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OBSERVATIONS ON THE EXISTENCE OF ENZYMES IN CANCEROUS GROWTHS.

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Apart from the unsolved problem of the initial source of cancer cells, it has always seemed to me a matter of great importance, especially from a therapeutic point of view, to settle definitely what is the exact relationship of the cells of a carcinoma to the surrounding tissues, for what constitutes one of their most deadly attributes is the manner in which they invade, destroy, and even replace them. Were it not for this characteristic, a cancerous tumour would be less formidable than it unfortunately is, and to the surgeon would present a more favourable field for operation. Why should it be that a small and apparently limited cancerous growth has, like the cloud no bigger than a man's hand, possibilities within it that no one can foretell? How is it that, before its increasing and advancing cells, every tissue in the body, be it muscle, bone, or nerve, has to yield and possibly eventually to disappear? To get an answer to these questions, it is necessary briefly to consider how a carcinoma invades the adjoining tissues. The general consensus of opinion is that it is by the lymphatic system, and that it is through the lymph spaces of the part attacked, that every structure is primarily penetrated. When this has taken place, what is the sequence of events? At first, probably, the cancer cells lie quite free in the spaces, being swayed hither and thither by the currents of the lymph stream. With their rapid increase by the proliferation so characteristic of them, a different state of matters is, however, established. They crowd the spaces, and in time actually expand them, so that sooner or later we have formed those alveoli packed with cells which are so often the dis-
tistinguishing feature of a cancerous growth. Looking to their origin, it will be at once apparent that the walls of these alveoli are really the pressed-out remains of the tissue whose lymph spaces the cancer cells have invaded, and to them the term stroma has been applied. At first this stroma contains the blood vessels of the part, but it is also permeated sooner or later by a number of new cells, some of which are small and round, while others are larger and more elongated. These are not cancer cells, but are partly the offspring of the fixed connective tissue corpuscles, and partly migrated leucocytes. They are really inflammatory, and they result from the irritation caused by the presence of the invading cancer cells. As time goes on, the stroma is subjected to more and more pressure, both from the increasing cancer cells and from the fibroid contraction which the above mentioned inflammatory exudate undergoes. The further result of these two factors, namely, the fibrous contraction of the inflammatory products, and the mechanical pressure of the cancer cells, is that the blood vessels in the stroma become obliterated, and very marked degenerative changes are set up. Accordingly, the centre portion of a carcinoma of the breast of some standing exhibits a distinctly fibrous appearance, being whitish in colour and speckled with small yellow spots. These latter are the cancer cells of the alveoli undergoing fatty degeneration from want of proper nourishment. I do not propose to dwell on the importance of these changes from a curative point of view, but there can be no doubt that by them the cancer cells have been rendered inert, a most instructive fact. All I would insist on is, that the section of a mammary cancer of some duration shows that one of the agencies by which carcinoma works in removing tissue and replacing it by its own cells, is mechanical pressure.

This agency, however, does not to my mind explain all that is seen in connection with the spread of carcinoma. The microscope shows us at the active margins of a growing mammary cancer, not only the acini being pushed aside by the infiltrating cells, but a very rapid disappearance of the epithelium. Not that this is undergoing conversion into cancer cells, for probably such a thing does not happen, but that it is apparently vanishing before the cells of the new growth. The same thing is seen in that glandular implication which is such a marked feature of the carcinomata. If an involved lymphatic gland is examined in an early stage, it will be found that the first manifestation of its being affected is at the periphery, in the course of the usual afferent lymph stream. Here will be found arrested groups of malignant cells, which may be showing the varied mitotic appearances of active division and proliferation. If some time is allowed to elapse, a later examination of the same gland will show that it is now composed of nothing but cancer cells, which have made their way in from periphery to centre, and have practically
replaced the glandular parenchyma and its lymphoid cells with a new growth of malignant tissue. Were observations to be made while this change was in progress, they would show that the glandular lymphoid cells took no active share in it, but that they disappeared entirely in the presence of the cancer cells. It is difficult to grasp that mere pressure can bring about this complete transformation of tissue, and attempts have been made to explain it, as well as the greatly increased vegetative capacity of cancer cells, by ascribing to them the possession of special properties. Thus, Virchow has favoured the existence of a seminum or spermatie influence, while others have supported the idea of an infective fluid of unknown composition but of great power. All of these suggestions have underlying them the principle that carcinoma spreads by some contagious excretion, which infects the adjoining tissues and practically changes them. I am not prepared to support this view, for, as I have already said, I do not think we have a conversion of epithelial cells into cancer cells, but I certainly hold that cancer cells have the power of acting on adjacent cells, and that this may be explained in another way, and more in keeping with the advances that have been made of recent years, on how cells may influence one another.

