ON THE LIFE-CYCLE OF *SPIROCHAETA*[1] *GALLINARUM.*

PRELIMINARY NOTE.

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(With 5 Figures and Diagram in Text.)

During the past two years I have been working on the morphology and life-history of *Spirochaeta duttoni* and, although successful in tracing parts of the life-cycle of this parasite, yet the work has been accompanied by many difficulties owing to the large percentage of ticks that are immune to infection.

Whilst working at the Institut Pasteur, Professor Marchoux kindly placed at my disposal a plentiful supply of *Argas persicus* infected with *Spirochaeta gallinarum* and, therefore, having such good opportunities of studying this parasite in both of its natural hosts, I took up the study of this closely related species and have been successful in following the main features of its life-cycle.

As, however, certain confirmatory evidence as to the true relationships of the spirochaetes is not complete and, moreover, is liable to be delayed, it has been thought advisable to publish the main results which have been obtained in the form of a preliminary note.

Two strains of *Spirochaeta gallinarum* have been employed in the following work. For the earlier observations the original strain of

[1] In employing the term *Spirochaeta* instead of *Treponema* we do not thereby express disagreement with the scheme of classification proposed by Dobell (1911). This point will be considered in a later communication.

spirochaetosis, brought back from Brazil by Professor Marchoux, was used; later, Dr Foley sent a large number of infected *Argas persicus* from Beni-Ounif-de-Figuig, Algeria, with which most of the subsequent observations have been made.

The spirochaete in the fowl.

The parasites have been examined in the living state and also in stained preparations. For fresh examination the blood film was maintained at a temperature of 40° C. by means of a thermostat and, by employing dark-ground illumination, the division of the spirochaetes could be followed through all its stages.

