responsible for the regulation of growth? For the regulation of traffic two lights are needed — a red and a green one. Could traffic be regulated with one light only? Certainly, it could. Red light, in this case, would mean "stop", while switching off the red light would mean "go". To regulate traffic with one light, one would thus need a switch with which to switch off the red light. Cells do contain such a switch, an enzyme, discovered by Monder, which can decompose retine.

But how could retine retard growth in the presence of an enzyme which decomposes it? Very simply, by being kept separated from the enzyme. Of course, separation at the small distances within the cell, means a high degree of order, a precise pattern. Regulation in cells is mostly achieved by separating substances, like enzymes and substrate, and the separation can easily be disturbed. What we have done by cutting out part of the liver of the rat was to create disorder and disturb separation on the cut surface, allowing retine and the enzyme to meet, whereupon the retine was destroyed releasing thus the liver from its inhibition.

Biochemists always ask the same primitive question: what

is the chemical nature of the substance in question? We expected our retine to be a ketone aldehyde and ketone aldehydes are easy to detect since they give instantaneously a red precipitate with2-4 dinitrophenylhydrazine. Our extract did not give such a precipitate but when the extract was stored the precipitate developed. Evidently the strongly acid reaction of the reagent split something, had split off the methylglyoxal from the complex. So our retine was a derivative of methylglyoxal in which the aldehyde was covered up, and the acid of our reagent has split off the methylglyoxal.

Retine, itself, has a strong yellow color, much stronger yellow than corresponds to its methylglyoxal content, indicating that the aldehyde was linked to a nitrogen which is known to enhance the color. Retine was thus analogous to the artificial cancer suhstances we produced before wich D. Egyud.

Does all this tell us what cancer may be? It suggests very strongly that cancer means essentially disorder which allows the retine to he destroyed by its enzyme for which it acts as substrate. This would explain also why cancer can be produced by such a great variety of agents; disorder is very unspecific and can be produced in an endless number of ways. If we cut ourselves, our wound heals because we created disorder and released inhibition. The cell division goes on till the wound is healed and order and balance is restored.

The medical problem is not what cancer is but how it can be cured. Does the described mechanism give us any hope of curing or even preventing cancer? I think it does. Retine, or its synthetic analogues can strongly suppress cancer growth. Our curves indicate that it can suppress growth the more the fewer the cancer cells are which compete for the retine. They indicate that retine could completely suppress the cancer of *one* cell. This is important because cancer or a metastasis begins with *one* cell only and we should be able to suppress multiplication of this one cell and prevent cancer or metastasis altogether. So we could hope that while the blood does contain

enough retine, no cancer or metastasis will be formed. To clear this point we would have to know how much retine the body contains. This makes it desirable to have a method which allows us to find out how much retine the blood contains. Retine ha an absorption at 330 mu and has a strong fuorescent emission around 400 which can easily be measured. This makes it possible to develop a method which allows us to tell exactly how much retine our blood contains. This estimation can be done in 1 ml, and is exceedingly simple. This opens the chance of being able to tell how much retine the blood contains and how much retine has to be injected to prevent cancer or prevent metastasis if cancer was found. There is then a hope that one will be able not only to cure, but also to prevent cancer.

All this is only hope at present, a well-founded hope which demands a great deal of work to be turned into a reality.

There is nothing I want more than to do this work. Very unfortunately, in our days, killing has preference over healing and the means to do this work are being denied to me by our governmental agencies, and at present my laboratory has no grant whatsoever. So while our army does the killing in far away countries, cancer does the killing at home.

Cancer is a most fascinating problem. One of those grand experiments which nature does for us is cancer which offers us a chance to look into nature's cooking pot. Diabets is such an experiment too, which has lead us to insulin and cancer may be another, which has lead us to retine. To understand the problem we must approach it with basic research, as a biological, and not as a clinical problem, searching truth for truth's sake. Basic research has this paradoxical property that it can produce the most wonderful and useful results till it searches truth for truth's sake and mostly become useless as soon as it tries to be useful.