PRELIMINARY NOTE ON SOME PARASITIC PROTOZOA FOUND IN CANCEROUS TUMOURS.

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THE disease designated under the general term of carcinoma has all the appearances of being caused by a living contagium. A carcinomatous tumour begins in a small group of cells, invades the nearest tissues, gradually infects the lymphatic glands and the inner organs, and finally, should the patient only live long enough, extends to every part of the body. Like all living contagia, cancer chooses its soil, avoids certain people, certain races even, and attacks others who, though apparently in perfect health, fall an easy prey to the disease. True, it is very rare to hear of a cancerous person infecting another; but this objection may be met with the answer, that the same has been asserted of diseases which are obviously contagious—such as tubercle—simply because those who have maintained that fact have formed their opinion on the unsafe evidence of clinical experience, instead of taking into consideration the accurate data furnished by experiment. Even were it proved that cancer does not spread by direct contact from one person to another, yet there are many facts to be gathered in medical literature, proving that a cancerous patient may infect one part of his body from another part. It was only natural that when bacteriology became the fashion a diligent search should have been made for micro-organisms in carcinoma; and though some observers described such organisms, it was conclusively proved by the accurate and painstaking researches of Ballance and Shattock that, in properly conducted experiments, carcinomata contain no vegetable parasites whatever.

Foiled in their first attempts, pathologists sought for the cause of cancer in some other organisms. Many endeavoured to settle the question by the direct inoculation of pieces of cancer into animals; but this method proved useless, and in our opinion is not calculated to lead to the discovery of the cause of cancer. Others diligently searched for them with the microscope, and a large number of papers have already appeared on the subject. The researches of Russell and Hutchinson in England; of Thoma, Steinhaus, Podissowsky, and Sawtchuko, Sjöbring, van Heukelom, and Noeggerath, in Germany; of Welch in the United States; and of Malassez, Darier, Wickham, Borel, and Sudakewitch in France, were mainly concerned in proving or disproving the presence of coccidia-like parasites in carcinomata of man. These papers we shall discuss when we publish our investigations in full; but we would state that it appears clear to us that most of these observers have failed to distinguish the parasites from other bodies enclosed in cancer cells, and that Sudakewitch was the first who clearly demonstrated the presence of parasites in carcinoma.

In the early part of this year one of us (R.) observed in carcinoma bodies which he had every reason to think were parasitic. Since that time we have been constantly investigating this question, and in May one of us had the opportunity of showing our preparations to Professor Metschnikoff, of the Institut Pasteur. This eminent zoologist at once agreed with us as to the parasitic nature of these bodies, and urged us to publish our researches. Indeed he made this task a comparatively easy one, as he placed at our disposal his skill as a zoologist, and kindly drew many beautiful illustrations from our preparations. We may perhaps be allowed here to offer him our very best thanks for this and many other marks of interest in our work.

The demonstration of the parasitic bodies in cancer pre-

sents but few difficulties. Pieces of carcinomata are plunged whilst still warm into the usual reagents, and carefully hardened according to the ordinary histological rules. We have used for this purpose a concentrated solution of corrosive sublimate, absolute alcohol, osmic acid 1 per cent., Fleming's solution, Müller's fluid, etc. The parasites may be demonstrated in carcinomata hardened by any of these methods, but our best results were obtained with Fleming's solution and absolute alcohol. The pieces (not larger than $\frac{1}{4}$ -inch at most) remain in Fleming's solution from 2 to 3 days, and are then washed in running water for twenty-four hours at least. They may then remain in absolute alcohol for an indefinite time, and when ready to be examined, must be saturated with paraffin according to the Naples method.

The parasites have a strong affinity for aniline dyes, and beautiful results are obtained by staining the tissue with a nuclear dye and counterstaining with aniline dyes. We have used for this purpose acid fuchsin, orange, benzo-purpurine, methyl-blue, methyl-violet, rose-bengale, eosin, and Biondi's reagents, and have also made use of Bizzozzero's method. With carcinomata hardened in alcohol, the best results were obtained by staining with Biondi's reagent; whilst specimens hardened in Fleming's solution showed the parasites best when first stained in a watery solution of methyl-green, and counterstained with Biondi's reagent, or by using logwood in the first place, and afterwards a 1 per cent. solution of rose-bengale.

We were not at first able to find parasites in all cases of carcinoma, but this was simply due to our then deficient technical knowledge. With improved methods of staining we have never failed to demonstrate these bodies in the numerous carcinomata of the breast, tongue, intestine, stomach, liver, rectum, bile-ducts, and epiglottis which the staffs at the Cancer, University College, and Middlesex Hospitals have kindly placed at our disposal. Our best thanks are due to Mr. J. W. Hulke, Mr. J. B. Jessett, Dr. Voelcker, Dr. Wethered, Mr. J. B. M. White, and Mr. Cooper, for providing us with the material on which our researches are based. We may at once state that we have been unable to demonstrate the parasites in normal or other pathological tissues, and that, so far, our efforts to cultivate these bodies, although not uninteresting, have not been successful.

