Further researches on some parasitic protozoa found in cancerous tumours. [Pt. 1] / by M. Armand Ruffer and H.G. Plimmer.

FURTHER RESEARCHES ON SOME PARASITIC PROTOZOA FOUND IN CANCEROUS TUMOURS.

By M. Armand Ruffer and H. G. Plimmer.

(Plate XXIII.)

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Section A.

In a former paper, published in this *Journal*[1] by one of us (M. A. R.), in connection with Mr. J. Herbert Walker, a short account was given of the work which had already been published on this subject. We think it unnecessary, therefore, to go over this ground again, deeming it sufficient here to mention the observations which we have been able to find in the various medical journals issued during the last five months.

Soudakewitch[2] has published a second paper, in which he maintains and extends his former conclusions. He describes and figures a large number of bodies discovered by him in cancer, and which he regards as intracellular parasites. Moreover, at the end of his work, he refers to the mode in which he thinks parasites may act as etiological factors in cancer. We must confess, however, that our observations do not warrant us, for the present, in accepting all Soudakewitch's conclusions. With regard to the figures which illustrate this highly interesting paper, we venture to doubt whether all of them really refer to parasites. At any rate, we would state it as our very definite opinion that the figures (Plate XII., Figs. 5, 18, 22) described as falciform bodies are not true falciform bodies, *i.e.* spores. Fig. 5 refers, in our opinion, to the chromatin bodies often met with in parasites, whereas Figs. 18 and 22 are not of a parasitic nature at all. They resemble in many particulars some of the bodies described by Podwyssozki and Sawtschenko, and we shall have an opportunity of discussing them again. We notice that so competent

[1] M. Armand Ruffer and J. Herbert Walker, "On some Parasitic Protozoa found in Cancerous Tumours," *Journal of Pathology and Bacteriology*, vol. i., 1892, p. 198.

[2] Soudakewitch, "Parasitisme intracellulaire des néoplasies cancéreuses," *Annales de l'Institut Pasteur*, tome vi., 1892, p. 545.

an observer as Metchnikoff[1] appears to be of the same opinion as ourselves, for he states that he has been unable to find evidence of real falciform bodies in the preparations submitted to him.

In *Virchow's Archiv*, V. Müller[2] criticises our researches, and boldly asserts that the bodies described by Ruffer and Walker were simply due to the endogenous formation of cells. It must be confessed that Müller's statement would carry more weight had he read the full paper, instead of limiting himself to reading the short preliminary note, with necessarily imperfect illustrations. He would have seen that, in the later work, the authors directed special attention to the differential diagnosis between these parasites and cells multiplying by endogenous division, even going so far as to depict some of these causes of error. The plates accompanying the paper would have shown him that the staining reactions of the parasites and of these endogenously-formed cells are quite different. As we find, however, that other observers in England appear to accept some of Müller's conclusions, we shall discuss this point at length later on, where we shall show that the nucleus of the parasite does not contain chromatin.

In the same number of *Virchow's Archiv* we find a paper on Cancer of the Uterus by W. Kürsteiner,[3] in which this observer, without committing himself to an opinion, depicts bodies which, we have little doubt, are of a parasitic nature; whilst in the same periodical, Eugène Burchardt[4] has described coccidia in a colloid cancer. We shall have to refer again to his valuable observations.

In the *British Medical Journal*, Metchnikoff[5] gives a short *résumé* of the work done on coccidia and disease, and confirms our observations, whilst one of us[6] (H. G. P.) contributed in the same number a short account of his independent investigations. In the same journal a communication had shortly before been made by one of us (M. A. R.),[7] in which the intranuclear stages of the parasite were described.

At the time of writing we receive a paper by Dr. Joseph Coats,[8] in which this observer sums up his opinion as follows:—"The existence

[1] E. Metchnikoff, "Remarks on Carcinomata and Coccidia," *Brit. Med. Journ.*, 10th Dec. 1892.