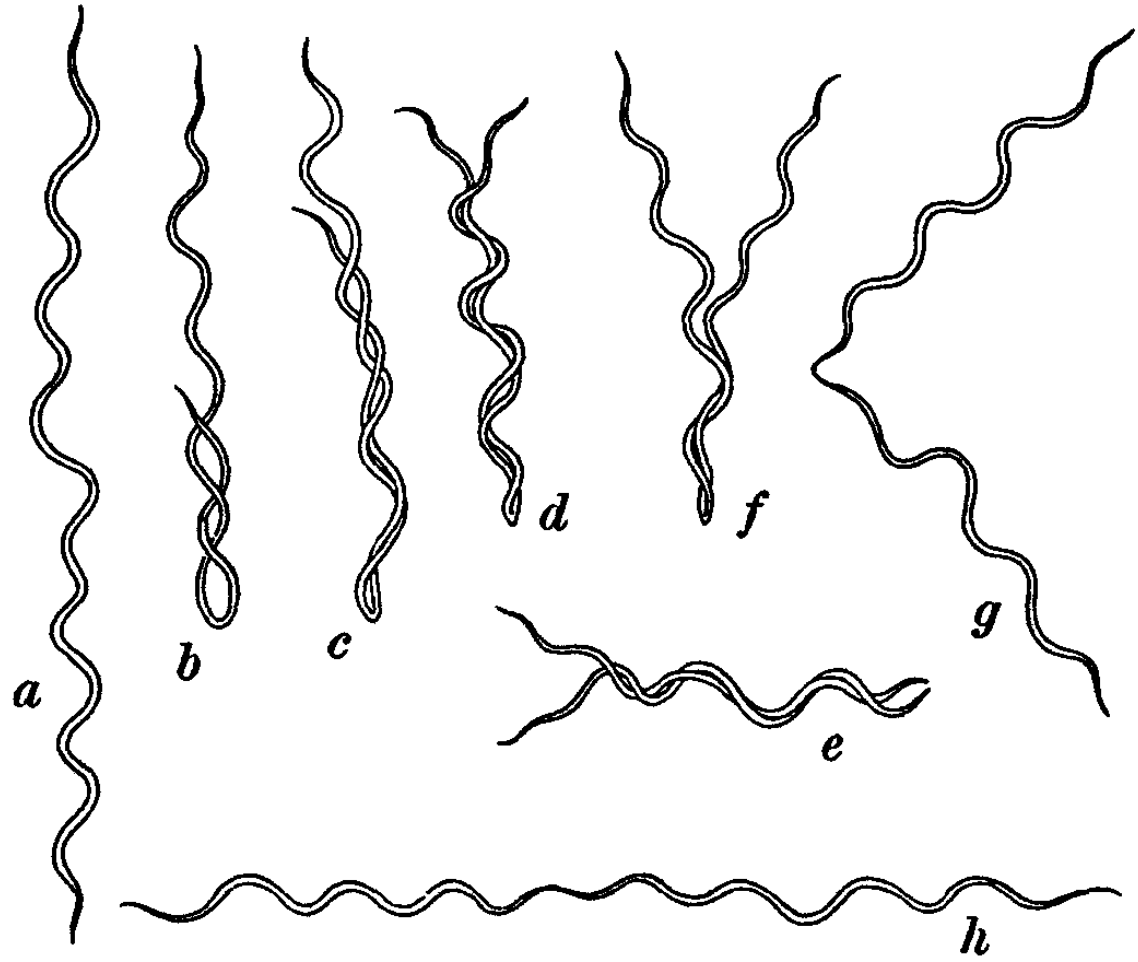

Fig. 1 *a–h*. Various stages in the flexion and transverse division of *Spirochaeta gallinarum*.

The details of division seem to differ in some respects from that previously described for blood spirochaetes (*e.g.* Mackinnon, 1909; Fantham and Porter, 1909), but resemble that of *Cristispira*, described by Gross (1910), and *Spirochaeta anodontae*, described by Bosanquet (1911).

The various stages are shown in the accompanying text figure (Fig. 1). One end of a long spirochaete (*a*) doubles back (*b*), the reflexed portion being closely applied to the main part of the parasite. The reflexed part gradually increases in length (*c*), all the time being closely wound round the other portion of the spirochaete (*b*, *c*), and thus the two ends approach each other and finally appear in juxtaposition (*d*). The appearance now presented by the parasite is that of two spirochaetes tightly coiled together, except that at one end of the coil the two threads are continuous, being flexed through an angle of 180°.

After having passed through this process, which takes place with considerable rapidity, the two halves of the spirochaete uncoil from each other (*f*, *g*) and separate at the point of flexion (*g*), thus producing two parasites. Sometimes the spirochaete breaks in two, before the daughter parasites have uncoiled from each other (*e*). In the former case the parasites may remain connected together for some time before finally separating (*h*) and in this case exactly resemble the forms of transverse division previously recorded for these parasites.

As the two halves of the dividing spirochaete are very closely wound together before separating, the process of unwinding and final separation into two halves simulates longitudinal division to an extraordinary degree and, in many cases, it required the most careful examination to decide whether one was dealing with a case of true longitudinal division, or the above described transverse fission. I have never observed longitudinal division in either this species or *S. duttoni*, and it seems probable that the so-called "longitudinal fission" might be explained as the final stage of this peculiar method of transverse division. As for those cases in which an apparently single, long spirochaete merely separates into two approximately equal halves, it is impossible to say whether they represent the last stage of the above-described division, or merely the separation of two distinct parasites which have been temporarily agglutinated together by their ends. It may also represent direct transverse division without any previous flexion of the spirochaete.

In any case, whatever may be the true interpretation of the latter forms, the division of *S. gallinarum* is certainly usually transverse, and as I have devoted considerable time to the observation of the parasite without ever having observed any sign of longitudinal fission, I believe that it is invariably transverse.

By means of examination with the dark-ground illumination, I have frequently observed the breaking up of the spirochaete into a number

of coccoid forms (? spores), in the manner described by Balfour (1911) for this species, and also by Bosanquet (1911) for *S. anodontae.* I can entirely confirm Balfour's description of this interesting process, which takes place at the crisis of the disease or after drug treatment.

It is observed much more frequently in the contents of the gut of an *Argas*, which has previously fed upon an infected bird. The spirochaete gradually assumes the appearance of a chain of beads (Fig. 2 *a–d*) contained within the transparent cell-wall.

After swimming about for some time in this form, the spirochaete appears to rupture at one end and the coccoid bodies escape into the surrounding medium, leaving an empty sheath behind them (*e*). In

