Klossiella muris.

By

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With Plate 10.

THIS parasite was first described by Smith and Johnson in 1902. There are only two other papers I know of on the subject, one by Woodcock of a critical nature, and one by Sangiorgi containing experimental facts of interest.[1] In all the above the only phases of the parasite described and mentioned are those in the glomeruli of the kidney, the epithelium of the tubules, and the urine. The first describers consider the schizogony that takes place in the glomeruli as a formation of merozoites, and the tubular phase as sporogony leading on to spores and sporozoites in the urine. Woodcock, on the analogy of Caryotropha mesnili, suggests that the tubular phase is one of schizogony leading to merozoites and that possibly the glomerular phase is one of schizogony leading to gametocytes. Sangiorgi, in a paper of which I have received an abstract from Woodcock, holds the same view with regard to the tubular phase, and says that he has infected mice with urine which contained cysts, but he seems unaware that these cysts contained definite products of division. Sangiorgi also states that he could not infect mice from mash of kidney-substance, and states that he considers the cysts in the urine to be oöcysts and describes the glomeruli of the kidney as containing bodies which he describes as sporozoites in a membrane or spore-capsule. He says that difficulty

[1] In addition to these Seidelin has lately published a paper describing a similar parasite in the guinea-pig in Africa, but mentions no other forms than those found by Smith and Johnson.

arises as to the phase of the parasite in which a new host is infected.

As will be seen, I consider the original describer is right in thinking the stage in the tubules to be sporogony, since cysts found freely in the urine of infected mice are practically in the same condition as those seen in the last stages of development in the kidneys. Sangiorgi's first experiment confirms this, but a criticism on the experiment must be raised as to whether his mice were previously infected or not. Examination of the urine would prove nothing in the early stages. I have several times missed infections in fresh specimens of the kidney, and found the parasite when I cut sections, cysts being nowhere present.

If the cysts are not spores, it is difficult to imagine how another host is to be infected on the analogy of coccidium. If cysts contain gametocytes and an insect host is concerned, there is only one I have heard of that is definitely attracted by urine, ants.

The schizogony in the glomeruli I consider to be one that produces gametocytes and to be secondary to the one producing merozoites which takes place elsewhere.

My great difficulty is the type of syngamy that takes place, but I have no doubt that this stage takes place in the cells of the kidney tubules.

I have found the parasite in about 40 per cent. of the white mice examined (25). I have an impression that it occurs more frequently during the summer months, but this may be due to differences in the source of supply of the mice. It is, however, possible that infection is brought about by an invertebrate host prevalent in summer, and, from what I have seen of the organism, I think this may take place as well as infection by the normal casual coccidial method.

Forms observed.

What is possibly the earliest form I have seen was in a mouse, which post-mortem showed no parasites in the kidney,

but had lived in a cage with others that were passing cysts. It consisted of a small gregariniform body about 12 μ long, free in the peripheral blood (Pl. 10, fig. 1). When stained by Giemsa, however, its morphology very closely resembles that of the merozoites of an intestinal coccidium of the mouse, and it is quite possible that one of these might have found its way into the blood-stream. I do not know if this mouse had coccidiosis of the intestine, but a very large percentage have.

The next stage I have seen was one in an arteriole of the kidney (Pl. 10, fig. 2). This consisted of a round body in an endothelial cell of the arteriole, with three nuclei in process of division. The diameter is about 8 μ. It may, however, be an early instance of either of the following types of schizogony.

In a similar position I have found a parasite definitely divided up into eight to twelve daughter individuals, these still being contained in an envelope formed by the remains of the host-cell (Pl. 10, fig. 3). This I take to be the schizogony of the parasite into merozoites. I have searched many sections of kidneys and other organs both in infected and uninfected mice, but up to the present I have not come across any other instances of the above stages. The daughter individuals in the above case have a definite nucleus with a well-marked karyosome. As the division in the other type of schizogony and also that into sporozoites are characterised by the great numbers of individuals produced, it is possible that this first type is comparatively infrequent in occurrence.

