Observations on Various Sporozoa.

By

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With Plates 31, 32, and 33.

CERTAIN problems propounded in the last few years demand for their solution a closer study of the intimate structure of the sporozoa than has hitherto been found necessary. The variations of nuclear form presented by these organisms call for especially close examination. For some of the higher members of the group this work has been well begun by Wolters,[1] who examined Clepsidrina blattarum, Monocystis magna and agilis in Lumbricus agricola, and Klossia in the kidney of snails. The description of the nuclear changes in the common gregarines of the earthworm is particularly complete, and since I have been able to confirm many of Wolters's observations it may be of interest if they are briefly reviewed here. He found in Lumbricus agricola both Monocystis magna and agilis constantly present, and almost to the exclusion of other species. In both, at every stage, a distinct nucleus was present. In the former it was relatively large and oval, in the latter of a rounded form. Under compression the nuclear membrane ruptured, but the contents did not escape, and when the compression was removed the nucleus tended to return to its primitive form. Thus the nucleoplasm appeared to be of a solid structure. In M. agilis this was also observed. The nuclear membrane was found to

[1] Max Wolters, "Die Conjugation und Sporenbildung bei Gregarinen," 'Archiv für mikroskop. Anat.,' p. 99, 1891.

be strong and sharply defined, and in the young parasite there was a single large nucleolus. Later the outline of the nucleus had become irregular and the nucleoli more numerous. In M. agilis, and in one instance in M. magna, Wolters encountered what he has named the flame-nucleus, a condition in which the nuclear membrane has disappeared, and the nuclear substance is prolonged at various points into the protoplasm of the body of the protozoon. As soon as the nucleolus has broken up into subdivisions the parasites are ripe for conjugation. It was found that after the syzygium was formed the nucleus of each parasite moved to the periphery, became elongated, and soon exhibited a typical nuclear spindle with the chromatin now massed together at the middle of the spindle. The chromosomes were very small. The division of each nucleus took place, and half of each nucleus was extruded as a polar body. Meanwhile the surfaces by which the parasites adhered to each other became altered in such a way that instead of the sharply marked line of division previously seen throughout a complete series of sections of a syzygium, there was at one part of the applied surfaces a communication through which the two parasites fused together. In each parasite the polar body was extruded on the surface opposite this communication, towards which, after the polar bodies had been formed, the two nuclei moved, and having reached the spot at the same time they fused together. After this a nuclear spindle was to be seen in each half of the syzygium, and could be distinguished, by its position close to the area of communication, from the spindles which were concerned with the formation of the polar bodies. Thus it appeared that the conjoined nuclei had undergone division, and that the daughter nuclei were again subdividing. The resulting two nuclei moved towards the periphery in each half of the syzygium, and there formed two spindles. This Wolters found to be the case in complete series of sections. These spindles were smaller than those of the polar bodies. By repeated subdivision these peripheral spindles and resulting nuclei increased in numbers and became surrounded by protoplasm constituting the sporogonia, which arranged themselves

peripherally, and also in spaces extending from the periphery into the remains of the body substance of the parasite.

The single nucleus of the sporogonia subdivided into eight, and meanwhile the sporogonia became surrounded by capsules constituting sporocysts (pseudo-navicellæ), the substance of which split up into eight crescentic spores, each of which contained a nucleus. Such are the chief results obtained by Wolters with regard to the Monocystides, and the thoroughness of the work and the beautiful drawings by Nussbaum, at whose instigation the work was undertaken, go far to establish the conclusions arrived at.

I will now detail some of the features I have obtained by examining the seminal vesicles of Lumbricus agricola, taken in the month of May. I have not had the opportunity of making such complete serial sections as Wolters, and in so far my criticism must be incomplete; still, the observations recorded below may be found of some interest. In this place it is advisable to describe the methods employed. Wolters found, and I have had the same experience, that Flemming's fluid did not give good results with gregarines. Wolters's results are chiefly based on the examination of sections of material fixed in saturated solution of picric acid. I have employed a method more commonly adopted, and which I have found most satisfactory, not only for gregarines, but for Coccidium oviforme and for animal tissues in general. Small portions of the tissue are placed for twenty-four hours in Foa's reagent, i. e. a mixture of equal parts of a saturated solution of corrosive sublimate in normal saline solution and a 5 per cent. solution of bichromate of potassium or Müller's fluid. Then the material is transferred for twenty-four hours to running water, and afterwards placed on successive days in 30, 60, and 90 per cent. alcohol. After that they are placed in absolute alcohol, and after saturation with chloroform are embedded in paraffin, care being taken that the bath does not reach a temperature higher than 50° C. The sections were cut with a Minot's microtome, and fixed on the slide with albumen and glycerine. After the usual process they were

stained with Ehrlich's acid hæmatoxylin diluted with distilled water, and when they had assumed a brownish pink colour were transferred to a bath of tepid tap water and left for at least two hours. Then for two or three minutes they were stained with a solution[1] of Grübler's water-soluble eosin, dehydrated, cleared by xylol, and mounted in the usual way.

