THE PARASITISM OF PROTOZOA IN CARCINOMA.

Being the Morton Lecture on "Cancer and Cancerous Diseases." Delivered in the theatre of the Laboratories of the Royal Colleges, London.

By JAMES GALLOWAY, A.M., M.D. ABER.,
F.R.C.S. Eng., M.R.C.P. Lond.,

Pathologist and Assistant Physician to the Great Northern Central Hospital; Demonstrator of Materia Medica to the London Hospital.

I.—INTRODUCTION.

MR. PRESIDENT AND GENTLEMEN,—The distinguished pathologist and surgeon who delivered the first Morton Lecture on "Cancer and Cancerous Diseases" suggested, in defining the wishes of the founder, that Mr. Morton probably looked forward to some practical result, possibly even the means of cure, as the outcome of his munificence. I need hardly say that this fortunate event is still far off; but it is evident that the Council of the College, in appointing one of the junior Fellows—already engaged in investigations on the subject in this laboratory—to give the lecture on this occasion, looked for some statement of research as to its etiology from the pathological standpoint, and implied that any crude opinions from the clinical aspect were not expected, but would be considered presumptuous and out of place.

Sir James Paget, on the occasion referred to, strongly expressed his conviction that cancer—speaking in the widest sense of the word—was a specific disease, comparable in many respects to the class of diseases to which syphilis, tuberculosis, leprosy, glanders, and actinomycosis belong.¹ Since that time much has been said in reference to the possible parasitic cause of cancer, and although a different class of micro-organism has been suggested as being possibly concerned in the production of the disease, the belief supported by Sir James Paget has grown apace.

In this way the subject of the present lecture was foreshadowed, and very little difficulty was felt on my part as to the choice of the particular line to be taken. At the time also when the choice of subject was under consideration recent and more accurate investigations of certain histological appearances in cancerous tumours had been published abroad, which brought the question of the influence of parasites on the formation of carcinomata, at all events, more prominently into notice than ever.

II.—PROTOZOA AS PARASITES IN ANIMALS: TYPE THE COCCIDIUM OVIFORME IN RABBITS.

As is now well known, the organisms which have been so frequently mentioned of late in connection with carcinoma belong to the division of the protozoa; and if I devote part of the time at my disposal to the study of a disease characterised during part of its course by the formation of tumours occurring in one of the lower animals, and undoubtedly caused by a certain protozoon, I shall carry out another of the suggestions of the lecturer already quoted, who pointed out that much light may be thrown on the formation of tumours in man by the study of growths in other organisms, and at the same time fulfil the purpose in my mind when I commenced this inquiry of obtaining a clear idea of some of the characteristics of the class of micro-organism which is now suggested as the cause of cancer.

The animal which I have chosen as the type of those infested by protozoa is the rabbit, partly on account of the ease with which it may be observed, and because an epidemic of the disease in question prevailed in the laboratory at the time when the investigation commenced, but principally because the life-history of the parasite which is the cause of the disease is very completely known, and may be looked upon as typical of many others allied to it in organisation.

Portions of this life cycle have been commented on so recently in certain quarters, that I shall draw attention mainly to the points appearing to be of special interest for the purposes of this lecture, and refer those interested to special in-

vestigations on the subject,² and especially to the recently published account by Dr. R. Pfeiffer.

This affection of rabbits is brought about by the influence of one of the sporozoa (*coccidium oviforme*, Leuckart) which infests the intestine, bile ducts, and livers of diseased animals in myriads. The disease is so common in certain hutches and warrens near London,³ that the keepers recognise it readily, and distinguish it by the "wet snout" which the affected animals exhibit. It is most fatal in young rabbits; which become affected as soon as they cease to suckle and begin to eat green food. They lose flesh rapidly, suffer from enteritis of more or less acute character, and many die in from eight to fifteen days after the initial symptoms. The adult animal is more rarely infected, and as a rule resists the disease. The development of the parasite which brings about this very fatal disease may be considered in two stages, external to and within the host.

III.—DEVELOPMENT EXTERNAL TO THE BODY.

The organism as it escapes from the alimentary canal consists of a firm, translucent cyst, enclosing a quantity of very granular protoplasm, which fills the whole cavity. The cyst, which is the striking feature of this period of development, is oval in shape, and measures about 36μ in length and about 22μ in breadth. Very soon after expulsion, and often while within the host, the protoplasmic contents contract, and form a sphere lying free within the cyst wall. Under suitable circumstances this ball of protoplasm pushes out projections, at first flattened, but soon becoming more distinct, till at length it divides into four distinct smaller spherules. Each of these protoplasmic masses becomes somewhat elongated and forms within itself two crescentic germs lying in its long axis, leaving unutilised a small nuclear mass. A wall of less density than the outer cyst is formed round each of these groups of two germs, and then this stage of development is complete.

In this condition the parasite seems very resistant to injurious influences, and is capable of remaining alive for at least six months.

