First annual report of the Cancer Committee to the Surgical Department of the Harvard Medical School, October, 1900.

FIRST ANNUAL REPORT

OF THE

CANCER COMMITTEE

TO THE SURGICAL DEPARTMENT

OF THE

HARVARD MEDICAL SCHOOL

OCTOBER, 1900

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CONTENTS.

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INTRODUCTION.

J. COLLINS WARREN.

Through the public-spirited and far-sighted generosity of the late Caroline Brewer Croft, the Surgical Department of the Harvard Medical School has been enabled to undertake a systematic investigation into the origin of cancer.

The plans for this research were formulated for the first time one year ago, and a body of investigators was organized by the committee of the corporation having the matter in charge. The members of this commission have worked diligently during the past year on some of the preliminary problems connected with such an investigation, and it has been thought best at the present time to present them in the form of an annual report to the Department of Surgery. In a review of the recent investigations on the nature of cancer,[1] the head of the department has endeavored to show that the disease has been steadily on the increase during the last fifty years, a period during which the careful preparation of vital statistics has made it possible to study such a question with tolerable accuracy.

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[1] Boston Medical and Surgical Journal, July 12, 1900.

Thanks to the generous aid of numerous friends who appreciate the importance of work of this character, and to the resources of a great university, we are enabled to plan these studies on a scale which it is hoped may constitute something of value towards the solution of so difficult a problem, and may stimulate others to take a renewed interest in the subject.

The work of the various men forming the commission and presented in this report speaks for itself. The Surgical Department acknowledges, with much appreciation, the assistance of the State Board of Health, for whom Dr. W. F. Whitney is at present studying the geographical distribution of cancer throughout the State of Massachusetts. It is also proper to state in this connection that Dr. J. D. Weis has been appointed to the Austin Fellowship in Surgical Pathology, and is at present in Europe, with a view of studying the nature of the blastomycetes, an organism whose name has been so much associated recently with the studies on this disease.

The presentation of this report emphasizes the policy of this department in devoting its energies, not only to the teaching of surgery, but to original research in some of the many inviting fields which are offered to well-trained and well-organized bands of scientific investigators.

STATISTICS OF CANCER.

W. F. WHITNEY.

The gross statistics have shown everywhere an increase of cancer. Those for Massachusetts have been carefully analyzed in various ways.

First, the death rate based upon the total number of deaths and the total population has been made. Second, the rate for each age period (decade) above thirty has been made. Below that age there are probably very few cases of cancer. Third, the ratio of deaths from cancer to the total number of deaths above thirty and for each age period, also to deaths other than from acute infectious diseases. In whatever way the subject was studied there was found a marked increase in the death rate from this disease.

The Massachusetts returns were also compared with those of the other New England States and Great Britain. There was a remarkable uniformity in the curves made from the report of the British Registrar General and those from Massachusetts.

The analysis of the internal forms of cancer, as far as they go, does not show any greater rate of increase than the external forms. This would tend to disprove the theory that the rate of increase is due entirely to better diagnosis, and, furthermore, it would seem that better diagnosis would have eliminated various syphilitic and tuberculous processes which formerly might have been returned as cancer.

As to locality, there does not seem to be any one portion of the State in which the disease is particularly prevalent. The studies in this regard, however, have not been completed.

I am not prepared, at present, to express any reason for this increase.

FIRST ANNUAL REPORT OF WORK ON THE ETIOLOGY OF CANCER.

EDWARD H. NICHOLS.

(*From the Sears Pathological Laboratory of the Harvara Medical School.*)

The following is the first annual report to the Trustees of the Croft Fund, under the conditions of which the work upon the etiology of cancer was begun at the Harvard Medical School. Since the work is in its preliminary stage and no certain results have been obtained, no attempt has been made to draw definite conclusions from the work of this year.

A brief summary is given of the general reasons for and against the theory that cancer is due to the action of a parasite. A short review of the writings of some of the leading workers upon the subject is made, and the results of my own work are stated. A special report by each worker of this commission upon his part of the subject will follow.

General Reasons Against the Parasitic Theory.

Within recent years many men have claimed that cancer is due to the action of a parasite. When the study of the subject was begun, a year ago, especial attention was given to examination of the claims of these men.

There are certain general reasons which make it unlikely that the cause of cancer is a parasite. Cancerous tumors are composed chiefly of masses of epithelial cells. If these tumors are due to the action of a parasite, the parasite naturally must be one which causes a proliferation of epithelial cells. Most parasites and irritants produce a proliferation of connective tissue only.

Moreover, secondary or metastatic areas of cancer commonly develop in patients suffering from cancer, and may appear in organs very distant from the original focus. The metastases may appear either in tissues in which no epithelium normally is present, or in organs which have a characteristic epithelium of their own. In either case the

metastases reproduce an epithelial growth of the same type as that of the original tumor. This indicates that the secondary nodules arise from masses of epithelial cells transferred from the original tumor by means of the lymphatic or blood vessels. Since the epithelial organs in which the metastases appear have a characteristic epithelium of their own, the secondary nodules in such organs, if the proliferation were due to the action of a parasite, should consist of epithelium analogous to the epithelium of the organ in which the metastases occur.

Finally, attempts to produce cancer in animals with material from human cancer have been failures.

General Reasons in Favor of the Parasitic Theory.

On the other hand, there are certain general reasons which seem to favor the theory that cancer is due to the action of a parasite.

There is a general impression that there is a relative increase in the frequency of cancer; that is, it appears as if there were epidemics of cancer. It is difficult to see why there should be such an increase, if the etiological factor was a constant one. Certain regions also are said to have a larger portion of cancer than others, and cancer is said to attack a series of residents in certain houses.

Also, cancer extends from the original site of the disease to distant parts of the body. This extension follows the line of the lymphatics or blood vessels. In this respect the formation of metastases in cancer resembles the metastases which occur in diseases known to be due to the action of bacteria.

Finally, patients suffering from cancer often develop a general cachexia which may be entirely out of proportion to the extent of the disease. This suggests the formation of some general toxic substance, which might be due to the action of some parasite.

Special Reasons in Favor of the Parasitic Theory.

Besides these general reasons for believing that cancer is due to the action of a parasite, there are certain special reasons which have led some men to this belief.

In many cancers in the protoplasm of the epithelial cells are found certain remarkable spherical bodies which have a definite structure and peculiar staining reaction, which are believed to resemble certain unicellular organisms (protozoa, blastomycetes) which under certain conditions are known to produce definite lesions in animal or human tissues. Because of this resemblance of these bodies and because of the constant presence, as they claim, of these bodies in the cells of cancer, it is believed that they cause the proliferation of epithelial cells which results in the formation of cancer. The same belief also is held by some men who have worked along experimental lines.

The men who believe that cancer is due to a parasite may be divided into two classes:

First, those who from morphological reasons alone believe that the cancer bodies are parasites.

Second, those who have obtained from fruits or human tumors an organism which morphologically is similar to the cell inclusions of cancer, and which when inoculated into animals produces a growth analogous to human cancer.

Morphological Basis.

In 1889 Thoma (1) described certain small unicellular bodies which were frequently seen in the *nuclei* of epithelial cells of cancer. The bodies were very refractile, and had protoplasm and nucleus, sometimes a nucleolus. From their size, shape, and composition he believed them to be parasites, probably coccidia. No experimental evidence of their parasitic nature was given, and there were no drawings to show the exact morphology.

In 1890 Russell (2) described certain bodies which he had found in forty-three out of forty-five cases of cancer. The bodies were spherical, homogeneous, or hyaline, with a

refractile capsule. Russell used fuchsin as a differential stain for the bodies, which consequently have been called Russell's "fuchsin bodies." Russell claimed that the bodies were parasites, reproduced by budding, and were the cause of cancer. He acknowledged, however, that he had found these bodies in other lesions than cancer. He offered no experimental evidence in favor of his claims.

In 1891 Ruffer and Walker (3) described bodies which occurred in the protoplasm of cancer cells, occurring as spheres, having a nucleus, protoplasm, and capsule. The nucleus did not stain like the nucleus of the cell, the protoplasm was homogeneous, mottled, radiate, or granular, the capsule often was double. The cells which contained these bodies might be normal or degenerated, but never were undergoing mitosis. They believed the bodies to be protozoa.

In 1892 Ruffer and Plimmer (4) continued the work begun by Ruffer and Walker. They at first examined various types of cancer, but finally confined their examinations to cancers of the breast, because these tumors were easily manipulated and contained cell inclusions in relatively large numbers. They described bodies similar to the bodies described by Ruffer and Walker, and claimed to find them most frequently in the advancing edge of the tumor and not at all in the degenerated portions. They said that the bodies were most numerous in tumors characterized clinically by a rapid growth. The cells in which the bodies lay did not show mitosis, but the adjacent cells did. They found so-called young forms of the bodies in the nuclei of cells, from which they escaped into the cell protoplasm. In the so-called adult forms the bodies had a nucleus, protoplasm, and double contoured capsule. They believed that the body reproduced by budding or by elongation of the nucleus and fission. They gave their technic in detail and added excellent plates. These plates showed great variation in the morphology of the body. They performed no experiments to confirm their views.

In 1892 Sawtschenko (5) described various bodies in the

protoplasm of cancer cells. The bodies varied in size, form, and staining reaction. He believed the bodies to be sporozoa, and claimed that, although there were different forms of sporozoa in the same tumor, these different forms represented different stages of development of the same organism. He described a remarkable series of steps in the development of the organism. His organism as shown by the drawings does not correspond to that described by the other writers. He offered no experimental evidence in favor of his claims.

In 1893 J. J. Clarke (6) read a paper in which he claimed that psorosperms were the cause of cancer, and also claimed to find them in sarcoma. According to Clarke, two-thirds of the mass of a round-cell sarcoma was due to parasites. He also claimed to see amœboid movements of the parasites. Later he showed sections of an early scirrhous cancer containing organisms which showed amœboid movements. A committee appointed by the Pathological Society of London did not confirm his claims.

Various other writers — Foa, Soudakewitch, Vedeler, and others — have worked on similar morphological lines, and believe that the cell inclusions in cancer are parasitic and causative, but have not confirmed their opinions by isolating an organism which produces similar lesions in animals.

In reviewing the work of these writers, the different methods of technic employed make it difficult to get an exact comparison. It is notable, however, first, that the morphology of the cell inclusions is represented to vary within very wide limits by individual writers, so that one must suppose either that there are different types of organisms present, that the organism is extremely pleomorphic, or that the writers have been unable to differentiate between the organism they describe and other forms of cell inclusions, perhaps due to various forms of cell degeneration.

Second, the morphology of the cell inclusions or parasites as described by different authors does not in the least coincide, so that one must assume either that various forms of organism may produce similar results, or that the writers describe entirely different structures.

Third, different authors draw entirely different conclusions as to the location in the animal kingdom of the bodies which they describe as parasites.

Fourth, it is evident that unless it is possible to determine the nature of the parasites, and unless the bodies correspond to some similar organism whose method of reproduction is known, any claims as to the manner of reproduction of the organism must be based upon uncertain facts and cannot be determined.

Experimental Basis.

Another series of observers have seen bodies in malignant tumors similar to those already described, and, having isolated analogous bodies from fruits or from tumors, have succeeded in cultivating them upon artificial media and have inoculated them into animals with various results.

Busse (19) saw in the cells of a soft sarcoma of the tibia bodies which on fresh examination showed a clear centre and a double contoured membrane. He cultivated these bodies upon artificial culture media, best on potato, and inoculated them into the bones of a rabbit. He produced apparently inflammatory processes, but in the pus found bodies similar to those seen in the sarcoma tissue. He also produced an adhesive peritonitis when the organisms were inoculated into the peritoneum of a rabbit. In cultures the organism did not show its original double-contoured form, but the original form reappeared when inoculated into animals.

Sanfelice (7) believed that the bodies in cancer cells were blastomycetes because of their morphological resemblance. He obtained a pure culture of a blastomyces, "saccharomyces neoformans," from the juices of fruits and cultivated it upon vegetable media. He believed that the organism in the fruit juice came "from the air." He inoculated this organism into various tissues of various animals.

Guinea pigs (8) inoculated in the subcutaneous tissue died in from twenty to thirty days, and showed a nodule of "sarcoma-like" tissue at the point of inoculation with enlarged lymph nodes and white nodules in the kidney, liver, and

spleen. If the inoculation was in the peritoneal cavity a neoplastic peritonitis with enlarged mesenteric lymph nodes was produced. The lesions consisted of proliferated connective tissue. The blastomycetes were present in the lesions in enormous numbers, lying chiefly in lymph spaces. The morphology of the blastomycetes in the tissues was unlike that of the blastomycetes in pure culture, but did resemble that of the cell inclusions of cancer, and, Sanfelice claims, they have the same staining reaction.