With regard to Wolters's description of polar bodies, I can only say that structures which are explicable only as of this nature are of frequent occurrence in M. agilis. They resemble flattened nuclei, and are placed beyond the surface of the parasite and lie between it and the capsule. A nucleus is usually to be seen within the parasite close to such bodies, and frequently remains of a spindle can be made out passing from the nucleus peripherally towards the polar body, as with Wolters I am inclined to regard it.

I have not been able to give sufficient time to the investigation to place me in a position to criticise Wolters's description of the fusion of the nuclei after extrusion of the polar bodies. I have been able to confirm Wolters's view of the origin of sporogonia in the main, but some modifications are, I think, required of the process as described by Wolters, who would appear to say that every nuclear division after the fusion and re-separation of the original nuclei proceeds by regular mitosis. Against this, such appearances as I have sketched in Pl. 31, fig. 1, may be objected. The drawing represents a syzygium of M. agilis. It was surrounded by a connective-tissue capsule. About the middle of each half of the syzygium is a mass (*a*) which I think can only be regarded as nuclear. These masses are composed of fine granules, most of which are coloured purple[2] by hæmatoxylin. Amongst these are coarser granules, which give with the same reagent a deep blue reaction characteristic of chromatin in both animal and vegetable cells. These chro-

[1] This was obtained by dropping a few drops of a strong alcoholic solution into a watch-glass filled with distilled water.

[2] This is Ehrlich's metachromatic reaction. "Metachromatisch d. h. in Einer dem angewöhnten Farbtone abweichenden Nüance farben," Ehrlich, 'Gesammelte Mittheilungen,' 1891, p. 2.

matic granules are arranged in lines (*b*) which radiate out into the substance of the parasite, in the body of which numerous similar granules can be seen, and they are often joined together by achromatic filaments. Besides these granules numerous typical spindles (*c*) and nuclei are to be seen, placed for the most part at the periphery of each part of the syzygium. I regard the granules as a phase of mitosis, and the purple granules of the nuclear bodies as chromatin in a modification previous to that in which karyokinetic activity begins. Wolters seems at one time to have held a similar opinion to mine in reference to the radiating strings of granules, and to have relinquished it, I think, on insufficient grounds. "Um diese Spindeln sah ich bei präparaten welche durch Flemmingsche lösung abgetodte waren, viele stark färbende Körnchen in der Substanz vertheilt, ebenso hier und da, auch weit ab von den Spindeln, in den Syzygiten. Ich war geneigt dieselben als chromatische Substanz anzusprechen. Spätere Untersuchungen an Hoden, die ich mit Umgehung dieser Lösung abtödtete und härtete, zeigten nichts davon, sodass ich von meiner ansicht zurück gekommen bin, ohne eine befriedigende Erklärung dieser Körnchen geben zu können." In a second drawing, Pl. 31, fig. 2, of part of the periphery of another couple of M. agilis I have represented some of these granules (*a*) on a larger scale. In this case some of the deep blue granules were surrounded by material which was coloured by the eosin of the stain. On comparing these with the sporogonia (*c*) lying at the periphery a close similarity of structure was observed, the body of the sporogonium staining in the same tone with eosin as the material investing the chromatic granules. The nuclei of the sporogonia all stained of the same deep blue as the granules, and they presented a great variety of form. In some mitotic processes seemed to have begun, and the same holds good for the sporogonia of Mon. magna, as is shown at (*b*) in the lower of the two in fig. 3. I am able to confirm Wolters's description of the formation of sporocysts and spores. Whilst speaking of sporocysts I may mention that I have encountered in the seminal vesicles

of Lumbricus agricola prismatic pseudo-navicellæ exactly like those described by Bosanquet[1] in the body-cavity.