The parasite consists of a central nucleus (Fig. 1), that is, of

Fig. 1.—Epithelial cell containing a parasite, with clear cyst wall and nucleus well defined; below this is the nucleus of the epithelial cell. The divergent rays from the nucleus of the parasite are not shown.

a central part which, according to the reagent used, takes up the stain more deeply than the remainder of the body, or else stains quite differently from it. This nucleus apparently does not possess a distinct membrane. From certain staining reactions it appears that the substance forming the nucleus resembles in its chemical reactions the nucleolus of the invaded cell, though there are certain points of difference between them. Around the nucleus the protoplasm is condensed (?) and from this centre extremely fine, delicate rays often extend to the periphery of the organism (Fig. 1). One might then think that the parasite consisted of the nucleus and central mass of protoplasm, furnished with long pseudopodia; but careful observation shows that these rays terminate at the periphery, and are continuous with the rounded outline of the parasite. We would suggest that some of these fine rays are simply an effect of the hardening reagents.

Scattered through the protoplasm, especially at the periphery, where they are sometimes arranged in a kind of ring, small, dark coloured particles are found, varying from four to twenty or more in number, and sometimes connected with each other by a delicate network (Fig. 2). We must confess that we

Fig. 2.—Two epithelial cells containing parasites; the cells are distended, and nuclei pressed against the cell wall. The parasite to the right has not taken up the stain, but shows the coloured particles often found.

are unable to give an explanation of these appearances, though we think they may in some way be concerned with the reproduction of the parasite. These particles must be carefully distinguished from the pigment granules, which are often found scattered in the parasite. In some preparations the nucleus gradually disappears, and the whole body takes a delicately mottled, greyish appearance. In others this mottled appear-

Fig. 3.—Epithelial cell containing several parasites.

ance is not visible, but the whole parasite seems to consist of a hard, white, highly refracting mass, which takes up no colouring matter (Fig. 2).

Around the parasite, but separate from it, there is a distinct double coloured wall, which stains far more deeply than the remainder of the cell, and which is probably formed from

the protoplasm of the invaded cell (Fig. 1). It is never absent, but can be demonstrated best in sections hardened in Fleming's solution, and stained with methyl-green and Biondi's reagent.

The size of the parasites varies to a great extent with that of the containing cell; but as the parasite grows, it distends its host so that the latter may become two or three times its original size. One epithelial cell may contain several parasites (Fig. 3); at the time of writing we have one under our

Fig. 4.—Several epithelial cells have here fused together, their contained parasites agglomerating into one cyst.

observation which holds seven of these parasites in its interior, each with its nucleus, surrounding protoplasm, and cyst wall. In some cells, though rarely, two parasites are close together, and are contained in one capsule, with the remains of a division between them; but in others several parasites are found in the same capsule. Moreover, it is not rare for several epithelial cells to fuse together, and again, for their contained parasites to get agglomerated in one cyst (Fig. 4). The resulting picture is at first utterly puzzling, and the problem is only to be solved by carefully observing the development of such cells in various sections.

Fig. 5.—Epithelial cell greatly distended, with its nucleus pressed and flattened against cell wall. It contains two parasites.

The parasites are contained in the protoplasm of the cell, but as they grow they displace its nucleus, and flatten it against the side (Fig. 2). At the same time, the protoplasm and nucleus of the epithelial cell undergo more especially degenerative changes, as shown by their reactions when in presence of staining reagents; but the nucleus of the invaded

cell remains for a long time absolutely normal. When degeneration is well advanced, the nucleus is converted into a dirty greyish mass, which, instead of standing out sharply from the cell, fuses into it by almost imperceptible gradations. (Fig. 6.)

The parasites are present in the cancerous epithelial cells only, and we have failed to discover them in the fibrous tissue surrounding the epithelial cells in the blood vessels or lymphatics. It is not improbable, therefore, that when outside the epithelial cells the parasites assume another form (spores), but at present we have been unable to demonstrate these to our satisfaction. The parasites do not invade the epithelial cells of the surrounding normal tissue; in the liver, for instance, the normal or degenerated liver cells never contain them. It is a noteworthy fact that they are found in the greatest number near the growing edge of the

Fig. 6.—Epithelial cells containing parasites undergoing degeneration. *a*. The parasites.

tumour, and although it is extremely rare to find a cell containing a parasite and undergoing indirect division, yet many of the surrounding cells undergo division. Here may be seen most beautiful and instructive examples of the atypical division of cancer cells described by Hanseemann; and, in fact, a special paper would be necessary to describe all the abnormal forms of division seen in our preparations. It would appear, therefore, that the presence of a parasite in an epithelial cell leads to the decay of that cell, and to the active proliferation of the neighbouring ones.

