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ON THE LIFE-HISTORY OF *HELICOSPORIDIUM PARASITICUM*, N.G., N.SP., A NEW TYPE OF PROTIST PARASITIC IN THE LARVA OF *DASYHELEA OBSCURA* WINN. (DIPTERA, CERATOPOGONIDAE) AND IN SOME OTHER ARTHROPODS.

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(With Plates IV—VI and 5 Text-figs.)

CONTENTS.

I. PRINCIPAL HOST OF *HELICOSPORIDIUM* AND ITS INFECTION.

THE usual host of this new parasite, *Helicosporidium parasiticum*, is the larva of a Ceratopogonid, *Dasyhelea obscura* Winnertz, which lives in the decomposed sap filling the wounds of trees—elm and horse-chestnut. All the material used for this study was derived from the wounds of two trees only: (1) an elm tree standing on the Caius College ground at Newnham, facing Church Rate

Walk, and (2) a horse-chestnut standing between the School of Agriculture and Downing College.

In addition to the new parasite, the larvae of *Dasyhelea* harbour several other parasites, two of which—(1) a gregarine, *Allantocystis dasyhelei* Keilin, and (2) a parasitic yeast *Monosporella unicuspidata* Keilin—have already been dealt with in my previous papers (1920 *a* and *b*).

To find the larvae parasitised with *Helicosporidium*, one proceeds in the following manner: to a small quantity of the semi-fluid exudate collected from the wounds of trees, is added ordinary tap water sufficient to cover the bottom of a wide dish. The mixture is then thoroughly stirred and is left for an hour or more to settle. The numerous larvae of various sizes found at the bottom of the dish are then transferred by means of a pipette to a dish containing clean water and standing on a black or dark coloured surface. On careful examination of the contents of this dish it is noticeable that while the normal *Dasyhelea* larvae present a whitish but translucent appearance, a few individuals are usually seen which are white and opaque. Isolated on a slide and examined under the microscope, all these opaque larvae are found to be parasitised either with *Monosporella*, or with the new parasite *Helicosporidium*, or very exceptionally with both organisms together. As the proportion of larvae parasitised with *Monosporella* is very small, almost all the opaque and milky specimens of *Dasyhelea* larvae are found to be infected with the new parasite.

If we examine under the microscope a parasitised larva, compressed between slide and coverslip, sufficient to prevent movement, we see that its entire body cavity is filled with small round corpuscles, 5 or 6 μ in diameter. These corpuscles occupy all the spaces between the organs of the larva and extend through all the segments including the head.

Being free in the body cavity of the larva, the parasitic corpuscles are always seen circulating or even gushing from one segment to another. This movement is purely passive and is produced either by the contraction of the segments of the host, by the more or less regular movement of its internal organs, or by the rhythmic contractions of the heart and the consequent plasma circulation. These passive movements of *Helicosporidium* are very easily seen in the head of the larva, where the parasites are less densely crowded on account of the restricted spaces between the strongly developed muscles of the mouth-parts. The post-abdominal segments on the contrary are filled to such an extent with the parasitic corpuscles that these form a solid mass occupying the whole cavity of these segments. The posterior portion of the larva becomes thus very turgid, loses its mobility, and becomes very fragile. By pricking such parasitised larva with a fine needle or even by gentle pressure on the coverslip a milky fluid gushes from the wound, and this fluid when examined with the microscope is seen to be a pure suspension of parasitic corpuscles (Pl. V, figs. 1, 2 and 3).

All the larvae which were recognised from their external appearance as being infected, had already arrived at such an advanced stage of infection, that

no stages of the multiplication of the parasite could be detected. To find the earlier stages of infection it was impossible to select the parasitised larvae with the naked eye. For this purpose each larva had to be examined separately under a high magnification, a very long and difficult task, because (1) the larvae are insufficiently transparent owing to their cuticle being lined with a layer of the fat body, whilst (2) the early stages of the parasites are minute and easily confused with droplets of fat or albuminoid corpuscles which often escape from the fat body of the slightly compressed larva.

After selecting living larvae which I suspected to contain the early stages of the parasites, I proceeded to make smears of their bodies, which after fixation and staining, revealed, with very few exceptions, the early stages of this parasite.

In this way I have collected a fairly rich material showing the various stages of the parasite. This material was studied in the form of smears as well as in sections of the larva.

As to technique, I may state that all classical methods of Protozoology were used. I have obtained the best results from the smears fixed in Schaudinn's fluid (with the addition of 1 per cent. acetic acid) and stained in iron-haematoxylin and from sections of the larvae fixed in Carnoy's fluid and stained also in iron-haematoxylin or in haemalum.

II. THE LIFE-HISTORY OF *HELICOSPORIDIUM PARASITICUM*.

1. *Localisation of the parasite.*

All the stages of the parasite are usually found free in the body cavity of the host. In several cases, however, especially when the infection was only recent, the parasites were found either in the fat body or in nerve ganglia. When they attack the fat body, the latter is rapidly destroyed and the parasites, attached to the fat droplets, escape into the body cavity. On the contrary when the nerve ganglia are infected, the infection remains for a long time localised; all the stages are then present simultaneously in the ganglia which become swollen and reduced to the neurilemma. It is interesting to remark that several successive nerve ganglia of the ventral chain may be infected, but the parasites are never found in the nerve commissures.

2. *Early stages and schizogony.*

The youngest stage of the parasite found in the tissue or in the body cavity of the host, is represented by small round corpuscles of 2 or 3 μ in diameter (Pl. IV, figs. 1, 2, 3); they are sometimes oval in shape, being then 3 μ long and 1·5 μ wide. The protoplasm of these corpuscles is homogeneous, being devoid of granulations and vacuoles. The nucleus, in the form of a spherical chromatic granule 0·5 μ in diameter is surrounded by a clear zone of protoplasm of 0·75 μ in diameter. This clear zone may be the real nucleus, while the chromatic granule is the nucleolus—this, however, could not be proved, as it was im-

possible to detect any nuclear membrane surrounding the clear portion of the protoplasm.

In this stage the parasite grows a little, and then divides into two (Pl. IV, figs. 3, 4, 5); the smallness of the parasite makes it very difficult, if not impossible, to follow in detail the mode of division. In some cases it appeared to me to be an ordinary amitotic division; in other cases on the contrary I could see a fairly clear mitosis. The two cells resulting from the division are of almost equal size and shape (Pl. IV, figs. 6, 7, 8 and 9); they are now more elongated and their protoplasm becomes more basophile. They grow a little, undergo a second division (Pl. IV, figs. 10, 11 and 12) and give rise to a small schizont (or morula) composed of four cells disposed in a tetrahedral manner (Pl. IV, figs. 13 and 14). In a few cases only I have observed all four cells symmetrically arranged quadrantally in one plane (Fig. 15). The schizont composed of four merozoites is usually slightly oval and measures 4μ by 3μ. These schizonts continue their development in two different ways: (1) either by breaking up into four merozoites (Pl. IV, figs. 16 and 17) which being set free, divide in their turn, or (2) by undergoing a third division (Fig. 18) and giving rise to schizonts composed of eight cells or merozoites (Pl. IV, figs. 19, 20). These schizonts, 4μ in diameter, are very basophile, so that it is often difficult to differentiate their nuclei. They undergo no further division, but break up into eight merozoites (Pl. IV, figs. 21 and 22), measuring $1{\cdot}7\mu$–2μ by 1μ which probably divide again in their turn.