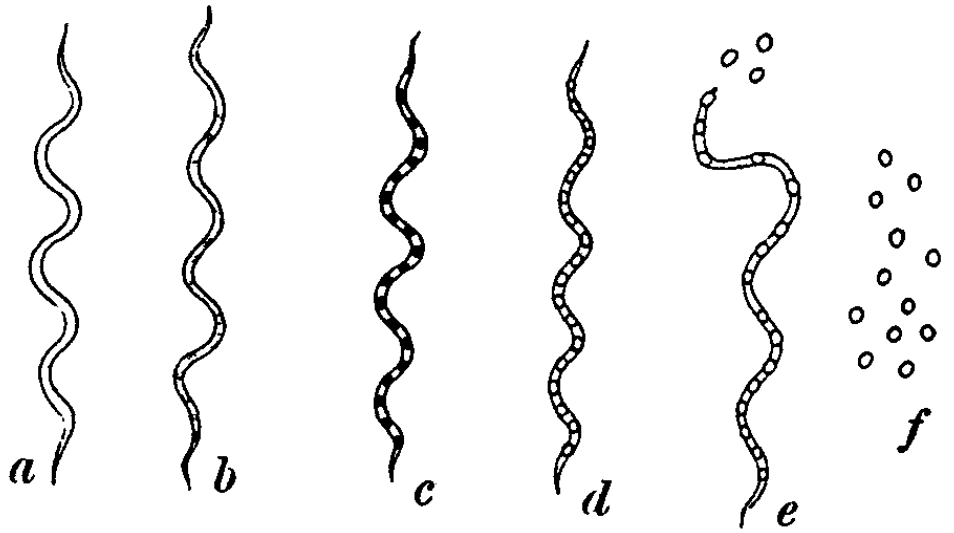

Fig. 2 *a–f.* Successive stages in the formation of the coccoid bodies (diagrammatic).

some cases the whole cell-wall seems to disintegrate before the coccoid bodies escape, but the final result is the same, viz. the liberation of a varying number of minute round or ovoid bodies (*f*).

The fact that these coccoid forms are produced in large numbers after drug treatment, or when the parasites are in unfavourable conditions, has caused them to be regarded previously as merely the result of granular disintegration. Apart from their regularity in size and appearance, which in itself precludes the possibility of their being regarded as degeneration products, the time of their appearance is just when one would expect them. Moreover, the appearance of degenerating spirochaetes is very different from that presented by segmenting forms, as, instead of the production of a row of coccoid forms of uniform thickness, either the whole spirochaete gradually dissolves away, or, as

the result of plasmolysis, one or more irregular swellings are formed along the length of the parasite (Fig. 3 *a–e*).

Some of these degeneration forms are very peculiar and have given rise to the belief in the existence of male, female and indifferent forms, which still exists among certain authors. One form of degeneration that is particularly common is the production of a large round cyst-like body about the middle (*a–d*), or at one of the extremities of the spirochaete (*e*). This is the result of the cell membrane of the parasite becoming swollen out like a bubble, as a result of the pressure of the contents. These forms have been repeatedly described as "cysts," but they are merely one of the results of plasmolysis.

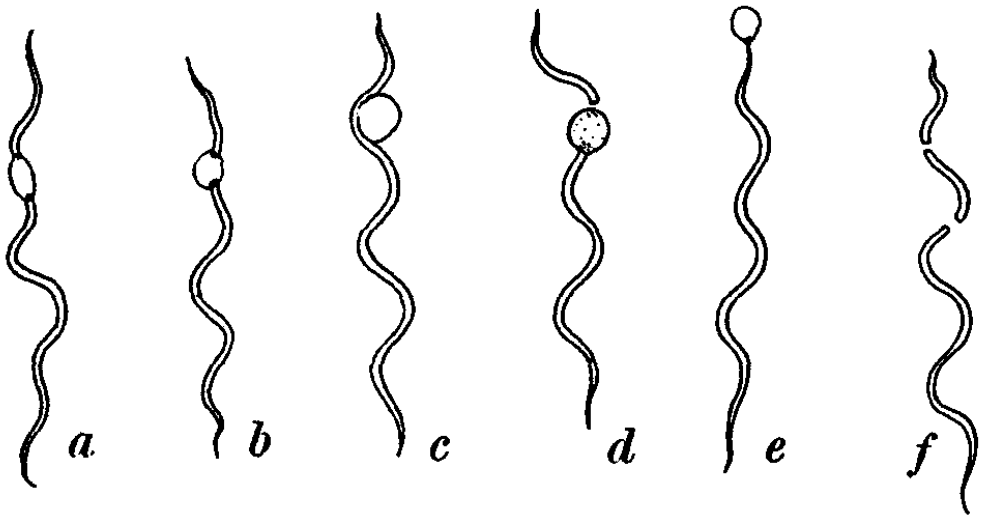

Fig. 3 *a–f*. Degeneration forms, showing the production of large cyst-like bodies.