[2] V. Müller, "Ueber Cellulaire Vorgänge in Geschwülsten" (Aus der pathologischen Anstalt in Heidelberg), *Virchow's Archiv*, 8th December 1892, Bd. cxxx., s. 512.

[3] W. Kürsteiner, "Beiträge zur Pathol. Anatomie der Papilloma und papillomatosus Krebs von Harnblase und Uterus," *Ibid.*, s. 463.

[4] Eugène Burchardt, "Ueber ein Coccidium im Schleimkrebs des Menschen u. seine Dauersporencyste," *Virchow's Archiv*, 2nd January 1893, Bd. cxxxi., p. 121.

[5] Metchnikoff, *loc. cit.*

[6] Plimmer, "A Note on the Parasitic Protozoa lately found in Cancer," *Brit. Med. Journ.*, 10th Dec. 1892.

[7] Ruffer, "Second Note on Parasitic Protozoa in Cancerous Tumours," *Brit. Med. Journ.*, 5th Nov. 1892.

[8] Joseph Coats, "An Address on Certain Considerations in regard to the Infective Nature of Cancer," *Brit. Med. Journ.*, 14th Jan. 1893.

of the bodies described, and the accuracy of the descriptions, cannot be denied, but the proof that they are parasites has by no means been furnished." There are several other points in Dr. Joseph Coats' interesting contribution, to which we shall have to refer as we proceed with this paper.

Our investigations have been made on various carcinomata of the tongue, skin, thyroid gland, omentum, peritoneum, uterus, vulva, and on cases of cancer of the breast in women, taken without distinction from forty-five consecutive amputations of the breast at the Cancer Hospital, Brompton, two from University College Hospital, and two from private operations. The reason why we limited ourselves after a time to carcinoma of the breast is, that we soon found it would be a thankless task to spend our time in examining cancers from various parts of the body, in order to trace the life-history of a parasite. It appeared to us to be evident, judging from our own investigations, that these parasites can be demonstrated, by appropriate means, in all cancers. Now it is quite possible that the various cancers of different organs may contain parasites differing in kind, etc., and we thought it would be preferable, therefore, to try and trace the life-history of the protozoon in one kind of tumour of one organ, in which we might reasonably suppose that we had always to do with the same parasitic organisms. The reasons which decided us in choosing cancer of the breast for our investigations are, first, its relative frequency, and, secondly, because, on account of its fairly large size, and its not being usually in free communication with the open air, it is easy to remove parts of it aseptically for cultivation and other experiments. Moreover, we soon found that carcinoma of the breast generally contains these parasites in large numbers, thus giving investigators a good field for studying the life-history of the protozoa.

For fixing purposes we have entirely given up alcohol alone, as the results, although occasionally very good, are uncertain. In some cases, indeed, we were unable to find parasites in alcohol specimens, when the examination of parts of the same cancer fixed in other media revealed them in large numbers. Osmic acid, 1 per cent., and the solution first recommended by Foà, namely, equal parts of saturated solution of corrosive sublimate in ·75 per cent. salt solution, and of 5 per cent. solution of potassium bichromate, gave excellent results. The osmic acid preparations, stained with eosin and hæmatoxylin, were extremely instructive, whilst those fixed with Foà's solution stained fairly well with Biondi's reagent, or with eosin and aniline blue.

For purposes of fixation we have also used, with striking results, a solution of chromic acid and spirit, which was first recommended, we believe, by Dr. E. Klein for the study of karyokinetic figures. The various staining reagents used will be discussed when we speak of the diagnosis of these protozoa. For the same reason that we found ourselves obliged to limit our studies to cancer of the breast, we must for

the present refrain from describing all the appearances presented by parasites in the cancers of other organs examined by us. Were we to do so, we should simply add a number of figures to our paper, which, however accurately photographed or drawn, would add little to our knowledge of the life-history of protozoa, and simply increase the present confusion. We must defer these descriptions until we have been able to examine a large number of malignant tumours of other organs. We would, however, draw attention to the following facts, which may throw some light on controversial points:—

