THE RELATION OF CARCINOMA TO INFECTION.*

BY

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[With Special Plate.]

BETWEEN the years 1905 and 1909 I published, in some instances in association with co-workers, a number of papers on cancer, recording observations on the ground of which it was maintained that the disease is due to some form of infection. Two of these papers I wish specially to recall. The first, published in the *Lancet* of January 25th, 1908, was entitled a "Note on the presence and significance of certain rod-shaped bodies in the cells of carcinomatous tumours." In this paper I claimed that the rod-shaped bodies revealed by the palladium methyl-violet method in cancer cells were living organisms and at least a phase of a parasite that causes the disease. The second paper, published in the same journal on June 5th, 1909, was entitled "Experimental evidence of the infective origin of carcinoma and of the transmissibility of the disease from the human subject to the mouse." In this paper it was recorded that carcinomatous tumours developed in seven out of thirty mice fed with incubated fluid from the pleural cavity of a case of malignant pleurisy, and with similar fluid from the abdominal cavity of a case of malignant peritonitis. On the ground of this result it was contended that a living virus had been transferred from the human subject to the mouse, and that cancer was therefore of infective origin. This is all I need say about these previous investigations, and I pass to the results of new investigations to which I have devoted any spare time I could snatch from official and other necessary work during the past two years.

The method of research is to test an hypothesis. By induction from a few established facts, we can discern, more or less accurately, where the next important truth lies, and we set out to look for it. If, after a thorough search, we cannot find it, we conclude it is not there, and we abandon the hypothesis. If, on the other hand, our search proves successful, we claim that we have established the hypothesis that we set out to test. This is the method that I have followed.

In the course of many years of bacteriological work applied to therapeutic immunization I developed some special culture methods, and, with their aid, observed some facts regarding the behaviour of bacteria which, although not altogether new, have attracted little attention. One of these facts has already proved of paramount importance for therapeutic immunization, because, unless it is taken into account, the bacteriological analysis upon which the success of therapeutic immunization primarily depends must very often be incomplete. This fact is that many bacteria that we regard as aerobes commonly assume, when their attack is prolonged, an anaerobic habit of growth, and can be cultivated only under anaerobic conditions. This applies, for example, to such important pathogenic species as *Micrococcus catarrhalis*, the gonococcus, *Streptococcus pyogenes*, *Streptococcus faecalis haemolyticus*, and several types of diphtheroid bacillus. Now suppose that an anaerobic bacterium of this kind gets within a living cell, what will happen? For the present we may consider the question only in regard to epithelial cells. In all probability these cells, like the mesoblastic elements, have a mechanism of defence against foreign invaders, but that this mechanism, if it exists, is sometimes overcome can be clearly demonstrated. Suppose then that a bacterium establishes itself within an epithelial cell and multiplies. What will happen? The first possibility is that the cell will die on account of the toxic action that must ensue. It is impossible that the cell can remain unaffected by the invader, but it is far from likely that in every instance it will succumb. We know that one action of many bacterial toxins upon cells is to stimulate growth. It is possible that some types of bacteria, if established within an epithelial cell, would have chiefly this effect. The cell would be stimulated to

divide, and would go on dividing, so long as the foreign element producing the toxin remained within it. It might even, after some thousands of divisions, acquire the habit of continued proliferation independently of the presence of the parasites.

With these few facts and possibilities before me, the hypothesis suggested itself that the rods I had observed within cancer cells by means of the palladium methyl-violet method might be anaerobic bacteria that had established themselves within the epithelial cells, and that the continued proliferation was due to the action of these bacteria. This is the hypothesis that I have been trying to test during the past two years, and, extravagant as it may seem on first view, the evidence that can already be brought forward in support of it is, to put it quite mildly, sufficient to arrest attention. There are at least four distinct methods by which the validity of this hypothesis may be tested. They correspond to four distinct classes of possibly procurable evidence that may serve to establish or to refute it.

The first is the method of direct examination of cancer cells. We can make microscopical preparations of carcinomatous tissues, and endeavour to determine if we can observe in the epithelial cells any bodies corresponding in their general characters to bacteria. A thousand negative observations of this kind are valueless before one set of positive observation made by a special technique that consistently gives the same result when widely applied. Many years ago I worked for months endeavouring to discover a process that would give a positive result, and at last elaborated the palladium methyl-violet method. It gave its answer, and I demonstrated the results before scientific societies and published a paper. The method is a permanent one, and the sections, some of which are under the microscopes to-day, are as clear as they were fourteen years ago. The bodies observable are minute rods, resembling bacilli: there is evidence that they occur chiefly within the nucleus, and that when they are thrown out into the cytoplasm, as they may be during division of the cell, they display a chemiotactic attraction towards the nucleus. The infected cells occur in special areas; throughout the greater part of the tumour it may be impossible to observe any of these rods. On the ground of these results, obtained fourteen years ago, I maintain that the evidence of the method of direct examination supports this hypothesis of epithelial cell infection.