In mice (12) death occurred in about eight days, with a general saccharomycosis and scattered nodules of proliferated connective tissue with many lymphoid elements. The blastomycetes generally were enmeshed in the tissues and not included in the protoplasm of the cells.

Rabbits (12) were more resistant. Some died in from thirty to forty-five days and showed nodules in the spleen, omentum, and kidney. The nodules showed a greater proliferation of connective tissue than occurred in guinea pigs.

He also inoculated many dogs. In some no lesion was produced, but in three cases he claimed to get an actual tumor production. His "positive" cases are three in number.

The first "positive" case (7) was a bitch which was inoculated in the breast and died after two months. The animal showed a nodule of new tissue at the point of inoculation, "like sarcoma." Inguinal lymph nodes were almost entirely replaced by similar tissue and there were similar nodules in kidney and spleen. Heart, lungs, brain, and cord showed nothing. In this tumor he saw blastomycetes sometimes in the protoplasm of the cells, sometimes free. He was unable to regain the organism on culture.

The second "positive" case (13) was a bitch inoculated in the breast with the same blastomyces which had been passed through a series of dogs. Directly after the inoculation the breast swelled, but the swelling disappeared in a few days. One month later a swelling appeared at the point of inoculation, which gradually increased, and the inguinal lymph nodes enlarged. The animal died after ten months.

At the autopsy a tumor, "adeno-carcinoma," was found affecting both of the posterior mammary glands, with similar tissue in the lymph nodes. He saw bodies which he believed to be blastomycetes, sometimes in the epithelial cells of the new glands, oftentimes free in the tissues. His descriptions and drawings show that his so-called blastomyces in this tumor does not correspond morphologically with the blastomyces as it appeared in the granulation tissue produced in other animals; the capsule, for instance, was entirely wanting. Sanfelice believes that this lack of correspondence is due to the fact that after the blastomycetes have remained in the tissues long enough they alter their shape and take a form which resembles the structures described by Russell as his fuchsin bodies. Attempts to obtain the organism by cultures on artificial media were absolutely unsuccessful. Sanfelice says that this is due to the fact that after the organism has assumed the characteristic form of Russell's bodies they will not grow upon artificial media.

In the third "positive" case (13) a dog was inoculated with the same organism in both testes. After a few weeks a tumor appeared, which involved the glans and showed a creamy discharge from beneath the prepuce. In this discharge were numerous organisms resembling Russell's bodies, but they could not be cultivated upon artificial media. The animal died after six months and at autopsy showed a tumor entirely replacing the testes, with nodules of similar tissue extending beneath the skin and involving the glans. There were large inguinal lymph nodes. Histological examination showed that the tumor was "adeno-carcinoma." There was no evidence of metastases in the internal organs or in the lymph nodes. Bodies were seen in the tumor similar to Russell's fuchsin bodies, most of them lying free, and very few in the epithelial protoplasm. Attempts to isolate the organism upon artificial culture media were absolutely unsuccessful. Animals were inoculated with an emulsion of the tumor tissue and with pus from the discharge, but as yet no positive results have been obtained.

Sanfelice inoculated other dogs (13) in the jugular veins

with the same organism. The animals became emaciated in two weeks and ultimately died. There were nodules in the kidney, and enlarged spleen and lymph nodes. The blastomycetes could be recovered on culture. The nodules in the kidney were "mesoblastic" and tended to involve the surrounding tissues, so Sanfelice says they cannot be spoken of as inflammatory, as they "do not resemble the granulomata of tuberculosis, glanders, or actinomycosis."

If dogs are inoculated in the subcutaneous tissue (13) the blastomycetes frequently produce nodules composed of proliferated connective tissue.

In cats (13) the organism produces an abscess or proliferation of connective tissue if inoculated in the subcutaneous tissue. If inoculated in the veins it produces a general infection, as in dogs.

Sanfelice also examined a primary cancer of the liver from an ox (11) and saw in fresh sections bodies like his blastomyces. He made cultures and isolated a blastomyces resembling the organism he had obtained from fruit juices, and he inoculated this organism, "saccharomyces litogenes," into animals. This organism has slight cultural differences from those of the neoformans. The inoculated animals showed nodules composed of young connective tissue, in the meshes of which were numerous blastomycetes.

Sanfelice believes that the parasite in cancer is due to blastomycetes and not to coccidia (12), because, although some stages of coccidia correspond morphologically to the form of the cancer bodies, other stages do not so correspond, whereas all stages of the blastomycetes do correspond morphologically to the form of the bodies seen in cancer.

Sanfelice also succeeded in isolating pure cultures of blastomycetes from human tumors and from tumors of cattle and swine (13). The blastomyces thus isolated, however, did not have pathogenic action.[1] Sanfelice states that the

[1] Wenn man nun auf solchen Platten zahlreiche Colonieen von Blastomycetes findet, so kann man wohl sicher sein, dass diese von den Geschwülsten und nicht etwa aus der Luft herruhren, denn in den 5 oder 6 Stunden, während welchen die Platten der Luft ausgesetzt waren, konnten sicher nicht so zahlreiche Blastomycetes auf die Platte gelangt sein.

blastomyces so obtained come from the tumors and not "from the air." He believes that the reason blastomycetes thus isolated produce no result in animals is that the organism is acclimated to human tissues and finds the conditions in animal tissues different, and cannot produce lesions in animals until after the organism has been passed through a series of animals and become accustomed to the new conditions.

He also claims (13) that the reason he is unable to obtain cultures of the blastomyces from his experimental cancers is that the organism, after long residence in the tissues, alters its form, so that morphologically it resembles Russell's fuchsin bodies, and in this stage is incapable of being cultivated upon artificial media.

Plimmer (14), of London, of late has worked along lines similar to Sanfelice's, and has obtained results which approximately correspond. He describes bodies which occur in cancer, usually in the cell protoplasm, rarely free, which have a nucleus, protoplasm, and capsule. He has devised a differential stain for these bodies. He claims that the bodies in cancer can be seen in process of division, by budding, fission, or segmentation. He believes that these bodies are parasites because they do not react like any known degeneration, because they are not in the degenerated parts of the tumor, but in the most actively growing parts, because they are not found in normal or inflammatory tissue, and finally because they can be isolated and grown outside of the body. He has found these bodies in 1,130 out of 1,270 cases of cancer examined. In a few cases, characterized clinically by very rapid growth, he found them in enormous numbers, and he has been unable to find them in other tissues.

His attempts to reproduce cancer in animals by inoculating them with bits of tissue from human cancer have been unsuccessful.

He attempted to isolate the organisms by cultures made upon various media, and finally succeeded in his attempts by using special media and growing the organism under anærobic conditions. He believes that the isolated organism

corresponds morphologically to the bodies included in cancer cells.

He inoculated animals with this organism. Some of the animals gave no results; others showed the bodies in the tissue without any reaction. Guinea pigs inoculated in the peritoneum showed diffuse lesions in the peritoneum, omentum, and internal organs. Histologically these growths were composed of "endothelial" tissue, and Plimmer characterizes them as "tumors."

Sanfelice and Plimmer both believe that cancer is due to a parasite. Both have isolated an organism, probably a saccharomyces or blastomyces, which they claim is identical morphologically with the cell inclusions in cancer. Sanfelice obtained his organism from the juices of plants and Plimmer obtained his from a human cancer. Both have inoculated animals and have shown that the organism can live and reproduce in the living tissues. They also show that the organism may extend along the lymphatic channels, or less readily by the blood vessels, may lodge in various organs, and produce a proliferation of tissue, — practically always, however, except for two cases of Sanfelice's, a proliferation of connective tissue cells. This proliferation may be so extensive as to lead to the formation of masses or nodules of considerable size, which they call "tumors," but they do not demonstrate the histological identity of these tumors with either sarcoma or cancer. Of Sanfelice's so-called "positive" cases, the first one may be excluded, because his own description of histological appearances does not prove its identity with sarcoma, but makes it probable that he had to do with proliferated connective tissue. The two cases described in the fifth part of his series of publications upon the "Pathological Action of Blastomycetes" (13) deserve more consideration. In one case, several months after the inoculation of a bitch in the breast, a tumor developed with metastases in the lymph nodes. This case is very striking. But it must be remembered that Sanfelice had inoculated a large number of dogs, 59, at the time his article was written, without producing any result beyond the proliferation of connective

tissue. Cancer is a common disease in dogs; *e.g.*, Fröhner states that of 643 tumors of dogs operated upon at the Berliner Thierärtzlichen Hochschüle during the interval between 1886 and 1894, 262, or 40 per cent., were cancerous. It is possible that the appearance of the cancer of the breast, after the inoculation of the blastomyces, was a coincidence and not the result of the inoculation. Sanfelice's failure to obtain the blastomyces by cultural methods, and the fact that he says that the morphology of the organisms in the tumor was unlike the usual appearance of the blastomyces in the tissues, make one very suspicious that such was the case.

The third positive case, where cancer of the genitalia followed inoculation of the testicle, also failed to produce cultivatable organisms and Sanfelice again says that the morphology of the organism was unlike that commonly assumed by the blastomyces in the tissues. His evidence that blastomycetes were the cause of the tumor does not conform to Koch's rule — that to demonstrate the pathogenic action of any organism, that organism must constantly be present in the diseased tissues, and so distributed as to produce the results; should be isolated in pure cultures; and should, by inoculation, produce the original disease in animals. Had Sanfelice been able to isolate his organism from this tumor, his position might have been tenable. As it is, one can say that his work is valuable in the way of increasing our knowledge of the pathogenic action of blastomycetes, but that his claim that blastomycetes can produce an epithelial proliferation, and an infiltrating tumor analogous with human cancer, is not proven. His work, however, is suggestive and should be continued until absolute results are obtained.

Max Schüller (15) claims to have found unicellular organisms, probably animal, in the cells of a giant-celled sarcoma and in cancer. He describes the bodies as refractile spheres, oval or round, three times the size of a blood corpuscle, with a granular centre and a capsule. He says that the bodies have protoplasmic processes which they extrude through pores. He claims to have grown these parasites outside the

body by putting tissue from the tumor in sterile tubes at body temperature, and says his organism appears as pearl gray or yellow colonies. He has been unable as yet to reproduce tumors in animals by inoculation.

Special Reasons Against the Parasitic Theory.

In opposition to the claims of the men who believe that cancer is due to the action of a parasite is the work of other observers who, working along morphological or experimental lines, have come to entirely different conclusions.

Pianese (16), in a very exhaustive monograph, describes in detail his work upon the character of the inclusions seen in cancer. He believes that the bodies are due to various changes in the cells themselves and that the inclusions are not parasites. He devised a special technic and his article represents an enormous amount of apparently very accurate work. His work accounts not only for the typical body with a dark centre and refractile protoplasm, and a double-contoured membrane, claimed by so many to be the " parasite," but he accounts for all of the peculiar types of bodies seen in the cells and protoplasm of cancer cells. Pianese believes that these bodies arise in various ways, and that no one process explains the origin of all of them. He says that different bodies arise either from degenerations of the protoplasm, degenerations of the nuclei, atypical mitosis, or from phagocytosis. His plates are very instructive, and he apparently explains the development of the typical cancer bodies.

Dean (17) used the same technic as Russell and showed that the so-called fuchsin bodies were hyaline degenerations. He also found similar bodies in inflammatory lesions; hence he concludes that Russell's fuchsin bodies are not parasites and are not pathogenic.

Lack (18) believed that cancer formation was due to the entrance into the lymphatics of normal epithelium which, carried to the various organs, lodged and proliferated indefinitely. To test this theory he opened the peritoneum of a rabbit and scraped the ovaries so as to set free ovarian epithelium. He killed the animal after fourteen months and

found a nodule the size of a cherry attached to the uterus with disseminated nodules on the liver, mesentery, and peritoneum. The diaphragm was infiltrated with similar masses, and there were nodules in the pleura and a mass in the mediastinum. Histological examination showed the infiltrated mass to be adeno-carcinoma. This experiment is of extreme importance, because cancer in rabbits is of extremely rare occurrence. If thiswork can be repeated it bids fair to throw much light on the etiology of cancer.

Conclusions.