I have not yet observed schizonts composed of more than eight cells. This multiplication, which forms an endogenous or schizogonic part of the life-cycle of *Helicosporidium* is very active and always results in the formation of an enormous number of unicellular corpuscles scattered throughout the body cavity of the host or invading its various tissues.

3. *Formation and structure of the spores.*

After a period of very active schizogonic multiplication the parasite passes into the second phase of its life-cycle, namely the formation of spores. The merozoite resulting from the schizogony increases slightly in size, becomes very basophile, and after two successive divisions (Figs. 23, 24 and 25) forms a morula of four cells tetrahedrally disposed. Of these cells, one grows more rapidly than the others and the whole morula completely loses its regular shape (Figs. 26, 27 and 28).

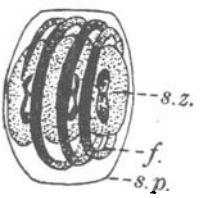
We now arrive at a very short phase in the life-cycle of the parasite, in which the latter undergoes some changes, the nature of which I was unable to follow clearly. However, by a few fragmentary observations and especially by the subsequent development, I think I have succeeded in reconstructing this missing stage, which I shall consider for the present as being hypothetical. Of the four cells which form the morula, one, the fourth, which lies now separately on one side and is much larger than the three other cells, finally

succeeds in surrounding them in the form of a ring. The three surrounded cells change their shape, becoming flattened, in the form of three superposed or parallel discs which occupy the centre of the ring formed by the fourth cell (Figs. 29 and 30). This cell then secretes an external membrane which envelopes the whole group of four cells, thus forming a spore.

We now arrive at a stage in which the spore is most frequently encountered and which is easily recognised. It is the barrel-shaped spore surrounded by a very fine and transparent membrane or sporocyst. Its largest diameter is between 5 and 6μ. When examined end-wise, the spore shows a central circular mass, strongly basophile, and surrounded by a highly refractive ring which fills the space between the central mass and the walls of the sporocyst (Pl. IV, fig. 31).

Examined from the side, the spore shows that the deeply-stained central mass is composed of three superposed discs, parallel to each other and to the flattened surfaces of the barrel-shaped spore (Pl. IV, fig. 32). These central discs are surrounded by a refractive ring which we now see from the side only.

Only by careful differentiation can the vesicular nuclei be detected in the central discoidal cells. These spores now undergo a further transformation: examined *in vivo* they show a spiral refringent band surrounding the three central cells and lining the sporocyst. The more detailed structure of this stage can be seen only in fixed and stained smears or in sections of the infested larva.



Text-fig. 1. Completely formed spore showing the three central cells or sporozoites—*s.z.*; a peripheral spiral filament—*f.*; and the external membrane or sporocyst—*s.p.*.

The protoplasm of the three central cells now loses more and more its basophile property, while in the refringent ring which surrounds these cells a chromatic substance appears which assumes the form of a spiral band with 3–4 turns surrounding the central cells of the spore (Text-fig. 1 and Pl. IV, figs. 33, 34, 35, 36 and 37). Viewed from the polar ends of the spore, the chromatic spiral band appears as a series of superposed chromatic rings surrounding the central cells (Pl. IV, figs. 38–42). During differentiation after staining with iron-haematoxylin, the chromatic spiral still retains a very dark colour, after all the rest of the spore is completely decolorised.

At this stage the nuclei of the central cells are distinct; the nuclei are variable in shape but are usually discoidal, their chromatin forming a peripheral ring which is connected with a central body or karyosome of an irregular form.

The parasite now invades the whole body of its host to such an extent that the latter dies, and as we have seen, the host's tissues are destroyed and replaced by a solid mass composed solely of these spores. For a long time I supposed that these spores represented the final developmental stage of the parasite, namely a resistant form, which, being set free from the dead host, were swallowed by other larvae of *Dasyhelea* which thus became infected.

However, a further study of the parasite showed this supposition to be wrong, for the spores above described were found to undergo further development in the dead body of their host.

4. *Development of the spores subsequent to the death of the host.*

The decomposing sap collected from the wounds of trees often contains dead *Dasyhelea* larvae which, on microscopic examination are found to be completely filled with elongated filaments 60–65 μ long, with pointed extremities (Pl. V, figs. 8 and 9). As these filaments show a close resemblance to the acicular spores of yeasts of the genus *Monosporella*, one species of which I have described as being parasitic in the larva of *Dasyhelea*, I was at first under the impression that the elongated filaments were the spores of a similar yeast.

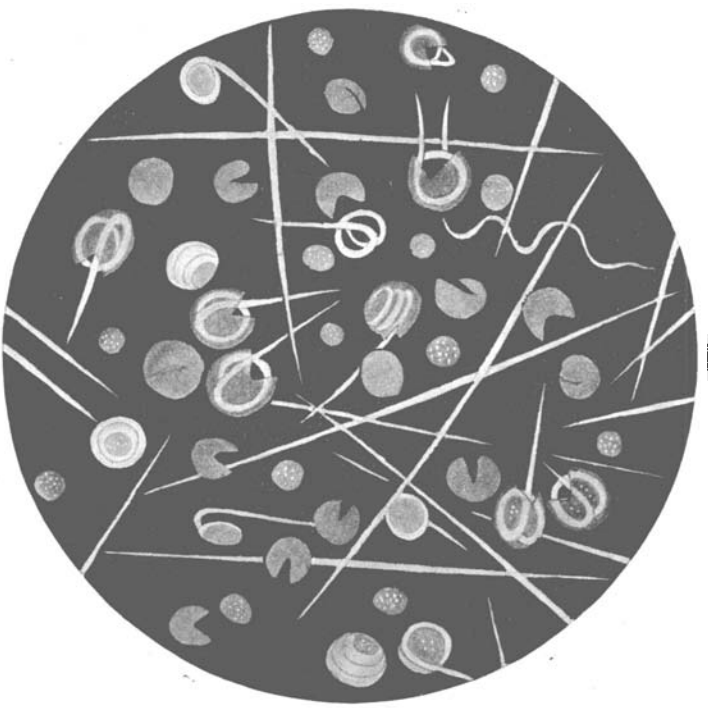
Subsequent observations showed me that such was not the case. These filaments have nothing to do with yeast, but actually belong to a later phase of the development of the spores of *Helicosporidium*. I have been successful in tracing the consecutive steps in the formation of these free filamentous structures from the barrel-shaped spores. If we isolate a parasitised *Dasyhelea* larva in a drop of ordinary tap water or in a small quantity of decomposed sap and leave it to itself it will soon die, being killed by the parasite. On *allowing the body of this larva, filled with the barrel-shaped spores, to dry* very slowly no noticeable change will occur in the spores; but if the dried body is moistened again, it swells up and a large number of filaments similar to those we have previously mentioned will appear among the barrel-shaped spores. The formation of these filaments does not necessarily result from a previous drying; it may occur also in the parasitised larva continuously submerged in water, but in this case the process takes place much more slowly. The successive drying and moistening of the spores appears, however, to hasten the formation of the filaments, and doubtless plays a very important part in nature, because the wound of the tree is necessarily exposed to alternating conditions of drought and moisture.