In the first place, we have examined carefully another[1] colloid cancer of the omentum and peritoneum, in order to ascertain whether the colloid degenerated cells resemble in any way the parasites described by us. Two remarkable facts were soon observed, firstly, that not in a single preparation could we find the slightest resemblance between a colloid cell and a parasite; and secondly, that the parasites themselves were never found in colloid cells, but always at the periphery, in healthy active parts of the tumour, where a number of healthy cancer cells surrounded them. E. Burchardt[2] says, in his paper on Colloid Cancer, that the parasitic cysts were "only to be found exceptionally in colloid cells. I have only seen this appearance three times, and, so far, this observation is not without value, as it shows that the cysts, as such, do not play a part in the formation of mucus"—a conclusion which we would not adopt straightway. In a cancer of the skin, which had grown deeply into the thyroid gland, we had occasion to compare the parasites with the normal colloid material of the gland, and also with the same material altered pathologically by the growth of the tumour. Nowhere did we find the slightest resemblance between the parasites and the colloid cells, or the matter lying between the cells or the spaces in the colloid material. The differences in staining reactions and in other characters never left us in doubt as to the true nature of every one of these structures.

With regard to epitheliomata of skin, tongue, and vulva, we would draw attention to the fact that, by using various methods, we have been able to demonstrate the presence of parasites in all. It is a remarkable fact, however, that in this form of tumour the parasites occur in large groups more often than in the other forms of cancerous tumours. One may cut many sections with a fruitless result, and then suddenly come across a field in which numberless parasites are present. This was especially the case in a rapidly growing epithelioma of the back removed from a young man by Mr. F. B. Jessett[3] of the Cancer Hospital. Here, in some fields, the cells contained numberless parasites, in groups of

[1] See also Armand Ruffer and Walker, *loc. cit.*

[2] E. Burchardt, *loc. cit.*, p. 127.

[3] We take this opportunity of again expressing our best thanks to the surgeons of the Cancer Hospital for the invaluable help they have given us in our investigations. We would especially express our gratitude to their able pathologist Mr. Fearnley, and to Mr. Le Quesne, their courteous resident medical officer.

from two to ten, or more; and yet in another section hardly a single specimen could be found.

In carcinoma of the body of the uterus the parasites were also seen, and as far as we could judge from the somewhat scanty material at our disposal they resembled those found in other cancerous tumours of other parts of the body.

We may now proceed to discuss some of the appearances which we have observed in carcinoma of the female and male breast, but we again beg to point out that we have no wish to describe here all the appearances we have seen, but only such as will help others in easily recognising them, and will illustrate the particular stage of the life-history of the parasite of which we wish at present to treat. The description of each form will, as far as possible, be given as we proceed to discuss its physiological meaning.

The most likely place to find the parasites is in a section passing through or near the growing edge of the cancer, *e.g.* its axillary border. Care must be taken that the parts selected be really from the *growing* edge of the tumour, for, as has been shown before, part of the periphery may already be encapsuled or extensively infiltrated with leucocytes, and thus the search may not be successful. The drawings represented in Plate XXIII., for instance, were all taken from the extreme edge of the cancer, that is from the actual growing edge of the tumour as it extends into the surrounding fat. Needless to say, the cancer cells in such a situation are young, extremely active, and by no means degenerated, and karyokinetic figures may be seen in almost every field.

At the periphery the parasites may be single or double, but as one proceeds inwards one may see, at no great distance from the periphery, whole groups of such parasites of different sizes and shapes. Fig. 8, Plate XXIII., is an exact representation of one of these groups, the cell containing eleven such parasites; we have, however, come across some cells containing 32 or more, but in such cases a process of multiplication, to be presently described, had taken place. The intranuclear forms also often become common now, whilst the various stages between the intranuclear and extranuclear forms may also be observed. As one proceeds towards the older parts of the tumour, the parasites gradually disappear, so that in the real fibrous part, or in the degenerated areas, none are met with. Near such parts it is not rare, however, to find a cell which has been converted into a mere bag by the numerous parasites contained in it. In one case, indeed, we saw a group of such parasites partly in and partly outside the cell, and, occasionally, some are found lying free.