Fig. 1.—a, Coccidium showing capsule full of granular protoplasm; b shows condensation of the protoplasm into one sphere, after two days' growth external to body; c, division of the single sphere into four daughter spherules, after four days' development; d, an empty ruptured cyst. (From photographs $\times$ about 500.)

IV.—METHODS.

For the purpose of noting the changes which I have briefly mentioned, the parasites obtained from recently killed animals may be placed on a cover glass and examined as a drop cultivation. The method which I have found most useful is that recommended by Professor Delépine, under the name of inter-lamellar films.⁴ In this way the cycle of development which I have described will be accomplished in less than a week, the transformation into four daughter cells being noticeable on the third day, at the ordinary temperature of a room.

On commencing these observations, the ordinary bacteriological processes were made use of, with very little result. Tubes of sterilised blood serum, bouillon and other media were utilised and the usual precautions observed. Subsequently the inoculated materials were placed in incubators at temperatures of 20°C . and 38°C . The more elaborate the precautions, however, the less result was obtained. The organism

² Leuckart, R., *The Parasites of Man*, English edition, 1886; Balbiani, G., *Leçons sur les Sporozoaires*, Paris, 1884; Pfeiffer, L., *Die Protozoen als Krankheitserreger*, Jena, 1891; Malassez, M. L., *Notes sur la psorospermose du foie, etc.*, *Arch. de Médecine expérimentale et d'Anatomie pathologique*, vol. iii, 1891; Pfeiffer, R., *Beiträge zur Protozoen-Forschung*, Heft i, Berlin, 1892; Delépine, S., for bibliography, *Trans. Path. Soc. Lond.*, vol. xli, 1890.

³ Hutchinson, J., sen., *Archives of Surgery*, vol. iii, 1891.

⁴ Delépine S., *Lancet*, vol. i., 1891.

¹ Morton Lecture, 1888. Longmans, Green, and Co.

flourished best when freely exposed to the atmosphere. And as R. Pfeiffer observes, the presence of carbonic acid and the change from the temperature of the body to the cooler external temperature aid in their development. A warm temperature and want of free aëration modify and even prevent the changes described, and influences of this sort no doubt account for the very great discrepancies as to time which may be noted between the accounts given by different observers.

I have drawn attention to these facts to show how readily this important external cycle of development may be influenced.

V.—DEVELOPMENT WITHIN THE BODY.

When an uninfected young rabbit swallows the parasite in the larval or resting condition just described with its food, the resisting capsules are acted on by the digestive ferments, so that the crescentic germs already mentioned become free. A new cycle of intense activity is now observed. These crescents become rounded and probably acquire the power of locomotion. Most of the naked amoeboid forms of the organism divide into numerous small crescentic sporules, which, in their turn, also become free, and it seems probable that this "endogenous sporulation" may be repeated. Thus myriads of young sporozoa are soon found in the alimentary canal, the gall bladder, and the bile ducts of the infected animal. In this stage the organisms are very readily destroyed on being removed from the body, showing a marked difference, so far as resistance is concerned, when compared to the organism in its resting stage.

The sporules, on being set free from the mother cell, have the power of entering the epithelial cells of the affected region, where they commence a process of growth and differentiation of their protoplasm, which ends in the production of the encysted parasite. It is said that several parasites may infect the same epithelial cell, but, in the adult condition, one parasite is seen to occupy the greater part of the distal region of the cell body, and no trace of others can be seen. In course of time the epithelial cell wall is ruptured, and the parasite escapes, without necessarily causing destruction of the host cell; it passes through the alimentary canal, gains access to the atmosphere, and thus meets the conditions necessary for recommencing the cycle of development.

Fig. 2.—Adenoma from rabbit's liver, caused by the *C. oviforme*; shows the arrangement of the growth, the condition of the epithelium, and numerous parasites enclosed in the cells, and lying free in spaces between the processes. (From a photograph $\times$ about 100.)

VI.—ANATOMICAL CHANGES PRODUCED IN THE HOST.

Let me now pass in review the changes produced by this parasite, whose life history I have described, and especially those in the liver where the disturbance is most obvious. The liver of an animal dead of the disease after the acute stage has passed, or killed when the condition has become chronic, is studded over with greyish-white areas, varying in size from

a pin's head to that of a pea, usually rounded but occasionally somewhat branched in shape, resembling the aspect of chronic tubercle, a likeness which is more close as each spot of disease is filled with material which is apparently caseous. On examining these tumours, however, by means of microscopic sections, they are found to consist mainly of epithelium and connective tissue, which is arranged in the form of a very complex adenoma. The area of the bile duct affected becomes widened, and the space thus formed becomes filled up with the much hypertrophied and convoluted mucous membrane. The outer margin of the growth is marked off by a layer of well formed connective tissue varying in thickness from the surrounding hepatic substance. Lining this fibrous layer is the epithelium of the bile duct, which in most places has not preserved its typical cubical form, but is somewhat embryonic in character. The most striking feature of the tumour, however, is the extreme complexity of the processes projecting from the mucous coat. These projections are very finely branched, and thus the characteristic convoluted appearance is obtained. They carry with them a small quantity of fibrous tissue, and usually very large thin-walled vessels. Filling up the interstices of this adenomatous structure are seen multitudes of parasites in many stages of development. Some are encysted, and in this condition may be observed lying free, or still contained within the epithelial