The examination of stained preparations has not added much to the information derived from a study of the living parasites. In most cases the films were fixed in osmic acid vapour, then hardened in absolute alcohol and stained either with Giemsa, or one of its modifications. Some films were fixed in Flemming's solution, or in sublimate alcohol and then stained with Heidenhain, or safranin and methylene blue (Hindle, 1909), the films never being allowed to dry. These latter preparations were of use as confirmatory evidence of the results obtained by a study of dried films.

The examination of the spirochaetes, no matter what method of staining was employed, has not revealed the existence of any differentiation in the contents of the cell. Neither chromatic granules nor anything even suggesting a nucleus can be seen and the whole

parasite appears to consist of a thick cell-wall (periplast) surrounding a homogeneous core which, from its staining reactions, seems to consist of chromatic material.

At certain periods, especially after being ingested by the tick, the endoplasm of the spirochaete breaks up into a series of darkly staining bodies (Fig. 2 *c*), which become rounded off in the form of minute coccoid bodies or spores, contained within the cell-wall (Fig. 2 *d*). These coccoid bodies then escape by rupture of the cell-wall and appear in the plasma as round, or ovoid, chromatic-staining bodies, the diameter of which is usually about 0·2 μ. The true nature of these bodies is problematical, for although in some respects they resemble the spores of bacteria—especially the *Disporea*—in their formation, yet the fact that they stain deeply and also multiply (see below), at once differentiates them from true spores. From their shape and subsequent behaviour, it is preferred to call them "coccoid bodies" and their nature will be discussed in a future communication.

The development of these coccoid bodies into spirochaetes has not yet been observed in the blood of the fowl, as the spirochaetosis of fowls rarely assumes the relapsing type. It has been followed, however, in the body of the tick and will be described under that section.

I have not been successful in observing the penetration of the spirochaetes into the red blood corpuscles in the manner described by Prowazek (1906), but from their behaviour in the tick it is quite possible that it may occasionally take place. The transformation of spirochaetes into the intracorpuscular bodies of Balfour (1909) has, therefore, not been observed, although these bodies have been met with occasionally.

A careful examination of a number of normal fowls has revealed the fact that occasionally intracorpuscular bodies, identical in appearance with those described by Balfour, may be found in the red cells, and consequently it is extremely probable that they are the products of nuclear degeneration. The fact that the nucleus, or its contained nucleolus, may bud off fragments into the cytoplasm is well known and also occurs in the red cells of normal fowls. That the appearance of numbers of these bodies is associated with an attack of spirochaetosis is no more significant than the fact that haemolysis accompanies an attack of piroplasmosis. It is known that nuclear degeneration of certain cells is associated with some diseases (*e.g.* rabies; Acton and Harvey, 1911) and, therefore, there is nothing improbable in the view that the intracorpuscular bodies of Balfour are of this nature. It is

important to note that Balfour's figures show that these bodies have exactly the same staining reaction as the nucleus of the red cell, and, moreover, are identical in appearance with the products of undoubted nuclear degeneration seen in normal fowls.

This nuclear degeneration seems to be a special feature of the fowl spirochaetosis occurring in the Sudan and in South Africa (Jowett), for it only rarely occurs in the blood of fowls infected with the strains of this disease from Brazil and Algeria respectively. With these latter strains of spirochaetosis the infected bird usually dies at the height of infection, or else recovers, and the infection rarely assumes the chronic form, necessary for the production of abundant nuclear degeneration of the red cells.

The spirochaete in the tick.

After being taken into the tick, some of the spirochaetes penetrate the wall of the gut and appear in the coelomic fluid. The time which elapses between the ingestion of the parasites and their appearance in the coelomic fluid varies from 2 hours to as long as 48 hours and, moreover, their appearance is by no means constant, for in some cases we have never seen any spirochaetes in the coelomic fluid, although the tick was examined at intervals for four days after having fed on an infected fowl. Usually the parasites only remain in the coelomic fluid a short time, as they bore their way into the salivary glands and gonads of the tick within a few hours.

As a result we have found numerous spirochaetes in the salivary glands and ovary, respectively, of a tick that had fed on an infected bird 6 hours previously. As this tick had been kept at a uniform temperature of 28° C. for at least three months previously, the spirochaetes in these organs must have come from the gut. Contamination during the dissection certainly did nor occur in this case, for the preparations show a complete absence of any of the characteristic gut-contents.

