Studies on Microsporidia parasitic in mosquitoes.

VIII. On a microsporidan, *Nosema aedis* nov. spec., parasitic in a larva of *Aëdes aegypti* of Porto Rico [1]).

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(With plate 4—5.)

Contents.

Introduction.

In the previous papers of this series, some microsporidian parasites of mosquitoes belonging to genera Anopheles and Culex were studied. While I have not observed any microsporidian occurring

[1]) Contributions from the Zoological Laboratory of the University of Illinois No. 348.

in mosquitoes of the genus Aëdes, a number of investigators reported several parasites which they considered as Microsporidia. Perhaps the first published paper is that of PARKER, BEYER and POTHIERE (1903) who found in the course of their study of the etiology of yellow fever, a protozoan parasite, *Myxococcidium stegomyiae*, which occurred regularly in *Aëdes aegypti* (*Stegomyia fasciata*) "contaminated by feeding on a case of yellow fever." Although they did not state that the organism was a microsporidian, its generic name leads one to suppose that they considered it a related form to the latter. These investigators fed the mosquitoes on "human blood, overripe banana or other fruits or syrup" and observed the organism in the lumen and epithelium of the alimentary canal of the insects. They claimed that they succeeded in tracing through a cycle of development of the parasite from the gamete to the sporozoite, which was undoubtedly influenced by the then newly discovered development of Plasmodium in the female mosquito. Their accounts on the so-called spore are very incomplete and the appended photomicrographs are unfortunately indistinct. It is, therefore, difficult to determine the true nature of the organism without reexamining the sections they had studied.

MARCHOUX, SALIMBENI and SIMOND (1903) reported their observations on *Nosema stegomyiae* from larvae and especially adults of *Aëdes aegypti*. They distinguished colorless and brown spores. The general appearance of these spores is that of a microsporidian, but the development as was interpreted by them is certainly unusual if not hypothetical and is of a type which has not been observed in any species of Microsporidia. According to these observers, the spores measured 4 to 7 μ long by 2 to 3 μ broad (in the fresh state?), while SIMOND (1903) reported in a separate paper that the length and breadth of the spore were 3 to 5 μ and 2 to 3 μ respectively. On account of the general appearance of the spore and a part of the change which the colorless spore was supposed to undergo during development, I have assumed it, in one of my papers (KUDO, 1924b), to be a microsporidian and placed it provisionally in the genus Plistophora.

LUTZ and SPLENDORE (1903) observed also in adult *Aëdes aegypti*, *Nosema lutzi* (*N. stegomyiae*), which was found either scattered or in polysporous cysts in the intestine of the host. The organism occurred rarely. The polymorphic spores were oval or pyriform and measured 3,5 to 7 μ long by 2 to 2,5 μ broad. These observers gave neither figures nor statement whether the parasites were also found in the host's tissue or not. The polar filament was not mentioned.

Since 1920, four European investigators noted the presence of Microsporidia in some species of Aëdes. Nöller (1920) observed sporogonic stages of *Thelohania* sp., which occurred abundantly in the fat body of a larva of *Aëdes nemorosus* collected in the vicinity of Hamburg. He did not see mature spores, but suggested that it was probably *Thelohania legeri* Hesse which, however, seems to be, as far as we know, a typical anopheline parasite. The same author saw further in larvae of *Aëdes nemorosus* and *A. cantans*, a Nosema which he thought identical with *Nosema culicis*. Martini (1920) spoke briefly of the presence of a microsporidian (*Nosema* sp.) in larvae of *Aëdes* sp. Wenyon (1926) mentions *Thelohania* sp. which was discovered by MacGregor in larvae of *Aëdes nemorosus* in England. This microsporidian was found in the fat body of the host and the fresh spores measured 6 to 7 μ long by 4 to 4,5 μ broad.