Fig. 3.—a, b, *Coccidia* occupying epithelial cells of the affected bile ducts; c, appearance presented after escape of the parasite.

cells of the tumour. Naked forms may be seen from the smallest homogeneous globules of protoplasm, in all stages of granularity of protoplasm, according to their age, up to the adult form of the encysted parasite in which the protoplasmic contents of the cyst become very coarsely granular. These younger forms are seen usually lying imbedded in the epithelial layer. If an animal still suffering from the acute form of the disease is examined, the crescentic spores due to "endogenous sporulation" may be seen if special precautions are taken.

Fig. 4.—a, b, Formation of crescentic spores within the daughter spherules, external to the host (after Balbiani); c, d, sporulation within the host, division of the spores into numerous crescentic segments. (After photographs by Pfeiffer $\times$ 1,000.)

If the animal recovers from the disease, the tumour just described commences—apparently in a very short time—to alter its appearance. Many of the parasites pass out of the body, the remainder show signs of granular and fatty degeneration, their cyst walls resisting longest and being for some time obviously empty. The fibrous boundary of the adenoma becomes thicker, contracts, and at length the lesion caused by the attack of the parasite heals, and its site is marked by a small concentric nodule of fibrous tissue.

In the small intestine the areas of mucous membrane

affected show similar changes to those described; the protozoa penetrate deeply into the glands, the epithelial cells become infected, the mucous membrane is thickened, and there results a hypertrophied, adenomatous condition of the particular area involved.

VII.—INOCULATION EXPERIMENTS.

Before passing from this division of my subject, I wish to draw attention to certain attempts which have been made to produce this disease by artificial introduction of the organism. Messrs. Ballance and Shattock attempted to infect certain animals by means of "psorospermial material," both by subcutaneous inoculation and intravenous injection.⁵ No note is made of the stage of development in which the psorospermial material was at the time. Mr. D'Arcy Power,⁶ by means of specially-devised methods, has sought in this laboratory to produce the disease by the implantation of coccidia on specially irritated epithelial surfaces; and has extended his experiments by the inoculation of material obtained from cases of Paget's disease of the nipple. Dr. R. Pfeiffer⁷ has taken coccidia which have passed through their cycle of development external to the body, and were thus presumably in a stage capable of causing infection, and injected them directly into the liver and into the veins of presumably healthy animals. All these experiments have yielded negative results, while the feeding of rabbits with ripe coccidia has brought about the disease in animals presumably healthy; and, so far as we can judge, infection by the alimentary canal is probably the only method of infection. These facts throw light on the difficulty of infection by these organisms if the path chosen is not the one in which they meet conditions propitious for their development, and give an explanation of the apparently non-contagious character of this disease.

I have ventured to draw your attention in some detail to the life history of this sporozoon as it is now so well known, but facts of similar significance may be derived from the study of sporozoa affecting man and other animals, especially invertebrata; and possibly even more guidance may be obtained in our researches into the cause of cancer, when the life history of such protozoa as those described by Laveran, Marchiafava, Golgi, Celli, and others in the blood of lower animals and man, becomes better understood.

VIII.—CONCLUSIONS AS TO PROTOZOA IN RABBITS.

It may in the meantime be taken as established in the case of the coccidium oviforme attacking the rabbit that:

1. A most important portion of the developmental cycle of this parasite takes place only external to the body, under aerobic conditions.
2. Influences occurring outside the body delay, and even prevent, the external sporulation of the parasite, thus interfering with its infective power.
3. The host cannot be infected by coccidia inoculated directly from animals already suffering, thus proving that the disease, though infectious, is so only in a very special way.
4. The parasite infects the host by passing into the alimentary canal, where it meets suitable conditions for its future development.
5. The parasite enters and grows within epithelial cells without necessarily destroying them, and causes great proliferation of the neighbouring epithelium.

IX.—THE QUESTION OF PARASITES IN CANCER.

Having drawn attention to the development of this well-recognised organism, and its effect on the tissues, I wish for the remainder of the time to consider certain appearances which may be recognised in carcinomata, and to inquire what evidence there is of their parasitic character.