The second method is that of artificial cultivation of the supposed bacteria. If there are living micro-organisms within cancer cells, it may be possible to obtain growths of them by suitable methods. Here again thousands of negative results are valueless against a positive demonstration by a special technique that gives consistent results in a series of cases. The evidence of this kind that I have obtained is, perhaps, not so decisive as that of each of the other three methods, but it is, I think you will admit, sufficiently striking. The results suggest that it is necessary to widen the hypothesis that is being tested. They would seem to indicate that not one species of bacterium but several species belonging to a group are capable of causing cancer.

It is necessary that you should understand the methods I have used. Only those now found to be of most service need be mentioned. I use haemoglobin agar slopes of various degrees of acidity, set up anaerobically by the pyrogallic acid method. This mode of culture is, however, only of limited service. An anaerobic fluid medium is essential. The one that I have now used for several months is prepared as follows: One part of fresh serum of the sheep is added to 5 parts of sterile normal salt solution in a large test tube and incubated for twenty-four hours. If the fluid remains clear, it is next decanted into small tubes, which are filled to about 1 in from the top. With the cotton-wool plugs inserted, the tubes are placed together in a suitable bottle, and the air in this is exhausted by means of the Geryk pump for two days. The tubes are then taken out and immediately sealed by dipping the plugs in melted hard paraffin and replacing them. The tubes are next incubated for ten days at least. They are then numbered, and the fluid in each is decanted into a series of similar tubes, which are immediately sealed with paraffin. The number of the original tube is written on each. A small amount of the deposit remaining in each original tube is then examined microscopically by the staining method used in the subsequent

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study of cultures, and the decanted fluid in the tube with the corresponding number is passed as fit for use, only if the deposit gives a negative result as regards the presence of micro-organisms. After a few days' further incubation the decanted tubes, if they remain clear, are put aside in the cold and are ready for use.

Cultures are best made from scrapings of a freshly cut surface of the tumour. Incubation of the solid and fluid media has generally to be continued for from two to six weeks. Any colonies appearing on the solid media are examined in the usual manner. Growth in a fluid medium shows itself as a deposit or as minute white particles adhering to the glass. Before examination the fluid is centrifuged. The tube is then unsealed by heating the plug in a Bunsen flame. The fluid is decanted into another tube, which is immediately sealed with paraffin. It is of advantage to wash the deposit with distilled water and to centrifuge a second time. The deposit is then stained and examined. I have found Gram-neutral red staining the most serviceable, but some of the bacteria studied have required heating of the stain in order to obtain a satisfactory result.

I have made cultures chiefly from mammary cancers removed by the surgeon. For most of the specimens I am very greatly indebted to Mr. W. J. Stuart. For others I have to thank Professor Alexis Thomson, Mr. Alexander Miles, Mr. J. M. J. Hartley, and, I believe, one or two others who forgot to give their names when sending me the specimens by post. In several instances, as might be expected, the tumours showed common bacterial sepsis. *Streptococcus pyogenes*, staphylococci and aerobic diphtheroid bacilli were the most frequent contaminating micro-organisms. By far the larger number of specimens were, however, free from anything that can, I think, be rightly regarded as bacterial contamination. In many instances tissues that appeared sterile under aerobic conditions yielded colonies of diphtheroid bacilli, and less frequently of staphylococci, under anaerobic conditions. On first view these growths might also be regarded as the result of simple contamination, but, as I shall presently explain, they may have a different significance. A large proportion of the tumours yielded no growths on surface media, either under aerobic or under anaerobic conditions, but gave a positive result in an anaerobic serum broth, or in an anaerobic serum salt solution medium. The actual number of cases examined does not matter at this stage of the investigation, because many of the earlier observations were tentative and carried out with a technique that has been abandoned. The following is a summary of the positive results to which, in view of the testimony of the other three methods, I believe there is reason to attach importance.

From eight carcinomata of the breast removed by the surgeon I have obtained, under anaerobic conditions in a serum broth, or in a serum salt solution medium, growths of bacillary bodies resembling the rods observed by means of the palladium methyl-violet method in sections of similar tumours. These micro-organisms would not grow on a haemoglobin agar surface, either aerobically or anaerobically. Stained by the heated Gram-neutral red method they appear as minute granular rods incompletely Gram-fast. Stained with an acid solution of methylene blue, they are seen to contain minute metachromatic granules. On this account they must be classed as bacilli of the diphtheroid group.

