Notes on Sporozoa.

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Smith and Johnson (1) recently described a new Coccidian parasitic in the kidneys of the mouse (*Mus musculus*). The seat of the infection is the renal epithelium of the convoluted tubules of the cortex and of the visceral layer of Bowman’s capsules. The enormously hypertrophied parasite-containing cells swell out into and completely occlude the lumen of the tubule, causing entire disorganisation of the affected tissue.

The diagnostic characters on which the new genus is based are as follows. The sporogonic cycle is characterised by the development of twelve to fourteen spherical spores, each about 16 \( \mu \) by 13 \( \mu \), and containing thirty to thirty-four banana-shaped sporozoites. Another phase of the life-history was also met with. This is taken by Smith and Johnson to represent either schizogony or the formation of microgametocytes, but actually which of the two is left an open question. As a matter of fact, the authors’ figures leave no doubt that the stage which they have described as sporogonic is nothing more nor less than merogony or schizogony, while the other part of the cycle is, in all probability, the commencement of gametocyte formation. As this new Coccidian presents certain very interesting features, I have thought it worth while to give a re-interpretation of Smith and Johnson’s
clear and careful drawings, the real significance of which will be readily manifest on comparing them with the figures of another Coccidian, Caryotropha mesnilii, lately described by Siedlecki (2) from a Polychaste, Polymnia nebulosa, where it inhabits the testis. The name Klossiella muris may quite well be retained, at any rate until the parasite is re-discovered and the number of its genuine spores and sporozoites determined, since, notwithstanding the resemblance between the schizogonic phase in the two forms, the very different habitat, and important distinctions in the manner of formation of the microgametocytes already preclude us from uniting the two genera together.

The drawings in Fig. A are reproduced from Smith and Johnson’s figures, and those in Fig. B are copied from Siedlecki’s paper. All are drawn the same size as the originals.¹ I will first give, as it were, a revised account of what is known of the life-history of Klossiella muris, and then proceed to justify my interpretation of the same, finally contrasting the genus with one or two other Coccidia. The authors’ designations of the various stages are enclosed in square brackets.

In Fig. A (1) we have one of the smallest trophozoites [sporonts] seen. Such a young form, commencing to grow, is from 8 µ to 11 µ in diameter, and lies in a vacuole (v.) in the host-cell. Its membrane, so far as it has one, is very delicate, and practically only a limit to the cell. Each individual contains from ten to twenty plastin granules (pl. g.). “N.” is the nucleus of the host-cell, and “n” that of the parasite. In the next figure, A (2), the trophozoite has become considerably larger (even allowing for the difference in magnification), and is now almost full-grown; it is, in fact, a schizont beginning merogony [mother-sporoblast]. Such an adult trophozoite or schizont

¹ A comparison of Smith and Johnson’s different figures would have been greatly facilitated if they had been drawn to the same, or multiples of the same, magnification; while Siedlecki does not give the magnification of his at all.
may attain a diameter of as much as 40 μ. In the one before us the nucleus has already divided up into several, each possessing one to four karyosomes (k.), with usually a certain amount of granular chromatin besides. Around each of these daughter-nuclei the cytoplasm segregates itself, and thus the parasite becomes (superficially) divided up into a number of uninuclear portions (Fig. 3). These buds next commence to grow out at the periphery (Fig. 4), forming daughter-schizonts, or, as Siedlecki terms them, “schizontocytes” (sz.c.) [daughter-sporoblasts]. The host-cell is by this time greatly hypertrophied, and consists for the most part of a very delicate, attenuated layer of protoplasm, enclosing the huge vacuole in which the Klossiella lies; on one side (at h. c.) it is rather thicker, and this portion contains the nucleus, also much altered and hyperchromatosed. The schizontocytes
are at length cut off, and become separate inside the remains
of the cell. According to Smith and Johnson, the central
part of the cytoplasm of the mother-schizont may be entirely
used up ("resorbed") by the daughter ones, as in Fig. A (6),
or some may be left over as a residual body [restiform body].
In Fig. A (6) the contents of each separate schizontocyte
[spore] have further divided up into a great number of
merozoites (m. z.) [sporozoites], all arranged in one direction,
and constituting, indeed, a typical merogonic "barillet."
The homogeneous-looking masses are simply deeply stained
daughter-schizonts, too opaque to show the merozoites inside.
It will be observed that the only "membrane" holding the
products resulting from one parasite together is the com-
pletely atrophied host-cell. Fig. A (5 b) shows a single
barillet of merozoites liberated from a fresh kidney; the
cluster is attached to a small secondary residual body (r. b.).
Our authors state that the membrane surrounding the mero-
zoites [i.e. the spore-membrane] is usually rounded, but of
no definite shape and quite structureless, and in optical
section appears only as a sharp line; moreover, it is easily
ruptured on pressure, setting free the enclosed merozoites.
In short, it doubtless represents, in its turn, the remains of
the schizontocyte, nearly all of which has been used up to
form the cluster. At (a) in the same figure are seen two
free, unstained merozoites [sporozoites], each about 7 $\mu$
by 3 $\mu$ and containing several little vacuoles, one of which is
often more prominent than the rest.