It is now more than forty years since Virchow⁸ drew attention to the structure of certain cells occurring in tumours which appeared to contain inclusions of extraneous origin. If reference is made to the plates illustrating his papers, it will be observed that most of the appearances seem to be due to what we now consider to be certain forms of cell degeneration, but a few are very similar to the bodies to which I wish

to draw attention immediately. At that time considerable discussion arose concerning these cell inclusions. The chief opinions then advanced in explanation of the bodies under observation were that they arose from the endogenous formation of cells, along with alteration of their nuclei, so as to give the appearance of homogeneous spheres (Virchow), or that they were due to the imbibition of albuminous or watery fluids, probably a part of a degenerative change (Henle, Bruch, and others).

The next series of researches of importance in the history of parasitic influences on cancer were undertaken as a result of the great additions which were made to our knowledge of pathological processes by the investigation of the pathogenic fungi. Many observers sought to discover a specific micro-organism belonging to the vegetable kingdom. I need not recall to memory how the results of these researches were disproved, but simply refer to them as a warning against the formation of opinions too hurriedly from insufficient data. The papers of Scheurlen,⁹ Kubasoff,¹⁰ and others are well worthy of perusal from this point of view.

After this period the question of the parasitic origin of carcinoma seems to have been considered in most quarters as one of those which for all practical purposes was settled definitively in the negative, and the affirmation of the belief in its possibility was looked upon as purely visionary, and to be regarded as a harmless though amiable delusion.

About the year 1888, however, the current of opinion began to change, taking a somewhat different channel. Increased attention was being paid to the lowest animal organisms, which had been long overlooked on account of the great interest which had been taken in the corresponding group of the vegetable kingdom, and the influence of these as possible factors in disease began to be more carefully studied. A number of monographs from French observers,¹¹ dealing with appearances to be observed in cancers of the skin and other organs, and also in certain skin diseases, attracted the most attention. The bodies described by these observers were claimed to be parasitic protozoa, and the names of such scientists as Balbiani and Malassez lent weight to these statements. At this time similar appearances were exciting attention in Germany and elsewhere, and also among British observers.¹² Many opinions were advanced as to the character of the bodies described, but were on the whole unfavourable to the hypothesis that the bodies under discussion at the time were of a parasitic character.

X.—CERTAIN CELL INCLUSIONS DESCRIBED AS PARASITES.

On looking over the numerous papers written on this subject,¹³ and especially by comparing the figures given by their authors, the conclusion which must inevitably be drawn is that the bodies described are of the most diverse character, and that undoubtedly many forms of cell and nuclear degeneration have been made to do duty as parasites, or are mentioned by the writers as having been described as parasites. I would venture to draw the attention of those interested in this subject to the papers by Russell, Steinhaus, Noeggerath, and by Podwysoski and Sawtschenko,¹⁴ as giving examples of the appearances referred to, along with others perhaps of a more significant character.

Before proceeding, let me mention certain cell inclusions which have to be kept constantly in mind as being likely to mislead in carrying out observations on this subject:

1. The appearance displayed when a cell, partly invaginated into the cell body of another, is cut transversely is apt to be deceptive. In carcinomata, when the cells are large—as in those of the skin—this occurs very frequently, and almost every microscopic field affords illustrations of the appear-

⁹ Scheurlen, *Deutsch. med. Wochenschrift*, 1887, etc.

¹⁰ Kubasoff, *Arch. f. klin. Chir.*, Band xxxix, 1889.

¹¹ Darier and Albarran, *Comptes rendus de la Soc. de Biologie*, 1889, etc.; Wickham, *Contribution à l'étude des Psorospermioses*, etc. *Thèse de Paris*, 1890.

¹² Hutchinson, J., jun., *Trans. Path. Soc. Lond.*, 1890, etc.; Bowlby, *Trans. Roy. Med. Chir. Soc. Lond.*, 1891.

¹³ For the bibliography of this subject refer to papers by Stroobe, *Centralblatt f. path. Anatomie u. allg. Pathologie*, Bd. ii, 1891; Ruffer and Walker, *Journal of Pathology*, vol. i, 1892.

¹⁴ Russell, *BRITISH MEDICAL JOURNAL*, vol. ii, 1890; Steinhaus, *Arch. f. path. Anatomie*, 1891; Noeggerath, *Beiträge zur Struktur und Entwicklung des Carcinoms*, 1892; Podwysoski and Sawtschenko, *Centralblatt f. path. Anatomie*, 1892.

⁵ Ballance and Shattock: *Trans. Path. Soc. Lond.*, vol. xlii, p. 380.

⁶ Personal communication.

⁷ *Loc. cit.*

⁸ Virchow, *Arch. f. path. Anatomie*, etc., Bd. i, 1847, Taf. ii, Fig. 5, k and l, and Band iii.

ance mentioned. The nucleus of the outer cell is frequently pushed on one side, and, being flattened out, gives the appearance of having been gradually pressed on by a foreign body growing within the cell. As portions of both nuclei usually lie in the same plane of section, the distinctive staining reactions given by the nuclear chromatin serves to identify the nature of this inclusion.