The spirochaetes in these organs and also in the Malpighian tubules bore their way into the cells (Diagram A), and after becoming more or less coiled up, often producing cyst-like forms, segment into a number of "coccoid bodies." These intracellular coccoid bodies multiply by transverse fission, especially in the cells of the Malpighian tubules and the ovary. As a rule they soon disappear from the salivary glands and therefore it seems probable that, as in the case of *O. moubata* (Leishman, 1910; Hindle, 1911), these organs are not usually responsible for the

infection, but that it is produced by the excretion of infective material which enters the open wound caused by the tick's bite.

In support of this view I might mention that, although the majority of *Argas persicus* do not emit their coxal fluid and excrement whilst attached to the fowl, yet a few were noticed to pass their secretion immediately after feeding. In these cases the excreted substance bathed the open wound caused by the tick's bite. The injection into a Java sparrow[1] of some of this material—coxal fluid mixed with excrement—from one tick was followed by a severe spirochaetal infection, and yet, in practice, it is generally necessary to feed numerous ticks on a bird in order to produce an infection. I believe that the reason of this latter fact is that, as a rule, only those few *Argas* which emit their coxal fluid and excrement whilst on the bird are responsible for the infection. The fact that spirochaetes may be found in the salivary glands is evidence that these organs may be infective, but as both the spirochaetes and the resulting coccoid bodies often disappear from this part of the tick, it is evident that it is not a favourable situation for their development.

Many of the spirochaetes remain in the lumen of the gut and it is in this situation that one observes the formation of the coccoid bodies with the greatest clearness. One to two days after the parasites have been ingested into the gut of the tick, by examination with dark-ground illumination, large numbers of spirochaetes may be observed undergoing this process of the formation of the coccoid bodies. As a result the spirochaetes rapidly disappear from the gut of *Argas* (in three to ten days, according to the temperature), whereas, when infected blood is merely kept in a sealed glass tube, the parasites may persist for as long as three months and show various stages of degeneration, some of which have been described above (Fig. 3).

The resulting coccoid bodies persist in the lumen of the gut and Malpighian tubules and are excreted together with the Malpighian secretion. When the crystals escape from the Malpighian tubule the intracellular coccoid bodies may also become free and thus the Malpighian secretion is continually infected, and when mixed with the coxal fluid it may enter the open wound caused by the tick's bite. The coccoid bodies would then be able to develop into spirochaetes in the blood of the bird, although this process has not actually been followed.

The development of the intracellular coccoid forms into normal

[1] These birds are not more susceptible to *S. gallinarum* than fowls. From their small size they are more convenient for inoculation experiments.

spirochaetes and also into fusiform bacilli has been repeatedly observed in the tick. If an infected *Argas* be kept at a temperature of 37° C., after about five days spirochaetes appear in the coelomic fluid, the lumen of the gut, and all the organs of the tick. By making a series of films commencing with the unincubated tick and ending with those containing fully developed spirochaetes, all the stages in the development of the latter forms from the coccoid bodies may be obtained.

The effect of heat on the development of the intracellular coccoid bodies is rather variable and does not always result in their development into spirochaetes. The first effect seems to be the production of growth and transverse fission, resulting in the production of large masses of