We have had an opportunity of examining two breasts affected with Paget's disease of the nipple. In both, the parasites, though not found in the eczematous part itself, were present in the actual breast tumour. The first deserves special mention, as it is interesting from many points of view.

The case was that of a middle-aged woman who had suffered from this affection for some time. The eczema was well marked, but clinically no tumour could be found in the breast itself. Nevertheless, the surgeon, Mr. F. A. Purcell of the Cancer Hospital, determined to remove it; the result proved the wisdom of this course. On dissecting it immediately after its removal we found a thin strand of dense fibrous tissue, extending for about three-quarters of an inch into the breast; this led to a rather soft tumour, not much larger than a small bean. The whole was carefully hardened, and sections made, in which the following interesting appearances were demonstrated. The nipple itself showed all the lesions usually found in this disease, but no parasites. In the fibrous tissue connecting the small tumour with the nipple, no parasites and no epithelial cells were met with until one came quite near to the tumour, when epithelial cells became visible here and there. The tumour proved to be a true scirrhus of the mamma, containing perhaps an excess of fibrous tissue. Here and there parasites began to appear as the section approached the centre, but the distal and youngest part of the tumour contained numerous parasites, and a correspondingly increasing number of cancer cells. As we shall see further on, these parasites differed absolutely from those described in Paget's disease by Wickham, Darier, and Malassez.

The number of parasites contained in a section varies not only with the part chosen for examination, but more especially with the case from which the tumour is taken. Some tumours are singularly poor in parasites, so that several fields may have to be examined before one is found even at the growing edge of the cancer, whilst in others almost every field, of a section not too far removed from the growing edge, contains them in abundance. The four cases in which we found them in the largest number were—

1. A carcinoma of the breast, very rich in cancer cells, removed by Mr. Heath from the breast of a middle-aged woman. The tumour was nearly the size of a fist, and appeared to have been only 4 months in attaining that size. This proved to be quite a mine of parasitic wealth.

2. Another tumour, greatly resembling the former, also from the breast of a middle-aged woman.

3. A tumour which had recurred for the fifth time in the cicatrix of the primary operation within three years.

4. A recurrent tumour of the breast from a woman in whom both breasts had previously been removed for carcinoma.

Generally speaking, we found that the faster the growth of the tumour appeared to be, as determined by its clinical and anatomical characters, the larger was the number of parasites.

It is not within the scope of this part of the paper to discuss the changes which take place in the cancer cells containing parasites in their interior; we may, however, remark at once that, in contradic-

tion to some authors, we cannot in anyway confirm the statement that the presence of parasites in cancer cells causes the latter to divide by karyokinesis. On the contrary, our investigations have shown us that whereas the cells in the neighbourhood of an infected cell are very often in a state of active division, the infected cell itself is, as a rule, perfectly passive as far as division is concerned.

In the first paper published by Ruffer and Walker, attention was drawn to the fact that leucocytes not infrequently invade the parasitic cysts and attack the parasites. In the breast, however, such a phenomenon is rarely to be observed. On the other hand, although we are unable to give a reason for this fact, we have observed this phenomenon again and again in abdominal cancers, more especially in metastatic growths in internal organs.

The same remark which we have made concerning karyokinesis occurring in infected cancer cells applies to other changes often found in such cells, and also attributed to the presence of parasites in them. We shall have an opportunity of showing, later on, that such changes (hypertrophy, hyperchromatosis, etc.) are often quite as common (if not more common) in non-infected as in infected cells.