Of these advances, the most important as bearing on our subject are those that have been made in connection with the physiology of fermentation, a term that now includes a number of manifold processes, all of which have these two features in common—(1) That they are accompanied by the decomposition of complex molecules, and (2) that they take place, directly or indirectly, through the agency of either vegetable or animal cells. Such cells are known as ferments. When they act directly, the chemical changes involved in the process occur only in the presence of the cell itself; when they do so indirectly, the changes accomplished are brought about by a fluid which is secreted by the cell, and acts apart from it. This difference in their mode of action has separated the large group of ferments into the two kinds of solid organised ferments, better termed ferment organisms, of which yeast is an illustration, and the dissolved unorganised ferments, which have been named by Kühne Enzymes, and of which pepsin is an example. These soluble ferments are a group of highly complex and interesting organic bodies. The first one to be detected in 1833 was diastase. This was followed by pepsin in 1836; and since then investigations have shown that they are very widely distributed in nature. Present both in animals and plants, they are apparently manufactured within the living organism itself by a metabolism of the proteids of the cells, and they are given off to the outside of the cells as a secretion. As to the mode of action of enzymes, this is not quite definitely settled. It may be by a chemical decomposition of the neigh-
bouring molecules, or it may be by mere contact, just as happens in the case of formic acid, which can be broken up into carbonic acid and hydrogen by finely divided metals like iridium and rhodium, the metals themselves undergoing no change in the process.

Be that as it may, enzymes can have a powerful chemic and hydrolytic effect on surrounding tissues, and by their influence they can bring about decomposition and changes in other cells. Further, there is this remarkable point about them, that in doing so they apparently exhibit no change themselves, and thus their activity can be practically illimitable. It may be that they are destroyed and are constantly reformed, but the fact remains that they in no way combine with the products of their reaction, so that when a quantity of material has been acted on by a definite amount of an enzyme, at the end of the process the original quantity of the enzyme is found to be the same.

I have for some time felt that whatever be the origin of the first cell or cells that start a carcinoma, the relation of their numerous offspring to the tissues they invade is that of a parasite, in the sense that they get their nourishment from them. By saying this, I hope it will not be understood that I support the parasitic theory of the cause of cancer. As I have written elsewhere, I hold the opposite view, and look for the cause of the disease rather within the body than without. But it has always seemed to me that the active and rapid growth of a carcinoma with its vegetative activity, as shown by the quick proliferation of its cells, could only be explained by assuming that the tissues of the affected organism served as pabulum for it. The complete disappearance of all the structures invaded by the carcinoma is confirmatory of this view. It is self-evident that cancer cells, if they are animal cells, as I feel sure they are, cannot live on purely inorganic matter, such as water, salts, or other chemical substances, in the same way that we know plants can do; and though they are brought, by means of the blood vessels in the stroma, into contact with that blood plasma which is the common nutrient material for all the tissues, yet it is not an unreasonable assumption that in addition they may be endowed with an extracellular digestion, which will allow of their reducing the solid tissues around them into a soluble form and then ingesting them by resorption. If this be so, I thought that it might be a useful inquiry to ascertain if any enzyme existed in cancer cells, and on laying my views before Professor Mc'Kendrick, in May 1897, he thought that investigations might profitably be conducted in this direction, and he suggested that I should seek the co-operation of his then Muirhead demonstrator, Dr. David F. Harris, whose ability in physiological chemistry rendered him specially suitable for undertaking the work. This I did, and I have here to acknowledge the valuable help and ready assistance I received from him.
I cannot do better than give here the report furnished to me by Dr. Harris.

"In June and July 1897 I received from Dr. Beatson several portions of cancerous tissue excised from the human subject, with the view of inquiring whether there was any evidence of the presence of a ferment secreted by the cells of carcinomatous growth. Dr. Beatson's idea was briefly that the cells of a carcinoma, having the power of steadily invading adjacent tissues, do so by means of some tissue dissolving ferment, presumably secreted by the cells of the tumour. Such a ferment would be of the nature of a proteolytic enzyme.

"I directed that the freshly excised pieces of the cancerous tissue should be at once placed in two to three ounces of Price's pure glycerine, after having been merely rapidly washed in normal saline solution, to remove blood or any other accidental contamination.

"I assume that this enzyme, like the majority of enzymes known to us, was also soluble in glycerine, and therefore followed in principle the method of von Wittich for its extraction. The mass of tissue having lain in the original glycerine for two or three days at the temperature of the room, was then ground up in a mortar in presence of the glycerine in which it had been lying, and of powdered glass or of fine sand, in order to disintegrate it. This was in some cases a matter of extreme difficulty, owing to the extraordinary toughness and denseness of the cancerous mass. The cellular débris, along with the glycerine, sand, or glass, was then scraped into a thin flask plugged with cotton-wool, and kept in a water bath for seven or eight days, at a temperature of about 40° C. After this the contents of the flask were filtered, and any enzyme soluble in glycerine should have been in the filtrate.

"It was assumed that an enzyme such as was being sought for, being proteolytic, would digest such material as washed fibrin from blood-clot. A few shreds of fibrin were therefore placed in the filtered glycerine extract, and kept for fourteen days at 40° C.