2. The occurrence of epithelial cells containing leucocytes is not uncommon, especially towards the margins of rapidly-infiltrating growths and in secondary deposits in lymphatic glands. As a rule the chromatin of the leucocyte takes up a nuclear staining reagent even more readily than that of the epithelial cell, which is often degenerating under the circumstances, and helps, with other characteristics, to show what has taken place.

3. The endogenous formation of cells (Virchow) must necessarily give rise to certain cell inclusions.¹⁵ In cases where there is a rapid formation of new cells, the hypothesis that direct division is greatly concerned in their reproduction must be granted on account of such considerations as the comparatively small number of mitotic figures (indicative of indirect division) in even a rapidly-growing cancer, and the huge increase of epithelial cells in such tumours as the coccidial adenomata of rabbits, apparently without any appearance of mitotic figures. It is in this way, no doubt, when division in the cell wall has not followed division of the nucleus, that the occurrence of cells in new growths containing two or more well-marked nuclei lying side by side or scattered throughout the cell body ("giant cells of cancer") originates.

4. A very interesting and much more misleading appearance is that produced by the asymmetrical mitosis which goes on to so great an extent in morbid epithelium.¹⁶ In this process, instead of the normal indirect division proceeding by the formation of two poles in the mother cell and the subsequent rearrangements and ultimate division of the chromatin threads of the nucleus so as to give rise to two separate daughter cells, there may be observed the formation of three, four, or more poles in the mother cell, each with its sheaf of achromatic striæ, followed by the splitting up of the mother cell chromatin into a corresponding number of daughter masses after having given rise to the most abnormal figures. In other cases the asymmetry of the division consists in the fact that one of two daughter cells takes far more than its own share of chromatin, and at the same time the larger share of the vitality of the cell. As the result of these abnormal processes most striking cell alterations occur. The daughter cell having the largest amount of chromatin very often seems to grow at the expense of the others, and becomes much hypertrophied. The nuclei in the neighbourhood, including those of the same generation, become pressed to the side, and ultimately, as the nucleus of the hypertrophied cell itself becomes degenerate, the appearance is presented of a large bladder-shaped cell, containing one or more chromatin particles, with a crescentic nucleus of another cell at its outer margin, presenting very much the appearance which one might expect from a parasitic inclusion. Certain other appearances of an equally misleading character, due to this fragmentation of the nucleus, are frequently met with.

5. The already well-recognised degenerations of cancer cells such as the colloid, keratinous, etc., and especially the various degenerations of the nucleus itself, such as the breaking up of the chromatin—processes allied, perhaps, to the wandering of the chromatin described under special circumstances by Messrs. Ballance and Shattock¹⁷—must also be borne in mind, though they are not likely to mislead.

The classification which I have thus made is not advanced as being perfect, but it is convenient, as in its divisions it is possible to place most of the cell inclusions which are met with in various cancers and which cannot be considered to be parasitic.

XI.—THE EXISTENCE OF A PARASITIC PROTOZON IN CARCINOMA.

In this way having accounted to myself for many of the peculiar structures described by various writers, there still

remained one series of appearances which remained to be identified. I now refer to the cell inclusions which have been noted by Sawtschenko¹⁸ and also by Sjöbring¹⁹ in their papers, though not with precision, and to which special attention has been drawn by Soudakewitch,²⁰ and particularly by the careful description and accurate illustrations of Dr. Ruffer and Mr. J. H. Walker.²¹ Later corroboration of these descriptions has been given by Foà,²² and within the last months by other observers.²³ After having investigated the matter for some time, and examined a large number of different cancers, I had become still more sceptical than at the outset of being able to identify any structure which might be considered with any degree of probability to be parasitic in character. When I was able to define this body, however, it became obvious that something totally different from the appearances already noted was under observation. Since that time I have examined a number of cancers from different regions—breast, liver, alimentary tract, skin, etc.—and I am glad to say that I am able to corroborate most of the descriptions of the various writers mentioned.

The illustrations of this structure which I am about to place before you will be observed to be in many cases from cancer of the breast; partly on account of the frequency with which this variety of growth is obtained from recent operations, and also because I agree with the remark of a pathologist who has paid great attention to the subject of cancer from the point of view of parasitism, that what holds true about such typical growths as those of the mamma may be regarded as being also applicable to most other varieties of cancer.

XII.—DESCRIPTION OF THE PARASITE.

1. *In the Cell Body.*—Taking, therefore, cancer of the breast as an example, if careful microscopic examination is made, there will be found lying, most commonly within the cell body, rounded or oval structures varying in most cases from 2μ to 10μ in diameter, having when large a very distinct capsule, and presenting a smaller body of variable shape, situated, as a rule, towards the centre of the capsule. From the capsule there may be seen passing towards the centre numerous fine radial striations, and the capsule itself occasionally seems to have similar markings. On the other hand, passing outwards from the nucleus towards the periphery may be observed processes of a somewhat different character; they are not nearly so regular, and appear to be prolongations of the nucleus.