Let us now turn to one of the phases of the life-history of the parasite during its existence in a cancer cell. The particular form of the parasite to which we wish to draw attention in this section makes its first appearance in the nucleus of the cell as a small, round body, staining well with (Plate XXIII., Fig. 2, *a*) various dyes. In specimens fixed with Foà's solution, and stained with Biondi's triple stain, it appears as a reddish body, but in all it can be seen that this body is absolutely independent of the other part of the nucleus. In other words, it is not *part of the nucleus,* but it is a *foreign body lying in the nucleus.* The nucleus in infected cells generally resents this invasion by becoming darker and smaller, more condensed so to speak. The chromatin network of the nucleus disappears, whilst its staining reactions also change. With hæmatoxylin it becomes much darker, whilst with the triple stain it assumes a dirty, greenish-brown homogeneous colour. At other times, however, the nucleus does not apparently greatly resent the presence of the parasite. The latter may be single or double, or a whole cluster may be present in the same nucleus.

The next change which takes place is that part, or the whole of the parasite, becomes clearer and lighter (Plate XXIII., Figs. 1, 2, 3), whilst in a still further stage a tiny nucleus makes its appearance in the centre (Plate XXIII., Figs. 1, 4), and at this time a distinct capsule can be made out around the parasite. The nucleus of one cancer cell may thus be invaded by a number of minute parasites, each one with its little nucleus, protoplasm, and capsule.

In other cases, especially when the parasite is single, it may acquire a goodly size (see Plate XXIII., Figs. 5, 6), and become fully developed

before it leaves the nucleus. Whether single or multiple, however, the parasites approach the periphery of the nucleus and escape into the protoplasm of the cell. When multiple, the wall of the nucleus actually gives way, and the contents are discharged. Fig. 6, Plate XXIII., is a photograph of a nucleus from which the contents are escaping into the protoplasm of the cell. It contains four fairly large parasites, and it is evident that its upper wall is on the point of giving way. Such appearances are best studied in osmic acid preparations.

We do not mean to imply that whenever the nucleus of a cancer cell contains a large number of young parasites, these are all formed from different parents, for, as we shall see in another section, there is a process of multiplication to be observed in the nucleus which, in some cases, does account for the number of the inclusions.

The young or adult parasites now assume the form of what may be called the normal, full-grown protozoon, which is found in carcinoma of the breast. It consists essentially—

1. Of a central, round, oval, or slightly irregular nucleus, sometimes connected by fine, delicate rays with the periphery ;

2. Of a variable amount of surrounding protoplasm, almost, if not quite, filling up the capsule ; and

3. Of the double contoured capsule surrounding the whole.

Other appearances seen in such parasites will be discussed later.

When one of the parasites has found its way into the protoplasm of a cell, it generally grows until it may become so large as to push the nucleus on one side, so that this latter atrophies. In this respect we have nothing to add to what has been stated by one of us (M. A. R.) in a former paper.

It is also a remarkable fact that when the nucleus has once got rid of the parasites, it appears to heal up in a wonderful manner. At any rate, one often sees cells in which the parasite is in the act of just slipping out, or has just slipped out of the nucleus, and yet the latter structure presents hardly any pathological appearances. How this recovery of the nucleus is effected is a matter for future study.

We would then sum up this section of our paper as follows:—In carcinomatous tumours of the female mamma some of the protozoa found therein inhabit the nucleus as also the protoplasm of the cancer cell. The protozoon often appears as a small body in the nucleus, and then develops gradually until it exhibits the characteristics of the full grown protozoon in the nucleus, or in the surrounding protoplasm of the cancer cell.

DESCRIPTION OF PLATE XXIII.

FIG. 1.—Osmic acid preparation, hæmatoxylin and eosin staining. *a*. Two small parasites lying in the nucleus of the epithelial cell. Verick Oc. 1, Obj. $\frac{1}{12}$.

FIGS. 2, 3, 4.—Same mode of preparation as Fig. 1. *a*. Small parasites lying in the nucleus. Same staining and magnification as Fig. 1.

FIG. 5.—Same mode of preparation as Fig. 1. *a*. Larger parasite with well-marked nucleus lying in the interior of the nucleus of an epithelial cell. Same staining and magnification as Fig. 1.

FIG. 6.—Same mode of preparation as Fig. 1. Four parasites leaving (*n*) nucleus of epithelial cell. Same staining and magnification as Fig. 1.