All the stages in the formation of these filaments are easily found by opening the body of the parasitised larva in a drop of water a few days after the insect's death (Text-fig. 2). The process whereby the barrel-shaped spore gives rise to filaments can only be followed, however, by examining smears prepared from the dead infected larvae, fixed with Schaudinn's solution and stained with iron-haematoxylin.

Such smears show very clearly that the filaments are the unrolled internal spirals liberated from the spores. The latter, under the pressure of the unrolling spirals, rupture at one end, and from the opening in the ruptured sporocyst a portion of the spiral protrudes (Pl. IV, figs. 42–47). The protrusion usually begins at one end of the spiral, which progressively unrolls and liberates itself from the sporocyst. At various stages of this unrolling the three central round cells are mechanically expelled from the spore, leaving the sporocyst completely empty.

In somewhat rare cases the protrusion of the spiral begins either with a loop (Pl. IV, fig. 44) formed by its central portion or with both extremities simultaneously (Fig. 46).

The same smears also contain many other spores devoid of a sporocyst (Text-fig. 3). The spiral filament of these spores is of a very irregular shape, the rings which form it being often loosened or the unrolled portion secondarily twisted. The irregular form of these filaments is undoubtedly due to mere mechanical pressure produced during the preparation of the smears.



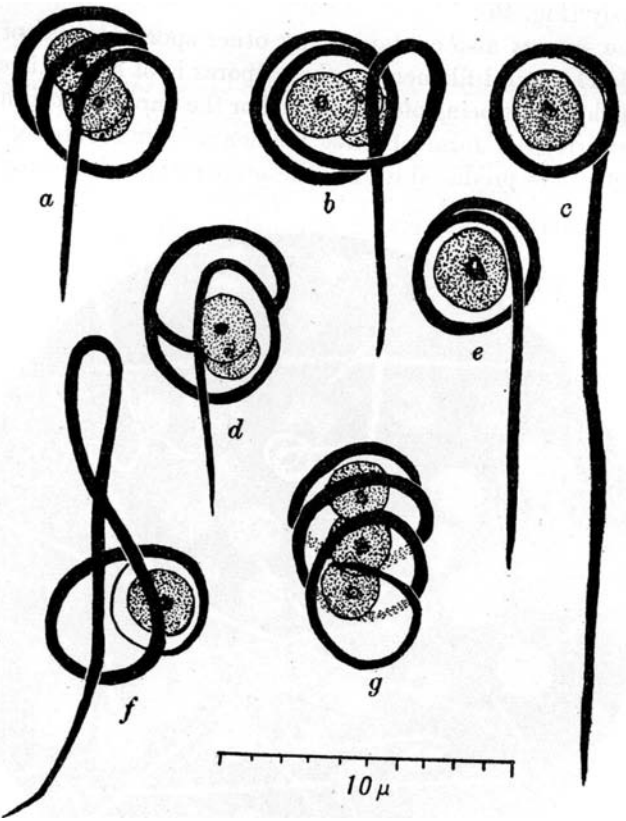
Text-fig. 2. A drop of fluid taken from the dead body of a *Dasyhelea* larva infected with *Helicosporidium*. The drop diluted with normal salt solution, shows different stages of the opening of the spores and unrolling of the spiral filaments.

The three liberated central cells, which are 2μ in diameter, remain much as they were when inside the sporocyst, with the difference that the refractive granules in their protoplasm are now more distinctly visible.

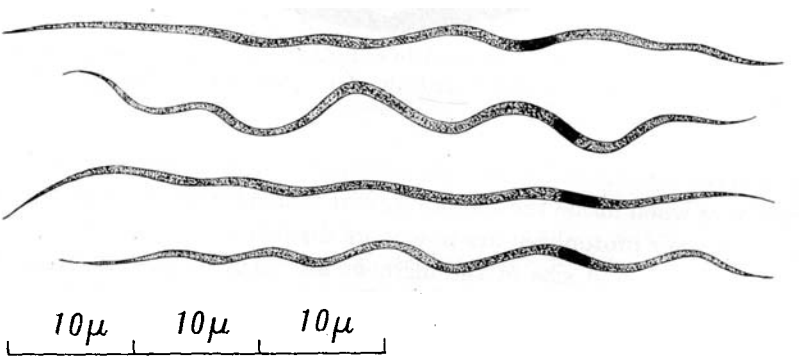
The structure and size of the filament are fairly uniform. Examined *in vivo* they are straight, needle-shaped (Text-fig. 2 and Pl. V, figs. 8 and 9), 60–65μ long and 1μ wide. They are very refractive, pointed at both ends, but the two extremities are not equally attenuated.

In fixed and stained smears, these filaments are more or less sinuous and much narrower, not exceeding $0{\cdot}65\mu$ in their widest portion (Text-fig. 4, Pl. V, fig. 7). This is undoubtedly due to the fact that the central axial

part of the filament takes the stain, while the peripheral refractive non-staining sheath either contracts or becomes invisible in refractive mounting



Text-fig. 3. Different forms of unrolling spiral filaments without sporocyst; such forms are often found in fixed and stained smears.



Text-fig. 4. Unrolled filaments in fixed and stained smears showing their nuclei.

media. In iron-haematoxylin the filaments stain very slowly, but once stained, they are very retentive of the dark colour and are decolorised with difficulty.

After differentiation in iron alum, the darkly stained axial portion of the filament presents a granular structure, and if the differentiation is more prolonged, until the filament becomes of a grey colour, a darkly stained chromatic body, of definite size and structure, makes its appearance (Text-fig. 4). This body, which is undoubtedly a nucleus, is 2–3 μ long and 0·6 μ wide, always lies in the wider portion of the filament, 15–18 μ from its extremity. By prolonged differentiation this nucleus can also be detected in filaments of the spiral form while still enclosed in the sporocyst (Pl. IV, fig. 41). We now arrive at the final stage of the life-cycle of the parasite: the distended cuticle of the dead larva being filled with a felt-like mass of entangled filaments mixed with small cells, the central cells of the spores and the empty sporocysts (Pl. V, figs. 8 and 9).