From twelve carcinomata of the breast removed by the surgeon I have obtained colonies of anaerobic diphtheroid bacilli upon a haemoglobin agar surface. Similar growths have been obtained from five other carcinomatous tumours, including a rodent ulcer recently excised by Mr. Miles. The rule has been that only one of these two forms has been obtainable from a tumour. The only other bacterium that has been found with any frequency in anaerobic cultures from what could be regarded as uncontaminated tumours has been a staphylococcus, not *pyogenes*, but one belonging to the mannite non-fermenting subgroup. Such results tell us nothing as to the relation of these bacteria to the growth of the tumour. As will be seen, however, they furnish an important link in the chain of evidence.

The third method is that of the experimental production of cancer. If this hypothesis that I have set out to test is correct, it may be possible to show that the bacteria isolated from human carcinomata have the power to produce tumours in lower animals. I have sought the

evidence of this method under no small difficulty. On account of the limited facilities at my disposal, I have been obliged to work only with mice. My experiments are still in progress, but already they have yielded many positive results.

A consideration that should, I think, be kept in mind in this connexion is that there must be a certain natural power of resistance to bacterial attack on the part of the epithelial cell, and that it may be only occasionally that this can be overcome. In the mouse, as in the human subject, there may be important factors that weaken the resistance of the epithelial cell to such attack.

I have varied my experiments in many ways, endeavouring to find a method by which it may be possible to produce tumours in a high percentage of animals. Simple hypodermic injection of the living cultures, injection into the mammary region, feeding, inoculation by scarification and genital tract infection have all been tried. Many of the experiments set up have not yet yielded their complete results. I should say that I have a large number of control animals, and that none of them have so far developed any tumour. The following are some of the results already obtained:

One of six mice inoculated by scarification in the ear with an anaerobic diphtheroid bacillus, isolated from a case of inoperable epithelioma under the care of Dr. Arthur Wilson, after about six months developed a large tumour at the junction of the neck and chest. It proved to be a rapidly growing carcinoma, probably arising from mammary gland tissue.

Four female mice were injected in the mammary region with a culture of an anaerobic diphtheroid bacillus isolated from a mammary carcinoma. Two of the animals were lost through the carelessness of a temporary custodian when I was on holiday. Of the two that remained, one died after five months; it showed no tumours. The remaining one developed great abdominal swelling, and was killed about ten months after the commencement of the experiment. It was found to have a large solid tumour of the pancreas. Both ovaries were also converted into large solid tumours, and there were secondary nodules in the liver, spleen, lung, and anterior mediastinum. All of these tumours have the same structure, that of rapidly proliferating epithelial cells without adenomatous arrangement.

Four female mice were injected in the mammary region with a culture of an anaerobic diphtheroid bacillus obtained from another carcinoma of the breast. No. 2 began to show abdominal swelling after nine months, and was killed two months later when in a moribund state. In the position of the left horn of the uterus there was a large abscess which had destroyed most of the tissues that might have previously occupied its site. There was a small tumour in the lung, and I suspected that the abscess had been superimposed upon a tumour of the ovary or uterus. Investigation of the tissues behind the abscess revealed the presence of adenomatous tissue, such as might have arisen from the epithelium of the uterus. The tumour of the lung has the same adenomatous structure. I think the evidence conclusively shows that this was a case of carcinoma of the left horn of the uterus that had become infected by a coliform bacillus. Mouse No. 1, after about twelve months, developed a very rapidly growing tumour of the neck similar to that already described in another mouse. The other two, which were killed and examined, showed no tumours.

Eight female mice were the subjects of the next experiment. Nos. 1 and 2 were injected in the mammary region with cultures of an anaerobic diphtheroid bacillus obtained from a carcinoma of the breast. No. 3 was similarly injected with a culture of a mannite non-fermenting staphylococcus. Nos. 4 to 8 were injected with both micro-organisms. After fourteen months nothing has happened to Nos. 1, 2, and 3. No. 6, after seven months, developed a rapidly growing tumour of the buttock and was killed about nine months after the experiment began. This tumour has the structure of an adeno-carcinoma. No. 4 was observed to be sickly after about twelve months, and was killed with chloroform. It was found to have a large tumour of the right kidney and a smaller one of the left. Both are rapidly growing epithelial tumours, with distinctly adenomatous structure. No. 8 was noticed to have a palpable tumour in the abdomen. This gradually increased in size and the animal was found dead about a

year after the experiment began. It had a tumour in the abdomen of the size and shape of a date stone, evidently taking origin from the pancreas. There was a secondary nodule in the spleen. Both tumours are composed of epithelial cells of the character of those that might be expected in a malignant tumour arising in the pancreas. The remaining two mice were noted for several weeks to be greatly swollen about the abdomen, and hard tumours were felt in both. The animals were recently killed, and both were found to have great enlargement of the uterus, with nodular swellings. In each case the structure is that of an adeno-carcinoma. The results of this experiment would suggest that the association of a particular species of anaerobic diphtheroid bacillus and of mannite non-fermenting staphylococci has some special potency in the production of tumours. It will be observed that all of five mice subjected to this method of experiment developed cancer.