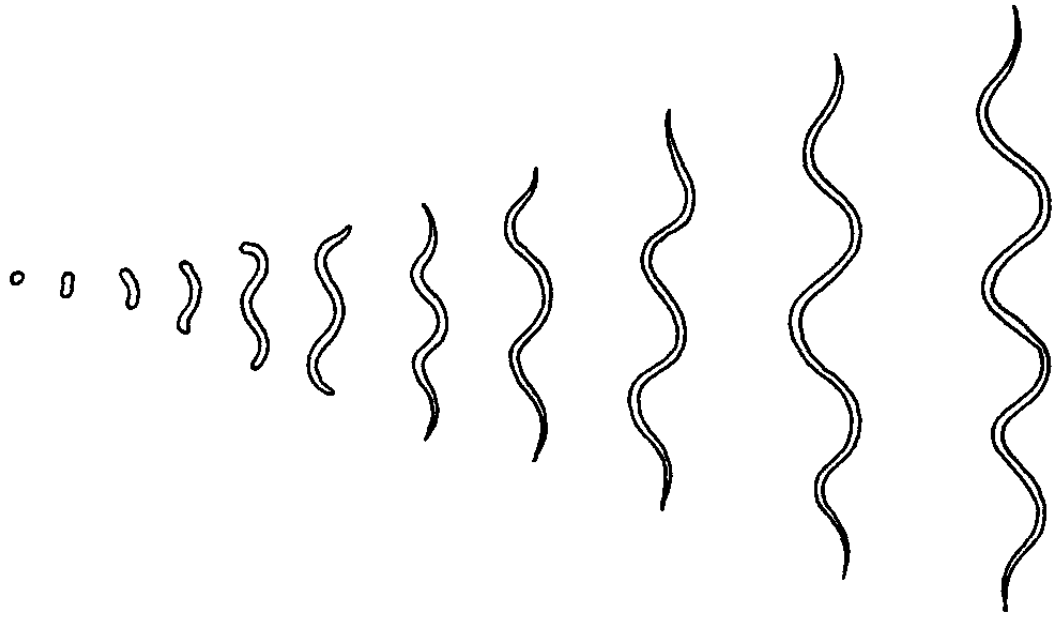

Fig. 4. Stages in the development of spirochaetes from the coccoid bodies.

coccoid bodies that simulate schizogony. Subsequently, certain of the coccoid forms elongate, first assuming a bacillus form, and then appearing as short spirilla (Fig. 4). Some of the bacillus forms merely elongate into long fusiform bacilli which are usually intracellular. The short spirilla escape from the cells into either the lumen of the gut and Malpighian tubules, or into the coelomic fluid, and there grow in length until they appear as normal spirochaetes (Fig. 4). The various stages in their elongation up to the normal spirochaete have been repeatedly observed in the tick and, therefore, it may reasonably be assumed that the same form of development occurs when the coccoid bodies are introduced into the blood of a fowl. As the spirochaetes appear in the coelomic fluid of a tick which has been incubated at 37° C. for a few

days, the parasites naturally make their way from this medium into all the organs, where they may easily be found.

The development of the coccoid bodies was also followed in the eggs laid by infected *Argas*. It was found that the eggs usually contain a considerable number of these coccoid bodies and as in this case there is absolutely no possibility of external contamination, many were kept at a temperature of 37° C. in order to follow the development of the spirochaetes. This was all the more important as the only experiments with the larvae hatched from the eggs laid by an infected *Argas*, seemed to show that the infection was not transmitted to the larvae. Blanc (1911) has used this as an argument against the view so ably put forward by Leishman (1910) that the granules (= coccoid bodies) represent a stage in the life-history of the spirochaete.

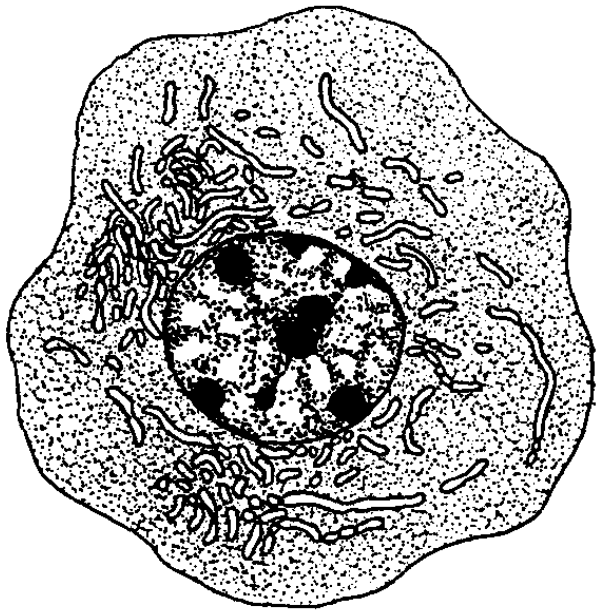

Fig. 5. Cell from the embryonic Malpighian tubule of an egg that has been heated for 5 days at 37° C. Showing the elongation of the intracellular coccoid bodies resulting in the production of long filaments.