At this stage the macerated cuticle of the larva breaks away and its entire contents escape into the surrounding medium, the decomposed sap of the tree. Smear preparations of this sap often reveal the above-mentioned filaments, while the central cells of the spores cannot be recognised in the crowd of various micro-organisms, yeasts, moulds, rhizopods, ciliates, etc., which usually inhabit the fluid.

5. *Supposed mode of infection of the host.*

A question arises now: which is the infective form of *Helicosporidium*? Is it the filament or the central cells of the spore? The cellular structure of the filament, its great resistance to external influences, and its resemblance to the infective stage of *Monosporella* suggest that it may be the infective stage. If this is the case, the central cells of the spore could only be considered as residual bodies.

On the other hand, the great number of the central cells (three times more numerous than the filaments) and their resemblance to the first stages of *Helicosporidium*, as seen in the body-cavity of the host, make it almost certain that they represent the real sporozoites or infective forms of the parasite. In this case the spiral filament may be regarded as a cell differentiated for the purpose of dehiscence of the spore and can be compared in respect to its function with the elaters of Mycetozoa, with the difference that, while in the latter they are of complicated structure and extrasporal, in *Helicosporidium* they are unicellular and intrasporal.

The sporozoites, after being swallowed by a healthy larva, penetrate probably through the wall of the alimentary canal into the body cavity of the larva, where they begin their endogenic multiplication or schizogonic cycle.

6. *Frequency of infection of* Dasyhelea *larvae.*

Several generations of *Dasyhelea obscura* occur in the course of a year, and the larvae of all the generations are equally subject to infection. It is, however, impossible to estimate the true rate of infection as this varies greatly and depends upon the condition of the wound of the tree at the time

when the material is collected. In rainy weather the larvae leave the flooded parts of the tree's wounds and penetrate into the fissures of the tree; in the meantime, the wound is thoroughly washed by the rain and is freed from the collected sap which usually contains dead and dying larvae infected with *Helicosporidium*. When the normal conditions are restored and the wound is once more covered with freshly exuded sap, the larvae crawl again from their hiding places and invade the wound. If the sap is collected at this time, very few infected larvae will be found. On the contrary, in damp weather with the absence of much rain, when the old sap remains in the wound for a prolonged period, the number of diseased larvae increases. Finally, the sap collected from the wounds and kept in jars in the laboratory gives a still higher proportion of diseased larvae, as in this case the non-infected larvae rapidly become infected from contact with their diseased companions.

7. *Stages of the host susceptible to infection.*

In all its larval stages *Dasyhelea obscura* is susceptible to infection with *Helicosporidium*. This is undoubtedly due to the feeding habits of the larvae remaining uniform throughout its life. Very small larvae, hardly 1·5 mm. long, were often found with the body cavity filled with spores of the parasite, the spores showing already completely formed spirals and the three sporozoites. In a single instance a full-grown larva, almost ready to pupate, showed only the schizogonic cycle of the parasite, a condition which indicates a recent infection. Between these two extremes all the intermediate phases are met with. Only a few pupae of *Dasyhelea* were found infected and this infection is almost certainly derived from the larval stage: a full-grown larva became infected just before pupating, when all its imaginal discs were already completely formed and the pupation took place before the parasite had time to make a destructive invasion. Such infected pupae are eventually killed by the parasite. In no case have I observed the parasite in the adult insect.

III. Other Hosts of *Helicosporidium parasiticum*.

1. *Mycetobia pallipes* Meig. (Diptera, Rhyphidae).

The larvae of *Dasyhelea obscura* are usually found associated with a number of other Dipterous larvae living in the same medium. According to their feeding habits, these larvae can be separated into two groups:

(1) Saprophagous larvae which like *Dasyhelea* feed upon the decomposed sap *e.g. Rhyphus fenestralis* Scop., *Mycetobia pallipes* Meig., *Aulacogaster rufitarsis* Meig. and the larvae of *Eristalids*.

(2) Carnivorous larvae such as *Systenus adpropinquans* Loew, and *Phaonia cincta* Zett.

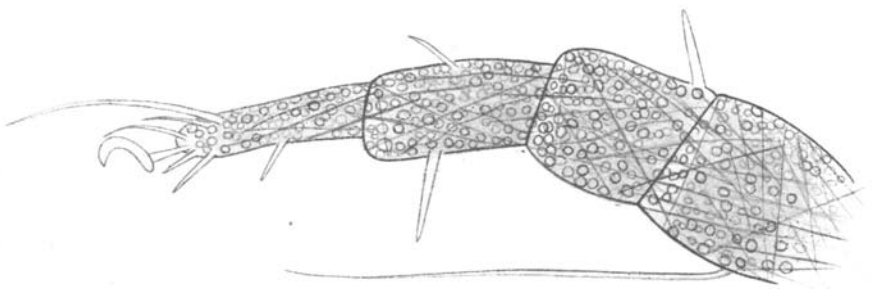
I have frequently examined large numbers of larvae of all these different species for various parasites and only once have I found the spores of

Helicosporidium in a specimen of *Mycetobia pallipes*, invading the peripheral portion of its fat-body.

2. *Hericia hericia* (Robin) Kramer (1899) (Acarina, Tyroglyphidae).

In addition to the above-mentioned Dipterous larvae, the exudate which fills the wounds of the elm tree contains also a very interesting mite belonging to the family Tyroglyphidae. This mite, *Hericia hericia* (Robin) Kramer, 1899, is undoubtedly the most frequent inhabitant of the exudate. It was discovered and very well described by Robin (1868) who found it in the exudate of elm trees in France.

The mite has been since found in England by Michael (1903, Vol. II. "Tyroglyphidae," pp. 31–38, Pls. XXIII–XXIV) who gives in his monograph of British Tyroglyphidae a complete description of all its stages. Concerning its habitat he writes: "This species usually lives in a semi-aquatic condition, wading in the sap which exudes from splits in the bark of elm trees, or under loose bark of these trees, and in the brown saccharine matter which collects there. In such situation it is often present in great numbers; it is also found,



Text-fig. 5. A leg of *Hericia hericia* heavily infested with *Helicosporidium* showing the spores and filaments

but less frequently, in similar situations on the oak. I have not found it on other trees, but it is quite possible that it may exist on them."

I myself found this mite abundantly, often covering the entire surface of the wound.

On many occasions I have carefully examined large numbers of this mite without finding a single parasitised specimen, but recently (October, 1920), whilst collecting the *Dasyhelea* larvae, I noticed a portion of the body of *Hericia* with two legs attached to it, the whole filled with filaments and a few complete spores of *Helicosporidium*. I believed at first that I was dealing with an empty skin of a dead *Hericia* which was invaded by a small *Dasyhelea* larva parasitised and killed by *Helicosporidium*, but on careful examination of other mites, I soon discovered eight entire specimens, three of which were alive, all showing *Helicosporidium* in different phases of its life-cycle.

