

No. 28.

(NEW SERIES.)

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

THE SEXUAL CYCLE OF LEUCOCYTOZOOM CANIS
IN THE TICK.

BY

CAPTAIN S. R. CHRISTOPHERS, M.B., I.M.S.
(Superintendent of the King Institute of Preventive Medicine, Madras.)

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA
BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT
OF INDIA, SIMLA.



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THE SEXUAL CYCLE OF LEUCOCYTOZOOM CANIS IN THE TICK.

INFECTION of vertebrates by protozoa is known to be commonly brought about by various invertebrate blood-suckers, but it is only the malarial parasites of man and proteosoma of birds that have as yet been actually followed with certainty through their cycle of development in such an intermediary host.*

The *Hæmogregarinidæ* have by many been considered, perhaps as a result of the work of Hintze¹ on *Lankesterella*, to be disseminated by casual infection; very few of the observations of writers who have described extra corporeal development of these forms being very complete or convincing.

If we except these doubtful instances, it is necessary to recognise that only in a single instance, *Lankesterella*, has a complete sexual cycle of a hæmogregarine been described, the cycle in this case not requiring an intermediary host.

The discovery of the sexual cycle of the mammalian hæmogregarine *Leucocytozoon canis* (James), a description of which is given in this memoir, is therefore of considerable interest, especially as unlike that of *Lankesterella* the sexual development of *L. canis* takes place in an intermediary host, the tick.

A certain number of communications describing development of hæmogregarines in intermediary hosts exist. Labbé,² whose work unfortunately I have not been able to consult in the original, has described the conjugation of sexual forms of *K. lacertarum* (Danilewski.) in the gut of a tick, an observation which my work tends to confirm. Development of *H. stepanovi* (Danilewski.), a hæmogregarine of the tortoise *Emys orbicularis* (L.) in the leech, *Placobdella cateingera* (Moqu Tand), is described by Siegel.³ The microgametes are small and oval, like those described by Hintze in *Lankesterella*; the fertilised ookinities reach the so-called œsophageal glands and form numerous sporozoites, which give rise to infection when the leech bites another tortoise. Siegel also notes that hereditary transmission may occur by sporozoites getting into the embryo leeches.

A short but important contribution is that of Durham,⁴ who notes in a species of toad common in Para the almost constant existence of two parasites, a *drepanidium*, which occurs in numbers in the peripheral blood, and *dactylosoma* (Labbé), which is confined to the internal organs. In citrated blood kept in

* The term "intermediary" is used in preference to "definitive" for the reasons given by Minchen¹⁷ in his "Sporozoa," page 254. Since writing the above I have been able to follow out the complete life cycle of *Piroplasma canis* in the tick.



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vitro all the parasites become free vermicules, but in large quantities of freshly drawn blood fixed with sublimate, Durham was unable to find any free drepanidia, and concludes that the vermicules of his drepanidium are not liberated within the host, but function only in extra corporeal development. He believes that the drepanidium and the dactylosoma are different forms of the same parasite, the former being the schizogonous form, whereas the latter is differentiated for sexual development outside the body. Durham also notes in ticks which had fed on these toads suggestive appearances of conjugation of the parasite, and the presence of cysts up to 60μ in diameter, though he had no time to follow this development in detail.

These conclusions of Durham's are in accordance with my own on *Leucocytozoon canis* where two kinds of vermicules occur, an encysted and a non-encysted form, the former of which leaves its cell host only under extra-corporeal conditions.

Billet⁵ has suggested an ontogenetic relationship between *Trypanosoma inopinatum* (Sergent) occurring in the leech *Helobdella algira* (Moqu Tand) and a species of Lankesterella in a toad, based on the fact that toads upon which leeches feed develop an infection of *drepanidium*.

Laveran and Negre⁶ have described a protozoon parasite in the tick *Hyalomma aegyptium*, which these authors think may possibly be developmental stages of *H. mauritanica* (Sergent), though the observations of Nicolle and Comte⁷ that hæmogregarines may be common where the ticks do not contain the cysts described by Laveran and Negre do not favour this view.