The observations made by Wolters on Clepsidrina blattarum are, unfortunately, scanty. Much remains to be done with regard to the earlier phases, both of the poly- and the mono-cystides. More importance attaches to Wolters's description of certain phases of Klossia helicina. This parasite is of great interest from the position it holds between the Gregarines and the Coccidia. From the fact that conjugation has not been observed, I, like L. Pfeiffer,[2] should be inclined to place it with the latter group. First described by Kloss[3] of Frankfort, it has since been described by A. Schneider and by L. Pfeiffer (loc. cit.). To the latter author I am indebted for many beautiful preparations of the parasite in Helix hortensis and Succinea Pfeifferi. Pfeiffer has arrived at some interesting conclusions based on the study of this parasite. Of these the more important are—1st, the phenomenon of multiple[4] infection, as many as fifteen parasites being found within a single epithelial cell; 2nd, that when multiple infection occurs only one of the parasites reached maturity; and 3rd, that the size attained by the parasite is determined by the size of the epithelial cells of the kidney of the species of snail infested by the parasite, though in all cases the sporogonia have the same dimensions, the number of sporogonia varying with the size of the parasite from which they are derived.

The general features of Klossia I have never seen better than in some common grey slugs which I examined in July, 1892. The slugs were found in a hollow in the rocks below the falls of the river Shin, in Sutherland. With them were

[1] W. C. Bosanquet, 'Quart. Journ. Micr. Sci.,' 1894, No. 143, p. 421, fig. 19.

[2] L. Pfeiffer, 'Protozoen als Krankheitserriger,' 1891, p. 72.

[3] Hermann Kloss, 'Senkenbergische Abhandlungen,' vol. i, 1855-6.

[4] L. Pfeiffer compares this with what occurs in the Sarcosporidia, the microsporidia in Coccidium salamandræ, and in the Coccidia of the kidneys of he goose and the dog.

numerous examples of Helix hortensis. The kidneys of the snails and the slugs were all alike infested by the sporozoa, but only in the slugs did I find swarm-sporing side by side with the ordinary mode of reproduction by sporocysts. One of these parasites, as seen in a teased preparation of a slug's kidney, is shown in fig. 4. The nucleus (*a*) was large and oval, and showed a single large nucleolus (*b*). In fig. 5 is represented a cell (*a*) as seen in a section, and containing a parasite subdivided into four sporocysts (*c*) (others were present out of focus), each of which contains six crescentic spores (*d*). In some of these a single nucleus could be made out. The spores were very large, averaging 12 μ in length. All the sections of the kidneys of several slugs showed a marked infection, and in most of the sections swarm-sporing was well seen. Fig. 6 shows an example of this. A much hypertrophied cell (*a*) contains a large sporing parasite, and six smaller parasites (*e*). The sickles (*b*) are very large, 20 μ in length. Some of them are undergoing farther subdivision.[1] The capsule of the parasite has ruptured, and at the point of rupture are some free sickles, which also are undergoing farther subdivision (*c*). The appearance of swarm-sporing in a fresh teasing is shown in fig. 7, where some detached sickles (*b*) are present. The colour reactions in these sections were not good, probably owing to the slugs having in the first instance been placed in Scotch whiskey, so that they will not serve as a basis for comparison with Wolters's descriptions; but since I have been able to find in Coccidium oviforme all this author encountered in Klossia, and also many additional features, I will pass to the consideration of this more familiar parasite. For examination I chose a highly infected liver in which the lesions were still in process of evolution. Fig. 8 shows an average appearance of a portion of the epithelial lining of a cyst as big as a small pea. All the larger parasites show signs of nuclear activity. The chromatin, in nearly every instance, gave a typical deep blue reaction to acid hæmatoxylin. The most abundant form

[1] The large sickles formed in swarm-sporing would thus appear to have the equivalents of sporogonia, not of spores.

in which Coccidium oviforme usually presents itself in sections is the spherical granular body represented in fig. 9 to the left. The granules (*b*) stain slightly with eosin, but remain transparent. They have, no doubt, the same signification as the Gregarina corpuscles[1] in the higher Sporozoa, i. e. they serve as stored food. The "dauerform" (L. Pfeiffer) of the parasite is represented in the same drawing to the right. In it the granules have disappeared, and the nuclear body (*a*), which is round in the spherical-granular phase (*a'*), is oval in the encapsuled parasite. In neither case, however, does the nucleus give the reaction of chromatin, but is stained by the eosin. One other phase of the parasite may be mentioned in passing. This is a small, dense, spherical body, devoid alike of granules and of nuclear body, and staining throughout without eosin. Sometimes a parasite possessing a thick oval capsule is found to have broken up into sickle-shaped swarm-spores, but the rule is that when the parasite multiplies whilst still within the body of the host, it has either no capsule at all or only the delicate so-called primordial capsule. It is to the changes which lead to subdivision of the parasite within the host that I wish to direct attention. R. Pfeiffer[2] first described swarm-sporing, but chiefly in fresh specimens, and without any detailed account of nuclear processes. L. Pfeiffer (loc. cit., p. 45) described distinct hæmatoxylin-stained nuclei in the process of swarm-sporing, and says, "Eingehendere Untersuchungen sind hier noch sehr nöthig, dar der mehr oder weniger akute Krankheitsverlauf von Massgebenden einfluss ist auf die Vermehrungsweise des zugehörigen Parasiten."

