patient, which is the main object of the sanatorium, and as a consequence of this the power to treat a larger number of patients. I may be permitted to conclude by expressing my thanks to the staff of the Brompton Hospital for their permission to publish the cases under their care and for their continuous encouragement and useful advice during the whole of my work.

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NOTE ON THE PRESENCE AND SIGNIFICANCE OF CERTAIN ROD-SHAPED BODIES IN THE CELLS OF CARCINOMATOUS TUMOURS.

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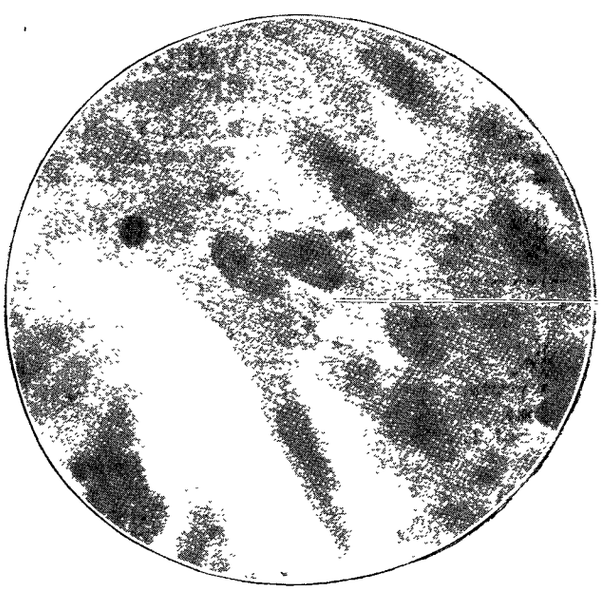
IN the course of investigations, of which some of the results have already been described,[1] I had occasion to stain sections of a carcinoma of the breast by the palladium methyl violet method, a staining process that has long been employed for nervous tissues in the laboratory of the Scottish asylums. The preparations obtained, besides serving for the purpose intended, revealed in the protoplasm of very many of the epithelial cells one or more rod-shaped bodies somewhat resembling tubercle bacilli. I have since applied the method to 36 tumours of various kinds and have found that similar rod-shaped bodies are constantly present in certain forms of carcinoma. I have observed them in all of ten carcinomata of the breast, in all of ten squamous epitheliomata, in both of two malignant adenomata of the sigmoid flexure, in a secondary cancer of the liver, in a cancer of the prostate, in a secondary cancerous growth in the dura mater, and in a tumour of the choroid composed of large epithelial cells. I have been unable to detect them in five adenomata of the breast, an adenoma of the cervix uteri, a papilloma, a uterine fibroid, a glioma of the brain, and a spindle-celled sarcoma, as well as in normal squamous epithelium, a mammary gland from a case of puerperal insanity, in a case of chronic mastitis, and in brain tissue.

FIG. 1.

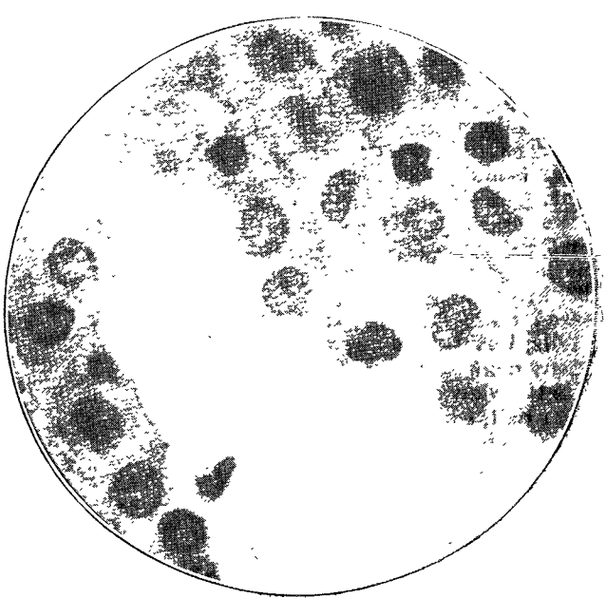


Section of a carcinoma of the breast showing three rods in protoplasm of epithelial cells. Palladium methyl violet method. × 800. The white line indicates the group of rods.