The development of *H. gerbilli* (Christophers), a parasite of an Indian field rat, *Gerbillus indicus*, has been described by me⁸ in the louse *Hæmatopinus stephensi* (Christophers and Newstead). Vermicules are freely liberated in the mid-gut, and in the body cavity large cysts are found; but as a result of work on *L. canis* in the tick I am doubtful whether the cysts have anything to do with the hæmogregarine.

Patton⁹ describes the active liberation of vermicules of *Leucocytozoon funambuli* (Patton) a parasite of *Funambulus pennantii*, one of the Indian palm squirrels in the gut of a louse (*Hæmatopinus*), which infests the species. He does not find the large cysts seen in *Hæmatopinus stephensi*, but figures (Pl. I, Fig. 16) a vermicule from the body cavity of the louse which has a close resemblance to some of the developmental forms of *L. canis* described later.

Of all work relating to the sexual forms of hæmogregarines, that of Hintze¹ on *Lankesterella minima* (Chaussat) is the most complete and convincing. According to this author *L. minima* undergoes both schizogony and sporogony in the body of its vertebrate host *Rana esculenta*. He describes in great detail

the process of sporogony. The microgametoblasts, the young mother-cells of the microgametes, are thin cells with finely granular protoplasm devoid of inclusions. When the microgametoblasts reach a length of 8 to 10 μ each of the chromatin bodies of which the nucleus is composed divide into two and scatter themselves through the substance of the microgametoblast. Later, taking a certain amount of protoplasm with them, the chromatin bodies separate off one by one in an irregular manner as microgametes. Unlike the microgametes of proteosoma those of *Lankesterella* are not whips, but small oval bodies endowed with amoeboid movement. The macrogametes which reach a length of 10 to 13 μ and a breadth of 2.5 to 3 μ , are plump forms with one end somewhat more pointed than the other and differ from the microgametocytes in possessing coarsely granular protoplasm. Prior to fertilisation the nucleus of the macrogamete divides and one portion degenerates, so that the usual reduction of chromatin in the female element prior to maturation is effected. In this division of the chromatin no mitosis appears to take place, the nucleus dividing directly; nor is any reception cone formed, but in the mature macrogamete a small canal is present to which the microgamete applies itself in the act of fertilisation. After fertilisation, the chromatin of the macrogamete splits up and arranges itself irregularly throughout the protoplasm. Forms with this arrangement are found especially in the blood vessels of the intestine. Eventually the fertilised macrogamete enters an epithelial cell of the gut and becomes encysted. The cysts, which are often quite numerous, have a diameter of 7 μ and are surrounded with a strong membrane; they contain sporozoites, but no secondary cysts (sporocysts). The chromatin bodies of the fertilised macrogamete come to the surface of the oocyst, but the actual formation of sporoblasts or sporozoites could not be followed. Eventually the cysts fall into the gut and pass out with the fæces; infection taking place, according to Hintze, by young frogs swallowing the cysts.

MATERIAL AND TECHNIQUE.

The schizogony of *Leucocytozoon canis* and its relation to other hæmogregarines has already been dealt with by me in a previous publication.¹⁰ The sporogony of the parasite I have since followed in the tick *Rhipicephalus sanguineus* (Latreille).

This tick in all its stages abounds on dogs in Madras, which are rarely found infested with any other species. Where dogs have much to do with goats or sheep, occasional specimens of *R. bursa*, or of *Hæmaphysalis fusca*, a tick of the goat, sheep, and buffalo, may be found. I have never found the ticks of oxen, *R. annulatus* and *Hyalomma aegyptium*, on dogs, though the latter species in Madras attacks cattle, horses, goats and sheep.

