PROBLEMS IN CANCER RESEARCH

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It is a pleasure to announce that a Department for Cancer Research has again been established in St. Louis by the Barnard Free Skin and Cancer Hospital. The material available is the laboratories and what can be developed from the more general biological side within them, and the clinical material.

The plan which is being used for the study of cancer divides the work into several distinct groups. One is a general study of the properties of the various cells of the body and the peculiarities of the mechanisms of growth, division, differentiation, and function of these cells. A second is a close study of the clinical course of the disease and the results of methods of treatment and diagnosis. A third is an attempted analysis of isolated facts now fully demonstrated, and their immediate relation to clinical and pathological data at hand.

For the immediate time, one problem which seems to be most pressing is the improvement of methods of diagnosis, especially the diagnosis of internal cancer. Early diagnosis of cancer of the breast has prolonged the life of many patients for at least ten or twenty years. The same is true for other superficial growths. Barring a few exceptional cases, methods for the diagnosis of internal cancer have not reached the same degree of perfection, as, for instance, cancers of the intestinal canal. If by chance a cancer of the intestine causes early obstruction, a diagnosis may be made and the treatment may be hopeful. How few cancers of this region give, however, this early symptom!

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Another of the immediate problems is the betterment of methods of treatment now available. The third and most important of all is the etiology of this disease. It is most important, because through its elucidation and even in its study all other problems must become simplified. Through it alone, barring some peculiar accident, can the hopes for ultimate success in treatment come. The greatest effort in cancer must be exerted, therefore, in this direction.

What is known about cancer today, aside from clinical considerations, is little more than was known many years ago. This is appalling but true, and the question arises: Is it not due to the fact that the general notions of the cell, its structure and properties have not materially advanced during this time? The problem of the etiology of cancer when resolved into terms of the cell may be quoted thus: Is the cancer cell a cell different from normal cells, or is it a normal cell suffering a continuous external growth stimulus? Since the application of the theories of cellular growth now in vogue have failed to answer this question, there remain open but two channels of attack; the hit and miss method, and a further probing into the nature of the mechanisms of cellular growth, division, differentiation, and function.

Cancer, no matter how it is taken, represents a break in the normal growth-regulating conditions of the body. Without a knowledge of the normal growth-regulating mechanism, it is to be expected that the etiology of cancer will remain obscure, unless it is proved that the disease is the result of some definite foreign agent or stimulus—parasite, or otherwise—which may be isolated.

It is known that the body is composed of cells. The minute metabolism of these cells, the mechanism of their formation, even the simplest constituents of their life are, however, based only on theories. The leucocytes are compared to the amoeba. One talks glibly of cellular metabolism, when he probably means body metabolism. Is this so-called cellular metabolism the work of the cell or is it the work of the organ or the body as a whole? In beginning a few years ago the study of the cells in tissue culture, it was of interest to note that the leucocytes, both the various mononuclear as well as the polymorphonuclear types, show
no ability to grow and divide like other cells. Their movements are passive reactions to external conditions. A connective tissue cell or a heart muscle cell suffering from the effects of certain toxic poisons can be made to resemble the small and large mononuclear cells of the inflammatory exudate. These latter cells have lost, also, all ability for further growth under the conditions in which they had previously grown or under any other conditions it has been possible to impose upon them. In actively growing culture many of these fixed tissue cells, unaffected by foreign poisons may also show such changes after they have been preyed upon by actively growing cells.