Among the parasites in actively extending lesions of the rabbit's liver some present a distinct "geflammte Kern," like that shown in fig. 10 (*c*). Such nuclei take only the eosin of the stain, or at most a slight tinge of purple at their edge. Most

[1] The chemical nature of these bodies is not yet determined. They dissolve n alkalies and mineral acids, and are not fat, nor do they contain lime. See Max Wolters, loc. cit.

[2] R. Pfeiffer, 'Beiträge zur Protozoenforschung,' Berlin, 1891.

of the nuclei which are preparing for subdivision show distinct radiating processes, which stain deep blue with acid hæmatoxylin. Such a nucleus is shown in fig. 11 (*a*). The food-granules in such parasites have become diminished in numbers, and in some cases stain more deeply with eosin. The next stage is shown in fig. 12, where the nucleus has subdivided into two parts with the formation of a typical spindle. Wolters was unable to observe spindle-formation in Klossia, but from the identity of some of the phases of nuclear structure in C. oviforme, and in Klossia as described by him, it is probable that in both as also in Gregarines, spindle-formation takes place. In this way two or three (fig. 1, *a* and *a'*) nuclei are formed. Fig. 13 shows the subdivision of a separated portion of the nucleus. A typical spindle (*a*) is present, and the chromosomes are like those of the Gregarines, extremely small. In many instances the subdivision of the nucleus appears to take place more rapidly, particles of nuclear matter being detached to the periphery along single achromatic filaments. The appearance results in a structure almost identical with that figured by Wolters in Klossia (loc. cit., pl. vii, figs. 10 and 11). When the peripheral nuclei are large the resulting subdivisions of the parasite may be termed sporogonia, for they undergo farther subdivision in the formation of sickles (see fig. 8, *b*). When the peripheral nuclei are small, sickles are formed immediately, as a comparison of figs. 14 and 15 suggests. Fig. 14 shows within a capsule (*c*) the optical section of a parasite with a central nuclear mass (*a*) and small peripheral nuclei (*b*). Fig. 15 shows a collection of sickles (*b*) within a cell (*a*). Sometimes the peripheral arrangement of chromatin takes the form of a zone of fine granules, as is shown in fig. 16, *a*. The peculiar behaviour of chromatin in Sporozoa may be illustrated by the fact that sometimes capsules are met with containing sickles stained only with eosin, though in perfectly similar capsules close to them the sickles have single nuclei well stained with hæmatoxylin. With regard to the phase of Coccidium oviforme, marked by the presence of peripheral bars of chromatin which I mentioned in a previous

paper, I may add that it arises as a modification of the peripheral distribution of chromatin already described, but here the particles are rod-shaped, and at certain stages are connected with a central nuclear mass by a beautiful arrangement of achromatic lines, as shown in optical section in fig. 17, where within the host-cell (*a*) the central nuclear mass (*b*) is connected by achromatic filaments with nuclear rods (*c*) at the periphery. When once this system of peripheral rods is constituted, the subdivision of the parasite by in-dippings of the surface would appear to be the rule. Fig. 18 shows in a surface view an example of such a parasite in which subdivision has proceeded to some extent. Some of the parasites with peripheral rods are of considerable size, and show evidence of having changed their form by active movement. The arrangement of the deep blue rods is then often most complex; this is shown in fig. 19, where the large parasite (*a*) touches the basement membrane on one side of a dip between two papillæ, and on the other the nucleus of a cell, so suggesting a previous conjugation of two parasites. It also has two dark bodies on its surface near its base. These bodies have an appearance quite similar to the polar bodies referred to in gregarines.

The general arrangement of chromatin in the swarm-sporing process in Coccid. oviforme thus agrees with Wolters's description of Klossia, and also with what L. Pfeiffer (loc. cit., p. 31) observed in coccidia: "Rasch folgen auf diese anflockerung des primären Kernes eine Reihe von Kerntheilungen wie sie die Bilder in fig. 12 wiedergeben. Die zahlreiche Tochterkerne liegen an dem Mantel der Parasiten Kugel dicht an und oft in schöner geometrischer Anordnung." As far as I am aware, the description of nuclear spindles in coccidia is here given for the first time. What may be the more minute features of the nuclear figures, i.e. with regard to the attraction-spheres, remains yet to be shown. The different behaviour of the parasites with peripheral rods of protoplasm as compared with such as the one shown, fig. 14, may perhaps prove to throw light on direct nuclear division described by Arnold, as compared with the more commonly observed

indirect division of cells. During the subdivision of parasites such as the one shown in fig. 18, when acid hæmatoxylin is used alone, bright crimson particles of chromatin are sometimes visible in the interior of the segments.