From this brief review, it is obvious that our knowledge on microsporidian parasites of the genus Aëdes is highly inadequate compared with that of those parasitic in mosquitoes of genera Anopheles and Culex. In the spring of 1929, I received a larva of *Aëdes aegypti* from Dr. Wm. A. Hoffman of the School of Tropical Medicine, the University of Porto Rico, San Juan, Porto Rico. It was found that the larva was infected by a microsporidian. Although the spore was only slightly larger than the average microsporidian spore, its structure was distinctly recognizable. The transformation of each sporont into a spore, indicates that it is a species which should be placed in the genus Nosema. The general form and appearance of the spore resemble somewhat those of *Plistophora* (*Nosema*) *stegomyiae* quoted above, but the dimensions differ conspicuously between the two. It differs also from the spores of *Nosema lutzi* or of any of the other known species of the genus in the appearance, form, dimensions and structure of the spore and in its host species, although comparison with many of them can adequately not be done because of the incomplete descriptions. The present microsporidian is, therefore, considered as a hitherto unrecorded species, and the name *Nosema aedis* is given to it.

Material and methods.

The larva which was fairly grown, was fixed in Bouin's fluid and preserved in 70 per cent alcohol. When it came to my hand, it was found that the fixation was excellent[1]). Under a low power

[1]) I am under obligation to Dr. Hoffman who discovered the larva and placed it at my disposal after careful fixation.

objective, numeorus isolated microsporidian spores could clearly be seen in the anal gills, which indicated that the infection was well advanced. A few posterior segments were cut off from the body and washed and emulsified in the distilled water. From the latter were made hanging drop preparations as modified by NEMECZEK (1926) and also several permanent smear preparations. To make out the polar capsule of the spore, a small amount of a solution (1:100) of methylene blue M. P. was added to a hanging drop preparation.

Since fresh spores were unavailable, no attempt was made to cause the extrusion of the polar filament, partly because the coiled filament was distinctly visible within the spore, which fact made certain that the organism was a microsporidian and partly because there was only one infected larva for the entire study.

The remaining part of the larva was sectioned (4 μ in thickness). For staining, GIEMSA'S solution, HEIDENHAIN'S iron haematoxylin or DOBELL'S alcoholic iron haematein was used. Some of the sections were first stained with HEIDENHAIN and eosin and studied. They were then restained after DOBELL'S method after complete decolorization. Such preparations showed numerous mature spores in which their structure was most distinctly noticeable.

The relation between the host and the microsporidian.

As to the behavior of the larva in life, HOFFMAN writes me that it was much less active compared with the other larvae which were found living in the same water.

The site of infection is the adipose tissue as is the case with the most species which occur in mosquito larvae (Fig. 59). The fat body of the thorax was most heavily infected. In the posterior region of the abdomen, the integumentary adipose dissue was free from infection, while that which was located in the general body cavity was heavily infected by the microsporidian (Fig. 59). The muscles, intestinal epithelium, gonads, Malpighian tubules and nervous system, were free from the parasite, although search was made for it in them. The gonads which were surrounded by infected fat bodies remained free from any stages of the microsporidian. There was a heavily infected fat body immediately below the left cephalic ganglion, but the latter appeared to contain no parasites.

Numerous isolated spores were noticed scattered throughout the body cavity, from the head (Fig. 60) to the hind-most segment of the sectioned portion (Fig. 59). The presence of numerous structures which coresponded to microsporidian spores in general appearance

in the anal gills as seen under a low power objective, was quite typical of a microsporidian infection as was noted above, by which one can discover a fairly advanced infection without dissecting the host larva. The dorsal vessel contained also isolated spores (two spores are visible in Fig. 59, D). It seems probable that the wall of infected host cells in the thoracic region became ruptured, due partly to the mechanical pressure of the parasite which increased in them and partly to the movements of the larva. These spores were apparently carried around by the circulating body fluid into various parts of the host body.

The majority of these isolated spores were surrounded by an irregularly reticulated mass which could be noticed in water (Fig. 1) and also in sections (Figs. 55, 56, 59—61). This covering mass is not part of the parasite, since the spores located within the host cells are not covered by it (Figs. 62, 64, 67). Further, not all the free spores are surrounded by it (Figs. 2—5). This mass does not take nuclear stains, but stains uniformly intensely with eosin. Its outline is irregular and aside from its reticulation, it is structureless. Several masses may anastomose with one another (Figs. 1, 55, 56, 61). It seems to be a mass of body fluid of the host which became adhered closely to the spores that were set free in the body cavity through the rupture of infected cell walls. Although similar appearance of isolated spores in the host's body cavity was noted in mosquitoes and other host animals (Kudo, 1921—1929), in no one of the cases was seen such a mass covering the parasites. Why this occurs in the present species is not understood. It must be considered as a specific reaction on the part of the host insect. Phagocytosis was also observed in the present case, as was noted before (Kudo, 1924 b), and recently by Weissenberg (1926) and Mattes (1928).