The nipples of a mouse with suckling young were smeared with the centrifuge deposit from an anaerobic serum broth culture containing a diphtheroid bacillus of the kind that will grow only in an anaerobic fluid medium. This growth was derived from a carcinoma of the breast. The idea underlying the experiment was that the bacteria might reach the mammary gland through the lacteal ducts. No mammary cancer developed, but, after about nine months, the mouse was found to have a large fungating tumour over its sternum. This tumour has the structure of a squamous epithelioma.

Several other experiments have not yet had time to yield their final result. On April 6th six mice (three adults and three half-grown ones) were injected hypodermically in the back with a primary serum salt solution culture of an anaerobic diphtheroid bacillus obtained from a carcinoma of the breast. Two of the three young mice died from pneumonia within eight weeks, and cannot be reckoned in the experiment. Of the four that remained, two have already developed carcinomatous growths. Two months after the commencement of the experiment, mouse No. 2 was observed to have a small fungating tumour of the skin at or near the site of the injection. This tumour steadily enlarged. Five weeks later the animal was found to be in a moribund condition, and it was therefore killed under chloroform. The tumour of the skin has the structure of a squamous epithelioma. The illness on account of which I was obliged to terminate the experiment was clearly dependent upon a remarkable lesion of the kidneys, both of which organs were enlarged, granular on the surface, and extremely pale. On microscopical examination I found that, scattered among the tubules, there were numerous small masses of rapidly proliferating epithelial cells. That the lesion is of the nature of an early carcinomatous one is, in my opinion, certain, but whether the new growths are primary or secondary to the squamous epithelioma of the skin is not clear. Mouse No. 1 was killed under chloroform two months after the experiment began because of an abscess in the region of the ear, which probably had nothing to do with the inoculation. The right ovary was found to be enlarged to about ten times its normal size. It shows a tumour, composed of large epithelial cells, rapidly proliferating. One of four mice, inoculated by scarification in the back with a culture of an anaerobic diphtheroid bacillus isolated from a rodent ulcer excised by Mr. Miles, has developed a spreading ulcer at the seat of inoculation, which after ten weeks has attained the breadth of about a quarter of an inch and a length of half an inch.

The fourth method of investigation is that of focal reaction and therapeutic immunization. If cancer is associated with bacterial infection it is possible that the injection of an exact dose of the corresponding bacterial toxin will be followed by a focal reaction in the tumour, and that upon the occurrence of such focal reactions there may be based a successful method of therapeutic immunization. In regard to this method of investigation also there is a consideration that requires to be borne in mind—namely, that, if this hypothesis is correct, it may be taken as certain that it is not always the same bacterial species that is at work, and that, consequently, a stock vaccine may fail to produce a focal reaction, simply because it is not the right one for the case.

Notwithstanding this limitation of the possibilities of success, positive results have been obtained in several cases. I shall briefly relate some of the most striking

facts observed. Dr. Arthur Wilson, who was the first to observe focal reactions of the kind I am about to describe, has permitted me to make use of the two following observations.

A gentleman, about 50 years of age, suffered from an epitheliomatous growth within the meatus of the ear. It was operated upon, but soon afterwards recurred. A more extensive operation was undertaken, as the glands in the neck had become involved. Again recurrence took place, and further operation was considered out of the question. From the discharge from the ulcer I obtained an anaerobic diphtheroid bacillus of some vigour, and from this I prepared the vaccine that was used by Dr. Wilson. When treatment was begun there was a long scar, consequent upon the second operation, extending from behind the ear downwards. In the line of this scar several hard secondary growths had developed. In addition, underneath the angle of the jaw there were two large nodules, each the size of a walnut. Discharge continued from the meatus. With each dose of vaccine the patient had temporary aggravation of pain and increased discharge tinged with blood. With continuation of the injections the amount of pain he suffered gradually diminished. The nodules in the line of the scar disappeared. One of them broke down and supplicated. It was swabbed out with iodine, and healed absolutely by first intention. The two large nodules in the neck, in front of the scar, also disappeared. The prospects in this case seemed exceedingly bright, when unfortunately the patient developed erysipelas in the wound, and from this he died. In view of the accumulation of observations pointing to the infective origin of cancer it is of considerable interest to know a tragic sequel to this case. The patient's wife nursed him through his illness and daily dressed his wound. Up till nine months after his death she was apparently in good health. She then sought advice on account of symptoms that required a pelvic examination. It was found that she had cancer of both ovaries and large secondary growths involving the intestine. Her death occurred a little over a year after that of her husband.