The females of both *R. sanguineus* and of *R. bursa*, when they have dropped from their host, crawl away, and if possible climb upwards to a height of many feet, eventually squeezing themselves into cracks where they lay their ova. *Hæmaphysalis fusca* on the other hand drops to the ground, and when this happens in a cage the gorged females of this species, unless rescued, are nearly always removed by the attendant with the fæces and urine. Whether from this or from other reasons *H. fusca* introduced among dogs tends to disappear, whereas *R. sanguineus* occurs in ever-increasing numbers wherever dogs are kept together without attention. *R. bursa*, though it does not spread among dogs like *R. sanguineus*, persists longer than *H. fusca*.

Neumann¹¹ gives for *R. sanguineus* a very extended distribution, and I have received specimens described as the ordinary dog tick from different parts of Bombay, Northern India and Burmah. It is an example of a species of tick which restricts itself to a single host, and may aptly be termed the dog tick of India. The question of varieties and further details of the life-history I intend to discuss shortly in a memoir upon *Piroplasma canis*, which is transmitted by this species of tick; at present it is necessary to refer to the life-history only in so far as it is likely to have relation to the development and transmission of *Leucocytozoon canis*. Female and male ticks of this species attach themselves to almost any part of the dog, but select especially the ears, the paws and the skin behind the neck. After a variable number of days, dependent apparently upon the vascularity of the part chosen for attachment, the females become fully gorged and drop off. As a rule two days are taken up before the female is half the size it will ultimately become, but the final stages of engorgement are much more rapid, and full repletion may take only twelve or twenty-four hours longer. The males remain behind when the females drop off, but should the dog die they readily detach themselves and at once seek a new host.

The eggs are laid in cracks and from these, in about a month, hatch out the larvæ which collect together in swarms and transfer themselves, often in masses of several hundreds, to any dog which happens to come in contact with them. After remaining on the host for three days the larvæ become gorged and drop off, crawling away into cracks where they become quite inert and apparently lifeless. From these in eight or nine days emerge the nymphs which again seek a host, and after remaining on the dog for about five days, drop off in a gorged condition and secret themselves in some crack where they again become inert and apparently dead. The adults, both male and female, emerge from the nymphal skins in about fifteen days and, after a brief rest, once more attach themselves to the dog.

Though they belong to different genera, the Cape dog tick (*H. leachi*), the life-history of which has been studied by Lounsbury¹² and Nuttal,¹³ and the

dog tick of Madras (*R. sanguineus*) have therefore very similar habits.

Oviposition commences as a rule about three days after the female has dropped from the host and is over by about the eighth day or even later, a few eggs often being passed by females which have shrivelled to one-third or one-quarter of their original size. In the young ova the protoplasm is finely granular and transparent, but after the ova have reached a certain size the protoplasm becomes more coarsely granular and more opaque. At a certain point in development numerous rod-shaped masses of chromatin appear and herald the formation of the large yolk globules which characterise the mature ovum. By the end of oviposition the ovaries are almost devoid of ova and shew only peculiar crinkled masses, which are the collapsed membranes left after the mature ova have fallen into the lumen of the ovary.

The oviducts during oviposition are of great length and contain ova at short intervals along their whole course. On entering the oviduct the ova are devoid of any hard envelope, but a shell is formed as they pass down the duct.

When laid, the ova are coated with a thick layer of oil derived most probably from the sebaceous gland-like dermal gland and cephalic gland both of which at this time are very large and conspicuous (*vide* Anatomy and Histology of Ticks, page 48), and this makes the ova adhere loosely together.

The method of dissection and the structure of ticks has been described in a previous publication.¹⁴ For the study of the developmental stages of *Leucocytozoon canis* preparations of the gut are made by dragging short lengths of it over the slide by means of needles. Preparations of ova are made by squashing the ovary and spreading in the usual way upon a slide, or by similarly treating eggs, either in the oviducts, or after they have been laid.

The examination of fresh preparations is often of use in detecting infection because owing to their movements, the vermicules and sporozoites can be detected with a dry lens of comparatively low power. Male ticks were examined either by dividing them vertically and afterwards dragging out the gut with needles, or by snipping off the posterior end of the body and squeezing out the viscera by pressing a needle over the body of the tick from the head, end backwards.

Nymphs were examined by spreading the gut, over the slide as in the case of the adult.