Without stopping to describe the phagocytic processes taking place in the fibrous tissues surrounding the tumour, we may come at once to the action of leucocytes in cancerous tumours. It has been known for some considerable time that leucocytes penetrate into epithelial cells, and we need hardly

Fig. 7.—Epithelial cells containing parasites undergoing degeneration. *a*. The parasites.

remind our readers of the elaborate theories framed by Creighton and Klebs, and based on their presence in such situations; but we may remark that our observations give no support to these theories.

These leucocytes belong to the small variety, possess a hard deeply-staining nucleus, and—thanks to the special method of staining adopted in our work—they are sharply differenti-

ated both from the epithelial cells and from the parasites contained in them. Now it is evident that the leucocytes enter in considerable numbers, chiefly, if not exclusively, into those epithelial cells which contain parasites. Moreover, it is at once apparent that the parasites so attacked gradually disintegrate under the influence of the ameboid cells, and it is a most instructive sight to see a parasite attacked from every side by three, four, or more leucocytes. After a time these wander into the interior of the parasite, and gradually destroy it. We had the pleasure of having this fact confirmed by the pioneer of phagocytosis, Dr. Metschnikoff.

Before ending this short account of our work, we must add a few words concerning some appearances found in cancers. It was suggested that colloid and other degenerations might simulate the parasites just described; accordingly, we examined several colloid cancers, and degenerated parts of other carcinomata; and we have no hesitation in saying that the parasitic protozoa cannot be mistaken for any kind of degeneration whatever, provided the sections be well hardened and stained. In sections in which both parasites and degenerated cells are present, the difference between the two at once strikes the observer; and, moreover, it is extremely rare to find parasites in the neighbourhood of degenerated parts. In many carcinomata some epithelial cells are invaginated into others, and there is no doubt that such cells often undergo degeneration, and have been mistaken for parasites. We are convinced, however, that those who will use aniline dyes will have no difficulty in diagnosing one from the other, as their staining reactions are absolutely different. In some preparations, indeed, we saw a parasite in the interior of an invaginated cell, but in these cases the external cell, the invaginated cell, and the parasite were sharply differentiated from each other.

These researches were carried out at the conjoint laboratories of the Royal College of Physicians (London) and Royal College of Surgeons (England). It is our pleasant duty to thank the Committee of the Laboratories for the facilities afforded us for carrying on the inquiry, and the Director, Dr. G. Sims Woodhead, for his judicious advice and criticism.

M. GRANCHER was elected a Member of the French Académie de Médecine on July 12th.

AN International Medico-Legal Congress will be held during the Chicago "Exposition" next year.

THE fourteenth session of the Congress of Scandinavian Naturalists was held in Scandinavia from July 4th to July 9th. It was attended by 560 persons, of whom 130 were Swedes, 77 Norwegians, 18 Finlanders, and 335 Danes. The four sections of the Congress devoted to medical matters were medicine, surgery, anatomy and physiology, and military hygiene.

CONGRESS ON TUBERCULOSIS.—The third Congress on Tuberculosis will be held in Paris at the end of July, 1893. The following are the subjects for discussion at present on the programme: (1) The part played by contagion and heredity respectively in the propagation of tuberculosis; (2) infectious diseases as determining factors of tuberculosis. The part played by certain of these in the localisation of tubercle; for example, that of gonorrhœa in the development of tuberculosis of the testicle, of influenza in the onset or aggravation of pulmonary tubercle, etc.; (3) periods of remission in tuberculosis; their duration, the means of recognising them, and of foreseeing their termination; causes of relapse; (4) various means of diagnosing bovine tuberculosis, particularly the determination of the question whether the inoculation of tuberculin affords a certain basis for the diagnosis of tuberculosis in bovine animals; (5) dangers that may possibly arise from the burial of tuberculous bodies; expediency of substituting cremation for earth burial; necessity of destroying tubercle bacilli in corpses; (6) new methods of prophylactic and curative treating tuberculosis based on its etiology; (7) utility of making the inspection of meat general. A prize of 3,000 francs (£120) will be awarded to the author of the best essay on "The Means of Diagnosing Latent Tuberculosis before its Appearance or after its Cure." Essays, which must be in French, and which must not have been previously published, should be sent before April 1st, 1893, to Dr. L. H. Petit, 76, Rue de Seine, Paris.