The process of nuclear division is not limited to the larger parasites. Some of the smaller (6—8 μ) intra-cellular coccidia present nuclei which are dividing indirectly, see Pl. 32, fig. 21, *a*. The chromatin is, as a rule, not arranged into chromosomes which can be separately distinguished, but is arranged in what under a magnification of 1000 diameters appear to be masses of material which stain purple or blue with hæmatoxylin. Such small parasites have no large food-granules, and in proportion as the nuclei multiply the cytoplasm and the chromatin increase in amount, giving rise to bodies such as those shown in Pl. 32, fig. 21, *b*, and in Pl. 31, fig. 19, *b*.

Achromatic filaments are distinctly recognisable in many of the parasites in this modification. The usual termination of the process is the formation of sporogonia which contain several minute nuclear masses embedded in eosin-staining protoplasm. The sporogonia subdivide into sickles of the ordinary form. Not infrequently, however, sickles are formed directly, i. e. without a sporogonium stage. Again, the growth of this non-granular form of coccidia may pass beyond the average size (fig. 19, *a*), and the chromatin becomes minutely subdivided and arranged at the periphery, thus producing the phase marked by peripheral rods, as shown in Pl. 32, fig. 21, *d*. Returning to the larger granular coccidia, I would here explain that regular mitotic figures, such as Pl. 31, fig. 12, are extremely few in number. Not so infrequent are typical but slightly irregular spindles, in which all the chromatin of the original nucleus is concerned. Examples are given in Pl. 32, fig. 20, *a*, *b*, and *i*. The whole of the chromatin may become active without the formation of a spindle, as shown in figs. *c*, *k*, and *l*. In the parasite represented in fig. *k* the peripheral collection of chromatin (2) gave a metachromatic (crimson) reaction, whilst the other (1) extremity stained dark blue. In the greater number of the parasites the main mass of the nucleus

remains unchanged for a time, the first indication of karyokinetic activity being the separation of hæmatoxylin-stained chromatin particles joined by achromatic filaments (figs. *d*, *e*, and *h*). When this is the case the unchanged part of the nucleus stains but faintly with hæmatoxylin alone, and when eosin after staining is used assumes a red colour. Sometimes the whole of the nucleus stains deep blue with hæmatoxylin at first without any appearance of nuclear filaments. In such cases the nucleus has the appearance shown in fig. *f*. Irregular mitoses are abundant. Such are shown in figs. *f* and *g*. In such mitoses the first portions separated from the main nucleus frequently give the crimson reaction to hæmatoxylin. Nothing is more striking than the growth of chromatin in coccidia. In the final multinucleate condition the coccidia possess more than a hundred times the amount of chromatin they had at the commencement of the process.

Once more reverting to the parasites with peripheral chromatin rods, it should be observed that in a few cases, which can only be found by patient search, the rods are replaced by fine granules of chromatin, as shown in fig. *m*. Others of the parasites with peripheral rods appear to break up into an immense number of extremely minute sickles without previous subdivision into segments. Finally, with regard to the segmentation of the parasites in the phase marked by peripheral rods of chromatin, it is sometimes seen that the subdivisions possess sickle-shaped outlines like the one seen in optical section in fig. *n*. It is to be observed, however, that such crescentic segments are not homologous with single sickles, but that each peripheral chromatin rod is the potential nucleus of a spore.

I would now turn to mention, and it can be but too briefly, L. Pfeiffer's recent work on the Myxo-, Sarco-, and Microsporidia.[1] In these groups Pfeiffer has added much to bio-

[1] See L. Pfeiffer, 'Protozoen als Krankheitserriger,' 1891, and 'Untersuchungen über der Krebs,' 1891.

For a general account of the Sporozoa and the groups included, together with figures of most of the important forms, see Lankester's 'Zoological Articles' (A. and C. Black), article "Protozoa."

logical knowledge, and has closely studied the effects of the parasites on their hosts,—or, in other words, their pathological effects. Since Dr. Pfeiffer has most kindly given me many beautiful preparations of Sporozoa belonging to this and other groups, I have been able to study, and, I may add, to confirm his results.