Text-fig. 5 shows a leg of a very heavily infested specimen of *Hericia*, the whole body of which is invaded with the spores and a large number of filaments.

All this shows clearly that *Helicosporidium parasiticum*, although a common parasite of *Dasyhelea obscura*, is by no means specific to this host, but occurs in other Dipterous larvae like *Mycetobia pallipes* and what is still more remarkable, in at least one other Arthropod, the Tyroglyphid mite *Hericia hericia* (Robin) Kramer, 1899.

IV. Systematic Position of the Genus *Helicosporidium*.

Now that we know the structure and the life-history of *Helicosporidium*, a question arises as to the systematic position of this genus. It seems to me very difficult to answer this question and all I can do at present is to discuss the relations between the new parasite and various forms of Protists.

1. Helicosporidium *and Cnidosporidia.*

One is tempted first of all to compare the genus *Helicosporidium* with the Sporozoa, especially those which have multicellular spores, as for instance Cnidosporidae, comprising the three Orders: Myxosporidia, Actinomyxidia and Microsporidia.

The trophozoite stage of *Helicosporidium* as well as its schizogonic cycle recalls in many respects those of the Microsporidia Monosporogenea Pérez (*e.g. Nosema bombycis*); on the other hand, the trophic stage, in the form of a small round cell, as well as the schizogonic cycle, cannot be used for establishing the affinities between the various groups of Protists as similar modes of multiplication can be found in widely separated orders.

On the other hand the development and the structure of the spores provide a series of much more important characters which have been used already with success in the classification of the Protozoa. A character which is common to all Cnidosporidia and *Helicosporidium* is the complicated multicellular structure of the spores, the latter in both cases being composed of heterogeneous elements. It remains to be seen, however, in how far the spore cells of *Helicosporidium* can be compared with those of the Cnidosporidia.

It may be assumed, for instance, that the three central cells or sporozoites of *Helicosporidium* correspond to the germ cells, sporoplasm or sporozoites of the Cnidosporidia, while the polar capsule of the latter corresponds to the spiral filament of our parasite.

It must be admitted, however, that this assumption, which at first sight appears to be reasonable, is based on very superficial points of resemblance, and it needs a critical examination. The germ cells or sporoplasm of the Cnidosporidia differ from those of *Helicosporidium* in that they are usually reduced to a single binucleated cell, instead of three uninucleated cells as in *Helicosporidium*.

Among the Cnidosporidia the uninucleated sporozoites are known in a few species of Actinomyxidia, but they are very numerous in each spore.

As to the filament of *Helicosporidium*, although it is formed from one

differentiated cell as is the polar capsule of Cnidosporidia, it differs from the latter in several essential characters.

The following table shows the points of difference between these two structures:

Spiral filament of *Helicosporidium*	Polar-capsule filament of Cnidosporidia
(1) Filament not enclosed in a polar capsule but lies free beneath the wall of spore.	(1) Filament enclosed in a capsule of which it forms a part.
(2) Filament always unrolls in the dead body of its host.	(2) Filament does not unroll until spores reach intestine of a second host.
(3) Filament unrolls slowly.	(3) Filament is shot out.
(4) Filament pointed at both ends and wide and ribbon-like in the middle.	(4) Filament pointed at one end only and very fine.
(5) Axial portion of filament is very chromatic, nucleus well formed in anterior third of filament.	(5) No chromatic axial portion, degenerated nucleus upon wall of terminal capsule.
(6) Filament robust, very resistant in all media.	(6) Filament fine and very fragile.

The foregoing shows clearly that the spiral filament of *Helicosporidium* is of a nature totally different from that of the polar capsule of the Cnidosporidia.

Other distinctive characters of *Helicosporidium* are: (1) the wall of the spore does not seem to be formed by a specialised cell, at any rate no trace of such a cell could be detected in the wall of the completely formed sporocyst; (2) the spore of *Helicosporidium* does not show a binary or ternary symmetry and pansporocysts are non-existent.

All this demonstrates clearly enough that there is no real affinity between the genus *Helicosporidium* and the Cnidosporidia.

2. Helicosporidium *and Haplosporidia.*

We may now compare the genus *Helicosporidium* with the Order Haplosporidia of Caullery and Mesnil (1905). Although this Order, conceived in its widest sense, is heterogeneous, all the forms which it comprises differ greatly from *Helicosporidium.* In their life-cycle they have a plasmodium stage and a cyst which surrounds the spores; these characters never appear in the life-history of *Helicosporidium.* The spores of the Haplosporidia are unicellular with one or two envelopes while the spores of *Helicosporidium* are composed of four cells of different structure. These differences are sufficient to show that there is no affinity between *Helicosporidium* and the Haplosporidia.

3. Helicosporidium *and* Serumsporidia.

It remains finally to be seen whether or not there are some relations between *Helicosporidium* and a few Protists temporarily placed in the Sporozoa but whose systematic position is still *subjudice.* The only group among these Sporozoa which may interest us is the group of Serumsporidia of Pfeiffer.

Under the generic name of *Serumsporidium*, Pfeiffer (1895) has described a certain number of parasites which he discovered infesting the body cavity

of several species of Crustacea, belonging to the genera *Cypris*, *Daphnia* and *Gammarus*.

The descriptions and figures of this author are, however, very superficial and incomplete, and it is hardly possible to get a general idea of the structure of the parasites which he has described.

Neither his descriptions nor his figures of some eight distinct species of this genus give the remotest indication as to the character which these species have in common. From his description one can only say that the few characters which are common to all his eight species are of no systematic value, namely (1) that these species live as parasites in the body cavity of Crustacea, (2) that they produce very numerous spores, (3) that their structure and life-cycle are equally obscure.

From the systematic point of view the term *Serumsporidium* has no more value than the term "blood parasites" applied to protozoal parasites of mammals.

It is almost certain that his "*Serumsporidium II* (Mülleri), nov. sp.," "*Serumsporidium gammari*" and especially "*Serumsporidium cypridis IV*, n. sp." are the spores of a gregarine liberated from the ripe cysts, which, being fragile, have become ruptured inside the body of the host.

From all his descriptions and figures those relating to his species "*Serumsporidium* (*Cytamoeba? Labbé*) *cypridis I*, nov. sp." (p. 12), are of especial interest to us, as three of the figures illustrating this species bear some resemblance to *Helicosporidium*. His figure 2, B. 7 (p. 12), for instance, recalls the morula of *Helicosporidium* composed of four cells, one of which is concealed behind the other three, while the Figs. 2, B. 8 and B. 9, resembles somewhat the spores of *Helicosporidium* which shows the three nuclei of the central cells only. It is, however, hardly possible to base upon these superficial resemblances any relationship between *Serumsporidium* and *Helicosporidium*.