Whilst the most typical form of these bodies is that of a straight or slightly curved rod, closely resembling the tubercle bacillus (about 3μ in length and 0 3μ in thickness), much smaller and also much larger forms may frequently be observed in sections in which the bodies in question happen to be numerous. The smaller and medium-sized varieties usually stain evenly of a reddish-violet tint but the larger forms are generally distinctly granular in appearance and may sometimes present one or more pale or colourless transverse bars. The edges are always smooth. The ends are generally blunt and there is no evidence of the presence of flagella. These rods are not stained by Gram's method. In sections stained by the Ziehl Neelsen method for tubercle bacilli they are invisible. In sections stained with carbol thionin or methylene blue only some of the larger forms can be detected as faintly stained bodies, generally lying in a ground work of the same tint. The rods are most commonly to be seen in the protoplasm of the epithelial cells and they are to be found in largest numbers in the most rapidly growing parts of the tumour (Fig. 1). In the most successful preparations they appear as reddish-violet bodies lying in a pale yellow protoplasm. They are also very commonly, though less frequently, to be observed in the nuclei of the epithelial cells. Regarding their occurrence outside these cells little can be said, because the special staining method also colours the elastic fibres which are generally abundant in these tumours and which, especially if fragmented, cannot be distinguished absolutely from rods similar to those that occur in the protoplasm of the cells. In some special preparations in which elastic fibres have not complicated the picture the rods have appeared to be almost exclusively intracellular.

In five carcinomata of the breast I have found these intracellular rods to be present in very large numbers. In some preparations a hundred or more may readily be counted in a single field under an oil immersion lens. Single epithelial cells may occasionally be observed with from 20 to 30, or even a larger number, lying in their protoplasm (Fig. 2). In five other carcinomata of the breast in

FIG. 2.



Section of a carcinoma of the breast showing numerous rods in the protoplasm of an epithelial cell. Palladium methyl violet method. × 800. The white line indicates the group of rods.

which these rods have been detected they appear to be present in comparatively small numbers. I have found them to be numerous in only three squamous epitheliomata; in seven other tumours of this kind in which they have been clearly recognised they occur only occasionally. They have likewise been observed only in small numbers in the other carcinomatous tumours in which they have been detected.

So far as I have been able to ascertain attention has not previously been directed to these peculiar bodies, either by those who have described parasites in carcinomatous tumours or by others. The evidence which can be adduced in support of the view that they are parasitic in nature is, I think, conclusive. They present features which prove them to be growing organisms and they have been cultivated in an artificial medium. The possibility of their being bacilli can be excluded at once. It can be shown that they arise from comparatively large rounded bodies which are certainly not bacterial organisms. It is further to be noted that they have not the characters of the spirochæta microgyrata which has been described as occurring in carcinomata of the mouse.

My observations lead me to conclude that these rod-shaped

[1] THE LANCET, August 10th, 1907, p. 358.

bodies represent a stage in the life cycle of the protozoan organisms described by Dr. Henry Wade and myself as occurring in certain carcinomatous tumours. We had previously observed such rods in the old silver-gold preparations but never in large numbers, and we were unable to attach any significance to them. In sections prepared by the improved ammonia-silver process and decolourised by cyanide these rod-shaped bodies tend to retain the black deposit for a short period subsequent to their development; at a later stage they are much more readily bleached by the cyanide. These preparations reveal in the protoplasm of many of the epithelial cells, especially in carcinatoma of the breast, more or less numerous spherical or oval bodies which were described in previous papers, and from these bodies the rods can be seen to originate. In palladium methyl violet preparations these globular bodies can also be recognised, often appearing distinctly nucleated, and various stages in their transformation into rods can easily be observed. The evidence of the histological preparations upon this point is confirmed by that derived from an agar culture from a secondarily infected gland in a case of malignant adenoma of the intestine, described by Dr. Wade and myself. This culture contains in the substance of the agar numerous spherical bodies with the staining reactions of those demonstrable in the protoplasm of some carcinoma cells, and the study of preparations of this culture stained by the methyl violet method has revealed the fact that these spheres become transformed into rod-like bodies identical with those that can be seen in the tissues. In a future paper I hope to deal fully with the subject of the life cycle of these protozoan organisms. If these rods represent merely a single phase in a complicated life cycle, it should be easy to understand how in many carcinomatous tumours they can be found only in small numbers whilst in other tumours of the same kind they are abundant. It seems to me probable from evidence collected that not one species of protozoan organism but several closely allied species are the pathogenic agents in the production of carcinomatous tumours. It at least appears that the forms found in carcinoma of the breast present certain characters which distinguish them from those that may be observed in squamous epitheliomata and in intestinal tumours, although the life cycles are essentially the same.