Dr. Wilson's second case in which therapeutic immunization has been tried is that of a lady who was discovered to have a carcinoma of the rectum, which was regarded as inoperable. Colostomy was performed. Dr. Wilson informs me that he has now given ten injections. The vaccine used was one prepared from a serum salt solution culture from a scirrhous cancer of the breast. Each dose has been followed by a general and a focal reaction. The general reaction has manifested itself simply in malaise, lasting generally for about two days. It has been found impossible to increase the dose above 0.04 mg. A focal reaction has been evidenced by a sensation of fullness in the rectum and by increase of discharge of mucus which has been tinged with blood. The patient has now gained nearly a stone in weight. The most gratifying feature of the case is, however, the abatement of her sufferings, which were acute up to the time when therapeutic immunization was begun.

Mr. W. J. Stuart has kindly given me notes of observations made in three cases treated by vaccines. Two were carcinomata of the breast with extensive recurrence after operation. In both of these it was clearly observed that a vaccine dose was followed by increased tenderness of the affected glands. No distinct improvement was noted as the result of treatment. The third case was one of carcinoma of the rectum, in which colostomy had been performed. In this patient there was a distinct general reaction after each dose, manifested by feelings of malaise and chilliness. The patient also reported signs of a focal reaction consisting in "a greater feeling of rectal drag and more rectal discharge on the second day." His general health remains good, but the rectal tumour seems to be growing. At a discussion on this subject that recently took place in Edinburgh, Dr. A. Graham Ritchie described violent focal reactions he had observed in several cases of advanced carcinoma under his care.

Through the kindness of the physicians of the West House, Royal Edinburgh Asylum, I have had an opportunity of making an observation in a case of ulcerating carcinoma of the breast in a female dement. Last summer injections were given of from 0.1 mg. to 0.26 mg. of a vaccine prepared from an anaerobic diphtheroid bacillus obtained from a breast cancer. Four successive injections were each followed next day by severe congestion of the tumour, accompanied by profuse haemorrhagic discharge. The most severe reaction followed the fourth injection of 0.26 mg. It was distinct after twenty-four hours, but subsequently increased in severity, lasting four days and terminating with severe haemorrhagic effusion from the ulcer. In all, ten injections were given. During this time the tumour and the ulcer diminished in size. The patient has gained weight, and though senile, remains in fairly good physical health. The tumour has ceased to grow.

In a case of uterine cancer, under the care of Dr. Robert Robertson, a vaccine prepared from one of the more vigorous strains was also used. The injections began

with 0.02 mg., and the dose was gradually increased to 0.08 mg. Haemorrhage had never been a symptom in this case. Each of the first four injections was followed next day by profuse haemorrhage from the tumour. The patient seemed to improve. The fifth injection was, however, followed, not by haemorrhage, but by severe malaise, which continued for many days. The injections were stopped and the patient was sent to Longmore Hospital for Incurables. What became of her I do not know. The interest of the case lies chiefly in the distinct focal reactions following the injections.

Two cases observed by Dr. Alexander Dey of Wooler are also of interest. He used a vaccine prepared from a primary serum broth culture of an anaerobic diphtheroid bacillus obtained from a case of carcinoma of the breast. The strength was only 1 c.cm. = 0.1 mg. The following is Dr. Dey's report on the first case:

A woman of 50, whose breast had been excised, and who, eighteen months later, had recurrence in the cicatrix and in the armpit, was feeling so generally ill that she spent most of her time in bed. After she had had three or four weekly doses she got such a general feeling of well-being and strength that she was able to be about with comfort. She always had, about thirty-six hours after the dose, what she called feverishness and discomfort, but after that, for the rest of the week, she was wonderful. The swelling in the armpit ceased to grow and the cicatrices looked healthier. I attempted to increase the dose slightly, but the reaction was so pronounced that I had to go back to the original dose (0.2 c.cm.), and on the last two occasions she had such severe headache and throbbing in her head that she became so alarmed that she would not let me give her any more. She admits, however, that she feels so much better that she knows the vaccine has done her good. So far the growth is certainly arrested. . . . The case, I consider, is highly encouraging on account of the definite arrest of the growth and the feeling of well-being the patient got after the reaction stage had passed. It was peculiar that the reactions got severer with repeated injections.