Hence we may conclude that in the cells of malignant tumors certain bodies are found, generally in the protoplasm of the cells; that these bodies are quite, but not absolutely, constantly present; that these cell inclusions vary greatly in size, shape, and color reactions with various stains. Certain observers, on morphological grounds alone, have believed these bodies to be parasites and the cause of cancer. From morphological appearances alone it is impossible to prove either that the bodies are parasites or that they are the cause of disease. One man has inoculated animals with a blastomyces obtained "from the air" and produced lesions composed of proliferated connective tissue. One man has isolated an organism, probably a blastomyces, from human tumors, has inoculated animals and produced connective tissue proliferation. In two cases, after inoculation of animals with blastomycetes, epithelial tumors have developed, analogous to cancer in men, but the evidence that these tumors were due to the action of blastomycetes is not conclusive.

Other men, on morphological grounds, believe that the bodies included in cancer cells are due to degenerative changes in the cells themselves, and that the cell inclusions are not parasitic or pathogenic. And one man in one case has apparently produced a tumor in an animal analogous to human cancer, by setting free normal epithelium.

To sum up, we can say that the theory that cancer is due to a parasite is not proven.

My own work upon the subject has been a study of a variety of tumors, in order to determine if the characteristic bodies claimed to be the cause of cancer were constantly present. A number of animals were inoculated with tissue from fresh cancer, in order to see if it were possible to reproduce cancer in animals. The attempt was made to isolate parasitic organisms from malignant tumors. Inoculation of animals with the blastomycetes of Sanfelice and Plimmer, kindly given to me by them, were made.

Morphology.

Forty malignant tumors were examined histologically. As different methods of hardening have been used by different investigators, pieces of each of the earlier tumors were hardened in a number of different fixing reagents in order to determine which method gave the best results. Absolute alcohol, alcohols of various strengths, Hermann's solution, Fleming's solution, corrosive sublimate, and Zenker's fluid were used. The best results were obtained with Zenker's fluid, and after the twenty-fifth tumor only Zenker's fluid was used.

For stains again very different methods have been used by different authors. The methods employed by Sanfelice and Plimmer were used at first. Sanfelice's method did not give satisfactory results in my hands. Plimmer's method of staining with Heidenhain's iron hæmatoxylin as a nuclear stain, and a mixture of acid fuchsin and orange G, or a solution of Bordeaux red, as a differential stain, gave fair results, but was uncertain and uneven in its results. The best results were obtained by using, at Dr. Mallory's suggestion, chloride of iron hæmatoxylin as a nuclear stain, and a mixture of 1 per cent. acid fuchsin, and a saturated aqueous solution of picric acid as a differential stain. This stain colors nuclei black, protoplasm a faint greenish pink, and connective tissue a brilliant red. Inclusions stain the central portion a brilliant red, the clear protoplasm a faint pink, and the periphery red, like the centre. The stain is easily manipulated, and is very constant and even in its action.

The technic is as follows: After hardening in Zenker's

fluid, the tissue was mounted in paraffin and cut. The paraffin was removed with xylol, followed by absolute alcohol. Corrosive crystals were removed by a weak solution of IKI for ten minutes.

The sections were then stained as follows:

1. Ten per cent. aqueous solution ferric chloride, two minutes.
2. Aqueous solution hæmatoxylin (1 to 2 per cent.), freshly made, two minutes.
3. Wash in water.
4. One per cent. solution ferric chloride until blue color is removed from protoplasm and nuclear stain is distinct (watch under the microscope).
5. Wash in water.
6. Aqueous solution ac. fuchsin one per cent., 1 part.

Saturated aqueous solution picric acid, 2 parts, two minutes.

7. Wash in water.
8. Dehydrate in 95 per cent. alcohol.
9. Xylol to clear, 3 changes, blotting dry between each change.
10. Mount in xylol balsam.

The 40 cases of malignant tumors examined included

		Cancer bodies present.	Cancer bodies absent.
Cancer of breast	16	13	3
" " upper jaw	1	0	1
" " lymph nodes	1	1	0
" " bladder	1	0	1
" " intestine	1	1	0
Secondary cancer omentum	1	1	0
Epidermoid cancer lip	3	0	3
" " penis	3	0	3
" " face	5	0	5
" " jaw (secondary)	1	0	1
" " tonsil	1	0	1
" " uterus	1	1	0

		Cancer bodies present.	Cancer bodies absent.
Sarcoma back	1	0	1
" pleura	1	0	1
" lymph nodes	1	0	1
" finger	1	0	1
Lympho-sarcoma lymph nodes . .	1	0	1
	40	17	23

Taking as typical bodies those which correspond to the description given by Sanfelice and Plimmer, *i.e.*, those which have a central portion which does not stain with nuclear stains, a more or less transparent faintly staining protoplasm, and a periphery, sometimes double, which stains sharply, it will be seen that the typical bodies are present in less than half the cases. In 16 cases of cancer of the breast the bodies were found in 13. In 5 cases of sarcoma the bodies were not seen once. In 13 cases of epidermoid cancer typical bodies were not seen. In 2 cases of cancer involving the jaw, the bodies were not present. In an epidermoid cancer of the uterus the bodies were not present in the cells of the cancer, but typical bodies were seen in epithelial cells of the mucous membrane, which showed no involvement in the cancer process.

In many of the tumors, however, in which no typical bodies were seen, other kinds of cell inclusion were seen, notably in the epidermoid cancers. These other cell inclusions occurred invariably in the protoplasm of the cancer cells, either in vacuoles or in close proximity to the nucleus of the cell. They were generally circular or oval without a definite membrane, were homogeneous or very firmly granular, and took a stain (with acid fuchsin and picric acid) of a bright pink color. They usually showed one or two dots, generally eccentrically placed, which took a nuclear stain. Sometimes no such central dot was seen.

In most of the tumors in which the typical bodies occurred they were few in number and were found only after long search. They lay usually in the protoplasm of the cells,

but occasionally were free. They were not seen in degenerated cells, but occurred in cells in which the protoplasm was well preserved. They often, however, did appear in the older part of the tumor, if the cells were well preserved. In one case one of the bodies lay in the protoplasm of a cell which was undergoing mitosis. At times several such bodies were seen in one cell; more often they were single. The number of the bodies was not greater in tumors which clinically were of rapid growth. In but two tumors were the bodies extremely numerous, and in one of these cases in some fields the bodies appeared in nearly every cell.

Inoculation of animals with tissue from fresh cancer. At first an attempt was made to inoculate animals with a bit of tissue from every cancer examined. It soon appeared, however that this was unprofitable, because of the impossibility of manipulating small tumors in such a way as to preclude the possibility of infection without destroying the tumor for histological purposes. Later inoculations were made only from such tumors as were received within two hours from the time of operation, which showed no ulceration, with its accompanying danger of septic infection, and which were of such a size as to offer certainty of aseptic manipulation.

The technic was as follows: The hands were prepared as if for a surgical operation. The tumor was incised with a sterile knife. The incised surface was seared with a heated metal, and then, with sterile forceps and small sharp-pointed scissors, a piece of tissue was removed through the seared surface and instantly dropped into the peritoneal cavity of a rabbit or guinea pig, and the wound was closed. In all, 9 rabbits and 3 guinea pigs were inoculated, chiefly with pieces of tissue from cancer of the breast. In spite of the care used, 3 of the earlier animals died of septic peritonitis, probably because of using cancer tissue which had been infected before operation. Three of the animals since have been killed at intervals of from 7 to 9 months. In one of the animals a small bit of tissue (5 mm. in diameter) was found involved in folds of the mesentery. The inoculated tissue was spherical in shape, rather soft and gelatinous, and

was encapsulated by folds of mesentery. Under the microscope the tissue is seen to be necrotic, although the outlines of the cancer cells still can be made out. There was no evidence of cancerous involvement of any of the tissues.

In one of the animals there was evidence of an old adhesive peritonitis, but no trace of the inoculated tissue could be found. The third animal showed no pathological change whatever. The other animals still are living and appear to be in good condition.

Cultures.

From many of the cases, where the cancer tissue was obtained within 2 hours of the time of operation, cultures were made by dropping a piece of the cancer, or by scraping the surface and dropping the scrapings into fluid culture media. The technic of removing the solid tissue was the same as that of removing the tissue for inoculation of animals. At first cultures were made from all the cancers; later only such tumors were used as gave certainty of manipulation without danger of septic contamination. In all, cultures were made from 13 cases of cancer of various sorts. In 3 cases, either because the tumor itself was contaminated or because of errors in technic, there developed a growth of ordinary pyogenic organisms. In the other 10 cases no growth at all developed.

Professor Sanfelice and Mr. Plimmer each kindly gave me pure cultures of the organisms with which they had obtained their results, and animals have been inoculated with cultures of each of these organisms.

Sanfelice's "saccharomyces neoformans" has been cultivated on various media. It grows very rapidly on any slightly acid or neutral medium which contains glucose or starch. It grows best on potato, and my experience has been that it grows more rapidly at room temperature than it does in the thermostat at 37° C.

Plimmer's organism grows very rapidly on similar media, best on potato, and better at room temperature than in the thermostat. I have not succeeded in growing it under anaërobic conditions.

A number of animals have been inoculated, of which only 5 have been thoroughly examined as yet. Several of the animals still are living.

Animal 1. Guinea Pig. — Inoculation with Sanfelice's blastomyces; .2 cc. of a 14-day-old culture of the organism in glucose bouillon were injected into the left anterior chamber of the eye. The cornea soon became cloudy. After about 10 days the eye bulged, the cornea ulcerated and finally ruptured, discharging thin pus. The eye collapsed and apparently was filled with very vascular granulation tissue. The animal was killed 7 weeks after the inoculation. At this time the eye was collapsed and showed an extensive ulcer in the middle of the cornea. Cultures were made from the blood and from the peritoneal cavity. No growth resulted. The internal organs showed no gross lesions. The axillary lymph nodes were enlarged, rather firm.

Histological examination showed that the eye was filled with very cellular granulation tissue. In this granulation tissue were many blastomycetes, generally free, but often included in the protoplasm of epithelioid phagocytic cells. The size of the blastomycetes varied within wide limits, and the morphology varied a great deal, showing what Sanfelice describes as young, adult, and degenerated forms. As a rule, the morphology of the bodies included in the epithelioid cells was quite unlike that of the cell inclusions seen in cancer cells. Rarely, however, bodies were seen which quite closely resembled cancer bodies. The staining reaction was entirely different from that of cancer bodies, the blastomycetes often remaining quite colorless, or at best taking only a very faint light pink color.

Animal 2. Rabbit. — Inoculated with Sanfelice's blastomyces, 2.5 cc. of a fourteen-day-old culture in glucose bouillon were inoculated in the ear vein. The animal was killed seven weeks after the inoculation, at which time it had become greatly emaciated. Cultures from heart's blood and peritoneal cavity gave no growth. The internal organs showed nothing abnormal except the pyramid of the left kidney, which showed a small circular yellow area, the size of the

head of a pin. The retroperitoneal lymph nodes were rather swollen, soft, and injected. Microscopic examination of the focus in the kidney showed that the centre of the area was necrotic and infiltrated with polynuclear leucocytes. About this area was a zone of epithelioid cells in which were a few newly-formed blood vessels. In the central focus were fairly numerous blastomycetes, lying free. In the granulation tissue about the central area were much less numerous blastomycetes, generally free, occasionally in the protoplasm of the epithelioid cells. As in the first case, the morphology and staining reaction of the cells did not correspond with those of typical cancer bodies.

Animal 3. Rabbit. — Inoculation with Sanfelice's blastomyces; 1.5 cc. of a two-weeks'-old culture in glucose bouillon was injected into the liver. At the time of the injection the syringe leaked and a few drops of the culture were spilled into the abdominal incision. The animal became greatly emaciated, and was killed seven weeks after the inoculation. The abdominal wound was healed, but in the cicatrix was a soft elastic mass 2.5 × 1 cm., very slightly movable. Cultures from heart's blood and peritoneal cavity gave no growth. The autopsy showed that the mass at the site of the scar was in the subcutaneous connective tissue, firmly attached to the scar. On section the nodule was grayish, rather firm, having in the centre an irregular cavity containing caseous semi-fluid material. The lymph nodes were not enlarged. Kidney and lungs appeared normal. Histological examination showed no change in lungs, liver, or kidney. The spleen was injected. The mass from the scar showed microscopically a cavity lined on one side with epidermis, the rest of the circumference being composed of epithelioid cells and young blood vessels. Surrounding the epidermis and its corium was a similar layer of granulation tissue. The cavity was filled with necrotic material and epithelioid cells with a few polynuclear leucocytes. Many of the epithelioid cells contained cell inclusions, many of which were leucocytes in various stages of degeneration. Some cells contained blastomycetes. Fairly numerous blastomycetes were seen

free. In the epidermal cells along one side of the cavity were occasional blastomycetes, generally free; rarely did they appear to be in the protoplasm. No mitotic figures were seen in the epithelial cells, and there was no evidence of cell proliferation. Kidney, liver, lung, and lymph nodes showed no change. The area was practically an abscess cavity surrounded by a thick layer of granulation tissue. The epithelial cells probably were due to infolding of the edges of the skin incision.