It was found, however, that not only could spirochaetes be obtained by maintaining the eggs containing coccoid bodies at a temperature of 37° C., but that also infection could be produced by feeding the infected larvae on a fowl. A large number of larvae, hatched from eggs laid by infected *Argas* from Algeria, were placed on a fowl. The bird showed parasites four days later and the next day died from the infection, with its blood swarming with spirochaetes. Another batch of larvae hatched from the eggs of the *Argas* from Brazil, but possibly mixed with some of the Algerian strain, also produced a fatal infection in

a fowl. With the first stage nymphs obtained from these larvae another fowl has also been infected and we are rearing these nymphs in order to see whether the infection is transmitted to their offspring, as in the case of *O. moubata.*

The study of the coccoid bodies in the developing eggs is of great interest for these forms seem to concentrate into particular regions of the embryo. The cells of the Malpighian tubule when in the embryo often contain enormous masses of the coccoid bodies, which, in some cases, are so numerous as to completely fill the cells. In this respect they agree with the similar stages of *S. duttoni* occurring in the developing eggs of *O. moubata* (Leishman, 1910). If the developing eggs were kept at a temperature of 37° C. either long fusiform bacilli or spirochaetes were obtained, according to the time at which one commenced the incubation.

If the eggs were placed in the incubator as soon as they had been laid the larvae did not hatch out until the 7th or 8th day and in the films made from them the simple elongation of the coccoid bodies into intracellular, long fusiform bacilli was observed (Fig. 5). No spirochaetes were found in the films made from the freshly hatched larvae, nor, in fact, from those made from the larvae after they had been kept in the incubator for another three or four days. Moreover, in these larvae the coccoid bodies and fusiform bacilli seemed to disappear after the 7th day.

On the other hand two batches of infected eggs that were allowed to develop at 28° C. for about a week before being placed in the incubator, both showed abundant spirochaetes in the larvae that hatched from them about four days later. In this case the various stages in the development of the coccoid forms into normal spirochaetes were the same as those occurring in the incubated adult tick.

It seems possible, from a consideration of the above-described results, that whilst the coccoid forms are intracellular they can either multiply by transverse fission or simply elongate into fusiform bacilli or short spirilla, identical in appearance with ordinary bacteria. In order to develop into spirochaetes it is necessary for them to escape from the cell into a fluid medium; in the case of the tick, either the coelomic fluid, or the contents of the gut. When the eggs are allowed to develop at 28° C. for a few days before being placed in the incubator, the developing spirochaetes are able to escape from the cells into the coelomic fluid, which is not formed until late in the development of the larvae. As a result, in this fluid medium they

grow up into normal spirochaetes resembling those occurring in the blood.

The inoculation of these coccoid bodies into Java sparrows has not always been followed by infection and there is evidently some other factor in their development which is not quite clear. One experiment has been made, however, which suggests that the coxal fluid may be of assistance in causing the development.

A number of eggs containing numerous coccoid bodies were crushed up and the resulting material divided into two halves. One part was mixed with some coxal fluid, carefully collected to avoid any possibility of it being contaminated by excrement, and the mixture then injected into a Java sparrow. The other half of the crushed eggs were directly injected into another sparrow. The first bird became infected five days later and died of the infection with its blood swarming with spirochaetes, whereas the second bird never became infected.

On another occasion the injection of eggs containing coccoid bodies that had been heated at 37° C. for 36 hours produced a very slight infection in a Java sparrow. Two injections of the coccoid bodies from eggs that had been heated at 37° C. for 24 hours were followed by negative results, whilst on another occasion an injection of similar material produced a slight infection. The results, therefore, are somewhat contradictory and show that there is still some undetermined factor connected with their development.

It is possible that when the coccoid bodies mixed with the coxal fluid enter the wound caused by the tick's bite, the spirochaetes multiply at the site of infection before entering the general circulation.

Up to the present we have been unable to cause the development of these coccoid bodies in culture media, but there is little doubt that they are capable of being cultured, for both Tunnicliff (1906) and Noguchi (1911) have succeeded with other species of spirochaetes. The former, working with *S. vincenti*, obtained cultures of the fusiform bacillus forms, which, in the case of *S. gallinarum*, are obtained in the cells of *Argas* that have been heated at 37° C. for a few days.

Therefore, it is possible that one of the stages of the spirochaete may be cultured without the spirochaete form being developed.

The discussion of the true position of the spirochaetes will be postponed until a more detailed account of this parasite is published.