I have heard that this case afterwards relapsed, and terminated in the usual way. The sudden or gradual increase of sensitiveness to the vaccine in this case and the preceding one are remarkable features which it may be difficult to explain. I have occasionally observed a similar occurrence in the course of other forms of therapeutic immunization. In these cases it can be satisfactorily met by simply diminishing the dose to about one-tenth, and it is probable that, when it occurs in cases of carcinoma, the same remedy will be found to be applicable. Dr. Dey's second case is more encouraging. He reported to me on February 24th last as follows:

The case was that of a working man who had been through the war, about 34 years of age, whom I sent to Mr. G. Grey Turner of Newcastle, suffering from what I considered cancer of the rectum, in the autumn of 1919. Mr. Turner operated and verified the diagnosis microscopically. Last May I sent him back to Mr. Turner, who found what he thought a recurrence, and sent him back to me to watch for any secondary growth before doing a colotomy. After I got the vaccine, I thought he was a good case, and have given him weekly doses till January, having increased the dose very slightly. In January I sent him to Mr. Turner, who examined him very carefully, and the recurrence which he found in May had disappeared, leaving a small nodule which Mr. Turner considered to be cicatricial tissue. . . .

The last case I wish to refer to is that of a large rodent ulcer in a female dement at the Royal Edinburgh Asylum. This patient has recently had six injections of a vaccine prepared from the anaerobic diphtheroid bacillus obtained from the rodent ulcer excised by Mr. Miles. To each of the first four injections she reacted in the tumour with congestion and increase of discharge, and after all except the first injection (which was probably too small) there was haemorrhage from the whole circle of the edge of the growth. The ulcer has now assumed a healthier appearance than it has ever been observed to have before. The formation of crusts has entirely ceased.

The results obtained by pursuing these four separate lines of investigation warrant, I think, the statement that there is strong evidence in support of the hypothesis that cancer is dependent upon cell infection by anaerobic bacteria. If this hypothesis can be accepted, a number of important interpretations of phenomena observed become possible, and with a word or two about these I will conclude.

There would appear to be a race of anaerobic bacteria comprising several species belonging to the large diphtheroid group or to the closely allied streptothrix group, which are capable of infecting epithelial cells and main-

taining within them a parasitic existence. Many facts point to the conclusion that we have to deal with a group of the higher bacteria that appear in several forms. There are some rounded or oval bodies that may occasionally be seen in the cultures which cannot be called either bacilli or micrococci, but which are not incompatible with the known polymorphism of some of the higher bacteria, especially the streptothrices. The interpretation of these bodies, already seen and described by several observers, will probably become clear as the result of further cultural and experimental work.

It is probable that bacteria of other groups are capable of acting in the same way. If this is so, cancer may have to be regarded as dependent upon a special mode of bacterial attack, rather than upon a specific infection. The effect upon the epithelial cell is to stimulate it to proliferate, and the result is a tumour varying in character in accordance with such factors as the type of epithelial cell, the bacterial species, and the reactive qualities of the host.

There are important conditions that render the epithelial cells more vulnerable and permit the initial invasion. Exposure to infection must be very common. Successful attack on the part of the bacterium must be comparatively rare; it is probably rendered possible only by the operation of some cause that weakens the resisting power of the cell. Long-continued proliferation of epithelial cells from any cause must render them more easily invaded by bacteria of the kind I have described. Many well known clinical facts are in harmony with this view.

Tumours that have developed to any considerable size are not uniformly infected; there remain only foci of infection, and the difficulty of obtaining successful cultures is increased by this circumstance. Invasion of the cell would appear to be effected, as a rule, by bacteria of comparatively vigorous growth and easy to cultivate, but when these bacteria adapt themselves to purely parasitic intracellular growth they become comparatively feeble and extremely difficult to grow in culture media. They cease to be capable of growth on any surface medium yet tried, but they can generally be cultivated in an anaerobic serum diluted with normal salt solution. Tumours can be produced in mice by both the vigorous and the feebly-growing strains. Focal reactions can be obtained in some cases, success probably depending upon the vaccine having been prepared from the same species as that in the case. The possible diagnostic value of such reactions is a question for future investigation. The prospect of being able to exercise some control over the growth of cancer by means of therapeutic immunization is distinctly good.

My attention has recently been drawn to the work of Loeb upon the influence of the endocrine glands on the growth of cancer in mice. It seems to me, however, that his results tell us nothing regarding the cause of cancer, although they may prove of much importance for its treatment.