Animal 4. Guinea Pig.—Was inoculated with Plimmer's organism; 1 cc. of a glucose bouillon culture was injected into the abdominal subcutaneous tissue. The animal was killed five weeks later, and was much emaciated. On the left side of the abdominal wall was a nodular swelling 2 × 3 cm., firm, indurated, not freely movable. Inguinal lymph nodes on both sides, especially the left, were enlarged. Cultures were made on potato and in glucose bouillon. Pure cultures of the original organism were obtained after three days. On section the tumor was seen to consist of rather œdematous tissue forming a discrete mass outside the abdominal wall, but firmly attached to and infiltrating the underlying muscles. The line of demarcation between the nodule and the abdominal wall was distinct, however. The infiltration of the abdominal wall formed a flattened swelling, which pushed up the peritoneum and projected into the abdominal cavity. There was no evidence of metastases in the peritoneal cavity. The retroperitoneal and inguinal lymph nodes were enlarged, and on section were composed of tissue closely resembling the chief nodule. Lungs, liver, and kidney appeared normal. Spleen and lymph follicles were enlarged. The microscope showed that the chief nodule was composed almost entirely of proliferated connective-tissue cells, with, in places, relatively numerous newly formed blood vessels. The blastomycetes were present in this granulation tissue in enormous masses. They lay usually free in meshes of the connective tissue. Many of the bodies, however, were included in phagocytic cells, sometimes one, sometimes several bodies being included in one such cell. The muscle fibres

of the abdominal wall had largely disappeared; the bundles of muscle which remained showed vacuolization and degeneration, but there was no evidence of invasion of muscle fibres by the blastomycetes. In places was considerable infiltration of the granulation tissue with polynuclear leucocytes. The lymph nodes were enlarged and the lymphoid elements had been almost entirely replaced by granulation tissue like that in the chief nodule, in which there were very numerous blastomycetes. Lymph sinuses were dilated, and contain very numerous blastomycetes, some free and some contained in phagocytic cells. In the spleen were numerous circular and irregular nodules of similar tissue containing blastomycetes. Liver was normal. In the kidney, chiefly in the cortex, were small circular nodules of granulation tissue containing blastomycetes. The lungs showed microscopic areas of the same. The morphology and staining reaction of the blastomycetes do not correspond to those of typical cancer bodies.

Animal 5. Guinea Pig. — Inoculated with Plimmer's organism, 1 cc. of a glucose bouillon culture 18 days old, in the peritoneal cavity. The animal was killed three weeks later. The animal was emaciated, and showed in the abdominal wall at the point of inoculation a firm circumscribed nodule, 1 cm. in diameter, quite firmly attached to the skin. The inguinal lymph nodes were enlarged. The peritoneal cavity looked normal, and there was no especial increase of fluid. The mesenteric and retroperitoneal lymph nodes were enlarged, and on section appeared firm, and pale pink in color. The liver showed no gross lesion. The kidney showed in the cortex numerous small circular areas like tubercles. Similar nodules were seen in the spleen. The lungs were injected, and seemed rather firmer than usual.

On microscopic examination the lungs showed numerous small irregular nodules composed of proliferated connective-tissue cells with a few new blood vessels. The nodules appeared to arise from the connective tissue of the alveolar walls. Blastomycetes were numerous in these nodules, usually free, sometimes included in the protoplasm of large

phagocytic cells. The blastomycetes could be seen budding in places. In parts of the granulation tissue eosinophilic leucocytes are numerous.

The kidney showed similar nodules chiefly in the cortex, affecting only the connective tissue between the tubules. There was no proliferation of renal epithelium, nor were there blastomycetes seen in renal epithelium.

The liver showed no areas of proliferation.

In the spleen were nodules like those already described, which appeared generally in the lymph follicles.

In the lymph nodes the lymphoid tissue was largely replaced by proliferated connective-tissue cells, partly as rather dense bands, partly as a loose mesh-work in which blastomycetes were lying in large numbers. In places this connective tissue was necrotic, and infiltrated with polynuclear leucocytes. In some lymph nodes much of the lymphoid tissue remained, and the blastomycetes were chiefly in the lymph sinuses, either free or in the protoplasm of epithelioid cells.

The nodule in the skin was confined to the corium, and did not involve the epidermis, which showed no sign of proliferation. The blastomycetes in this nodule were practically entirely free.

The staining reaction and morphology of the blastomycetes in the experimental nodules did not correspond to those of typical cancer bodies.

The most of the work of the year has been largely preliminary, and is not sufficiently advanced to enable me to draw definite conclusions. Typical cancer bodies have been found generally present in certain types of cancer.

They never have been found in epidermoid cancer. Attempts to produce cancer in animals by inoculating them with bits of tissue from human cancer so far have uniformly failed. No attempt to isolate an organism from human cancer has succeeded. Inoculation of animals with the organisms of Sanfelice and Plimmer has resulted in the formation of nodules composed of proliferated connective-tissue cells and newly formed blood vessels (granulation tissue),

but no tumor resembling cancer of human beings has been produced.

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PLATE I.

Cell inclusions in protoplasm of epithelial cells from adeno-carcinoma of breast.

Stained with iron hæmatoxylin, acid fuchsin, and picric acid.

Camera lucida.

FIGURE 1. — Zeiss comp. oc. 8; apochromat. 2. mm.: apert. 1.30.

FIGURE 2. — Zeiss comp. oc. 6; apochromat. 4. mm.: apert. 0.95.

FIGURE 3. — Same as 2.

FIGURE 4. — Same as 1.

Plate I.

Cell inclusions in protoplasm of epithelial cells from adeno-carcinoma of breast.

Stained with iron hæmatoxylin, acid fuchsin, and picric acid.

Camera lucida.

Figure 1. — Zeiss comp. oc. 8; apochromat. 2. mm.: apert. 1.30.

Figure 2. — Zeiss comp. oc. 6; apochromat. 4. mm.: apert. 0.95.

Figure 3. — Same as 2.

Figure 4. — Same as 1.

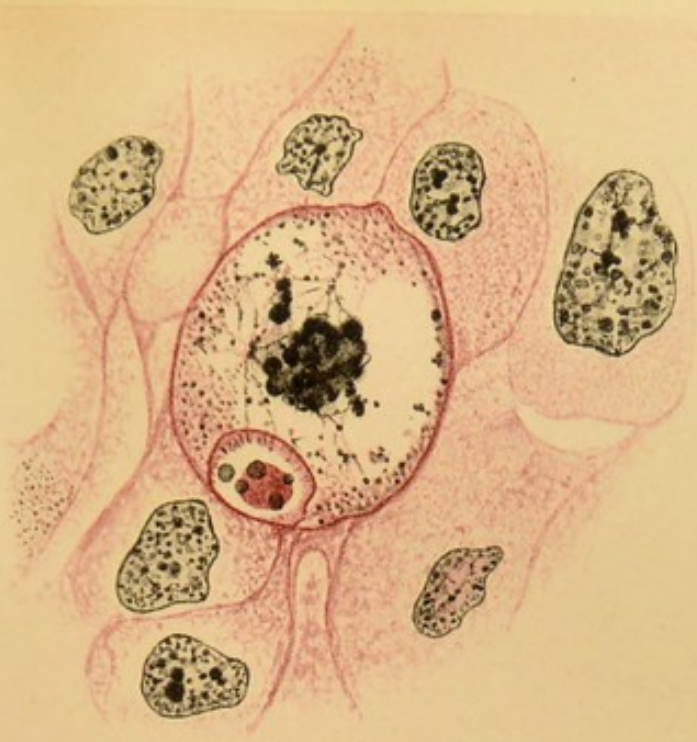


Fig. 1.

Fig. 2.

Fig. 3.

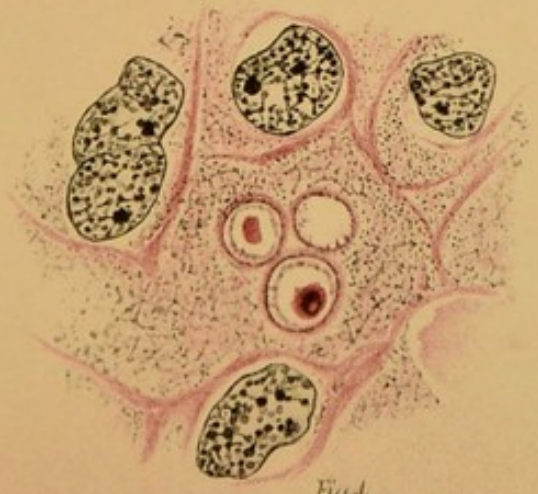


Fig. 4.

PLATE II.

FIGURE 1. — Same as in Plate I., — Figure 1.
FIGURE 2. — Same as in Plate I., — Figure 2.
FIGURE 3. — Same as in Plate I., — Figure 3.
FIGURE 4. — Cell inclusions in protoplasm of epithelial cells from adeno-carcinoma of breast.
FIGURE 5. — Cell inclusions in protoplasm of epithelial cells from adeno-carcinoma of breast.
FIGURE 6. — Cell inclusions in protoplasm of epithelial cells from adeno-carcinoma of breast.

Plate II.

Figure 1. — Same as in Plate I., — Figure 1.

Figure 2. — Same as in Plate I., — Figure 2.

Figure 3. — Same as in Plate I., — Figure 3.

Figure 4. — Cell inclusions in protoplasm of epithelial cells from adenocarcinoma of breast.

Figure 5. — Cell inclusions in protoplasm of epithelial cells from adenocarcinoma of breast.

Figure 6. — Cell inclusions in protoplasm of epithelial cells from adenocarcinoma of breast.

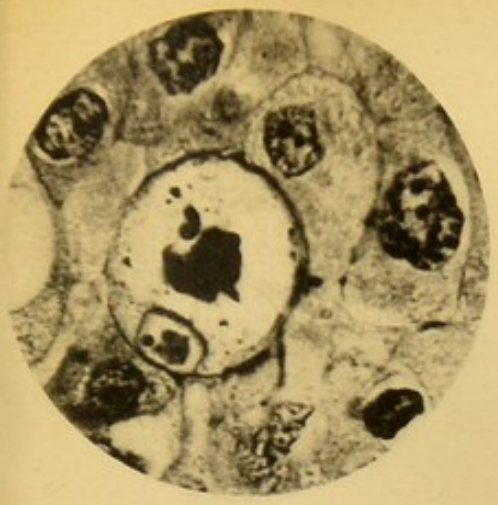
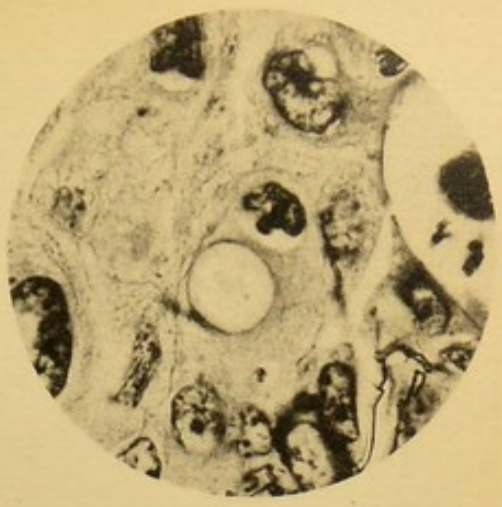


Fig. 1.