With regard to the Myxosporidia, the description of the epidemics they have caused from time to time in some of the rivers of Germany forms a most interesting part of Dr. Pfeiffer's work, and is worthy of the close attention of pisciculturists. Barbel, pike, and perch were chiefly attacked. "The sick barbel present a striking appearance from the presence of discoloured tumours in the skin, and of deep crateriform ulcers on the head, the hinder part of the body, and the tail: the ulcers have a widely infiltrated base." Fig. 22 shows part of one of Dr. L. Pfeiffer's sections of a myxosporidial tumour of a barbel. The growth is alveolar in structure, the alveolar walls being composed of fibrous tissue. The contents of the alveoli consist solely of parasites. In the upper part of the figure is a portion of the periphery of a reticulated parasite from which sporocysts have separated; at the edge of the parasite is a nuclear spindle. The thread-cells of the sporocysts are stained deeply. Some of the sporocysts are devoid of definite characters; whether they are young forms, or residua after the escape of the single amœboid spores, is a question I have not been able to determine. L. Pfeiffer shows that these tumours begin as an infection of striped muscle-fibres, and some of the preparations demonstrate this point most distinctly, the young parasites in all stages of existence being visible within muscle-fibres at the periphery of the tumour.

Writing of the effects of Sarcosporidia, Leuckart has said, "Although the tubes occasionally occur in immense numbers close to one another, so that the flesh looks as if half of it consisted of psorosperm tubes, yet they seem usually to cause no special uneasiness. In many cases, however, the phenomena of paraplegia, retarded respiration, and even suffocation, are

observed as associated with the presence of the tubes, and may with some probability be referred to this cause."[1]

L. Pfeiffer has demonstrated the nature of these fatal cases, showing that they are the result of the escape of the sickles from the muscle-fibre in which they are developed, and their entrance into the surrounding muscle-fibres, in which they appear as groups of minute round cells, and growing to a certain size constitute a new Miescher's tube, which, unless the host acquires a greater resisting power, again ruptures and liberates its swarm-spores.

I have been able, both in Dr. Pfeiffer's preparations and in others of my own, to confirm these observations. Pfeiffer has further shown that an emulsion of Sarcosporidia injected into rabbits causes an intense inflammatory reaction and toxic phenomena, and this is in keeping with what is seen in the progressive infection of muscle referred to above. The free spores seem to have a marked attraction for leucocytes (chemiotaxis), and thus the process has been termed by Pfeiffer "myositis sarcosporidica."

When this progressive infection is limited to a certain region, instead of inflammatory changes tumour formation is seen. Thus in horses L. Pfeiffer has found that tumours included by Kölliker in a group termed "Muskelknospen" are determined by sarcosporidia. Some of Dr. Pfeiffer's sections show this in, I think, a most conclusive manner. The growth possesses a stroma of fibro-cellular tissue, in which three zones may be described: an inner containing dense foci of degenerated material surrounded by giant-cells, &c.; a middle zone containing numerous Meischer's tubes, from which the sickle-spores are escaping; and an outer zone containing many muscle-fibres infected by Sarcosporidia.

One effect of a chronic progressive infection by Sarcosporidia has been most exquisitely shown by the same observer. On the œsophagus of sheep, white cysts, of which the largest are as big as horse-beans, are sometimes encountered. The larger (older) cysts are provided with a fibrous capsule. Dr. Pfeiffer's

[1] Leuckart, Hoyle's (1886) translation, p. 202.

sections show that the capsule is formed by a reactive inflammatory process, and that between its fibres lie compressed muscle-fibres (fig. 23, *a*). Those muscle-fibres which are embedded in the deeper, i.e. older part of the capsule, are distended by young sarcosporidia, which present a definite nucleus and a clear protoplasm (fig. 23, *b*). Next to the capsule come large spaces (fig. 23, *c*), filled with sickles. The spaces result from the distension of muscle-fibres and the consequent separation of the fibrous fasciculi of the capsule. It may be noted that some of the sickles in the spaces close to the capsule are subdivided into segments. I may add that whilst the sickles contain particles of chromatin throughout the whole of their substance, the youngest parasites have a definite nucleus and a clear protoplasm. L. Pfeiffer has thus shown that progressive inflammatory changes, cyst- and tumour-formation may be determined by Sarcosporidia.

Finally Dr. Pfeiffer's sections of the muscles of frogs show that like the Myxo- and Sarco-sporidia, the Microsporidia occur within muscle-fibres. Fig. 22 shows the remains of a muscle-fibre containing some of these parasites. One group of spores are not yet liberated from the parent cell, and present a central nuclear spot and an unstained peripheral region. The free spores are minute (2 μ) bodies, most of them slightly curved, so that they present the strongest possible resemblance to a collection of vibrios.

I cannot close this article without expressing my warm thanks to Dr. L. Pfeiffer for the liberal manner in which he has answered my request for specimens and material.

LONDON; December 27th, 1894.