4. *Helicosporidium and Mycetozoa.*

Helicosporidium has no affinities with the Mycetozoa, several species of which have been already found parasitic in insects. It differs from the Mycetozoa in the absence of the plasmodium and flagellate stages and by the complicated structures of the spores, which, on the contrary, are simple in Mycetozoa.

V. Conclusions.

The foregoing evidence shows clearly that the genus *Helicosporidium* differs markedly from all the actually known Protists, and that it forms a new type which may be temporarily included in the group of the Sporozoa. It is possible that the discovery of other new forms of Protists, parasitic in insects, will throw more light upon the systematic position of *Helicosporidium* or will lead to the finding of a connecting link between this new genus and the already well-known forms.

The genus *Helicosporidium* may be characterised as follows:

Helicosporidium n. g.

Diagnosis. Parasitic protist; trophic stage in the form of a small round cell 2–3 μ in diameter, with small spherical nucleus. Schizogonic multiplication very active. Schizonts forming a small morula of 4 μ in diameter, composed of four or eight merozoites, which become free.

Spore (5–6 μ in diameter) is composed of four cells surrounded by a thin wall or sporocyst. Of the four cells, three, which form the real sporozoites, are discoidal, occupying the centre of the spore. The fourth cell forms a peripheral spiral filament which surrounds the central cells.

The spores open inside the dead body of their host, by the unrolling of the spiral filament, and the sporozoites are thus liberated.

The spiral filament when unrolled is 60–65 μ long, pointed at both ends and 1 μ thick at its widest portion; its nucleus 2–3 μ long, lies 15 μ from one end of the filament. This filament is of a very resistant nature. One species known.

Helicosporidium parasiticum n. sp.

Diagnosis. The same as that of the genus.

Habitat. Body cavity, fat body and nervous system of the larva of *Dasyhelea obscura* Winnertz (Diptera, Nematocera, Ceratopogonidae). Occurs also in *Mycetobia pallipes* Meig. (Diptera, Nematocera, Rhyphidae) and in *Hericia hericia* (Robin), Kramer, 1899 (Acarina, Tyroglyphidae). The hosts inhabit wounds of elm and horse-chestnut trees, Cambridge, England.

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EXPLANATION OF PLATES IV—VI.

Helicosporidium parasiticum.

PLATE IV.

All the figures of this plate relate to the smears fixed in Schaudinn's solution with 1 per cent. of acetic acid added, and stained in iron-haematoxylin and eosin. Owing to the small size and complicated structure of the spores, high magnification was used for this study: Apochromatic imm. object. 2 mm.; N.A. 1·4 with Comp. oculars 8 and 12. For the sketches which were made with the camera lucida, comp. ocular 18 was used; the scale of magnificatien common to all the figures is shown in the plate beneath the figures.

Figs. 1, 2, 3. Trophic stages of parasite.

Figs. 4, 5. First division of the parasite and formation of two cells.

Figs. 6–9. Different forms of bicellular stage.

Figs. 10–12. 2nd division and formation of schizonts of four cells.

Figs. 13, 14. Schizonts composed of four cells, tetrahedrally disposed.

Fig. 15. Exceptional forms of four cellular schizonts with merozoites radially disposed.

Figs. 16, 17. Breaking up of four-celled schizonts into four merozoites.

Fig. 18. Nuclear division in schizonts of four cells.

Figs. 19, 20. Schizonts composed of eight cells.

Figs. 21, 22. Breaking up of eight-celled schizont into eight merozoites.

Figs. 23, 24, 25, 26. Three successive stages in formation of morula of four cells tending to formation of the spores.

Figs. 27, 28. Further transformation of these cells showing one cell growing more rapidly and surrounding the three other cells.

Fig. 29. Obscure stage showing a central mass surrounded by a ring.

Fig. 30. Hypothetical figure showing the three central discoidal cells surrounded by the fourth cell.

Fig. 31. A young spore, end view, showing a central darkly stained body composed of three superposed discoidal cells, surrounded by a very refractive ring, and by a thin wall or sporocyst.

Fig. 32. Side view of a similar spore showing clearly the three central cells.

Fig. 33. Further development of the spore (as shown in Fig. 32), showing the spiral filament formed in the refractive ring surrounding the three central cells or sporozoites.

Figs. 34–37. Spores viewed side-wise, similar to that illustrated in Fig. 33, showing some variation in size (due to fixation) and in the appearance of nuclei of the central cells.

Figs. 38–40. End view of a similar spore, the figs. 39 and 40 do not show the sporocyst, this being due to contraction by the fixative.

Fig. 41. Spore stained and much decolorised, showing a nucleus in the spiral filament.

Fig. 42. Spore seen from one end in a smear where almost all the other spores are already open (derived from the dead larva).

Figs. 43, 44. Ruptured spores showing the protrusion of a portion of the filament.

Fig. 45. Protrusion of the filament through the opening of a ruptured sporocyst.

Fig. 46. A case of protrusion of both ends of the spiral filament.

Fig. 47. More advanced stage of protrusion of the filament and its unrollment.

PLATE V.

Photomicrographs taken by Mr L. E. Robinson.

Fig. 1. Living larva of *Dasyhelea obscura* heavily parasitised, pressed between the slide and a coverslip until the posterior end of its body was ruptured, projecting an enormous mass of spores. The latter can be seen still filling the body cavity of the larva and reaching into the head. × 75.

Fig. 2. The upper portion of the same larva under a higher magnification. × 150.

Fig. 3. Another larva, filled with spores of *Helicosporidium* and ruptured in its middle, showing the spores inside and outside the body. × 150.

Fig. 4 × 800. Fig. 5 × 300. Fig. 6 × 250. Fig. 7 × 500. Fig. 8 × 100. Fig. 9 × 400.