I have endeavoured to ascertain what becomes of the rods, but as yet only a few facts have come to light. At first it seemed probable that these bodies are motile forms which escape from the cell in which they have originated and which, after travelling some distance, infect other cells, but this hypothesis on being tested has failed to obtain any confirmation. The evidence, indeed, very strongly supports the view that the rods, instead of tending to escape from the cell, seek to penetrate the nuclear membrane and that they undergo a further evolution within the substance of the nucleus. Not only may the rods frequently be seen to abut upon the nuclear membrane, but they may sometimes be observed to have partially penetrated it, either by movement or by means of end-growth. The occurrence of characteristic rods wholly within a nucleus is quite common and occasionally several may be seen in one nucleus.

In conclusion, I would say that if pathologists will apply the palladium methyl violet method to a few properly fixed and somewhat recently obtained carcinomata of the breast and squamous epitheliomata, I am confident that they will quickly be able to confirm and to extend these observations which I have here briefly recorded. I have to express my indebtedness to Mr. F. M. Caird, Mr. David Wallace, and Dr. M. B. Hannay for most of the tissues used in these investigations.

APPENDIX.

The palladium methyl violet method.—The reagents required are saturated solution of palladium chloride in 1 per cent. citric acid in water, 1 per cent. solution of methyl violet 6 B or 5 B, saturated solution of iodine in 2·5 per cent. potassium iodide, equal parts of turpentine and benzole, equal parts of pure anhydrous aniline oil and benzole, benzole and benzole balsam. The tissues should be fixed in 5 per cent. formalin in ¾ per cent. salt solution (and preserved in the same fluid or in alcohol), or for 24 hours in Heidenhain's sublimate solution, with subsequent removal of the mercury by means of iodine in the usual way. Cut thin sections by the dextrine freezing method. Place the sections overnight in the palladium solution. Wash them in three changes of water and then place them for from 10 to 20 minutes in the methyl violet stain. Wash the sections shortly in water and transfer them to the iodine solution, in which they should remain for from 10 to 20 minutes. Next transfer the sections to a bowl of water. In this they may be left for an hour or longer without suffering harm. Steel needles must not be used in these operations. Take a section up from the water upon a perfectly clean slide. Carefully remove water from around it by means of a towel. Next lay the slide upon the table and with a piece of smooth blotting or filter paper (folded double) blot the section in the same manner as one dries a sheet of wet manuscript. Immediately afterwards, without allowing it to dry completely in air, pour over the section some drops of a mixture of equal parts of turpentine and benzole. Renew this turpentine-benzole after a few seconds and then place the slide upon the heater (described below), where it must remain at a temperature of about 60° C. until completely dehydrated. If the turpentine-benzole tends to evaporate off the section add more by means of a pipette. When dehydration is complete the previously black and opaque tissue assumes a dark blue and faintly translucent appearance. Generally from 15 to 20 minutes are required. When the section seems dehydrated remove the slide from the heater, allow it to cool, and then pour off the turpentine-benzole. Decolourise with aniline-benzole. Renew this two or three times. Avoid breathing on the slide as the smallest trace of moisture in the aniline-benzole will cause complete decolourisation of the section. When the dye ceases to come away wash the section in several changes of pure benzole and mount in balsam in benzole. It is essential that the section should be completely dehydrated on the heater. Any spot in which moisture has been allowed to remain will be decolourised by the aniline-benzole. A heating apparatus of a very simple form is sufficient for the purposes of this method. I use a small spirit lamp placed below a tripod stand, on the top of which there is a thin metal plate, and upon this again two small iron bars laid parallel to each other and at such a distance as just to allow the two ends of a microscopic slide to rest upon them. By such an arrangement heat is transmitted only by the two ends of the slide and the turpentine benzole is driven to the centre. Tissues that have been in alcohol or in formalin for over two years do not, as a rule, stain deeply enough, and must then be regarded as unsuitable for the application of this method.

ON THE RELATIONSHIP OF CANCER CELLS TO THE DEVELOPMENT OF CANCER.

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AND

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IN the present communication we wish to record some observations made in the Cancer Research Laboratories, University of Liverpool. The observations in question relate to the propagation of cancer in mice. The tumours utilised are derived from a growth originating sporadically in a mouse, and most generously placed at the disposal of the Liverpool Cancer Research Committee by Professor Ehrlich of Berlin. The tumours upon which these observations have been made are of exceptional virulence. They are graftable from one mouse to another—that is to say, if small fragments of the growth be removed from an animal these fragments will grow when placed under the skin of a healthy individual. So far as can be ascertained from purely cytological examination of the process, the new tumour in inoculated mice appears to proceed directly from the cells belonging to the original tumour which have been mechanically transferred. The grafted tumour, in fact, apparently arises from the implanted cells, and not through any alteration of the tissues of the new host which surround the graft.

For purposes of investigation portions of these tumours were removed from mice and subjected for periods of from