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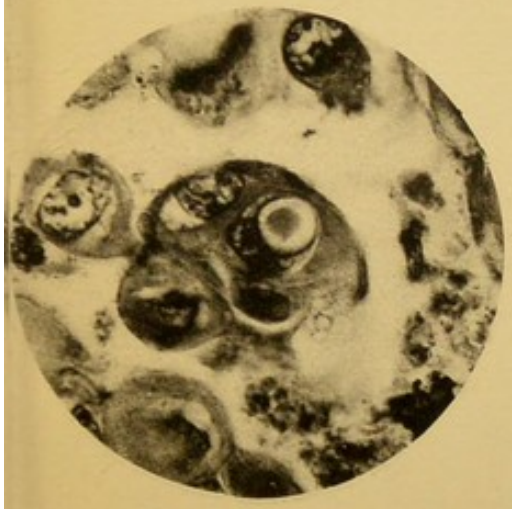

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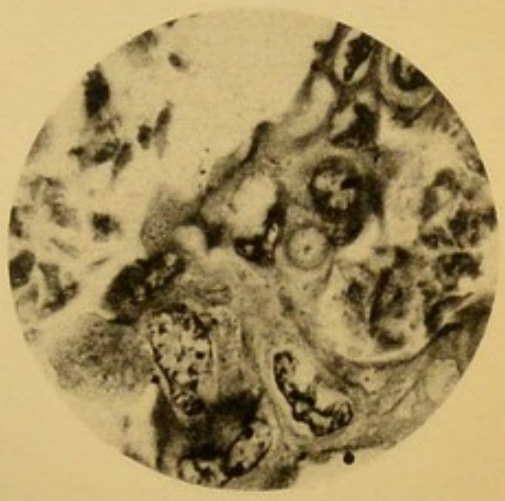

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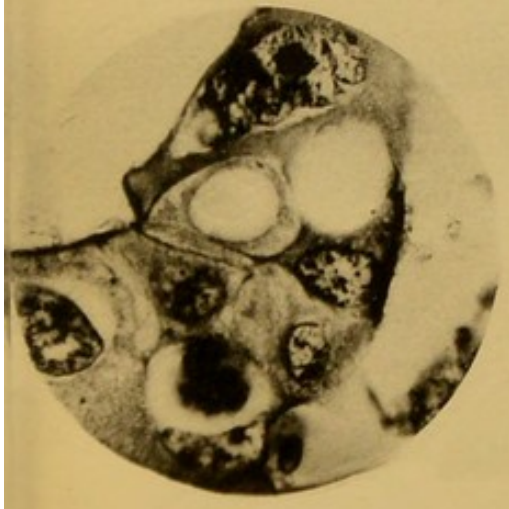

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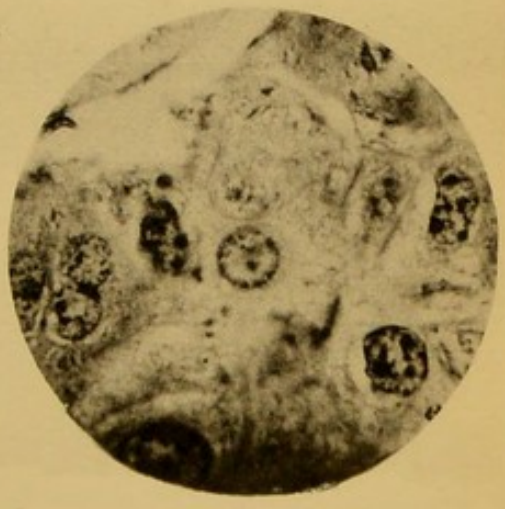


Fig. 6.

PLATE III.

Guinea-pig inoculated with Pfimmer's organism, killed after four weeks. Skin is reflected. Right abdominal wall and abdominal viscera are removed. Left abdominal wall in situ, and shows nodule of granulation tissue just to the left of the median line. Nodule infiltrates entire thickness of the abdominal wall. Chain of enlarged lymph nodes extends from the nodule into the inguinal region.

Plate III.

Guinea-pig inoculated with Plimmer's organism, killed after four weeks. Skin is reflected. Right abdominal wall and abdominal viscera are removed. Left abdominal wall in situ, and shows nodule of granulation tissue just to the left of the median line. Nodule infiltrates entire thickness of the abdominal wall. Chain of enlarged lymph nodes extends from the nodule into the inguinal region.

Fig. 1.

ON THE PRESENCE OF THE SO-CALLED "PLIMMER'S BODIES" IN CARCINOMA.

R. B. GREENOUGH.

(*From the Clinical Pathological Laboratory of the Massachusetts General Hospital.*)

This work was taken up in October, 1899, under the direction and with the assistance of Doctor Nichols, for the Department of Surgical Pathology. Doctors Whitney and Wright, of the Massachusetts General Hospital, kindly put their material at my disposal, and this report is based upon the results of the examination of twenty-one cases of carcinoma, nineteen of which were cases of carcinoma of the breast.

The method of examination was as follows: The material was taken, as a rule, directly from the operating room, and a record kept of the precise anatomical locality from which the specimens were removed. A rough chart of the area involved was preserved in most of the cases and the tissues from each area were separately fixed and hardened. Where material was sent into the laboratory the topographical relations were obtained as well as they could be made out, and a record kept in these cases also.

Fixation. — As previous experience had shown that Zenker's fluid was more satisfactory than Hermann's as regards the staining character of the tissues, no attempt was made at first to use Hermann's fluid, although the latter is recommended by Plimmer himself as the best fixation fluid.

Later in the year, however, a second attempt was made with Hermann's fluid and with perchloride of mercury, but again with no obvious advantages over Zenker's fluid. It is to be regretted that in this matter of fixation Plimmer's recommendations could not be carried out, but the results with Hermann's fluid were so universally unsatisfactory that no reliance could be placed upon the action of the fixed tissues to the stains.

Hardening. — After twenty-four hours in Zenker's fluid

the specimens were washed for twenty-four hours and placed in alcohol 7 per cent., to which about 2 per cent. of tincture of iodine had been added. From this solution they were taken up through stronger alcohols and embedded in paraffine. Sections were made with the Minot-Blake microtome, a section of 2 μ being as thin as could be cut in the majority of the cases, on account of the amount of connective tissue in the specimens. Sections were then made from all of the different regions of the material, and these sections were stained according to Plimmer's directions, with iron hæmatoxylin, and counter-stained with either orange G and fuchsin or with Bordeaux red. In the main, therefore, the procedure adopted by Plimmer was followed as far as possible, and in the matter of fixation only was it found necessary to depart from his directions.

The number of sections stained and examined from each area of a given case varied. A dozen or more sections were often made before one was obtained which satisfied all the requirements, and the number of areas thus examined ranged from one to ten. In most cases a control was made with the methylene blue and eosine staining, and this stain was sufficient in many cases to demonstrate the characteristic appearances, though not so clearly as they are shown by Plimmer's special stains.

The 19 cases of carcinoma of the breast examined showed in each case the characteristic appearances of the bodies described by Plimmer. In four cases they were so numerous as to be present in almost every field of the microscope. In other cases a long search was required through many specimens before a single characteristic figure could be found. In the majority of cases, however, the so-called bodies were readily identified.

Bodies. — The appearances which have been called Plimmer's bodies are described by him as being "round bodies of very diverse sizes, from .004 to .04 mm. in diameter." A central darker staining portion is present, surrounded by a lighter zone, and outside of this a capsule. The characteristic staining property of these structures is that they take

up protoplasmic rather than nuclear stains, particularly in the central portion, and in the sharply staining "capsule," and that a rayed appearance may be noted at times in the lighter staining periphery. This is the description given by Plimmer in his article in the "Practitioner," and it is such appearances as these that are figured in the plates which accompany that article. Such appearances I have found in all of 19 cases of breast carcinoma, and the description given would apply as well to these specimens as to his plates.

Situation of Bodies. — The bodies in these cases were invariably found in the cancer cells often lying in a position symmetrical with the nucleus and of about the same size as the nucleus — at other times being smaller or larger than the nucleus and occupying a polar or peripheral situation. They were found to be more abundant in the parts of the tumor which had suffered the least from degeneration, either before or after the removal of the specimen. In consequence of this, the periphery of the original tumor or one of the smaller metastases was the region in which the most satisfactory specimens were found. Furthermore, those cases which showed the most rapid growth were by no means the ones which showed the most satisfactory specimens of bodies, and of the four cases in which the bodies were most numerous, three were clinically characterized as being of slow growth. In fact, where the cancer cells were heaped together in columns of 20 or 30 the bodies were, as a rule, difficult to find; while in cases of a scirrhous type, where the cells were arrayed in columns of one or two cells, the detection of the bodies was much easier.

The diversity of size of these structures was notable, as remarked by Plimmer, the gradation from small forms to large in the same specimen making it quite difficult to draw any hard or fast line, and place a limit of size, beyond which they could not be considered characteristic. Furthermore there are in nearly every case of carcinoma hyaline or homogeneous intracellular structures which coincide with these bodies in all but the central staining area, and perhaps the rayed periphery. In other words, they are not to be sharply

Plate IV.

Figures 1, 2, 3. — Sections of carcinoma of breast. Single cells containing typical "Plimmer's bodies."

Figure 4. — Single carcinoma cell containing a body with large central staining area and a sharply defined periphery.

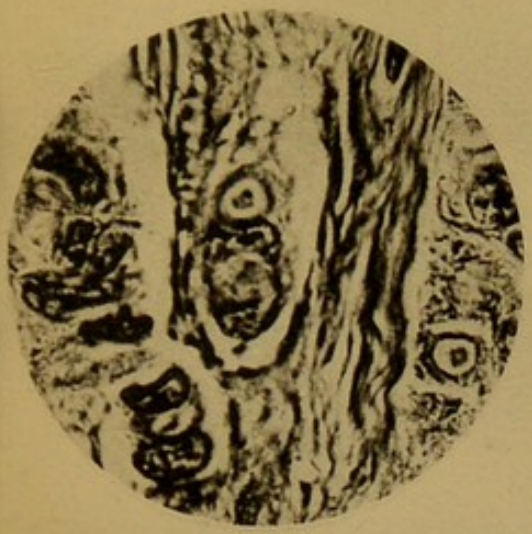
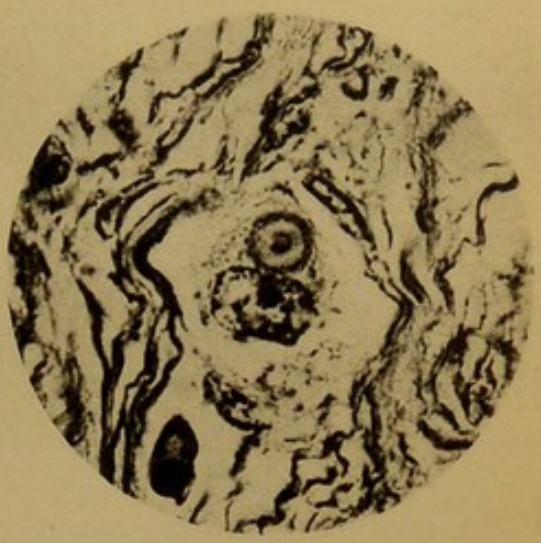


Fig. 1.



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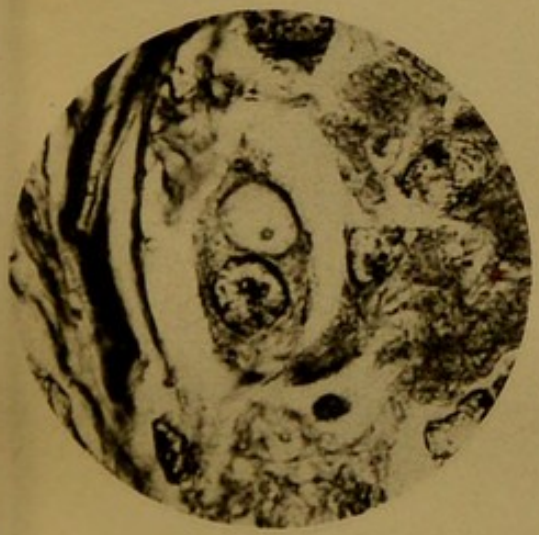

Fig. 3.

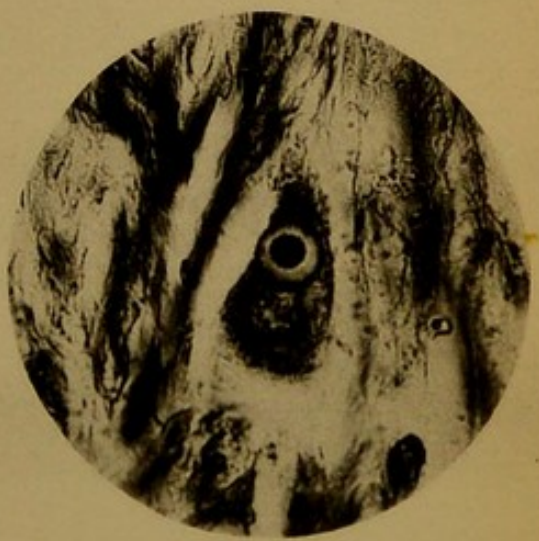

Fig. 4.

PLATE V.

FIGURE 1. — Single carcinoma cell containing a body in which the central staining area presents the appearance of budding.

FIGURE 2. — Single cells containing typical Plimmer's bodies.