Fig. 5.—From cancer of the breast. Cells showing parasites contained in the cell body $\times$ about 800. p, Parasite; n, nucleus.

Fig. 6.—Parasites enclosed within the cell body $\times$ about 800.

These bodies occur usually one in a cell, but there may be more; and in some cases eight or ten of small size may be seen lying closely together in a cluster. In a successful preparation each of the small ones may be noticed to contain the usual nuclear substance.

¹⁸ Sawtschenko, *Centralbl. f. Bakter. und Parasitenkunde*, Bd. xii, 1892.

¹⁹ Sjöbring, *Fortschrit. der Medicin*, Bd. viii, 1890.

²⁰ Soudakewitch, *Annales de l'Institut Pasteur*, 1892.

²¹ *Loc. cit.*

²² Foà, *Centralbl. f. Bakter. und Parasitenkunde*, Bd. xii, 1892.

²³ Burchardt, *Archiv. f. path. Anatom. und Physiolog.*, January 7th, 1892. Vide criticisms in the December (1892) number of the journal quoted.

¹⁵ Butlin: *Trans. Royal Med. Chir. Soc.*, 1876-77.

¹⁶ Hansemann, *Arch. f. path. Anatomie*, B. I. cxix, cxliii, cxlix, 1890-92.

¹⁷ Ballance and Shattock, *Trans. Path. Soc. Lond.*, vol. xxxix, 1888.

2. *In the Cell Nucleus.*—Similar structures of smaller size may be observed lying inside the nucleus of the epithelial cell. In this situation the capsule, which is so very characteristic of the intracellular inclusion, is very slight, and indeed appears to be absent in most cases. The nuclear inclusions may be single, or may also occur in small groups.

Fig. 7.—Cells from different cancers of the breast, showing various forms of parasites in the cell protoplasm $\times 1,200$.

Occasionally the bodies may be seen partly within and partly without the nucleus, as if they were passing out from the nucleus into the cell protoplasm. In this reference I would draw attention to an observation of Dr. Ruffer's²⁴ who has been able to show that in certain cases the nucleus seems to become filled up with numerous small parasites which escape into the cell protoplasm after having burst through the nucleus. The presence of the intranuclear forms does not seem to be so common as the intracellular variety, and for some reason they appear more plentiful in certain cancers than in others. The features shown by many preparations strongly favour the idea that in some cases the inclusions multiply readily within the nucleus, and ultimately free themselves from the nucleus and gain access to the surrounding protoplasm.

Fig. 8.—a, b, c, Cells showing single parasites occurring within the nucleus $\times$ about 800; d, numerous parasites in the nucleus and in the cell protoplasm $\times$ about 1,000.

3. *In the Intercellular Spaces.*—Bodies of similar character may be observed in much smaller numbers lying in the intercellular spaces, and more rarely still they may be seen lying two or three together in lines amongst the fibrous tissue at the margin of an alveolus, that is to say, in lymphatic vessels. It is difficult to say whether the latter appearances are accidental, but there seems to be no doubt that the bodies are of the same character as the intracellular and intranuclear forms already noted. They give the reaction which so readily distinguishes the body under consideration from the globules of altered chromatin which are so often seen in cancer and in other conditions, and with which, perhaps, they might be confused in unstained preparations.

4. *Position in the Growth.*—The position of these bodies is a fact of important significance. They occur with greatest frequency in rapidly growing cancers, and in those cases where there is the least sign of cell degeneration. In the case of cancer of the breast they are found most numerous at the outer margin of the mass of cancer or in the outlying alveoli, and in recently infected lymphatic glands. On approaching the centre of a mass of growth, or where degeneration has commenced, they begin to be less readily recognised, whereas the many varieties of so-called "cancer parasites" to which I have drawn attention become more and more numerous. The disappearance of these characteristic forms must not be taken as necessarily implying destruction, for it must be borne in mind how resistant are the adult coccidia in the case of the rabbit, and how they continue to develop in situations where their destruction by decomposition would seem inevitable.

It will be gathered from what I have said that I have described these bodies with so much detail for the purpose of

concentrating special attention on them, as the only bodies yet found which show any probability of being parasitic. Their occurrence within the cell as a distinctly foreign substance, their appearance so strongly suggestive of an organised structure, the staining reactions which they give so distinct from those presented by the normal contents of cells, their great analogy in this latter respect, and especially in their behaviour within the cell, and possibly also external to it, to well-known species of sporozoa recognised as parasitic in animals, all point forcibly to the conclusion that these bodies, though not necessarily coccidia, are nevertheless protozoa, and are parasitic in cancerous epithelium.

Fig. 9.—a and b, Groups of cells containing intracellular parasites $\times$ about 1,000; c, cancer alveolus from edge of rapidly growing carcinoma of breast, showing numerous parasites $\times$ about 400.

XIII.—METHODS.