DISCUSSION.

Dr. JAMES A. MURRAY (Director, Imperial Cancer Research Fund, London) said that he would state the objections that occurred to him against the argument that Dr. Ford Robertson's diphtheroid bacillus was the cause of cancer. In the first place, mammary tumours in mice were common. It was interesting that none had occurred in Dr. Robertson's controls, but that did not exclude the possibility of their spontaneous development in the inoculated animals. It was well known, however, that any form of chronic irritation might give rise to cancer, and Dr. Murray was quite prepared to admit a non-specific relation between tumours and bacteria. A point against the specificity of Dr. Robertson's organism was the very great variability in the length of the incubation period in the experiments—that is, from a few weeks up to eighteen months.

He had seen the bacteria-like structures in the tumour cells when they were demonstrated at Oxford fourteen years ago; he held then, as he still did, that they were not bacteria. There were so many intracellular structures, such as mitochondria, which could be mistaken for organisms, and under pathological conditions the intracellular appearances might alter considerably. No evidence had been forthcoming to prove that the rod-like structures were bacteria. Dr. Murray mistrusted metallic impregnation methods for the demonstration of special structures,

FIG. 1.—Rod-shaped micro-organisms in serum salt solution culture from a cancer of the breast. Ziehl-Neelsen staining. ($\times 1,000$).

FIG. 2.—Mouse showing tumour of buttock, seven months after subcutaneous injection with anaerobic diphtheroid bacillus isolated from a cancer of the breast.

FIG. 3.—Section of tumour shown in Fig. 2.

FIG. 4.—Spreading ulcer on back of mouse, eight weeks after inoculation (by scarification) with culture of an anaerobic diphtheroid bacillus isolated from a rodent ulcer.

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FIG. 1.—Section from the liver of a monkey the subject of an experimental infection, showing a patch of endophlebitis in one of the branches of the portal vein.

FIG. 2.—Section of the lung of a monkey the subject of an experimental infection, to show the almost complete obliteration of one of the branches of the pulmonary artery in the neighbourhood of one of the haemorrhages.

FIG. 3.—Section of the spleen from a monkey the subject of an experimental infection, to show the great increase in the number and size of the trabeculae and a vein (a) showing endophlebitis.

FIG. 4.—Section of the spleen from a normal monkey to compare with Fig. 3.

They were very uncertain in their action. One never knew whether the whole or only part of a structure would be impregnated. One part of a section might stain beautifully, while similar tissue near by might be only partly coloured or not at all. Much concerning the minute structure of cells was still obscure. A combination of morphology and staining reactions was needed in deciding histological questions. Another point against Dr. Robertson's theory was that there was no constant relation between the site of inoculation and the site of the tumour. Scarification of the ear did not, for instance, explain the occurrence of a tumour in the mammary gland or uterus. Dr. Murray thought that the failure of the site of the tumour to correspond in all cases with the site of inoculation, together with the variability in the incubation periods, was strong evidence that Dr. Ford Robertson's bacillus was not a specific cause of cancer.

Mr. McADAM ECCLES (Surgeon, St. Bartholomew's Hospital, London) said that Dr. Ford Robertson's paper was of the greatest importance, and was another link in the chain of evidence of the infective origin of carcinoma. As an operating surgeon frequently dealing with carcinomata, such scientific investigation of the cause of carcinoma was of particular and practical importance. All the material investigated appeared to have been from a neoplasm which might have been infected from the surface, the mamma through the ducts in the nipple, the rodent ulcer, etc. If similar results were to be obtained from a carcinoma which had arisen in a "buried" organ, say a testis, it would be a very striking fact.

The infection of and subsequent development of an epithelial growth in so many mice was of the greatest value. He ventured to throw out the suggestion that because mice were the animals most easily infected with later growths, might they not be a possible source of the bacteria which infect the human, seeing also that they were with us everywhere, and did not hesitate to run over and help themselves to our food? Had any of the tumours arising "spontaneously" in the mammae of mice shown any of these distinctive bacterial-like structures?

Dr. FORD ROBERTSON, in reply, said he desired to acknowledge the kindness with which Dr. Murray had criticized views that were completely at variance with those advocated by him for many years. The evidence he had put before them, and that of the twenty-one microscopical preparations laid out in another room, remained, however, untouched by anything that had been asserted.

THE PATHOLOGY OF GASTRIC AND DUODENAL ULCER.*

BY

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[With Special Plate.]