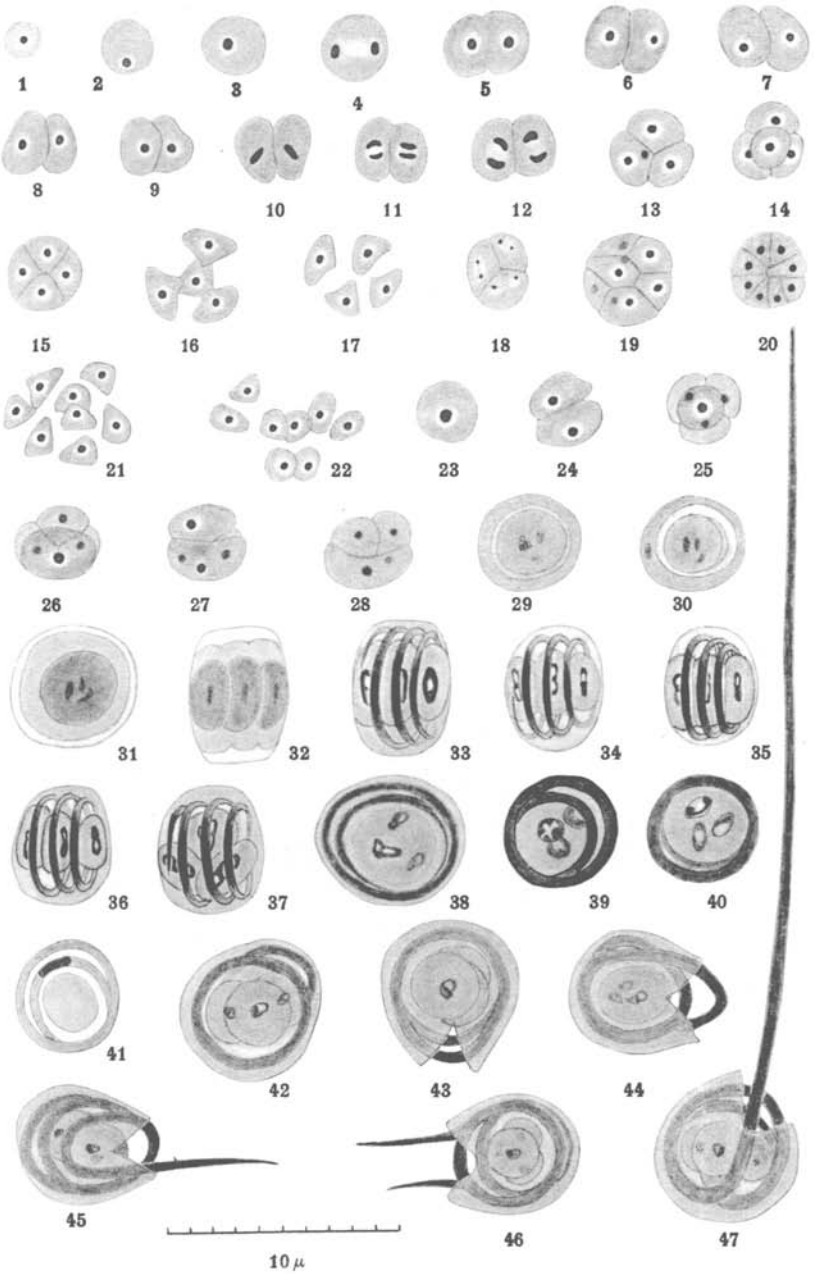
Fig. 4. Smear, fixed and stained, showing the spores with central cells and spirals.
Fig. 5. Transverse section of *Dasyhelea* larva very heavily infected with *Helicosporidium*.
Fig. 6. Transverse section of *Dasyhelea* larva not heavily infected with *Helicosporidium*.
Fig. 7. Spores and unrolled filaments of *Helicosporidium* in fixed and stained smears.
Fig. 8. Dead body of *Dasyhelea* larva infected with *Helicosporidium*, the spores of which are open and the spiral filaments free and unrolled. The body of the larva is ruptured by the pressure of a coverslip and shows an enormous number of escaping filaments.
Fig. 9. A portion of the same mass of filaments under a higher magnification.

PLATE VI.

Life-cycle of *Helicosporidium parasiticum*.

Figs. 1–9. Different phases of schizogonic multiplication, formation of four- and eight-celled schizonts.
Figs. 10–13. Formation of four-celled morula which develops into the spores.
Fig. 14. Hypothetical stage showing the three central discoidal cells surrounded by the fourth cell.
Fig, 15. Young spore before the formation of spiral filament: (*a*) side view, (*b*) end view.
Fig. 16. Mature spore: (*a*) side view, (*b*) end view.
Figs. 17–20. Different stages of the opening of the sporocyst, unrolling of the spiral filament and liberation of sporozoites.

Stages 1–16 occur in the living larva of *Dasyhelea*, while the stages 17–20 are found only in the dead body of the host.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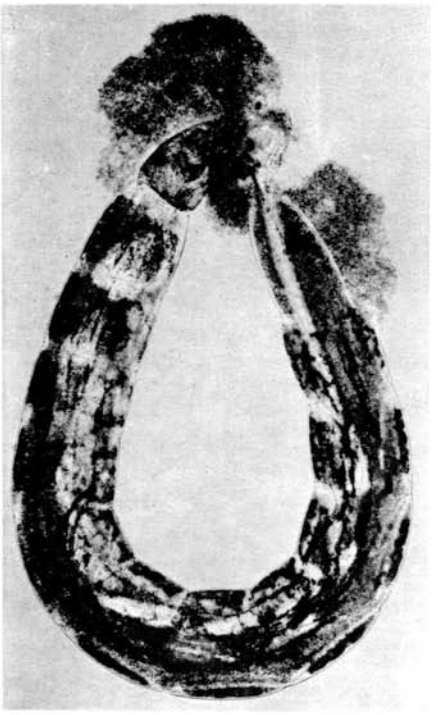

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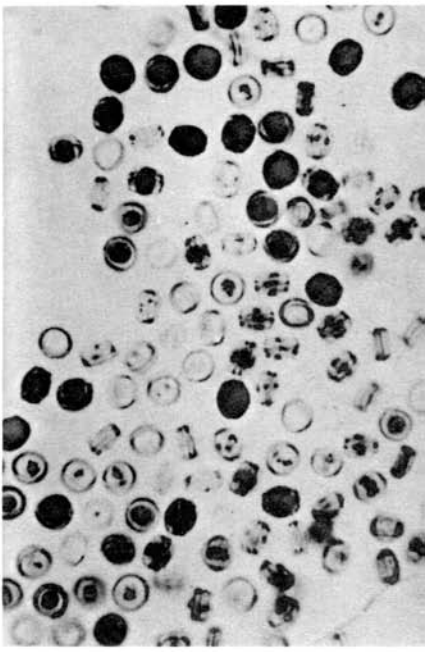

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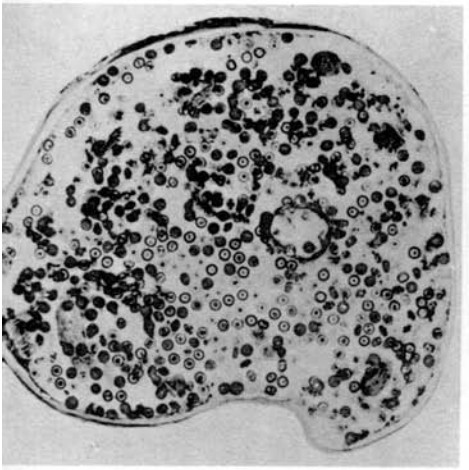
L. E. Robinson, photo.