It may be well in this place to say a few words about the methods adopted which have been found most useful in obtaining good histological results, and thus to keep clear of some of the errors of observation which I have alluded to as being possible.

At an early period of this investigation it occurred very naturally to me that cancers had been probably the most frequently investigated, from the histological standpoint, of all abnormal tissues, and yet these bodies had not been described. I examined many old specimens of cancers prepared by myself in this laboratory for an investigation on totally different lines, and I also examined a number of excellent preparations kindly lent me by a friend, and prepared some years ago, when the question of parasitism was not being discussed; in none of them could I obtain satisfactory evidence of the parasite under consideration. The cancers from which the preparations were made were hardened by immersion in Müller's fluid or chromic acid, and spirit. Being now accustomed to recognise this parasite, faint traces of them were observed in places, but nothing satisfactory nor convincing. The reason is, I believe, that the material was not properly prepared for the purpose.

Methods of Fixing.—The way in which I now attempt to proceed is to obtain the material as soon as possible from the operating table, fresh specimens being much more satisfactory to handle afterwards than material obtained from the post-mortem room. Pieces of small size ($\frac{1}{4}$ inch square) are then placed, one set in Flemming's fixing solution, and another set in corrosive sublimate, or in Foa's fixing solution. After remaining in the fixing solution for a sufficient time, the pieces of tissue are thoroughly washed, hardened in successive strengths of alcohol, imbedded in paraffin, and cut in the usual manner.

Methods of Staining.—The staining reagents which have been found most useful are hæmatoxylin (Ehrlich) alone, or with some ground stain (rose bengale, eosin, etc.), and the Biondi triple stain. The reason for having the preparations fixed in two different ways is that Flemming's solution fixes the cell elements more sharply and definitely than any other not containing osmic acid, and if the parasites are present in the piece of tissue to be examined one feels sure of finding them. The staining reaction, however, with an osmic acid fixing reagent are not very brilliant, and for this reason it is well to have the other series of preparations at hand to obtain staining reactions if required.

Flemming's solution, followed by hæmatoxylin, gives, to my eyes, as good results in the way of sharpness of definition as can be obtained; the epithelial cell nuclei take up the hæmatoxylin in the normal way, but do not bear the same tone of blue as that shown by the parasites; in the case of the "Biondi" reagent—so strongly recommended by Dr. Ruffer and Mr. Walker—the distinctive colouring is much more striking and beautiful, and its results are well seen in the beautiful drawings accompanying this paper.

I would add in reference to the matter of staining, that the secret of discovering the bodies is in the fixing and hardening of the preparations;

²⁴ Ruffer, BRITISH MEDICAL JOURNAL, vol. ii, 1892, p. 993.

no precautions should be thrown aside in carrying out these processes. If this is well done the observer need not seek far for stains if he wishes simply to see the parasite. A perfect differential stain to pick out the bodies alone is still to be found. The nuclear stains, such as methyl green, hæmatoxylin, are not very suitable for that purpose; like the coccidia in the rabbit's liver, the parasite seems to prefer general protoplasmic stains, and, acting on this principle, the best results of which I am aware have been obtained by Mr. Plimmer, who has used aniline blue as the special stain for the organism.

In all well-prepared specimens there is no difficulty in distinguishing the parasite from the ordinary forms of cell degeneration. It may be of interest to remark in this connection that when the coccidia in the rabbit's liver were being first investigated certain pathologists declared the appearances of this now well-known parasite as being given rise to by alterations in the surrounding liver tissue.²⁵

During this investigation I have had the opportunity of investigating over fifty cases of cancer of various organs, and in most of them, especially those more recently examined, there has been no difficulty in identifying the parasite. Taking the cases coming under my notice along with those mentioned by other observers, it has now been identified in practically all varieties of carcinoma: there are still one or two forms in which researches have not been recorded to my knowledge—for example, "rodent ulcer." At first many of my results were unsatisfactory, and still, in certain cancers, they are more difficult of discovery than in others. In my experience, they have proved most difficult of identification in colloid cancers of the alimentary tract, and in carcinomata of the skin. Taking the case of the breast once more as an example, if any difficulty is experienced in seeing them readily, in all probability the reason is that there has been some carelessness displayed during the process of hardening.

The material at my disposal has been obtained partly by the kindness of my friends at the London Hospital, and partly from cases coming under my own observation at the Great Northern Hospital.

XIV.—SARCOMA.

In reference to the question of sarcoma, it can only be said at present that parasites cannot be so easily identified by the methods I have described. Although it seems probable that parasites do exist in sarcomatous tumours, no definite opinion can yet be expressed. The subject is now under consideration.

XV.—CONCLUSION.