FIGURE 3. — Large carcinoma cell with numerous vacuoles, resembling "parasite spores."

FIGURE 4. — Carcinoma cell containing two bodies of different sizes suggesting a growth by budding.

The photographs were prepared by Mr. L. S. Brown in the Laboratory of the Massachusetts General Hospital. The lenses used were Zeiss Apochromatic 3 mm. $\frac{1}{12}$, with a No. 4 Projection Ocular, and give an amplification of about 1000 diameters. The specimens were procured from cases of breast carcinoma.

Plate V.

Figure 1. — Single carcinoma cell containing a body in which the central staining area presents the appearance of budding.

Figure 2. — Single cells containing typical Plimmer's bodies.

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Figure 4. — Carcinoma cell containing two bodies of different sizes suggesting a growth by budding.

The photographs were prepared by Mr. L. S. Brown in the Laboratory of the Massachusetts General Hospital. The lenses used were Zeiss Apochromatic 3 mm. $\frac{1}{40}$, with a No. 4 Projection Ocular, and give an amplification of about 1000 diameters. The specimens were procured from cases of breast carcinoma.

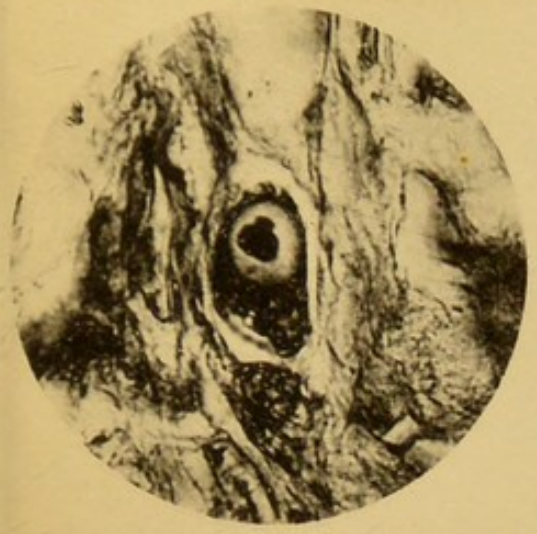

Fig.1.

Fig.2.

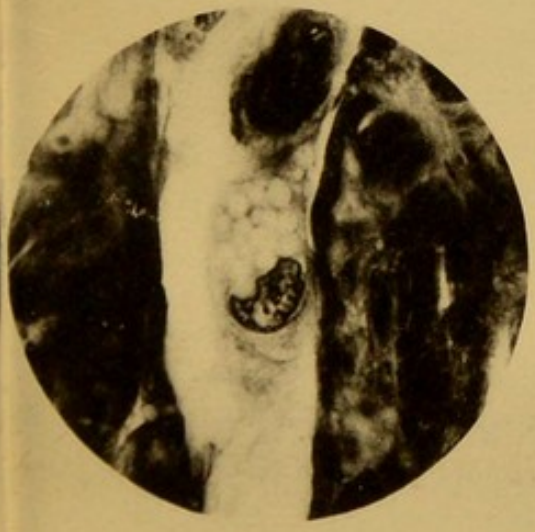

Fig.3.

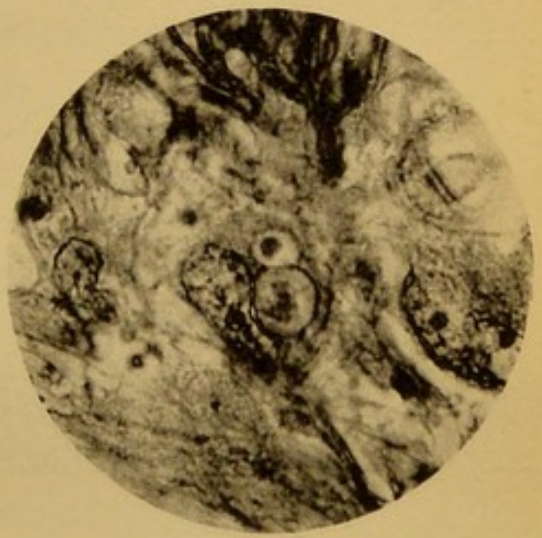

Fig.4.

TUMORS AND SPOROZOA IN FISHES.

E. E. Tyzzer.

In this paper I shall try to give, in brief, a few facts concerning the sporozoa that infect fishes. In addition to this, I shall present a species which I found, and which I believe has never been described. Although it may seem to have little bearing on the etiology of cancer, it may, however, be of interest to you to know where this class, sporozoa, belongs in the animal kingdom — what these organisms are like, and what their life history.

I wish to state in the beginning that but few of the general facts concerning these organisms are of my own observation, but are taken largely from the monograph of R. R. Gurley, M.D., of the United States Fish Commission. In classification I have followed Delage and Hérouard in their work on "The Protozoa."

In considering the branch protozoa, we find it divided into four classes: the rhizopods, the flagellates, the infusoria, and the sporozoa. The sporozoa are unicellular, ameba-like organisms, destitute of appendages (such as cilia or flagella), which multiply by spore formation, and which are invariably parasitic. They possess no pulsating vacuole, as do many of the amebæ. Of all the class sporozoa, the myxosporidia form the only group, so far as is known, that infects fishes. Besides fishes, the myxosporidia are also found with the crustacea and certain of the insects. They may be found in practically any of the tissues or natural cavities of their host. The individual species, however, seem to infect certain tissues or cavities in preference to others.

In the life history of the organisms there are two well-defined stages: the myxosporidium stage and the spore stage. The young myxosporidium, after it is "hatched" from the spore, is a very active, irregular, ameboid, or vermiform organism, generally possessed of two nuclei. It at once penetrates a cell of its host's tissues — perhaps an epithelial cell or perhaps a red blood cell. Now follows a period of

growth in which the organism distends and at last bursts the cell which has harbored it. On becoming free, the myxosporidium continues to grow and the nuclei multiply, division taking place by mitosis. As it increases in size the movements are less active and the nuclei are scattered throughout the whole organism. It is at this stage ready for sporulation. Before describing this process it would be well to describe the spore.

With each species the spores are characteristic, and thus it is by the spore rather than by the adult ameba that the myxosporidia are classified. The myxosporidium or ameba stage may be indistinguishable in species that are quite unlike in the spore stage. The typical spore, let us say, consists of several parts, one essential and other accessory parts. The essential portion is the "sporoplasm," which is in time to develop into the perfect ameba. The sporoplasm always contains one or more (usually two) nuclei, and it occupies a position in the spore which is called arbitrarily "posterior." The accessory parts are those concerned in the "capsules," which are situated at the end opposite the sporoplasm, or "anterior" extremity of the spore. These capsules are highly refractile and are continuous with a duct opening anteriorly on the spore. They each enclose a coiled thread-like body, or "filament," which under certain conditions is extruded through a duct opening on the surface of the pore. The filaments execute active movements, which may result in the movement of the entire spore. This apparatus, consisting of capsule and filament, bears a striking resemblance to the nematocysts of the coelenterates. Their function, however, cannot be such. A great many theories have been offered as to the function of these filaments. Observations on this subject are so unsatisfactory that not much information has been gained. It is certain, nevertheless, that some spores are slowly propelled by the action of the filaments. The census of opinion is, that they are organs either for the attachment or for the dissemination of the spores. The surface of each spore is composed of a thick "shell," which may be bivalved or otherwise. In certain species the

shell is drawn out posteriorly in a long, tapering process, the "tail."

To go back to the process of sporulation: around each of the many nuclei of the adult myxosporidium a mass of clear protoplasm is differentiated. This mass, with the included nuclear material, is termed the "pansporoblast." At the periphery of this there is a delicate surrounding membrane. Each nucleus divides and subdivides by mitosis until the pansporoblast contains perhaps a dozen nuclei. The mass divides subsequently into two equal parts, the "sporoblasts," each containing three nuclei. The remainder of the nuclei take up their position with the membrane surrounding the mass. Each sporoblast now consists of unequal parts, one large and two small, each containing a nucleus. The larger part ultimately forms the sporoplasm and takes up the larger part of the spore; the two smaller parts are concerned in the formation of the capsules. The capsules first appear as transparent vacuoles. Into these project little "buttons" of protoplasm, which elongate, are pinched off at the base, and finally become filaments. These are coiled within the capsules, which are now possessed of a definite membrane. In the above manner the whole substance of the myxosporidium may be converted into spores.

The spore stage with the myxosporidia does not so much represent a period of rest as it does one of multiplication and dissemination. Another question is: "How do the spores gain entrance to the host?" Such localities as the gills, the alimentary canal, the skin, and the urinary bladder have been mentioned by various observers as probable points of entrance. It is probable that the different species elect different points, and it is possible that, in the more general infections, the spores are spread throughout the body by way of the blood and lymph channels.

Pathology.

As a matter of fact each species develops its own characteristic pathological process. The tissues of the fish as a rule react but slowly, but there are described, nevertheless,

epidemics in which the processes have gone on so rapidly that the fish soon succumb and that too in large numbers. In all the literature upon this subject I have nowhere found described a neoplasm such as is ordinarily designated a tumor; nor have I found evidence of proliferation of epithelium in any case. In the lake-pike, a round-celled sarcoma is described by Ohlmacher, but he found no organisms, although he had personally examined many preparations of the myxosporidia. This tumor, which he described, started apparently in the connective tissue near the vertebral column, and metastases were found throughout the mesentery and involving many of the viscera.

A New Species of Myxosporidia.

I will now describe an organism which I found infecting several different species of fishes. My attention was first called to a diseased condition of the muscles of young herring by Dr. Linton. He had observed this condition a year previous, but had not investigated the matter. Examination of the fish showed small white cysts, one to two millimeters in length, lying between the muscle fibers of the myotomes. (See Plate VI., figs. 1, 2, and 3.) With a little pressure the cyst contents — a white, creamy mass — was squeezed out. Under the microscope this substance proved to be made up of small quadrilateral spores. Alewives and other fish were also found to be infected quite commonly with the same organism.

The spores are quadrilateral when seen face on, and in profile are oval. The four corners are a little protuberant and are directed slightly forward. The spores vary little in size, averaging about seven to seven and a half μ in breadth. In a given fish there was but little variation in shape, but as they were afterwards found in several species of fishes, the shape varied considerably. Thus in the young scup the corners were so drawn out as to give the spore an almost stellate appearance. There is also a tendency of the spores to occur fitted together in clumps of four or eight. (See Plate VI., fig. 6.) Each spore contains four capsules, very delicate, pale green in color, radiating from the anterior ex-

tremity toward the four corners. (See Plate VI., fig. 5.) From the four capsules were extruded, at times, the four filaments, whose vibrations caused the spore to move gradually forward. After a time the filaments would shorten and ultimately return into the capsules. Each capsule is surrounded by a clear space, the "perivesicular space." The remainder of the spore is taken up by the sporoplasm, and the whole surrounded by the shell. Four furrows radiate from the anterior extremity outwards to the sides, causing the spore to appear quartered. Posteriorly there lies an oval body, the nature of which I have not yet been able to make out with certainty. It takes a nuclear stain and it may be justifiable to consider it a "macronucleus" belonging to the sporoplasm. The spore ends anteriorly in a little projection through which the filaments pass.

The spores are stained by the ordinary aniline dyes, but the nuclei stain with difficulty. It is most satisfactory to use such stains as carbol fuchsin, or Loeffler's methylene blue, followed by a decolorizer. The capsules stain readily but are as readily declorized. With acetic acid the filaments are extruded and become fixed and the four nuclei at the corners are brought out.

In examining many different species of fishes, they were found in the alewife, the scup, the herring, the menhaden, the hickory shad, and the cunner — in all, six different species. In these the young fish were most commonly infected. It is probable that this percentage would have been increased if a more careful examination had been possible. In old fishes the tissues seem to be getting the better of the spores, and the latter were found to be quite degenerated. Owing to the difficulty of keeping a census of the sea, I can give no opinion as to the mortality of the infected fish. When kept in an aquarium they seem to thrive well until something more serious happens to them.

In studying sections of the tissues of small fishes, cysts were found so small that they were not discernible to the naked eye, yet no earlier stage of the organism could be found. There are cells which at times include one or more of the

spores, and whether these are sporoblasts or phagocytic cells I cannot say. They have every characteristic in common with certain cells found in the blood, having a lilac-stained protoplasm, and an irregular nucleus situated invariably at one side near the periphery of the cell. There is at times a definite cyst-wall composed of connective tissue, and at other times none. The connective tissue may grow among the spores inmeshing them in a network.