The nucleus of the heavily infected host cells shows striking hypertrophy (Fig. 57) as compared with that of an uninfected cell (Fig. 58). This is a well-known phenomenon characteristic of a microsporidian infection, although exact causes involved in bringing about this change are still unknown.

The Schizogony.

The schizogony was well advanced, and a host cell in which the infection was at the beginning stage could not be found. Among the actively dividing schizonts, one finds uninucleate bodies with reticulated cytoplasm (Fig. 9) which appear to be the youngest stage

found in the material. The nucleus is distinctly vesicular and contains usually an eccentric endosome, which appearance is similar to that of a mature spore (Figs. 1—5, 45—51). In spite of the comparatively large number of schizonts undergoing divisions, the complete prozess of nuclear division during the schizogony which I noted in *Thelohania legeri* (KUDO, 1924), *Stempellia magna* (KUDO 1925), etc., was not recognized in the material on hand. From the few cases such as indicated in Figs. 10 and 11, it may be said that the nucleus seems to divide amitotically, preceded by the division of the endosome and followed by rapid or delayed appearance of nuclear membranes between them. The divided nuclei assume terminal positions (Figs. 12, 13) and finally the whole schizont divides into two uninucleate schizonts (Figs. 14, 15). Frequently the nuclei divide once more, which results in production of tetranucleate schizonts that seem to divide into four uninucleate bodies. How many times the division outlined above, is repeated, cannot be determined.

The uninucleate schizont seems to increase in size. Its nucleus divides into two which remain adhered to each other. During the division, small chromatic granules become prominent and are connected by achromatic network (Fig. 20). A number of achromatic fibers become stretched in the direction of the division and several chromatic granules appear in two groups on them. The observation concerning the nuclear division is here also fragmentary so that further description is impossible. The process results in an increase in number of the nuclei in the binucleate stage and is somewhat similar to the corresponding stage present in *Stempellia magna* (KUDO, 1924b, 1925). Stages in which one to eight pairs of nuclei occur, are encountered (Figs. 23—29). These apparently divide to form a large number of binucleate schizonts such as are shown in Figs. 30 and 31. The two nuclei in such a schizont become highly vesicular and in each several large chromatic granules become prominent. This is followed by the disappearance of the separating nuclear membranes and the nuclear contents become fused into one. During this fusion, several chromatic granules seem to be extruded into the cytoplasm through the nuclear wall (Figs. 32, 33). The uninucleate body thus produced, is the sporont.

The sporogony.

The nucleus of the sporont moves towards one end. Its cytoplasm is at first uniformly reticulated (Figs. 34—36), but becomes, with the elongation of the sporont, differentiated into two regions. The

extremity in which the nucleus is located, is occupied by a condensed cytoplasm in which a vesicular nucleus is conspicuous, while the other part becomes coarsely reticulated (Figs. 37, 38). In the latter region an oblique longitudinal line was noticed in several individuals (Figs. 38, 40, 44). In the cytoplasm, there appears later a polar capsule (Figs. 41, 43) and the spore membrane becomes differentiated around the whole. The process as outlined here, agrees with that observed in *Stempellia magna* (KUDO, 1925) with the exception of the total absence of deeply staining granules in the developing polar capsule, but is quite different from that which was noted in *Thelohania legeri* (KUDO, 1929) in which the sporoblast nucleus divides into two, one of which remains as the nucleus of the sporoplasm, while the other seems to directly control the formation of the polar capsule and the filament, disintegrating eventually.

The spore.

The mature spores are broadly pyriform. Viewed in water in a hanging drop preparation, they show the following structure (Figs. 1—5). In the broad posterior part, there is a clear space in which a nucleus is distinctly visible. The latter is spherical or ellipsoidal in form and vesicular in structure. There is ordinarily a single endosome which is connected with the nuclear membrane by a number of fine strands. Attached to the membrane, are seen small granules. The remaining part of the spore is reticulated or transversally striated, without manifesting any further differentiation.