Such is the so-called sporogony of this Coccidian. With
regard to the other phase of the life-history (Smith and
Johnson's two figures of which I have not thought it neces-
sary to reproduce) a few words will suffice at present, since it
in no way affects the question of the sporogony of the phase
above described. The authors term this the "glomerular" stage of the parasite, since it is found in the epithelium of
Bowman's capsules, whereas the other form principally occurs
in the convoluted tubules. As the glomerular form was only
found in kidneys already infected with Klossiella, we can,
I think, agree with Smith and Johnson that the two are in some way related.

The chief difference between them is that in the former there is no "budding" nor anything analogous to the formation of schizontocytes. As the young parasites grow the (at first single) nucleus divides successively to form a great many, evenly distributed throughout the cytoplasm. The latter then segments up around these daughter-nuclei, and there result numerous "falciform bodies," which are, however, not nearly so sickle-shaped as the merozoites, but more of an elongated lozenge form. Each of them is about 7 μ by 2 μ, and possesses a rather small nucleus, centrally situated. The further history of these bodies was not followed; the authors suggest that the process may represent either schizogony or microgametocyte-formation, saying that the position is a favourable one for the development of either phase, but they do not decide between the two hypotheses, though perhaps, on the whole, rather inclined to support the latter. Nothing in the nature of macrogametocyte-formation was noticed.

I propose now to summarise my reasons, most of which will be, I think, already evident, for considering that the more fully-described part of the life-cycle of Klossiella is, in reality, only the schizogonous phase—serving for auto-reproduction, and not the sporogonic phase—producing resistant spores capable of transmitting the species to a fresh host. The spore-forming cyst, or oocyst, in the Coccidia is the result of fertilisation of a macrogamete by a microgamete, and may be looked upon as the final stage of the life-history undergone in the host. Representing, as it does, the termination of growth, the large macrogametocyte up to the time of maturation is contained within an atrophied host-cell, from whose shrunken and shrivelled remains it is set free prior to fertilisation. After conjugation (indeed in some cases before, e.g. in Coccidium proprium and C. faurei) a cyst-membrane is rapidly secreted round the oocyte (now the sporont), which becomes thick and tough and affords protection to the developing contents. Obviously, no further increase in size
is possible. Moreover the sporont is typically extra-cellular during the whole course of sporogony. Compare this with what we find in Klossiella. In Fig. A (1) we have a young form possessing, at most, a very delicate membrane, and lying in a vacuole in a host-cell that as yet shows hardly any effects of the parasitic invasion. Again, this young “sporont” grows from 10 μ to as much as 40 μ! Further, in the nuclei and nuclear division in a Coccidian sporont—in fact, while the sporogonic cycle lasts—there is no sign of karyosomes. When, as in C. proprium, these are retained in the ripe gametes and are thus present in the oocyte, they are invariably left behind in the residual cytoplasm of the latter and take no part in spore-formation; and even their retention up to this stage is unusual. The presence of karyosomatic nuclei is, in short, essentially a mark of schizogony, be it male, female, or indifferent in type; and it is a feature in the multiplicative stages before us (Fig. A 2, 3, 4). We will leave out of account the markedly peripheral origin of the “buds,”—although peripheral budding is characteristic of endogenous reproduction,—since in polysporous types (Klossia, etc.) there is a tendency to a similar mode of origin of the sporoblasts, with the formation of a central “reliquat kystal.” Let us pass on to the “spores” themselves. There is now no doubt about the occurrence of polyzoic spores; Cyclospora itself and the re-investigated Eucoccidium (“Benedenia”) octopianum are instances of it,—so it is quite possible that, in these cases, there may also be a more or less “barillet”-like arrangement of the sporozoites, such as is often met with in merozoites.

Here, however, the resemblance between the bodies seen in Fig. A (6) and spores ceases. Besides the very important facts that they are not enclosed in a definite oocyst and are still within the host-cell (the former of which, at any rate, would be without analogy in the order), there is another reason why these bodies cannot be regarded as representing true spores. This is their varying and indefinite shape—or rather their shapelessness,—together with the extremely delicate nature
of the envelope enclosing each cluster of germs. A Coccidian sporocyst is always quite definite in form and fairly tough and resistant, and generally consists of two valves which separate under the action of the new host's digestive juices (sometimes, this can be effected artificially) to liberate the sporozoites. Nothing of the kind is mentioned in Smith and Johnson's account; the authors simply state that the membrane is very delicate, and easily ruptured on pressure. As I have above suggested, it much more probably represents (together with a small amount of residual material) the remains of a daughter-schizont, most of which has gone to form the merozoites. Between these and sporozoites, in the fresh condition, there is little difference, so that I need only add that if my interpretation is correct, the germs in Fig. A (5 b) belong to the former category and not to the latter.1

Of course the novel, and at that time unexampled variation which distinguishes schizogony in Klossiella from the usual method, might, to a certain extent, mislead the authors in interpreting their observations. Apart from this, however, the above-mentioned very characteristic facts relative to the general course of development of a Coccidian parasite and its relation to the host-cell ought to have rendered them suspicious in accepting the observed stages as constituting sporogony. As it happened Siedlecki (l. c.) very soon afterwards described a similar modification of merogony in Caryotropha. The resemblance between the process in the two genera is most striking, and I have above used this author's terminology in interpreting the phase as it occurs in Klossiella.