The second type of schizogony is very different, the daughter individuals being very numerous, fifty to sixty being a moderate estimate. This division takes place usually in the cells forming the capillaries in the glomeruli of the kidneys, but on rare occasions (three)[1] I have seen it occurring in the endothelial cells of the arterioles of those organs (Pl. 10, figs. 9, 10).

As first seen, the parasite in this stage is a small uninucleate body bulging into the capillary, the granules in it giving it a greenish-grey tinge when stained with iron-

[1] Since the above was written, I have seen two other instances; in one the parasite had only two nuclei, in the other division was complete.

hæmatoxylin and orange (Pl. 10, figs. 4, 5, 6). This nucleus divides apparently fairly rapidly till we get a large mass occupying quite a large portion of Bowman's capsule in section, and containing nuclei too numerous to count easily, but about fifty to sixty in number (Pl. 10, figs. 7, 8). Around these the protoplasm segments, and we get a large number of gregariniform bodies, each with a nuclear area, containing two small masses of chromatin, and some residual protoplasm (Pl. 10, figs. 11, 12, 13). These chromatin granules are marked by their ovoid shape, and lie side by side with their long axes parallel to that of the organism. The mass of merozoites lies in a capsule formed by the thinned-out remnants of the host-cell, and on the bursting of this into Bowman's capsule the organisms are set free to wander down the tubule of the kidney, where they enter the cells of the convoluted portion in, I believe, the condition of gametocytes or gametes.

The next stage is the one somewhat difficult to interpret. There is no doubt in my mind that the normal condition is a double infection of the cells, the two individuals being at first indistinguishable from one another. Single infections are also found, but I think the balance of evidence is that these do not develop farther in a normal way. There is certainly often found growth, which is sometimes marked, and even budding apparently occurs, but this latter is generally internal instead of the normal external type.

Again, in advanced cases where spores are freely found in the urine, one frequently finds cast-off epithelial cells containing uninucleate small parasites. Another point in favour of this view is that in tubules containing parasites well on in the sporoblastic stage and further, it is common to find cells containing small uninucleate parasites. When one considers that the parasites of the epithelium of the tubule must arrive there practically simultaneously, for it is rare to find two bodies undergoing schizogony in one Malpighian tuft, it is difficult to understand the great difference of development unless due to syngamy having occurred in the advanced cases.

What is the type of syngamy? There seem two possibilities. First, though the observed instances are not too numerous, that it resembles that of Adelea ovata,[1] where the nucleus of one of the two associated and closely applied individuals divides into microgametes (four), one of which fertilises the other individual.

Second, that complete fusion takes place between the cytoplasm of the individuals, and their nuclei divide, though fusion of the chromatin does not take place till a much later stage.

That anisogamy of the type of coccidium with many microgametes formed separately from the macrogametes does not take place, I am practically certain.

The evidence for either of the above two views is not very definite. In favour of the first are the facts that forms in one cell sometimes show differences of nucleus and cytoplasm, the one showing signs of division in one and not in the other, while the latter is more finely granular in the parasite with the dividing nucleus and coarse in the other (Pl. 10, figs. 17, 18, 19). In later stages when apparent fusion has taken place and division has proceeded, there can sometimes be observed a body closely resembling a bud, but more definitely separated from the main mass, which contains irregular masses of a chromatin-like staining substance (Pl. 10, figs. 22, 23, 26, 29).

In favour of the second view is the fact that all through the enlargement of the parasite after fusion there seems to be two types of chromatin present, shown by different staining reactions, the one retaining iron-hæmatoxylin well, and staining deeply with acid hæmalum, while in the other the staining intensity is less marked. These masses often lie close to one another in pairs, and the dark stained one seems to divide first. This condition is retained until the bud is finally separated from the mother individual. This, however, may be due to differences in the condition of the chromatin, division of nucleus, etc. (Pl. 10, figs. 26, 27).