This spore seems without doubt to belong to the myxosporidia. Its mode of encystment, however, resembles that of the sarcesporidia which are found in the domestic animals and some birds. How the cysts increase in size, as they certainly do, with the growth of the fish, remains a mystery. This is not the first instance in which the development of such organisms cannot be followed. In a case similar to this, one observer suggested that the sporoblast existed free from any form of ameba and independently went on with the process of sporulation.

Plate VI.

Figure 1. — Small cysts in a young menhaden.

Figure 2. — Larger cysts in a young herring.

Figure 3. — Cyst in which the connective tissue is enmeshing the spores — scup.

Figure 4. — Smear stained with carbol fuchsin — scup. The nuclei and capsules have both retained the stain.

Figure 5. — Smear stained with Loeffler's alkaline methylene blue — alewife. The capsules are darkly stained.

Figure 6. — Smear showing characteristic clumped arrangement of the spores. Nuclei dark, capsules light.

Fig. 1

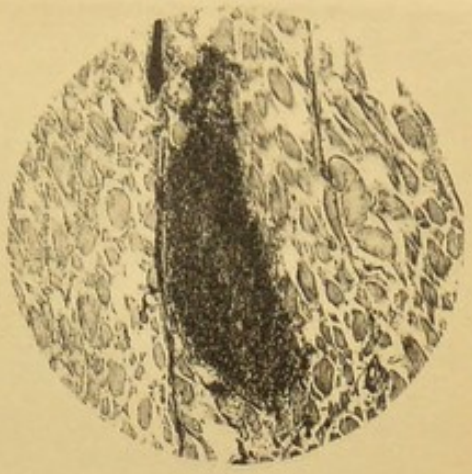

Fig. 2

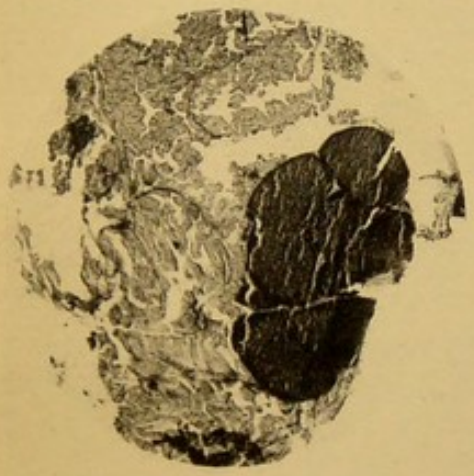

Fig. 3

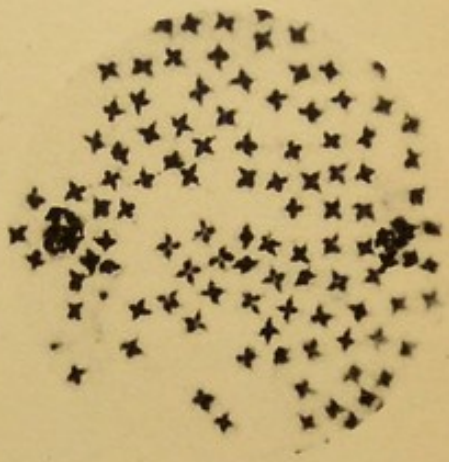

Fig. 4.

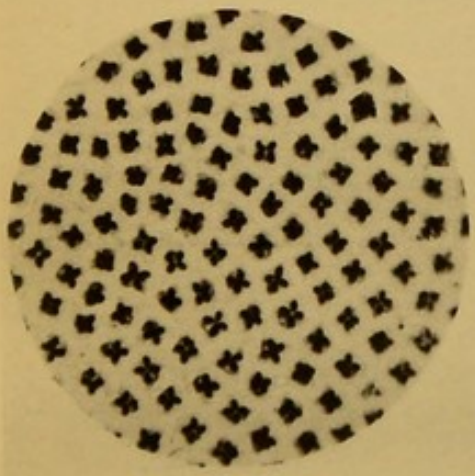

Fig. 5

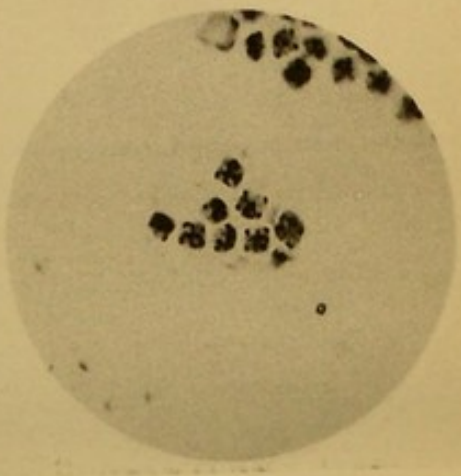

Fig. 6.

THE RECONSTRUCTION IN WAX OF A NODULE OF CANCER.

EDWIN A. LOCKE.

The following work was undertaken at the Sears Laboratory during the present year at the suggestion and under the personal direction of Dr. Nichols. To Dr. Mallory I am also indebted for many valuable suggestions.

My aim has been the construction in wax of an exact model, on an enlarged scale, of a minute portion of the growing edge of a carcinoma. Together with a careful microscopical study of a series of sections, this work in the study of the general morphology of cancer was undertaken in the hopes that it might throw some light upon the general theories of the course of the growth. Besides emphasizing certain things shown by microscopical examination, it was hoped that it might make evident some new features of the growth. While obviously impossible in such a model to show the cellular structure, it is, if reasonable skill and care in technique be exercised, a comparatively easy task to reconstruct with almost absolute accuracy the columns and masses of cells.

The method followed is the so-called "Reconstruction Method of Bom," so long used in embryological studies. This consists (1) in the cutting of the tissue into serial sections, (2) the drawing of these sections on paper with a definite magnification, (3) the transferring of these drawings to wax plates, (4) the cutting of these plates in accordance with the drawings, (5) the piling up of the wax into a model, and (6) finally the fusing of the plates.

A small portion of the growing edge of a scirrhous cancer of the breast was taken, fixed, and hardened in Zenker's fluid, imbedded in paraffin, cut in serial sections $\frac{1}{100}$ mm. in thickness, and stained with methylene blue and eosine. In this series I was able to follow in 74 consecutive sections one part of the growth from its outer limits to its point of division from the main tumor. It appears as a collection of many columns of cells closely surrounded by very dense connective

tissue not unlike a capsule. The space between the columns is occupied by a loose connective-tissue stroma containing a few blood-vessels, and densely infiltrated by lymphoid cells. This I selected as offering the best opportunity for study by the reconstruction method. For this purpose it was first necessary to draw on paper the sections in outline by the aid of a camera lucida, a uniform enlargement of 102 diameters being arbitrarily taken. The sharp differentation of the neoplasm from the surrounding fibrous stroma greatly facilitated the tracing. With the completion of each drawing the next section was placed in the field in such a position, through manipulation of the mechanical stage, that the projection exactly fitted the drawing. Then after the removal of the drawing, the projection could be drawn, and the next section adjusted to it in a similar manner. This method established a uniformity in the drawings, and consequently an adequate standard by which to build up the tumor.

The next step consisted in the transforming of these outlines, by means of carbon tracing-paper, to wax plates of proper thickness. The thickness of the sections together with its diameter and that of the drawing being known, the required thickness of the plates could be readily obtained. These plates were of pure wax, and made by the following method: A large pan 24 by 14 inches was partly filled with water and placed on a water bath in such a manner that it could be heated or cooled at will. With the temperature of the water a few degrees above the melting-point of the wax, a given amount of molten wax would, when added, rise to the surface and assume an absolutely uniform thickness. A Bunsen flame applied to the surface readily drove out any bubbles present. Rapid cooling gave the best results. When sufficiently solid, they were removed and cut into the designed sizes. Previous to the building up of the model, each one was cut out according to the drawings made. With the addition of each layer, the numerous small masses representing cross-sections of the columns of cells were held in place by the insertion of small bits of wire about 1 cm. in length heated moderately. Constant refer-

PLATE VII.

Lateral view of wax model of a small nodule from the edge of a scirrhous breast cancer with an enlargement of 102 diameters (or volume 1,061,208 times the original).

Model represented in cut one-eighth natural size.

A. — Tumor.

B. — Space occupied by the fibrous stroma.

C. — Fibrous tissue of the breast.

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A. — Tumor.

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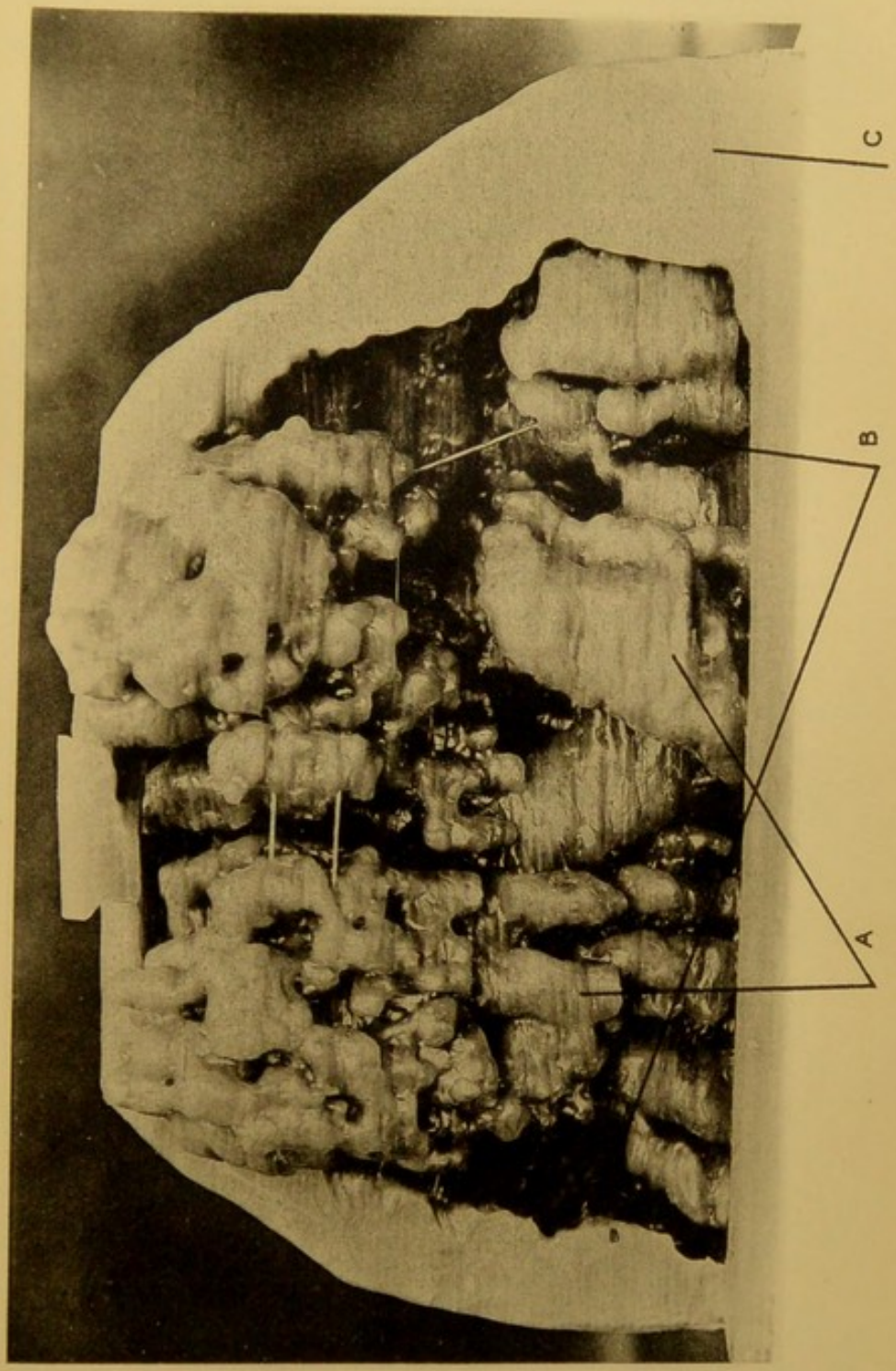
C
B
A

ence to the sections under the microscope served as a check both to the fitting of the layers and to the subsequent fusing. The layers in every case fitted almost perfectly, a sufficient evidence, it seems to me, of the accuracy of the work. Finally, by means of a small moulding-iron heated in the flame, the layers were fused into a solid mass.