The further questions which arise—namely, Can the parasites be observed "undergoing reproduction," and can they be observed developing outside the body? What influence have they on epithelial cell multiplication? and, finally, have they a causative influence on cancer?—are now the points to be investigated; and it must not be considered a reproach if as yet no definite answer may be given. It will be remembered that although the very characteristic coccidium oviforme was recognised so long ago as the year 1839 by Hake in this country, it is only within the last few months that the description of the processes, apparently completing our knowledge of the anatomical changes in the life history of this organism and accounting for the enormous rapidity of its multiplication within the body of its host, have been made known by Dr. R. Pfeiffer.

Speculations on my part as to the rôle of this "cancer organism," although they are tempting, are better avoided; they will occur to all those who have studied the subject; in the meantime I am satisfied if I have placed the facts so far as they are known before you. The prospects of the research are encouraging, if it is only from the point of view of opening up the possibility of a cause of cancer, more hopeful as to curative measures than that suggested by the hypothesis which has so long held the field.

I fulfil a pleasant duty, in conclusion, by thanking the Committee of the Royal Colleges of Physicians and Surgeons for the permission they have granted me to pursue the investigations on which the remarks I have made are based in this laboratory; and another most pleasurable task remains—to record my obligations to my friends Dr. Ruffer, Mr. Plimmer, and Mr. Walker, who have been engaged in similar investigations, and especially to Dr. Sims Woodhead for much useful criticism during the course of my work here.

²⁵ Lang, *Archiv f. path. Anatom.*, etc., Bd. 41.

OPERATIVE SURGERY IN NEW YORK.—The number of deaths directly due to surgical operations in New York during 1891 is given by the *New York Medical Journal* as 133. Of this number, however, 10 per cent. are said to have followed circumcision, and as in these cases the operators were presumably rabbis, it seems hardly fair that they should be included in the surgical "butcher's bill." Among the strictly surgical cases laparotomy was responsible for the largest number of deaths (76, or 57 per cent.).

A REPORT

ON THE

PHYSIOLOGICAL ACTION OF CHLOROFORM,

WITH A

CRITICISM OF THE SECOND HYDERABAD CHLOROFORM COMMISSION.

By W. H. GASKELL, M.D., F.R.S.,

Lecturer on Physiology in the University of Cambridge,

AND

L. E. SHORE, M.D.,

Demonstrator in Physiology in the University of Cambridge.

(Concluded from page 171.)

CRITICISM OF THE PROOFS OF THE COMMISSION THAT THE FALL OF PRESSURE IS NOT DUE TO WEAKENING OF HEART.

We have already, in the beginning of this paper, referred to the conflict of opinion upon the question whether, and in what way, chloroform affects the heart directly, and, seeing that the chief papers of late years have been collected together and published by Lawrie in the Report of the Hyderabad Commission, it is unnecessary to do more than refer to the evidence in that book as to the present state of the question. On the one hand, a large array of observers look upon it as proved that chloroform weakens the force of the contractions of the heart by its direct action upon the heart; on the other hand the Hyderabad Commission, as represented by Brunton, considers that chloroform only affects the heart in combination with an asphyxial condition of the blood, and, as represented by Lawrie, denies *in toto* any direct action of the drug upon the heart under any circumstances. To our minds it is perfectly clear that the weakening effect of chloroform on the heart is the chief, if not the only, cause of the fall of blood pressure seen upon administration of the drug, and it is difficult to understand how the Hyderabad Commission could have arrived at the conclusions they have published, for their experiments and their large number of curves confirm again and again the observations of others, and point directly to heart failure as the cause of the fall of blood pressure.

The report of the second Hyderabad Commission consists of five parts, in Part v of which all the manometer experiments are described in detail, and remarks made upon a large number of the individual experiments. This description of experiments and the remarks upon them, together with the tracings, form the most valuable part of the report, for they give the facts upon which the conclusions of the Commission are based. In their report the Hyderabad Commission do not state what is the reason of the fall of blood pressure so characteristic of chloroform poisoning; they imply, however, that such fall is not due to any direct action on the heart, but to the paralysis of the vasomotor centre through the drug. The reasons for such a belief are given on p. 137 in the discussion which follows Experiment 178.

"Experiment 178 is very like Experiment 162, in which the heart's action was temporarily arrested every time the respiratory centre was paralysed. As in 162, there was no further fall of blood pressure during the arrest, and the beats on each side of the stop were ample and strong. The most remarkable instance of this is seen at Fig. 13. For more than two minutes there were only 17 very slight pulsations recorded, and for over a minute of this tracing there was no pulsation at all. There was no fall of pressure during the arrest, and the Ludwig tracing shows how strong the beats on each side of it were. No better proof could be afforded than is to be found in these two experiments that direct weakening of the heart is not the cause of the fall of the blood pressure, which is inseparable from chloroform narcosis.

"Experiments 64, 65, 162, 178, and 186 prove three important points:—1. A general fall of blood pressure, whether sudden or gradual, is not in itself dangerous. 2. The fall of blood pressure, which occurs in chloroformisation with regular breathing, is due solely to narcosis of the vaso-