In Fig. B are reproduced some figures of Caryotropha for comparison with those in Fig. A. In (1) the host-cell (a spermatogonium) and two of its neighbours are greatly hypertrophied and have fused into a single mass containing

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1 Unfortunately it is impossible to tell from fig. 6 (the stained preparation) whether the germs have a karyosome in the nucleus or not, which would have conclusively settled the question.
the schizont. The cytoplasm of the parasite is left clear; its large karyosomatic nucleus is seen at (n), while at (N) we have the enlarged spermatogonial nuclei of the altered cells. “Sp. g.” represent normal spermatogonia around. The parasite, though not full grown, is, of course, relatively much older than the young Klossiella schizont of Fig. A (1). The next stage of Caryotropha depicted, seen in Fig. B (2), shows a condition intermediate between Figs. A (4) and (6). The mother individual has divided up into daughter schizonts or schizontocytes, ten or more in number, which are separate, but have not yet commenced to form merozoites. From Siedlecki’s account it is evident that these daughter-individuals have arisen in a manner perfectly analogous to their origin in Klossiella. He says that each of the nuclei resulting from the division of the original nucleus of the parasite pushes out at the surface of the body (surrounded,
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doubtless, by a "bud" of cytoplasm), and between them deep grooves extend inwards, so that at length the whole schizont becomes cut up into several portions—the schizontocytes. He does not add whether any residual cytoplasm may be left over unused or not. A small point distinguishing the schizogony in this genus is the unusually minute size of the karyosomes, which are present in the daughter-nuclei only as one or two granules. I think the last doubt will be removed by a comparison of Figs. B (8) and A (6), especially if we regard each of the clusters in the latter as showing up like it does in Fig. 5 (b). In both cases all the "barillets" are enclosed by the partly or entirely atrophied cell or cell-mass, and by that alone. The only slight difference is that in those of Caryotropha the remains of the daughter-schizonts seem to have more completely broken down than they have in Klossiella, leaving no distinct enclosing membrane. It is, however, most likely that in older clusters of the latter genus the delicate investment around each also naturally breaks down, as, indeed, it must do if the essential object of schizogony, namely auto-infection, is to be attained.

The marked correspondence between the schizogonic process in the two forms does not appear to be maintained in microgametocyte-formation. In Caryotropha this resembles schizogony to a surprising extent, and serves to emphasize the complete homology of the two kinds of reproductive germ. Briefly stated, a number of microgametocytes of the second order (strictly comparable to schizontocytes) are intercalated between the original microgametocyte (of the first order) and the ultimate male gametes. The microgametes themselves arise from these daughter-microgametocytes exactly as if they originated in the usual manner from the microgametocyte of the first order, as, e.g. in Coccidiium. Until ripe and ready for liberation they are all contained within the atrophied host-cell, just as are the clusters of merozoites. So far as can be gathered from Smith and Johnson's account nothing of the kind occurs in Klossiella; but this form, on the other hand, would appear to possess a
differentiation in another direction which is not met with in Caryotropha. In the latter there is no sign of an early differentiation of sexuality. The merozoites (representing the end term of schizogony), which grow into microgametocytes of the first order or macrogametocytes, respectively, are in no way different from the indifferent ones which become ordinary schizonts; that is to say, there is no male or female schizogony accompanied by the formation of male or female merozoites such as we find in certain cases (Adelea, Cyclospora). Now in Klossiella the "glomerular" form mentioned above almost certainly represents either male or female schizogony, leading to gametocyte-formation, and this view is supported by the authors' remark that the phase was only found in kidneys already strongly infected with the other stage, i.e. when merogony, we may assume, had almost run its potential course. In the absence of any further knowledge of the parasite it is impossible to say with certainty which sex the lozenge-shaped bodies above described represent; whether, in other words, they will grow into micro- or macrogametocytes. Smith and Johnson are inclined to think they may become the former, and suggest that they give rise to the actual gametes only when attached ("accoles") to a female element; they did not, however, observe this process taking place. Their shape somewhat recalls that of the male merozoites of A. mesnili as figured by Perez (3). Whether, if we accept these as male elements, the female merozoites (becoming macrogametocytes) are similar to the indifferent ones (as in A. mesnili, again), and whether they are formed in the same complicated manner, or by simple schizogony, are facts which have still to be ascertained. In any case the rediscovery of Klossiella muris, and the working out of its complete life-history, would probably furnish some very interesting and important additions to our knowledge of the Coccidia.
References.