ONE aspect of the production of gastric ulcer has recently come before me in doing experimental work with an organism mildly pathogenic for monkeys. The organism, a streptothrix of the species *actinomyces*, was obtained from a case of acholuric jaundice, and has been described in the *Journal of Pathology and Bacteriology*, Edinburgh, 1920, xxiii, 357. If a culture in broth be injected intraperitoneally into a monkey (preferably not rhesus) the animal suffers no inconvenience for a month or two; subsequently, however, it becomes less active, tends to sit crouched in a corner, and gradually fails in health during a period which may be as much as a year. There is not, so far as my observations go, any anaemia, but the animal is certainly paler in the late stages, may be puffy under the eyelids. The appetite is preserved throughout. Rigors are sometimes seen. There is usually some tenderness elicited over the spleen, and this may be enlarged and tender.

* A paper read in the Section of Pathology and Bacteriology at the Annual Meeting of the British Medical Association held in Newcastle-on-Tyne, July, 1921.

One of these monkeys, a male callithrix of moderate size, was injected on three occasions intraperitoneally with a broth culture of the streptothrix. The animal preserved its condition and kept up its weight for seven months, after which it quickly failed, and was killed thirty-two weeks (224 days) after the first injection. During the period of observation the animal was more apathetic than the controls, and for the last four months sat crouched in one corner of the cage. On palpation of the abdomen tenderness was first noticed six weeks after the first injection, and, except on one occasion, could always be elicited, more usually over the spleen, sometimes over the liver, and once or twice elsewhere. The spleen was felt throughout, but could never be said to be enlarged.

Blood counts, taken several times during the experiment, showed a gradual increase in the red cells and a slight increase in haemoglobin, not in excess of the control animals. An increase in the mononuclear count was also shown in this and the controls. The fragility was not raised. The necropsy, made immediately after death, showed no peritoneal abnormality. The spleen was extremely small and shrunken. On opening the gut the contents were black and pultaceous, mostly in the caecum and first part of the colon. There were several circular ulcers of the stomach with sharp borders not confined to any area, measuring up to nearly a centimetre across, showing blood altered by gastric juice. The black substance in the colon gave the reaction for occult faecal blood. The small intestine showed several hyperaemic and haemorrhagic patches, and a similar patch on the point of ulceration was found in the appendix. There was ulceration of the first part of the ascending colon. The spleen was in a marked condition of fibrosis with thrombotic changes in some of the veins. The liver was very pale and fatty, and showed towards the edge in one part two small areas suggesting contracting infarcts, and on the under surface were two shrunken, slightly haemorrhagic patches suggesting an area of ischaemic atrophy. On section the liver showed general fatty change and much bile staining near the bile passages. The lungs showed numerous haemorrhagic areas, and small haemorrhagic areas were also to be seen in the mesentery. Cultures made from the spleen, liver, and lung, heart blood and pericardial fluid, were negative. Shortly, this animal showed a chronically inflamed and fibrotic spleen with thrombo-phlebitis, multiple gastric ulcers, and haemorrhagic embolic lesions of the liver and lungs.

The venous blood from the spleen may return to the portal vein by two channels, either by the splenic vein or by the vasa brevia of the stomach wall; this latter channel is probably the normal one for a large amount of splenic blood. In fact, Mayo quotes 30 per cent. as the proportion of splenic blood that returns in this way (W. J. Mayo, *Contributions to Medical and Biological Research*, dedicated to Sir William Osler, ii, 991). The gastric veins must therefore be freely open to any substances, embolic or not, that may emerge from the spleen. If emboli are infective, it is not difficult to imagine that some may attach themselves to the wall of the vessel and produce a weakening and haemorrhage from it.

Acholuric jaundice is a condition that usually gives rise to no gastric symptoms beyond vomiting, and I have only been able, so far, to find one reported case that suffered from haematemesis (Box, *Proc. Roy. Soc. Med.*, Clin. Sect., 1913, vi, 8); but acholuric jaundice is very nearly related to splenic anaemia, and both are undoubtedly due to an infective agent whose site is the spleen. Why in the one type we should see progressive inflammation of the spleen with anaemia and jaundice, while in the other there is anaemia and haematemesis is not known. Be that as it may, the facts are that from an inflammatory lesion in the spleen both types follow, for splenectomy in the earlier stage cures both diseases. In the clinical group of cases known as splenic anaemia there is an enlarged inflamed spleen and a tendency to periodic gastric haemorrhage which may be so great as to be fatal. The origin of the bleeding is sometimes from dilated oesophageal varices, sometimes from lesions in the stomach which are described as erosions; such lesions, however, give rise to such blanching haemorrhages that we must presuppose a leakage from one of the large vessels of the stomach wall. It is curious that clinically such haemorrhages are seldom followed by the symptoms that we have come to look upon as characteristic of gastric ulcer, and must be related to