EXPLANATION OF PLATES 31—33,

Illustrating Mr. J. Jackson Clarke's "Observations on Various Sporozoa."

FIG. 1.—Section through a syzygium of Monocystis agilis, × 500 diams. From the nuclear masses, *a*, in the central part of each half of the syzygium numerous moniliform threads, *b*, of chromatin are directed towards the periphery, where are many small nuclei and nuclear spindles, *c*.

FIG. 2.—Section through part of the periphery of a syzygium of Monocystis agilis, × 1000. From left to right are (1) the connective-tissue capsule, *a*, (2) sporogonia, *c*, (3) part of the body of the parasite with gregarina corpuscles, nuclear spindles, *b*, and particles of chromatin, *a*, round some of which a covering of protoplasm has collected.

FIG. 3.—Two sporogonia of Monocystis magna, × 1000 diams. The nucleus of the lower of the two shows achromatic filaments, *b*, joining the particles of chromatin. The body, *a*, of the structure stained with eosin.

FIG. 4.—A free example of Klossia from the grey slug, × 1000 diams. Fresh specimen. Nucleus, *a*, nucleolus, *b*. × 1000.

FIG. 5.—An epithelial cell in the kidney of a grey slug containing a parasite subdivided into sporocysts. Some of the sickles contain a single nucleus. There are no residual bodies. × 1000 diams. From a section.

FIG. 6.—A cell, *a*, from the kidney of a slug containing a parasite in the process of swarm-sporing. There are six smaller parasites, *e*, in the protoplasm of the cell. *b*. Large sickle-shaped sporogonia. *c*. The same dividing. *d*. Undivided central part. × 1000 diams.

FIG. 7.—A free Klossia showing swarm-sporing with some sickles, *b*, detached. Fresh teasing. × 750 diams.

FIG. 8.—Part of the epithelial lining of a coccidial cystadenoma of a rabbit's liver. *a*, *a'*. Parasites with two and three nuclei respectively. *b*. A parasite subdivided into sporogonia. × 1000 diams.

FIG. 9.—A round granular and an oval encapsuled coccidium. *a*, *a'*. Nuclear body (red). *b*. Cytoplasm. *c*. Definitive capsule. 1000 diams.

FIG. 10.—An intra-cellular coccidium with a "Geflammte-Kern" at *a*. × 1000 diams.

FIG. 11.—An intra-cellular coccidium, the nucleus, *a*, of which shows radiating bars of chromatin. × 1000 diams.

FIG. 12.—Intra-cellular coccidium, the nucleus, *c*, of which is dividing by regular mitosis. *b*. Cell-protoplasm with food-granules. *a*. Host-cell. × 1000 diams.

FIG. 13.—A coccidium which shows, besides the main nucleus, a nuclear spindle. × 1000 diams.

FIG. 14.—An encapsuled coccidium (capsule, *c*) with peripheral particles of chromatin, *b*, which are connected with a central nuclear mass, *a*, by achromatic filaments. × 1000 diams.

FIG. 15.—An epithelial cell, *a*, of the rabbit's liver filled with nucleated sickle-spores, *b*. × 1000 diams.

FIG. 16.—An intra-cellular coccidium with numerous minute chromatin granules at *a*, the periphery.

FIG. 17.—An intra-cellular (host-cell, *a*) coccidium showing close-set peripheral rods of chromatin (*c*) connected with a central nuclear mass (*b*) by achromatic filaments. × 1000 diams.

FIG. 18.—A coccidium with peripheral chromatin rods seen in a surface view, and showing the mode of subdivision. × 1000 diams.

FIG. 19.—Part of the epithelial lining of the rabbit's cystadenoma showing large free parasite with peripheral rods and granules of chromatin, and at *x* a multinucleated parasite. × 1000 diams. The free parasite is seen in surface view.

FIG. 20.—*a*. Granular coccidium with a slightly irregular nuclear spindle.

b. A parasite with a nucleus similar to that shown in Fig. *a*, but with granules of chromatin at the left half of the spindle.

c. Granular coccidium, the nucleus of which has become altered so that it presents a collection of granules of chromatin.

d. Granular coccidium, of which the outer part of the nucleus is separated in an irregular spindle (1), whilst the central part remains unchanged (2).

e. A similar condition to that shown in Fig. *d*.

f. and *g*. Irregular mitoses.

h. A condition similar to that shown in Figs. *d* and *e*. The central unaltered part (2) of the nucleus stained with eosin; the peripheral part (1) (irregular spindle) stained with hæmatoxylin.

i. Irregular spindle.

j. Elongated nucleus stained densely with hæmatoxylin.

k. Irregular mitosis. Left extremity of the chromatin band stained blue, right extremity crimson. Acid hæmatoxylin alone.

l. Irregular mitosis, granules stained purple.

m. A large parasite in a phase equivalent to that with peripheral arrangement of rods, the latter replaced by granules of chromatin.

n. Large parasite with peripheral rods undergoing segmentation, one of the segments crescent-shaped. Optical section.