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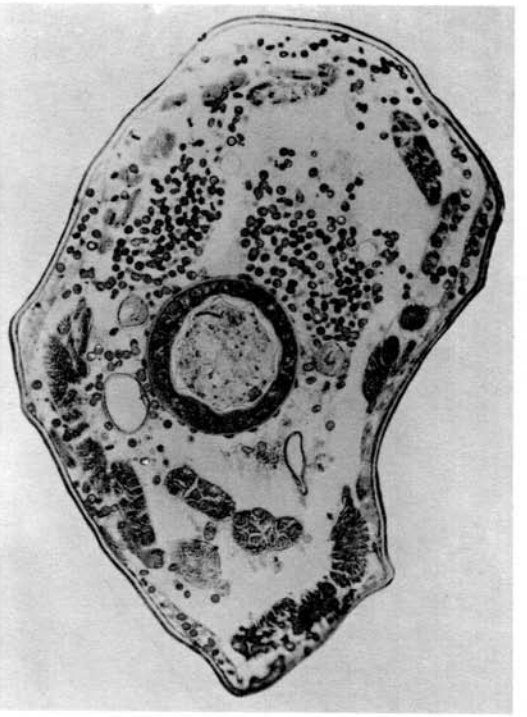

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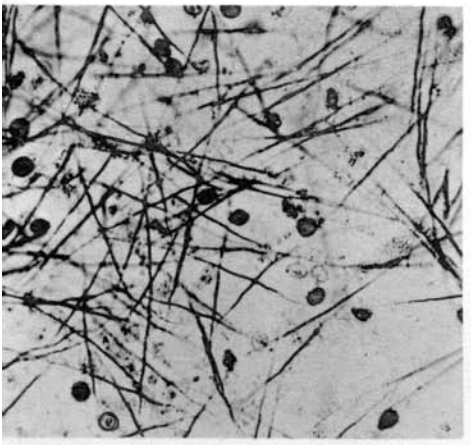

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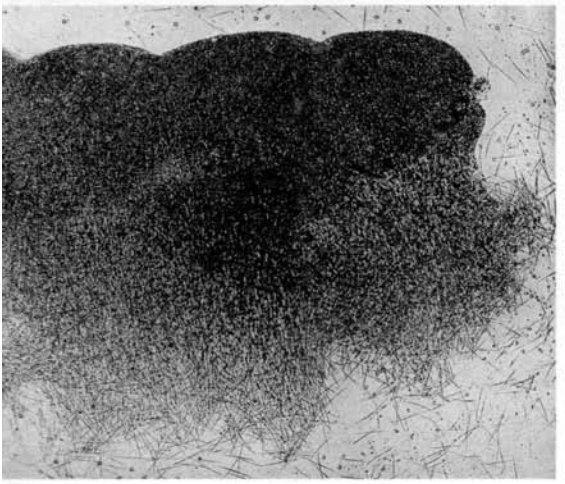

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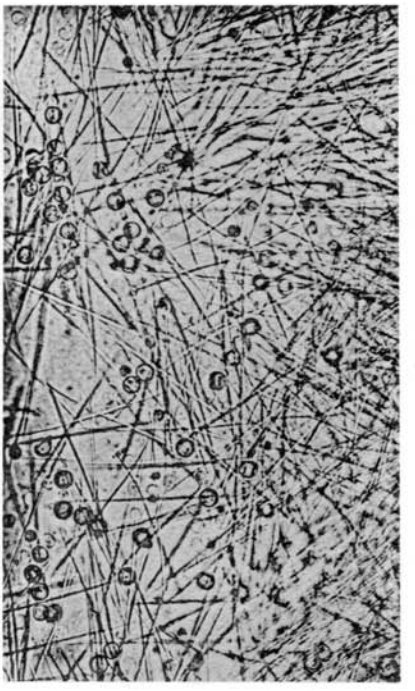

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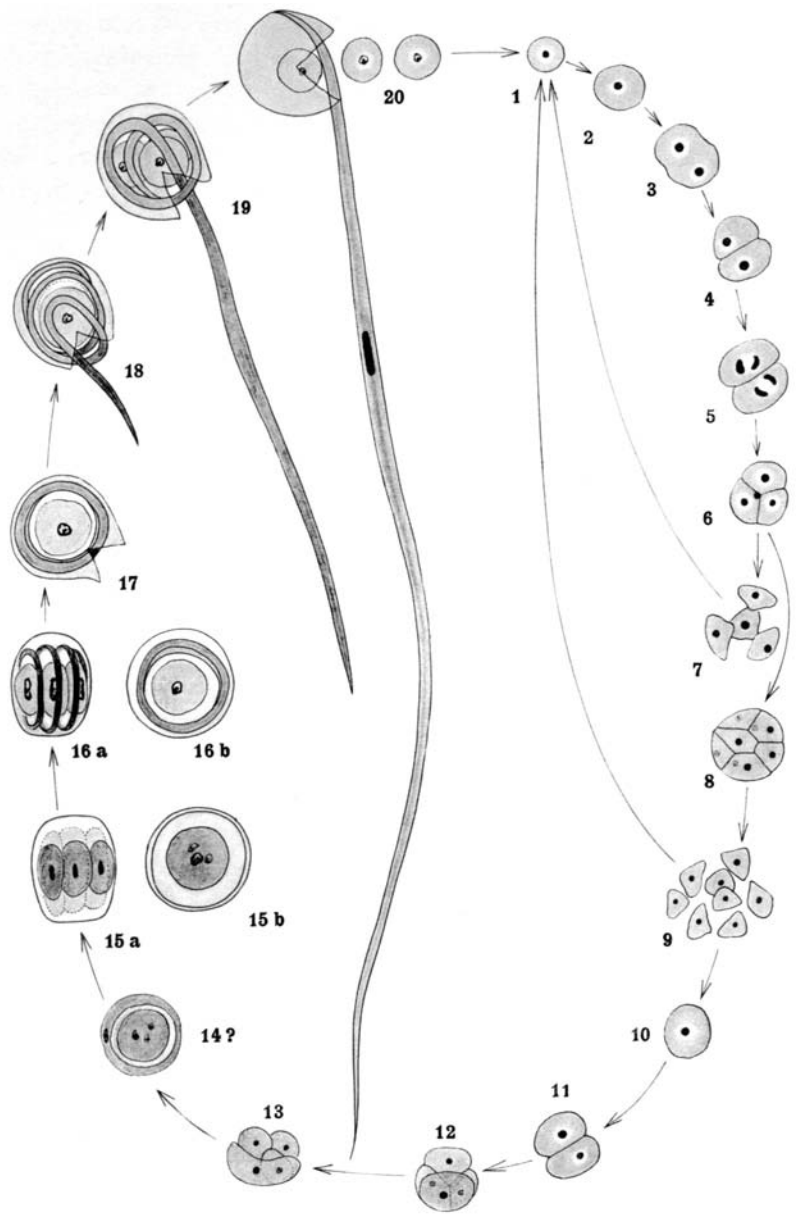

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