When stained temporarily with a solution of methylene blue. the nuclear structure is more clearly seen (Figs. 6—8). In the reticulated cytoplasmic mass, a polar capsule with the faintly visible polar filament and the deeply staining cytoplasm become distinctly visible, as these two structures stain in different manner. The longitudinal axis of the polar capsule does not coincides with that of the spore and the former, therefore, is more closely situated toward one side than to the other. The polar capsule is in reality longer than that illustrated in Figs. 6 and 8 and extends further down toward the posterior end as is visible in Fig. 7 in which the sporoplasm nucleus lies above the posterior margin of the capsule.

The majority of spores fixed in BOUIN's fluid and preserved in 70 per cent alcohol, measure in water 7,5 to 9 μ in length and 4 to 6 μ in the largest diameter.

In the section preparations which were stained with GIEMSA's stain (Figs. 48—50), DOBELL's alcoholic iron haematein (Figs. 45—47,

51, 52) or HEIDENHAIN's iron haematoxylin (Figs. 55, 56), the structure of the spore described above is far more clearly observable. The nucleus is located near the posterior margin of the spore, is vesicular and contains an endosome which is usually eccentric. Achromatic fibers which connect the latter with the membrane are noticeable. The cytoplasm of the sporoplasm which takes stains intensely occupies the posterior region of the intrasporal space and surrounds further the posterior part of the polar capsule. Figs. 51 and 52 represent two views of a single spore. Fig. 51 is an upper surface view showing the greater part of the sporoplasm with its nucleus, while Fig. 52 is the view at a lower focal plane, in which are visible a long polar capsule to the right extending nearly to the posterior margin of the spore and the sporoplasmic projection at the left. Figs. 65 and 66 are two photomicrographic views of a single spore. The sporoplasm is seen in both figures, but the polar capsule is fairly distinctly seen in Fig. 66. The nucleus of the sporoplasm is most clearly visible in Figs. 61 (A, B) and 67 (S), while the polar capsule is noticeable in Figs. 62 (S), 63 (S) and 64 (S).

The spore membrane is uniformly thin and there is no indication that it is composed of two valves as was noted in *Thelohania opacita* (KUDO, 1924 a, 1924 b, 1925 a). It is noted in spores suspended in water (Figs. 2—5) and also in spores scattered in the body cavity in sections due to the covering mass (Figs. 55, 56, 61, C).

The spores found in stained and mounted sections are, as is always the case with other species of Microsporidia, slightly smaller than those viewed in the water, apparently due to its refractivity which is similar to that of the mounting medium and also to a certain amount of shrinkage which accompanied the manipulation connected with sectioning, staining and mounting. They measure on an average 6,5 to 8 μ long by 3,5 to 5 μ broad.

Discussion on the structure of the microsporidian spore.

Notwithstanding the appearance in the last few years of studies on Microsporidia by several European investigators such as ZWÖLFER (1926), WEISSENBERG (1926), POISSON (1928), MATTES (1928), DEBAISIEUX (1928), SCHWARZ (1929) and others, there still exist diverse views and opinions concerning the structure of the microsporidian spore. Since these papers were not available at the time when I had considered the subject (KUDO, 1924 b), and since my observation of the spore of the species under consideration seems to clarify certain confusion, I shall here take up a discussion of the subject once more.

It is generally recognized that the microsporidian spore is, as a rule, minute and covered by a relatively thick spore membrane which obscures a clear view of its contents. The spores of Microsporidia which have come under the observation of the above-mentioned recent investigators were unfortunately less than 7 μ in length. It is seldom that one encounters a microsporidian, the spores of which approach 10 μ in length. Yet there have been a general tendency among the investigators to believe that all microsporidian spores are of similar structure.

Judging from the results of observations on several species of Microsporidia which I have studied since 1910 by employing an essentially similar technique, I am, however, led to believe, as I remarked before (KUDO, 1920), that structural variations exist among the spores of different species. In the Myxosporidia, another member of Cnidosporidia, the structure of the spore of various genera and species varies. For examples, the number of polar capsule varies from one to four, the position of the sporoplasm or sporoplasms varies from terminal to central, etc. The structural difference in microsporidian spores does not seem to be so great as that which is found in Myxosporidia, yet one can point out some variations.

From the published records on various species of Microsporidia which appeared up to the present, the following "types" of the spore are enumerated. Whether this differentiation holds good or not depends entirely upon the accuracy of the observations of the investigators referred to.