21.—A portion of one of the papillary processes of a coccidial tumour seen in section. *a*. Young parasites with division of nuclei. *b*. Larger but still not granular coccidia with dividing nuclei. *c*. Capillary vessels and basement membrane.

Fig. 22.—Section of a myxosporidial muscle-tumour of a barbel. From a preparation made by Dr. L. Pfeiffer. × 1000 diams.

Fig. 23.—Section of a sarcosporidial cyst from a sheep's œsophagus. From a preparation made by Dr. L. Pfeiffer. × 1000 diams.

Fig. 24.—Microsporidia in frog's muscle. From a preparation made by Dr. L. Pfeiffer. × 1000 diams.

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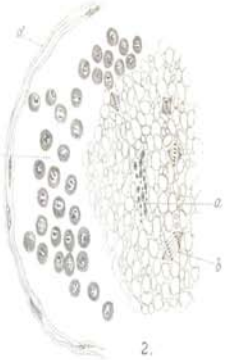

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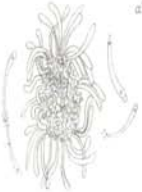

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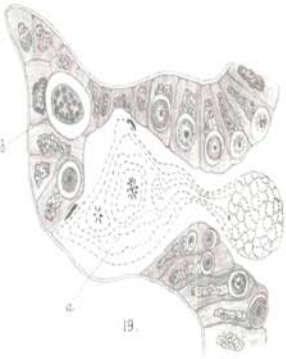

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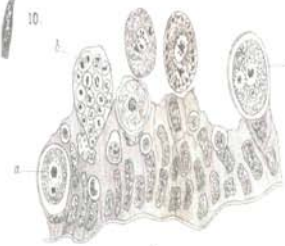

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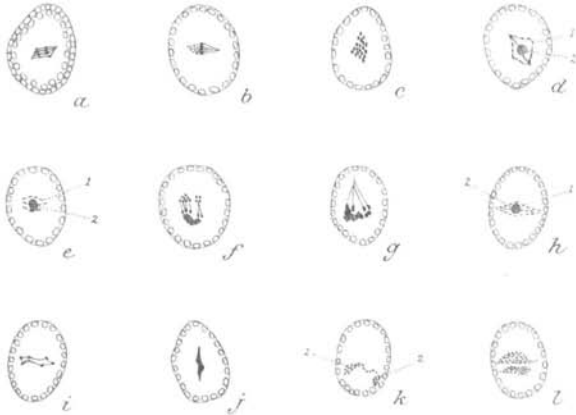

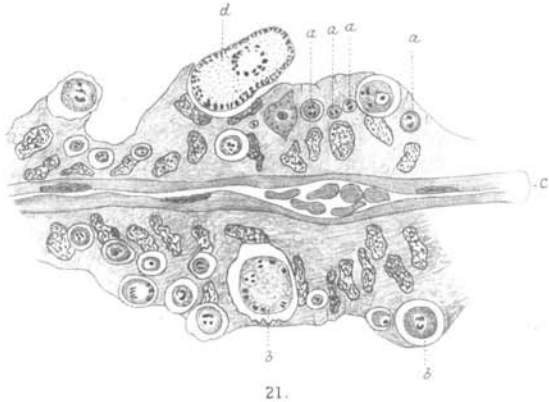

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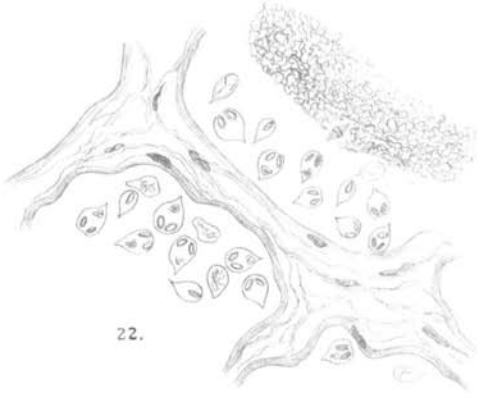

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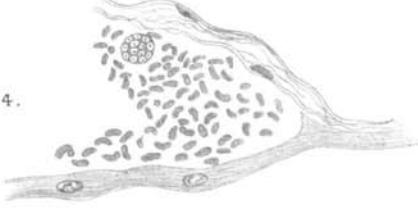

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