Whichever of the above views is right, there is no doubt

[1] Further observations tend to this view.

that fusion or association of two gametocytes takes place, and that after this nuclear division follows until there are twelve to sixteen nuclei arranged round the periphery of the parasite. The nuclei gradually travel outside the line of general contour of the parasite (Pl. 10, fig. 30) and along with some of the cytoplasm are finally budded off one by one. There can often be found a renal cell containing two or three buds and the remainder of the parasite still undivided (Pl. 10, fig. 31). By the time all the buds have formed, which are generally twelve to sixteen in number, the renal cell has become tremendously enlarged and often dilates the renal tubule to many times its original diameter. It is still, however, attached to the basement membrane by a fine point of apparently altered protoplasm. This fine attachment is seen at very early stages of infection of the cell; in fact, nearly all infected cells project freely into the lumen of the tubule (Pl. 10, figs. 15, 32).

The nuclei in these buds or sporoblasts then divide, enlargement takes place, and a cyst wall forms round the sporoblast till we finally get a spherical body containing about twenty-five nuclei, each of which latter consists of three granules of chromatin arranged in a line about an equator of the sphere and at right angles to the plane of that equator (Pl. 10, fig. 35). Around each three granules of chromatin the protoplasm segments, and we get a spore cyst containing about twenty-five sporozoites with a certain amount of residual matter left at one pole of the cyst (Pl. 10, figs. 36, 37, 38). The capsule containing these spores then bursts, and the spores travel down the tubules through the papilla and ureter to the bladder in which situations (except the ureter) I have found them in sections. In the urine they can be easily found if the infection is at all heavy.

General.

The later stages closely resemble those described by Christophers in the sporogony stage of Leucocytozoon

Canis in its invertebrate host. Miss Porter also in her description of the Leucocytozoon of the mouse mentions having found a schizogony stage in the bone-marrow. Is it possible that the organism we are dealing with is the same as this latter Leucocytozoon? We have only to postulate that in a large infection we might get a large number of parasites undergoing the gametocyte-forming schizogony in the arterioles, and when these were set free in the blood-stream they would probably be taken up by leucocytes. From Miss Porter's description the schizogony stage she saw was possibly that into merozoites.

Another interesting supposition also arises in regard to this parasite. Have we here a link between the hæmogregarines and the coccidia? We have only to presuppose that gametocyte formation takes place largely in the arterioles instead of in the Malpighian tuft capillaries, and we get the blood-stream charged with parasites in a condition suitable for sporogony in a blood-sucking host. The question of the greater (?) incidence of the parasite in summer also bears on this point.

From the medical standpoint of view the possibility of a protozoon being a parasite of the arteries seems to me to be of great importance.

Methods.

Fresh specimens were always examined, the tissue being teased in normal saline. This was useful for general diagnosis, but slight or early infections might easily be overlooked.

Smears, after teasing, were also made and fixed by Schaudinn's method. These were good for small forms.

Most of the work was done with serial sections, the tissue being fixed with corrosive sublimate and glacial acetic. The staining of these and the smears was with iron-hæmatoxylin or acid hæmalum.

EXPLANATION OF PLATE 10,

Illustrating Mr. A. C. Stevenson's paper on "Klossiella muris.

Reference Letters.

P. Parasite in its various forms. *C.* Host-cell or its remains. *N.* Nucleus of host-cell. N^2. Possible degenerate nucleus of host-cell. *R.* Red blood-corpuscles. *in.* Nuclei of tissue-cells. *m.* Possible remains of microgametocyte. *Bc.* Bowman's capsule.

[Magnification × 1200.]

Schizogony Cycle.

Fig. 1.—Free merozoite? in the blood-stream of a mouse which had been exposed to infection.

Figs. 2, 2*a*, 2*b*.—Young form in endothelial cell of kidney arteriole, showing division of nuclei. Three sections through parasite. This may be a form leading to that shown in fig. 3 or those in figs. 9 and 10.

Fig. 3.—Complete division of parasite into merozoites in endothelial cell of arteriole of kidney.

Figs. 4, 5, 6.—Small forms in the cells of capillaries of a glomerulus.

Fig. 7.—The same larger, nuclear increase.

Fig. 8.—Large form.

Figs. 9, 10.—Forms as in fig. 8, but with more nuclei, in the endothelial cells of the arterioles of a kidney.

Fig. 11.—Commencement of division forming gametes.