The examination of larvæ is difficult. In the present instance they were ruptured with needles and their body juice smeared upon the slide.

THE SPOROLOGY OF *Leucocytozoon Canis* (JAMES).

In the gut of females of *R. sanguineus* (Latreille) fed on dogs severely infected with *Leucocytozoon canis*, development of the parasite in all stages,

from the liberation of free vermicules to the formation of sporozoits on the breaking up of the oocyst, can be followed. Development in the tick therefore represents the sporogony of this parasite.

Fortunately no parasite that can be confounded with the developmental forms of *Leucocytozoon canis* exists normally in this species of tick in Madras, and a large number of observations extending over many months have established the direct relationship of vermicules in the gut of the tick with the presence of the parasite in the dog. In no instance have vermicules been found on ticks fed on uninfected dogs, but in every case where females of *R. sanguineus* are fed on infected dogs vermicules are present in the gut, and their number bears a close relation to the number of parasites in the blood of the dog upon which the tick has fed. Still more, certain proof is afforded by the fact that it is quite common to see vermicules in the act of leaving their capsule.

Since blood is taken in by the female tick throughout a period of from two to four days, it is to be expected that all the parasites will not be in the same stage of development, but this is true only as regards a minority of the parasites for, as already indicated, the final stages of engorgement are very rapid, and at least two-thirds of the blood in the fully fed female tick is, as a rule, taken in during the last twenty-four hours. Development, therefore, in the majority of forms has started, roughly speaking, from the time when the tick left its host, and the majority of parasites are at about the same stage of development.

In the gut of ticks examined immediately after removal from the dog, encapsulated parasites, or vermicules in their first stages of development, are seen. At the end of twenty-four hours most of the forms have penetrated the cells of the gut wall and are seen to be undergoing the different stages of fission which precede the formation of the sexual forms. By the third day conjugation bodies and the first stages in the development of the fertilised oocyst are conspicuous. On the fourth day mature oocysts are present, together with a certain number of sporozoits. The latter increase in numbers up to about the sixth day, by which time most of the oocysts have disappeared.

The liberation of vermicules.—James¹⁵ has described the liberation of a vermicule from a leucocyte *in vitro*, but this phenomenon must be rare, for I have failed on several occasions to find vermicules even in citrated blood kept for forty-eight hours. On the other hand I have already noted¹⁰ that vermicules are freely liberated in the gut of the tick, *R. sanguineus*, and that very few forms fail to become free.

Wenyon¹⁶ has drawn attention to the frequency with which a U-shaped nucleus is present in *Leucocytozoon canis*, and suggests that this arises from a looped shape of the parasite within the cyst; but in my specimens it is nearly always possible to detect one or two fine cross strands of chromatin joining the limbs of the U, so that the U shape of the nucleus is more apparent than real

(Fig. 1). *Leucocytozoon canis* may sometimes be bent upon itself, but it is very noticeable that in the majority of cases, when the parasite is first liberated, it is a stumpy form differing from the encapsuled form only in not possessing a capsule. It is only later that the parasite gradually elongates and becomes an active attenuated vermicule.

When the gut contents of ticks are examined immediately after they have left their host, many encapsuled forms are seen, but most of these are no longer contained in leucocytes, the persistence of the appearance of a capsule under these conditions being convincing as to the presence of a capsule proper to the parasite itself. In some cases only an indistinct capsule can be made out, in others there is no capsule, though the parasite, except that it stains more freely, is otherwise unchanged; the appearances at this time suggesting that the encysted vermicule does not leave its capsule until this has been partly or wholly dissolved by the digestive juices of the tick. When the parasite is free from its capsule changes begin to occur in the arrangement of the chromatin, and a greater amount of protoplasm passes behind the nucleus (Fig. 3), a vermicule being eventually formed possessing transparent protoplasm and an elongated oval nucleus (Figs. 4 to 7), the chromatin of which is arranged in a coarse reticulum. Later the chromatin is often arranged in the form of almost separate rings (Fig. 8), an arrangement which I have already referred to as figured by Patton in the case of a vermicule found in the body cavity of *Hæmatopinus* (Patton,⁹ Pl. 1, Fig. 16).