The model thus constructed represents only the beginning of the study undertaken, and from this alone clearly no general conclusions can be drawn. Its value lies chiefly in the illustration which it gives of the relation of various parts and the character and manner of growth. More clearly than by any other method it demonstrates the alveolar character so typical of carcinoma. Nowhere did I find nests of cells which sooner or later did not unite with other portions of the tumor. Each column is an outgrowth. These columns are very irregular, extremely variable in size, and appear to have no general law governing their direction. A single small clump of cells may within the limits of a few layers become a complicated mass, honeycombed by bonds of connective tissue and growing in many directions. In many places the outgrowth ends abruptly, indicating that the cancer may extend by mere expansive growth as well as by infiltration.

REPORT OF CULTURE EXPERIMENTS MADE WITH CARCINOMATOUS TISSUE, 1899 AND 1900.

OSCAR RICHARDSON.

(*Clinico-Pathological Laboratory, Massachusetts General Hospital.*)

These culture experiments were made to demonstrate whether the bodies found in the cells of carcinomatous tissue could be grown or not. The investigation was made parallel to one by Dr. R. B. Greenough on the pathological histology of the same material. The material was obtained chiefly from the surgical departments of the Massachusetts General Hospital and the tissues were taken and the cultures made as soon as possible after the time of operation. This procedure insured a fresh clean warm tissue in most instances and gave the most favorable conditions for making cultures.

The cultures were made under the strictest asepsis, all instruments and the glass plate on which the piece of tissue rested while the culture was made being cleaned in the Bunsen burner flame. The cube of tissue was seared on all its surfaces and an opening made into the central portion of the mass from which the cells of the new growth were curetted out as a semi-fluid pulpy mass in which the tissue cells were freely liberated. This material was then directly placed in sterilized tubes of media of various kinds and under aërobic and anaërobic conditions to be described.

Media.

Carcinoma Bouillon.— The tumor mass was taken from the breast and stripped of as much fat as possible. A few pieces of muscle clear of all fat were included. This material was chopped finely, weighed, and made up in the same proportions as ordinary bouillon. Then it was boiled thoroughly and filtered and brought to a boiling condition again, when peptone and salt were added as in ordinary bouillon. It was next carefully neutralized and boiled again for a short while and while hot, 2 per cent. of glucose and 1 per cent., of tartaric acid added. Then it was allowed to cool in a flask, then

boiled again and filtered and tubed hot. The tubes of the culture medium were then sterilized in the Arnold sterilizer three times, one half hour each time. When a tube was to be inoculated it was sterilized for one half hour just before inoculation.

Blood Serum, Plain Bouillon. — Prepared according to directions in Mallory and Wright's "Pathological Technique."

Bouillon. — Made like plain bouillon mentioned above, except that in place of beef the tissue from a cystic breast was used.

Bouillon and Agar. — Made from the mucosa of the intestine of a hog at the suggestion of Dr. Warren.

The small intestine of the hog was taken in fresh condition without cutting and washed thoroughly with running water until the water ran perfectly clear. The intestine was then laid open and the mucosa scraped off with a glass slide. One hundred and fifty gms. of mucosa were easily obtained from the small intestine of one hog.

Agar. — 250 c.c. of the mucosa was taken and 500 c.c. of distilled water was added. This was boiled for one half hour. It was then allowed to cool, was boiled again, then neutralized and boiled again. After filtering, it was boiled and 5 gms. of peptone and 2½ gms. of salt added and the material was then neutralized again and the boiling continued for twenty minutes and it was then filtered. One-half of this bouillon was then taken and 1½ per cent. of agar added and the medium boiled until the agar was dissolved. It was neutralized (if necessary) and then cooled to 68 degrees C. A well-beaten egg was then added and the medium boiled until the egg thoroughly coagulated. It was then filtered, tubed, and sterilized by the fractional method, then slanted.

Bouillon. — The other half of the bouillon was tubed and sterilized by the fractional method.

In one or two instances the tissue from a normal breast and from normal lymphatic glands was placed in sterile tubes of media and inoculated with cells from carcinomatous tissue.

Again, pieces of the tumor with adjacent tissues were placed in sterile tubes without the addition of any other

medium. In one or two cases the blood from the adjacent tissues of the tumor mass was added to the medium inoculated with the carcinomatous cells.

Methods of Cultivation.

Aërobic. — The usual test tube method, the carcinomatous tissue being distributed in or on the medium.

Anaërobic. — Fluid cultures were made after the method of Dr. J. H. Wright described in the "Centralbl. f. Bakt. u. Parasitenk," January, 1900, and the "Journal of the Boston Society of Medical Sciences," January, 1900.

Solid cultures. The test tubes, after inoculation, were placed in a sealed jar containing pyrogallic acid, according to Buchner's method.

Sub-cultures. With some of these the plate method of Petri was used. The records of the details of the culture experiments are to be found in the following table:

	1899.	Carcinoma Bouillon.		Plain Bouillon.
		Aërobic.	Anaërobic.	Aërobic.
Carcinoma lymphatic glands with involvement of the humerus.	July.	Negative.	Negative.	.....
Carcinoma lymphatic gland. Dr. Conant.	Aug. 4.	.....	Negative.	.....
Carcinoma lymphatic gland.....	Aug. 5.	.....	Negative.	.....
Carcinoma breast. Periphery growth. Dr. Elliot.	Aug. 10.	.....	Negative.	.....
Carcinoma of breast. Periphery growth. West surgical department.	Aug. 20.	.....	Negative.	.....
Carcinoma of breast. Culture from gland.	Aug. 24.	.....	Negative.	.....
No. 1. Carcinoma breast. Cultures from periphery and centre of tumor and from axillary gland. Dr. Mixter.	Sept. 30.	.....	Negative.	.....
Carcinoma breast. Periphery growth. Dr. Warren.	Oct. 4.	.....	Negative.	Negative.
No. 2. Carcinoma breast. Periphery of growth. Dr. Warren.	Oct. 6.	.....	Negative.	.....
Carcinoma breast. Periphery growth. Dr. Warren.	Oct. 9.	Negative.	Negative.	.....

	1899.	Carcinoma Bouillon.		Plain Bouillon.		Blood Serum.
		Aërobic.	Anaërobic.	Aërobic.	Anaërobic.	Aërobic.
No. 3. Carcinoma breast. Periphery of growth. Dr. Warren.	Oct. 19.	.....	Negative.	.....	.....	.....
No. 4. Carcinoma breast. Periphery of growth. Dr. C. B. Porter.	Oct. 25.	.....	Negative.	.....	.....	.....
No. 5. Carcinoma breast. Periphery of growth and gland. Blood from tissues of breast added in all cultures. Dr. C. B. Porter.	Oct. 27.	Negative.	Negative.	Negative.	Negative.	.....
No. 6. Carcinoma breast. Periphery of growth. Dr. C. A. Porter.	Nov. 7.	.....	Negative.	.....	.....	.....
No. 7. Carcinomatous peritonitis. From fluid. Dr. Warren.	Nov. 7.	.....	Negative.	.....	.....	Sterile.
No. 9. Carcinoma breast. Periphery growth. Dr. Warren.	Nov. 16.	.....	Negative.	.....	.....	.....
No. 10. Carcinoma breast. Periphery growth. Dr. H. H. A. Beach.	Nov. 20.	.....	Negative.	.....	.....	.....
No. 11. Carcinoma breast. Cheesy matter tumor. Dr. C. B. Porter.	Nov. 23.	.....	Negative.	Negative.	.....	.....
No. 12. Carcinoma breast. Periphery of growth. Dr. Warren.	Nov. 25.	.....	Negative.	Negative.	.....	.....

	1899.	Carcinoma Bouillon.		Plain Bouillon.		Blood Serum.
		Aërobic.	Anaërobic.	Aërobic.	Anaërobic.	Aërobic.
No. 13. Carcinoma breast. Periphery growth. Dr. Warren.	Dec. 5.	.....	Negative.	.....	.....	.....
No. 14. Carcinoma breast. Lymphatic gland. Dr. H. H. A. Beach.	Dec. 8.	.....	Negative.	.....	.....	.....
No. 16. Carcinoma breast. Periphery growth. Dr. H. H. A, Beach.	Dec. 16.	.....	Negative.	.....	.....	.....
No. 17. Carcinoma nose. (Cocci in cover glass from blood at operation.) Dr. Warren.	Dec. 21.	.....	Negative.	.....	.....	Staphylococcus Pyogenes Aureus.
Carcinoma breast. Lymphatic gland. Dr. A. T. Cabot.	Dec. 29.	.....	Negative.	.....	.....	.....
No. 19. Carcinoma breast. Periphery growth. Dr. H. H. A. Beach.	1900. Jan. 2.	.....	Negative.	.....	.....	.....
No. 20. Carcinoma breast. Periphery growth. Dr. C. B. Porter.	Jan. 10.	.....	Negative.	.....	Negative.	.....
Carcinoma breast. Lymphatic gland. Dr. C. B. Porter.	Jan. 15.	.....	Negative.	.....	.....	.....

	1900.	Carcinoma Bouillon.		Plain Bouillon.		Blood Serum.	Bouillon. Mucosa Pig's Intestine.		Agar. Mucosa Pig's Intestine.	
		Aërobic.	Anaërobic.	Aërobic.	Anaërobic.	Aërobic.	Aërobic.	Anaërobic.	Aërobic.	Anaërobic.
No. 21. Carcinoma breast tumor mass. Periphery growth. Dr. Mixter.	Feb. 15	.....	Negative.	.....	Cystic breast Bouillon. Negative.	.....	.....	Negative.	.....	Negative.
Lymphatic gland.....	.....	.....	.....	.....	.....	.....	.....	Negative.	.....	Negative.
Lymphatic gland. Piece put in a tube and left under anaërobic condition. Negative.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Piece from breast young woman inoculated from tumor mass.	.....	.....	Negative.	.....	.....	Negative.	.....	Negative.	Negative.	.....
Piece from normal breast inoculated from diseased breast.	.....	.....	Negative	.....	.....	Negative.	.....	Negative.	Negative.	.....
Piece from tumor mass.	.....	.....	Negative.	.....	.....	Negative.	.....	.....	.....	.....
Tumor mass. Piece put in a tube and left under aërobic condition. Negative.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

	1900.	Carcinoma Bouillon.		Plain Bouillon.		Blood Serum.	Bouillon. Mucosa Pig's Intestine.		Agar. Mucosa Pig's Intestine.	
		Aërobic.	Anaërobic.	Aërobic.	Anaërobic.	Aërobic.	Aërobic.	Anaërobic.	Aërobic.	Anaërobic.
Lymphatic gland and surrounding tissue.	.....	Negative.	Negative.	.....	Negative.	.....	.....	.....	.....	.....
Normal sterile gland inoculated from tumor mass.	.....	.....	Negative.	.....	Negative.	.....	.....	.....	Negative.	.....
Piece of normal breast inoculated from diseased breast.	.....	.....	Negative.	.....	Negative.	.....	.....	.....	.....	.....
Piece from breast young woman inoculated from tumor mass.	.....	.....	Negative.	.....	Negative.	.....	.....	.....	.....	.....
Lymph gland. No. 21.	Feb. 15.	.....	.....	.....	.....	.....	Negative.	.....	Negative.	.....
Tumor mass. No. 21.	Feb. 15.	.....	.....	.....	.....	.....	.....	.....	Negative.	.....
Carcinoma stomach. Peritoneal fluid. Dr. M. H. Richardson.	Mar. 17.	.....	Negative.	Plain Bouillon and Agar made from a cystic breast. Negative.	.....	.....	.....	Negative.	.....	.....
Carcinoma breast. Periphery growth. Dr. Harrington.	July 14.	.....	.....	Negative.	Negative.	.....	.....	.....	.....	.....

All of the above cultures were observed for ten days or over longer periods of time and in their examination the condition of the medium was noted, microscopical examination of unstained and stained slides made, and portions of the inoculated material in some instances were hardened, cut, and stained for microscopical examination.

Contaminations.

In many of the cultures, especially those made in carcinoma bouillon and under anaërobic conditions, no microorganisms were seen. The microscopical examination simply showed disintegrating cells. In other cases, after a day or two, bacillus and coccus forms occurred similar to those usually found in contaminated cultures. Over long periods of time all cultures became contaminated. In one or two instances sub-cultures, from the original cultures showing microörganisms, were made on plates and tubes under aërobic and anaërobic conditions, but nothing which could be identified with the so-called carcinoma bodies seen in sections of hardened tissue was demonstrated.

Previous to this series of culture experiments, we had made in this laboratory a number of attempts to obtain a characteristic growth from carcinomatous tissue, but they were all negative.

The result of our investigations is, that in the cases recorded above we were unable to grow from carcinomatous tissue anything which could be regarded as a specific infecting organism.