Fig. 12.—Complete division.

Fig. 13.—Same from a smear, showing residual protoplasm.

Sporogony Cycle.

Figs. 14, 15, 16.—Showing double infection of the cells of the convoluted tubule of a kidney by small forms.

Figs. 17, 18, 19.—Double forms of larger size, showing slight differences in the cytoplasm and nucleus.

Figs. 20, 21.—Single forms in a cell.

Fig. 22.—Single form in a cell: early nuclear division. At one side of the parasite is a mass of homogeneous protoplasm containing chromatin granules possibly the remains of a microgametocyte. Similar masses are seen in figs. 23, 26, 29.

Fig. 23.—Form with two nuclei.

Figs. 24, 25.—Two sections through one parasite. Chromatin mostly in granules, some suggestions of a mitotic figure.

Figs. 26, 27.—Sections through two parasites in one tubule, both in the same section, showing differences of nuclear staining.

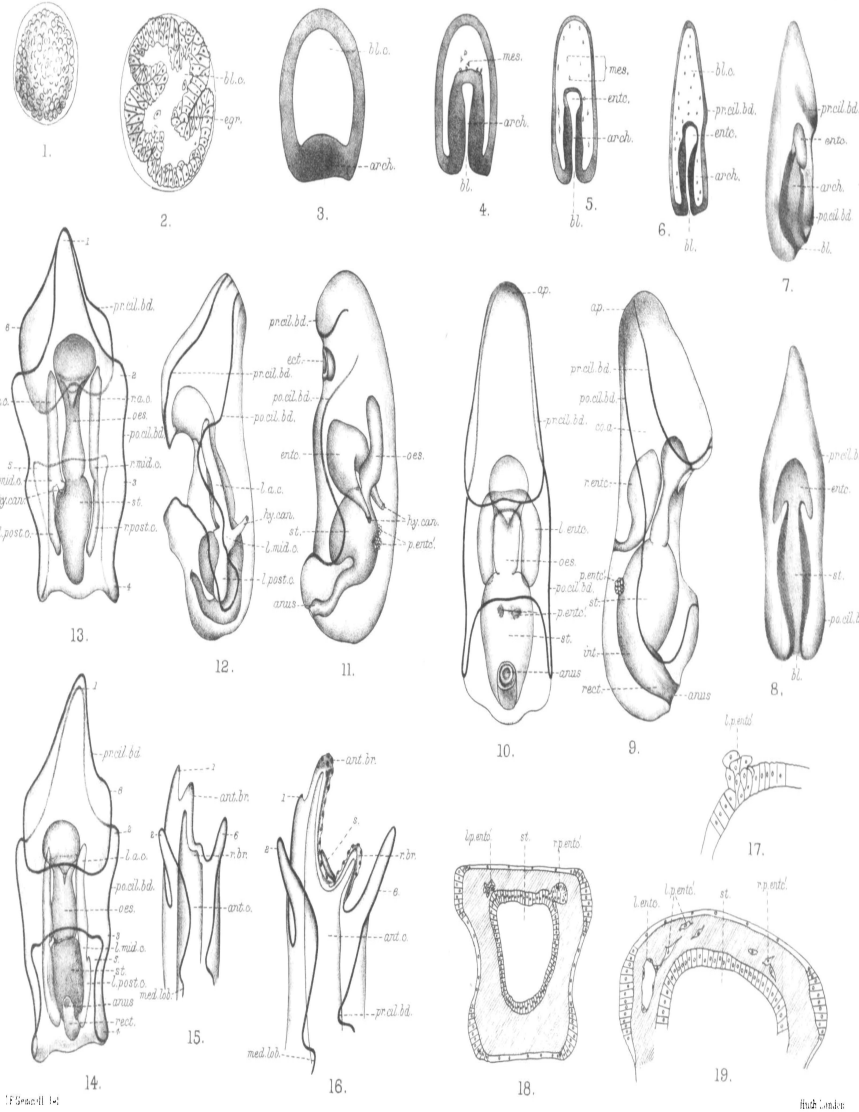
Figs. 28, 29, 30.—Increase of nuclei and commencement of budding.