The formation of the male and female elements.—In the gut of ticks examined twenty-four hours after they have left their host, many of the vermicules have embedded themselves in the protoplasm of the gut cells, especially in the younger cells which possess reticular protoplasm more or less free from the products of digestion, and for the most part they have become stouter (Figs. 9 and 10), the bulkiest forms measuring 11.5μ by 6μ . They are as a rule situated not very far from the nucleus of the epithelial cell of the gut, and the protoplasm of the cell is often somewhat condensed around them. Though sometimes nearly circular they are usually of an oval shape with one side rather flat and the other much more convex (Fig. 9). The protoplasm is no longer homogeneous, but contains vacuoles, the chromatin meshwork which is conspicuously opened out, is in many instances massed upon either side of the parasite, the intervening space being bridged as a rule by a few strands. So far as I have been able to ascertain, this arrangement of the chromatin does not end in its reduction as in *Lankesterella*, but is the prelude to actual fission of the organism (Fig. 11).

Vermicules lying in pairs (Fig. 12), derived from fission of the bulky forms just described, are in fact numerous, and parasites in the act of separation

are not infrequently seen. These show a pink staining material which lies mainly between the vermicules, but sometimes also surrounds them. The vermicules that have resulted from fission differ from those already described in that their protoplasm is more granular and contains refractile vacuoles. They frequently show a trace of the pinkish material already mentioned at one or, more rarely, both poles. Such an appearance is almost certain evidence that a vermicule exhibiting it has resulted from fission.

In many cases fission appears to be repeated several times, since groups of four and even eight vermicules are often seen lying in a pocket in the protoplasm of a cell (Fig. 13). To ascertain exactly what takes place at this time is difficult, but there is no doubt that the mature sexual forms are the result of at least one act of division of the original vermicules.

The sexually mature parasites are large vermicules measuring 13.5μ in length. The commonest type are stout forms with an oval nucleus, but thin forms with chromatin arranged along the length of the body are not infrequent. It is doubtful, however, whether these appearances are constant characters of the female and male elements, for, as will be seen later, in the conjugation forms, differences in the component vermicules are not distinguishable. In addition to the refractile droplets, which are very characteristic of the mature sexual forms, the protoplasm contains also a few small chromatin granules, and a darkly stained portion which is noticeable in the conjugation forms. The chromatin in the stout forms is arranged in an irregular meshwork, but in the narrow forms is more or less broken up and massed irregularly (Figs. 14 and 15).

Conjugation and the formation of the oocyst.—The vermicules first attach themselves to each other by one end, the chromatin of each coming towards the point of contact. They then arrange themselves alongside of each other and blend, forming characteristic conjugation bodies (Figs. 16 to 19). The conjugation bodies which are oval or round forms measuring from 7.6μ to 10μ in short and long diameter are at times very numerous, and it is easy to follow every step from the coming together of the two vermicules to the formation of the oocyst and the casting off of the protoplasm of the male form. In the early stages the line of demarcation between the two forms can still be followed, but later this becomes imperceptible. In some cases one vermicule is more packed with refractile vacuoles than the other (Fig. 17), but usually the appearance of the two halves is exactly similar. In each half the chromatin granules and dark staining portion of protoplasm can be distinguished, and where the protoplasm of the two vermicules has come together, one or sometimes two large vacuoles are usually left (Figs. 16 and 17).

So soon as the vermicules have come together, the two chromatin masses,

which are at first separate and situated on the periphery of the conjugation body, assume a very homogeneous appearance. In this condition they join together to form a single mass (Fig. 18). The chromatin now opens out in an irregular manner (Fig. 19) and passes towards the surface of the cell, the major portion of it being extruded in the form of two large and very darkly staining masses (Figs. 20 to 23). A portion remains behind and forms the origin of the chromatin of the oocyst. At the same time that the chromatin masses are extruded, the body of one of the vermicules, presumably the microgamete, is thrown off; and in it can still be recognised the refractile vacuoles, the broken up and now diffused granules of chromatin, and the dark area of protoplasm already noted. The mass is always devoid of even a trace of a nucleus (Fig. 21), differing in this respect from certain degenerated vermicules, referred to later, where the nucleus is still seen as a collection of deeply stained droplets.