"In this manner the following were examined:— 1) Seirrhus of mamma, (2) carcinoma of rectum (male), (3) carcinoma of rectum (female), (4) carcinoma of axillary glands (female), and (5) carcinoma uteri. The results of these "digestion" experiments were in all cases negative. In no flask was there any disappearance of the fibrin, and in no flask was there any appearance of proteose or peptone. Hence, as examined by the glycerine method of extraction, we seem entitled to conclude that the cells of a carcinoma do not secrete a proteolytic enzyme."

Besides, however, this inquiry carried out by Dr. Harris, it seemed to me that in our present uncertainty as to the exact nature of carcinoma, that the possibility of the disease being due to a yeast, as the Italian observers San Felice and others
maintain, should be kept in view, and that search should be made for the special enzyme associated with yeast, namely, invertin or invertase, which has the property of converting cane sugar into glucose (dextrose). This was done for me by Dr. Ernest Fortune, the experiments being carried out as follows:

Pieces of the tissue under examination were minced in a mortar (previously sterilised) along with a 10 per cent. solution of cane sugar. The mixture was then neutralised with a solution of sodium bicarbonate, and placed in a water bath at a temperature of 50° to 52° C. In certain of the experiments, for anti-septic purposes, the tissue was first bruised in chloroform water (B.P. 1898), and, after standing for twelve hours, the chloroform water was poured off and added to an equal quantity of cane-sugar solution. The tissues examined included scirrhus mammae, sarcoma, adenoma, breast tissue, and carcinoma of the liver (secondary). In all cases, means were taken to prevent the introduction of bacteria from without.

The cane sugar solution was tested with Fehling's solution at periods varying from two to forty-eight hours, and in only two instances was the Fehling reduced. In ten other experiments there was no evidence that cane sugar had become inverted. In the two instances where inversion occurred, this could be explained by the accidental introduction of bacteria, in some of whom probably invertase may exist.

Negative as these results are, I am not prepared to accept them as final, and the inquiry is still being prosecuted. All investigators into the action of enzymes, and their presence in tissues, have acknowledged the difficulty there is in extracting them from the protoplasm of the cells in which they are known to exist. It is possible that the portions of cancerous tissues used were of too long growth, and that the contained cells had undergone that fatty degeneration which, I pointed out, occurred in the centre of old mammary cancers. It may also have happened that sufficient time had not been given for the extraction of the enzyme. Both these points will require attention in future. The difficulties in the carrying out of the inquiry are, I know, great, not only because of our not having that chemical knowledge of enzymes which will allow of a definite precipitant being used, but also because of the small quantities of them which are generally present. Another factor that has to be reckoned with in such observations, is the possible presence of bacteria in the tissue experimented on. This would certainly be a disturbing element, for there are many enzymes secreted by micro-organisms, an everyday illustration of which is seen in the liquefaction of gelatine by certain bacteria. Cancerous tissue that has ulcerated is specially liable to this fallacy.

Difficult though it may be to isolate any enzyme in cancer cells, I am of opinion that there are grounds for continuing the
search. With the presence of one or more enzymes established in cancerous growths, a great deal in connection with the carcinomata that otherwise seems unaccountable would be explained. Thus it would, I consider, clear up those variations in the rate of progress of cancer that are seen in different individuals and at different ages, for then would come into play that resisting power of the tissues which depends on their suitability, or otherwise, to serve as pabulum for the advancing cells. If they do not yield easily to the digestive action of the cancer cells, then the disease will be slow, for the new growth must suffer from want of nourishment, and its cell proliferation will accordingly be lessened. On the other hand, if the tissue cells are easily assimilated, then the growth will be strong and vigorous, just as happens in the liver and submaxillary glands, where the glycogen present furnishes abundance of nutriment, and the secondary manifestations of the disease that occur there often assume most striking dimensions. From a therapeutic point of view, also, it will be readily seen an enzyme would be of great assistance to us, as it might allow of medicine coming to the help of her handmaid surgery, and possibly supplementing local removal in such a manner that a more permanent cure might be relied on. The pathological chemistry of carcinoma has not been perhaps sufficiently worked out, and when we are awakening to the fact that nearly all the metabolism and tissue changes in the body are really local fermentations,—in fact, that this body of ours is practically a ferment organism which daily transforms quantities of food-stuffs, and yet only slowly and imperceptibly disappears itself,—it is not unreasonable to surmise that cancer cells, even though they may be cells that have "gone wrong," are still subject to the laws of nutrition that hold good elsewhere.

BACILLUS TYPHOSUS AND BACILLUS COLI COM- MUNIS: A CRITICAL COMPARISON, WITH SOME DESCRIPTION OF A NEW METHOD FOR THEIR DIFFERENTIATION, AND ITS APPLICATION TO THE DIAGNOSIS OF TYPHOID FEVER.

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In the great kingdom of the micro-organisms there are no two which resemble each other so closely as the Bacillus typhosus and the Bacterium coli commune.

In fact, so many striking points of similarity exist between them, that no less authorities than Rodet and Roux have committed themselves to the opinion that the pair are simply varieties

1 Deutsche med. Wochenschr., Leipzig, 1891, S. 1416.