FIGS. 7, 8, 9, 10, 11, 12, 13.—Groups of parasites (Mehrling's-Infektions) in epithelial cells. Notice that each parasite has its own capsule. *n*. Nucleus of epithelial cells. Figs. 7, 9, 11 from osmic preparations. Hæmatoxylin and eosin staining. Same magnification as Fig. 1. Figs. 8, 12, 13, from a cancer hardened in chromic acid and spirit. Eosin and aniline blue staining. Zeiss Oc. 4, Obj. $\frac{1}{20}$.

All the figures were drawn from carcinomata of the breast.
For particulars see text.

20th January 1893.

(*To be continued.*)

Fig. 1.

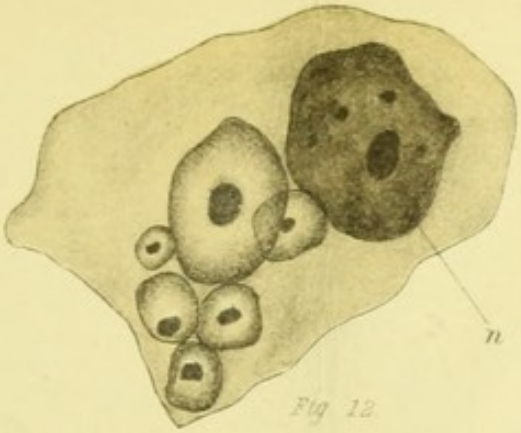

Fig. 12.

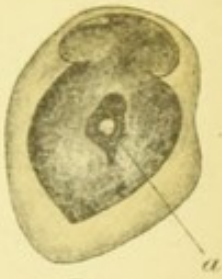

Fig. 2.

Fig. 3.


Fig. 4.

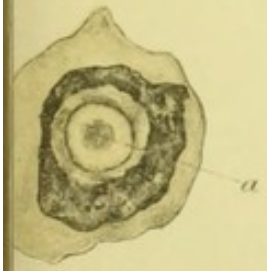

Fig. 5.

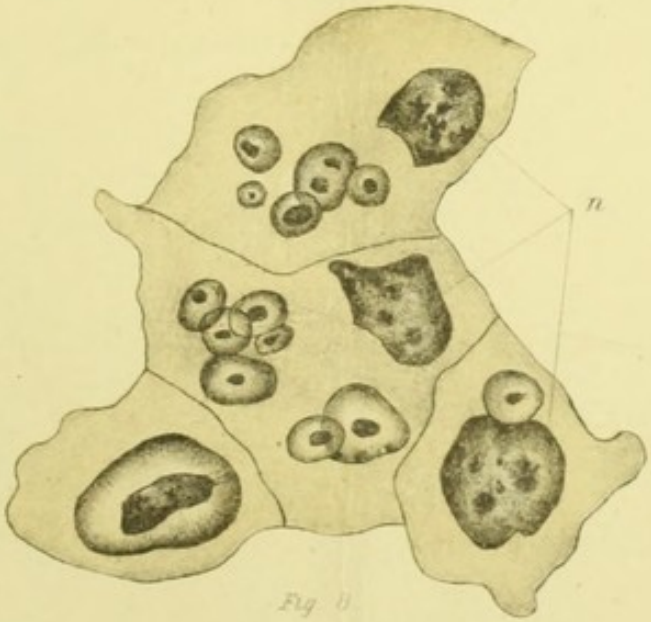

Fig. 11.

Fig. 6.


Fig. 11.


Fig. 7.

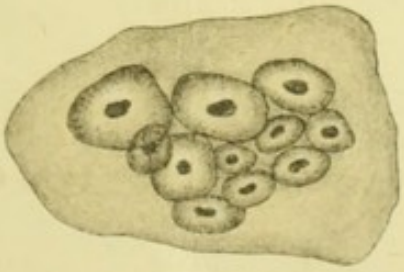
Fig. 13.


Fig. 10.


Fig. 8.