The newly formed body, the oocyst, which enlarges rapidly and quickly, assumes a very characteristic appearance, is a body with a clear central area and an outer rim of very conspicuous dark blue protoplasm, the chromatin, in the form of irregular fragments, being distributed near the periphery (Figs. 20 to 23). As the oocyst increases in size the chromatin becomes more and more broken up, forming as a rule an imperfect and irregular reticulum. In some forms the fragmentation is extreme and very fine particles of chromatin are dusted over the surface, in others the reticulum is much coarser. After the oocyst has reached a diameter of 14μ the central clear area becomes less conspicuous, and the surrounding protoplasm much more voluminous; the chromatin has also collected into a number of defined masses, which are arranged more or less regularly. At this stage the oocyst, either from being endowed with amœboid movements, or from their being easily distorted, is often very irregular in shape (Fig. 25).

It is not unusual to find groups of three or four oocysts in close contact. In such cases the individuals of the group may be all in the same stage of development, or, as often happens, they may exhibit a regular series of stages, suggesting the fertilisation of a number of females by successive wandering males.

The formation of sporozoites.—Shortly after the oocyst has assumed the appearance of a large amœboid body with voluminous protoplasm and scattered masses of chromatin, it breaks up to form the sporozoites. A single form has been seen, in which immature sporozoites were present, lying in what appeared to be an extremely delicate cyst wall (Fig. 24), but I am very doubtful if this represents the usual method of the formation of the sporozoites, the oocyst breaking up into naked sporozoites not contained within any cyst wall.

The number of sporozoites found in groups is usually from twelve to fourteen (Fig. 26), but groups have been seen containing many more. It is possible that

these larger groups have originated from three or four oocysts in close proximity to each other.

The sporozoits resemble somewhat the original vermicules, but they differ in being for the most part narrower forms with a very compact almost globular or short oval nucleus. Occasionally very stumpy forms are seen (Fig. 28). The presence of one or two large non-refractile vacuoles in the protoplasm is a characteristic feature. In the fresh state they exhibit a very curious spring-like action, bending slowly into a curve and straightening themselves with a jerk.

Sporozoits are most frequent in the lumen of the gut; this is probably due to the fact that, though the vermicules originally embed themselves in the young cells near the basement membrane, they are carried during their development by the growth of the cell towards the lumen. The large oocysts themselves, if not actually free in the lumen, are not far removed from this situation, and the sporozoits when liberated for the most part therefore find themselves already in the lumen.

The ultimate fate of the sporozoits and the method of re-entry of the parasite into the dog.—As the female tick after repletion does not again suck blood, the passage of the sporozoits into the ova seems a necessity, if the parasite is to regain an entrance into the mammalian host. It is to be noted also that the sporozoits are formed throughout the whole period of oviposition. Yet in the ova I have never been able to find sporozoits, nor indeed have I ever encountered these except in the gut.

Failing to find sporozoits in the egg I thought it probable that infection took place by sporozoits, formed in the gut of the nymph, gaining entrance into the dog when the tick in its adult stage fed again. Unfortunately for this hypothesis experiments have shown that, though in the nymph parasites exhibit the early stages of development, they have, in the two batches of nymphs examined, completely disappeared by the fourth day.

In the larva and in the male tick I have been unable to trace any development; so that at present the method by which the parasite re-enters its mammalian host is very uncertain.

That the shrivelled ticks should be consumed with food is a possible, though improbable, method of infection. If Durham's⁴ observation that the young ticks consume the contents of the mother be correct, it allows of another explanation, but I am unable to confirm Durham in this respect, and the exact method of infection has still to be ascertained.

SUMMARY AND CONCLUSIONS.