Fig. 31.—Sporoblasts separating from the main mass of a parasite.

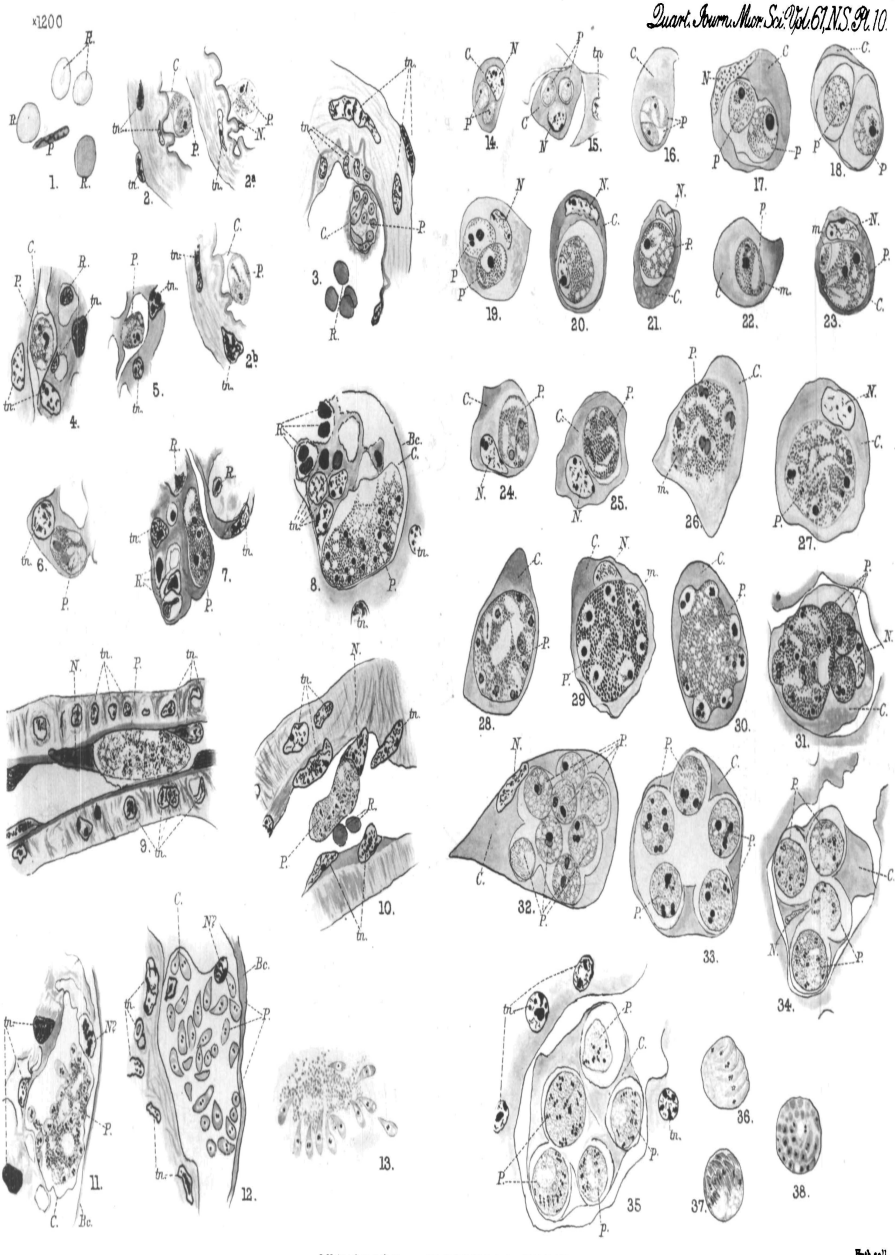
Figs. 32, 33.—Complete separation into sporoblasts. Increase of nuclei.

Figs. 34, 35.—Further increase of nuclei. In fig. 35 arrangement of nuclei about the equator of a sporoblast.

Figs. 36, 37, 38.—Sections through three spores showing sporozoites.



GEMMILL — PORANIA.



STEVENSON — KLOSSIELLA MURIS.