The polymorphonuclear leucocyte is a cell differentiated in a special locality. Lymphocytes undoubtedly are found in the same locality as well as in lymph-nodes. Whether the large number of mononuclear cells of the chronic inflammatory area or about cancers have this origin is still a very much open question. In fact, the work of Maximow has not been confirmed. (See Stockard (1).) That these cells contain enzymes and bacteriolytic substances which are liberated when they break down is, again, well established. What is not known are the conditions under which these cells live in the areas about a cancer and whether they do liberate these substances under ordinary conditions there. It seems evident, therefore, that they might as well be considered the result of active growth or the presence of toxic substances, as to be thought of as antagonists to these conditions. In chronic inflammations, it is generally where the bacteria are most active that they are most prevalent. In the tissue culture it is the actively growing tissue cells that show the true antagonistic action to growing bacteria. The lymphocytic cells and the rounded-off connective tissue cells intermingle in the cultures with the bacteria. The connective tissue cells, the heart muscle cells, and other actively growing cells cease to grow when they come within the zone of bacterial action. The bacteria stand them off as they also stand off the bacteria (2).

This absence of an absolute knowledge of many of the most important functions of body cells must in itself make it very unsafe, therefore, to draw any far reaching functional conclusions
from morphological data. It makes it absolutely necessary to
draw one's conclusions from the clinical facts alone. This applies
not alone to treatment, but also to other experimental results.
The laboratory in Saint Louis is well situated in this regard in
that it is a definite part of the clinic, and also allows the broader
type of biological research which in the light of present knowledge
must form an intimate part of the cancer laboratory.

PROBLEMS UNDER CONSIDERATION

1. Studies on oxidation in actively growing and differentiated
cells, especially in reference to their growth

In a previous communication (3) before this society evidence
was given by the author to show that connective tissue cells
and simple mesenchymatous and embryonic muscle cells show
no evidence of an internal organization suitable for growth.
They act more like homogeneous fluid systems, leaving aside
the nucleus and the astral center. Growth in them can be
explained entirely as a surface tension phenomenon, the energy
of the process being effected by the formation of a substance
within the cell, which is insoluble in circulating body fluid, but
soluble in dead cells, fibrin, and products of disintegrating cells.
A cell forming this substance and brought in contact with fibrin
suffers a decrease in surface tension along the line of contact.
The further details of the organization peculiar to growth is
determined by these contacts. The order of the contacts of
a growing and dividing heart muscle cell is different from a
rhythmically contracting one.

For a long time it has been known that actively growing
tissue cells, such as those taken from young embryos and
sarcomata commence their growth in the culture within one or
two hours or much earlier than adult tissues. This is seen in
a study of tissues of embryo chicks. The cells from a frag-
ment of the heart of a two, three, or four days old chick embryo
will commence to grow almost at once when placed in the culture.
About fragments of a ten day old embryo heart such activity
is not observed until after six or twelve hours, and about frag-
ments of a fifteen day old chick embryo heart after fifteen or twenty hours; while about young adult heart fragments this latent period is often as long as twenty-four or forty hours.

In a previous publication (4), the writer reported a study of the relation of oxygen to these changes in the cell. A method was developed which allowed accurate regulation of the amount of this gas in the chamber about the cells. The tissues especially studied were fragments of the heart and body wall of fourteen, fifteen, and sixteen days old chick embryos. It was found that the growth was as active in an atmosphere of 9 per cent oxygen as in pure oxygen, and ceased in atmospheres which contained slightly less than 6 per cent of this gas. It became of interest this year to see whether the more actively growing tissue might not show variations in these figures. The question that arose was whether the less actively growing tissues did not have to form or in some other way liberate the substance or substances which actively change surface tension or produce energy. The short latent period in the more actively growing cells suggested a supercharging of this substance or substances.

In 1903, Fletcher (5) showed that a muscle of the adult frog will give maximum contractions every five minutes for a period of two hours in an atmosphere of pure nitrogen. In the work on organization of isolated rhythmical, contracting heart muscle cells in the tissue culture, the author has found evidence to show that the chemical changes of muscular contraction are similar in certain stages to those of growth. If muscle will contract for a time without oxygen the question that arises is: Will not cells fully differentiated for growth show the same reaction (6)?