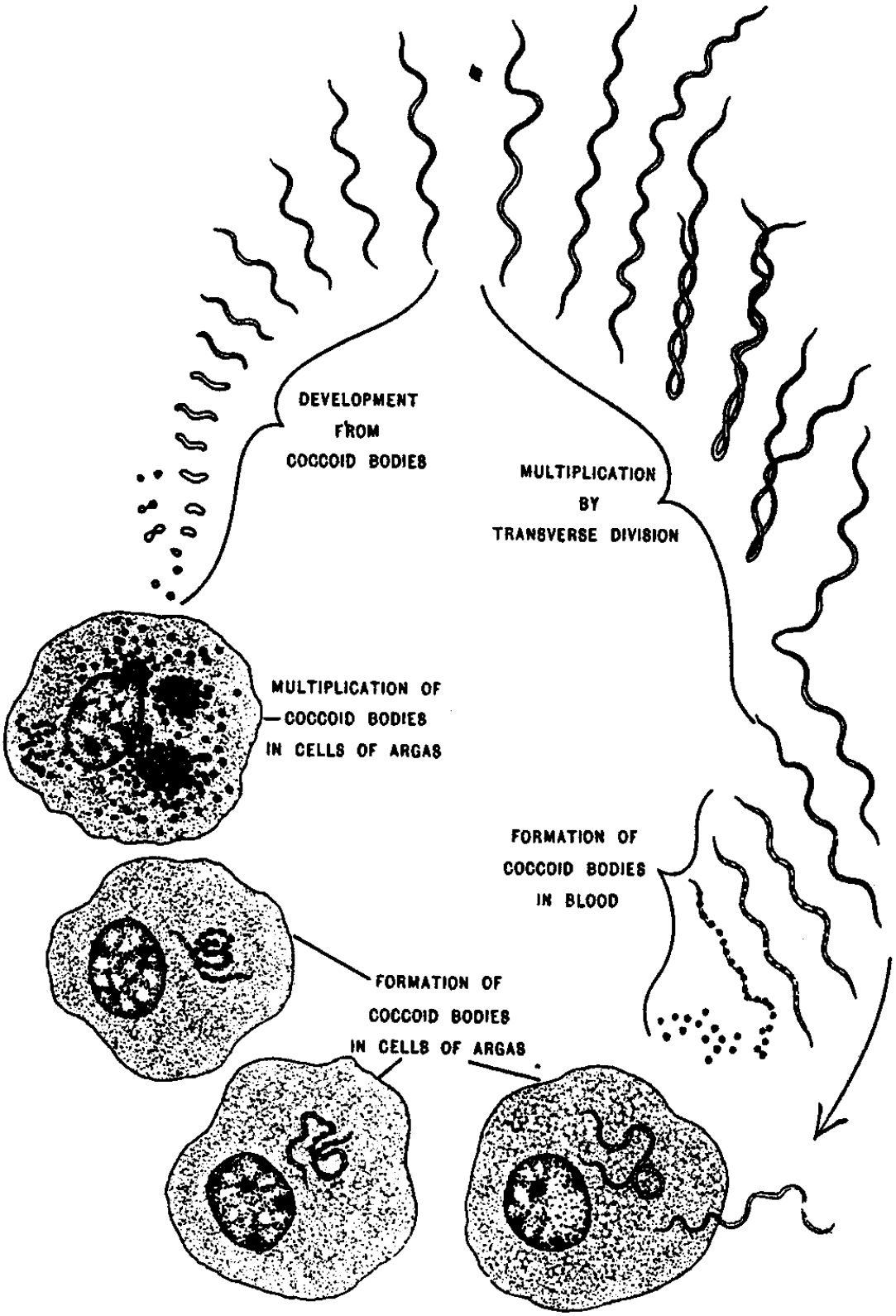

Diagram *A*. The life-cycle of *Spirochaeta gallinarum* (diagrammatic).

SUMMARY.

The life-cycle of *S. gallinarum* may be briefly summarised as follows:

Commencing with the ordinary parasite in the blood of the fowl, the spirochaete grows until it reaches a certain length (16 μ–19 μ) and then divides by the peculiar mode of transverse division described above. This process is repeated and is probably the only method of multiplication of the parasite within the blood. When the spirochaetes disappear from the circulation some of them break up into coccoid bodies which, however, do not usually redevelop in the fowl. When the spirochaetes are ingested by *Argas persicus*, some of them pass through the gut wall into the coelomic fluid. From this medium they bore their way into the cells of the various organs of the tick and there break up into a number of coccoid bodies. These intracellular forms multiply by ordinary fission in the cells of the Malpighian tubules and gonads. Some of the coccoid bodies are formed in the lumen of the gut and Malpighian tubules. The result is that some of the coccoid bodies may be present in the Malpighian secretion and excrement of an infected tick and when mixed with the coxal fluid may gain entry into another fowl by the open wound caused by the tick's bite. They then elongate and redevelop into ordinary spirochaetes in the blood of the fowl, and the cycle may be repeated.

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