In the cycle which has been described there are some points in common with that of *Lankesterella*, but in the formation of the sexuals form and in the

details of conjugation, there are very considerable differences. If one considers the original vermicules to represent the microgametoblasts and macrogametes of *Lankesterella*, one must in the case of the male element suppose that it is formed by repeated fission of the mother-cells. In the case of the macrogametes division more than once is unlikely, and even the formation of two forms each capable of development is improbable. It is possible that one of the forms degenerates. This view is favoured by the fact that a considerable number of degenerated vermicules, in which the chromatin is in the form of droplets, are seen about the time that conjugation is in progress. The group depicted in Fig. 29, in which a degenerated vermicule is seen separated from a fertilised oocyst by the pink substance characteristic of fission, also supports this view. On the other hand, in the groups of fertilised oocysts, only the cast-off bodies of the males are seen, so that if one of the products of division degenerates the other must move away from its original position.

Another view regarding the nature of the changes seen may of course be taken, namely, that the fission in the gut of the tick is a special schizogony resulting eventually in male and female forms. But the first hypothesis seems to be more likely.

The characters of the oocysts, and the fact that each of the limited number of sporozoites apparently represents a sporoblast, recall *Lankesterella*.

As the sporozoites represent sporoblasts it is possible that they further sub-divide, but there is no evidence to support such a view, and their formation would appear to complete the sexual life cycle of *Leucocytozoon canis*, the merogamous cycle of which I have already described elsewhere.

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EXPLANATION OF PLATE

- Fig. 1. Two encapsulated parasites in the gut of the tick. $\times 2,000$.
 Fig. 2. Parasite in which the capsule is no longer to be made out. $\times 2,000$.
 Figs. 3 and 4. Formation of the vermicle. $\times 2,000$.
 Figs. 5, 6, and 7. Vermicles of the first stage with clear protoplasm. $\times 2,000$.
 Fig. 8. Vermicle with chromatin arranged in four separate rings. $\times 2,000$.
 Fig. 9. Vermicle which has embedded itself in a cell of the gut and is preparing for fission. The cell is a young epithelium cell freed from digestion products. $\times 2,000$.
 Fig. 10. Swollen vermicle embedded in a cell of the gut. $\times 2,000$.
 Fig. 11. Vermicle in the act of fission within a cell of the gut wall. $\times 2,000$.
 Fig. 12. Two vermicles of the second stage, the result of fission. $\times 2,000$.
 Fig. 13. A single stout form and a group of four vermicles (microgametes) embedded in a cell of the gut. The cell contains a number of black granules, the result of digestion. A stout vermicle in the act of fission is seen lying among the granules immediately beneath the group of four vermicles. $\times 1,000$.
 Fig. 14. Thin form of sexual vermicle. $\times 2,000$.
 Fig. 15. Stout form of sexual vermicles. $\times 2,000$.
 Fig. 16. A conjugation body. Early stage. $\times 2,000$.
 Fig. 17. A conjugation body. Early stage. Shows one vermicle much more loaded with retractile vacuoles than the other. $\times 2,000$.
 Fig. 18. A conjugation body. Later stage. Shows the blending of the two chromatin masses. $\times 2,000$.
 Fig. 19. A conjugation body. Late stage. Shows the chromatin opening outwards. $\times 2,000$.
 Fig. 20. A fertilised oocyst with two extruded masses of chromatin and the protoplasm of the cast-off male vermicle. $\times 2,000$.
 Fig. 21. The same showing the absence of any trace of a nucleus in the cast-off male. $\times 2,000$.
 Figs. 22 and 23. Nearly mature oocysts. $\times 2,000$.
 Fig. 24. Formation of sporozoites. $\times 2,000$.
 Fig. 25. Fully mature oocyst showing irregular form. $\times 1,000$.
 Fig. 26. A group of sporozoites resulting from the breaking up of an oocyst. $\times 2,000$.
 Fig. 27. Usual form of sporozoite. $\times 2,000$.
 Fig. 28. Stumpy form of sporozoite sometimes seen.
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