The tissue so far tested has been cells from the hearts of chick embryos of various ages, ranging from four to fifteen days. Fletcher's experiments were repeated with contracting hearts and heart muscle fragments of these embryos isolated in the tissue culture. These cells will contract for from twenty to twenty-two hours in an atmosphere of nitrogen. In the same oxygen-free atmosphere, the cells from fragments of four and five days old chick embryos will grow from four to six hours. This
growth commences, however, always after a considerably longer latent period than that in an atmosphere containing oxygen. It is active for this short time, after which the cells invariably disintegrate rapidly. The period of growth in these hanging-drop cultures is very much longer in air; the early disintegration is rarely or never seen; and when it occurs, it is always later. To obtain a similar growth about the fragments of the heart of ten day's old chick embryos 1.8 per cent oxygen was required and about fragments of fifteen days old chick embryos 4.5 per cent. These experiments have not only been applicable to the problem in question, but have given a method of differentiating functionally the actively growing young embryonic cells from those showing greater degrees of differentiation. In the case of these cells they have also shown the necessity of oxygen for the maintenance of structure. Whether this method may be used for differentiating any actively growing tissue is a problem yet to be considered.

STUDIES OF PLANT CANCERS

Another method which has appealed to us in attacking the cancer problem has been the study of plant cancers. These represent an active proliferative growth, the etiology of which is known. Chambers (7) has undertaken this problem. He was interested during last summer in investigating the metabolic activity of the organism, Bacterium tumefaciens, in culture, and in the course of this study noted that these organisms produce alkali in the culture even in the presence of sugar, which they also cause to disappear rapidly. Smith, by a different method, had noted that ammonia is formed in the cultures of these organisms. Harvey (8) more recently, also, noted that the tissue of these tumors is more alkaline than normal plant tissue. The work of Chambers would indicate, therefore, that the increase in the OH-ion in the tissue of these tumors is due to the etiological agent, which produces alkali in the cultures at least. Chambers is now attempting to see if under the condition imposed on them by the plant, they also form it.
These facts attracted our attention to the work of Menten (9), by which an alkalosis was demonstrated in the blood of cancer patients; and this question is now under investigation. The various methods of testing were critically reviewed and the indicator method was selected. The tests are made on a dialysate of venous blood, which is taken under oil and kept unexposed to air throughout the whole procedure. The results so far obtained have been interesting. In the early part of the disease, these patients show no evidence of change in the reaction of their blood when it is taken from a region far removed from the cancer. A definite alkalosis is observed only when the cancer has grown to considerable extent and involves neighboring glands and tissues. The blood of a patient with a beginning carcinoma in the lip is normal. A definite $H$-ion change in the blood is not seen until the glands of the neck are involved, and then increases gradually as the disease progresses. The same is true for carcinoma of the intestine. A small annular carcinoma of the intestine with no or but little glandular involvement may show by the indicator method no change in the alkalinity of the blood. Sharp changes are always seen, however, when liver nodules are present. The CO$_2$ relations in the blood of these patients is now being studied. It is possible that by this means much concerning the significance of this alkalosis may be determined. The anemia may also have an effect.

The importance of these experiments is still to be ascertained. That the alkali is probably produced in the malignant tumor is indicated, however, in the study of one patient suffering from a sarcoma of the forearm. Blood taken directly from a vein leading from the tumor showed a pH of 7.5, while in the other arm the blood pH was 7.3, or normal. This case illustrates the difficulty of using such a method for a clinical test. The body compensates more readily for acid than alkali, but at the same time there is a rapid elimination of alkali by the kidney. In the case of acid, the compensating mechanism gives evidence. To what extent this can be used for determining the presence of alkali is yet to be determined. In the case cited, there was evidently alkali being given into the blood by the tumor, but it
was not detectable in the other arm. There was no glandular involvement in this case.

What the further studies of this reaction will open up and the further studies of the bacteria of the plant tumors may reveal is a question which the future alone can answer. It seems, however, that this method may yield something important.

REFERENCES

(9) Menten, Maud L.: The alkalinity of the blood in malignancy and other pathological conditions; together with observations on the relation of the alkalinity of the blood to barometric pressure. J. Cancer Res., 1917, ii, 179-211.