1. The spore is ovoidal and contains two polar capsules, one at each end of the spore. The sporoplasm is located between the two polar capsules. Thus it is comparable with the spore of Myxosporidia belonging to the family Myxidiidae. Example: *Telomyxa glugeiformis* after LÉGER and HESSE (1910).

2. The spore is cylindrical. There is no polar capsule (?). The polar filament is composed of the basal rod-like portion and the filament, occupying the greater part of the spore. The sporoplasm is located at the posterior extremity of the spore. Examples: *Mrazekia argoisi* after LÉGER and HESSE (1916), *M. piscicola* after CÉPÈDE (1924) and *M. niphargi* after POISSON (1924).

3. The spore is pyriform. The polar capsule is conspicuous and median or lateral in its position, occupying the anterior two-thirds or nearly the entire length of the spore. The sporoplasm is situated at the posterior region and may surround a portion of the polar capsule. Examples: *Thelohania acuta* after SCHRÖDER (1914), *Plisto-*

phora macrospora after LÉGER and Hesse (1916), *Stempellia magna* after KUDO (1921 a, 1924 b, 1925), *Nosema cyclopis* and *N. infirmum* after KUDO (1921 b), *Thelohania vandeli* after POISSON (1924) and *Nosema aedis*.

4. The spore is ovoidal or ellipsoidal. There is no polar capsule. The polar filament is coiled in the posterior cavity of the spore. The girdle-like sporoplasm is located near the middle of the spore and surrounds the polar filament. Examples: *Plistophora longifilis* after SCHUBERG (1910), *Glugea hertwigi* after WEISSENBERG (1913), *Plistophora blochmanni* after ZWÖLFER (1926), *Plistophora simulii* after DEBAISIEUX (1928), and *Thelohania ephestiae* after MATTES (1928).

5. The spore is ovoidal or ellipsoidal and is similar in its structure to that of the last type, except that the filament is coiled within a polar capsule. Examples: *Nosema bombycis* after STEMPELL (1909) or KUDO (1916), *Thelohania giardi* after MERCIER (1909) and *Nosema nonagriae* after SCHWARZ (1929).

It is interesting to note here that the great majority of the spores of *Nosema aedis* were, as was mentioned above and stated in the section dealing with the spore, of the third type, yet some spores showed structure that should be placed under either the fourth or fifth type. The abnormal spore shown in Fig. 53, contains a coiled filament in the posterior two-thirds, and a narrow band of sporoplasm near the anterior end and toward one side. Whether the polar capsule is present or not, cannot be determined positively, but it appears to be similar to the spore described under the fourth type. The spore shown in Fig. 54 presents, on the other hand, a structure which was stated under the fifth type.

Speaking in a general way, it would seem that there is in a cylindrical or pyriform spore an ample space at its posterior end for the sporoplasm (types 2 and 3) and that in an ovoid or oblong spore possessing a comparatively long filament, the sporoplasm shifts its position towards the middle where the diameter of the spore is largest and assumes a complete or incomplete ring around the polar capsule (type 5) or the coiled filament (type 4).

As to the number of the nucleus in the sporoplasm, observations vary a great deal. A single vesicular nucleus was noted in *Thelohania acuta* (SCHRÖDER, 1914), *T. varians* (DEBAISIEUX, 1919), *Stempellia magna* (KUDO, 1924 b, 1925), *Thelohania pyriformis* (KUDO, 1924 b, 1925 a), *T. legeri*, *T. indica* and *T. obscura* (KUDO, 1929) and *Nosema aedis*.

A single compact chromatic granule which was considered as the nucleus of the sporoplasm was reported in *Plistophora longifilis* (SCHUBERG, 1910), *Nosema bombycis* (OHMORI, 1912), *Thelohania corethrae* (SCHUBERG and RODRIGUEZ, 1915), *Nosema apis* (KUDO, 1921), *N. baetis* (KUDO, 1921 a), *N. cyclopis* (KUDO, 1921 b), *Thelohania vandeli* (POISSON, 1924).

One or two compact chromatic granules were reported to occur in the sporoplasm of *Nosema bombycis* (LÉGER and HESSE, 1907), *Toxoglugea* [1]) *mercieri* (POISSON, 1924), *Plistophora blochmanni* (ZWÖLFER, 1926).

Two compact nuclei were considered to be present in the sporoplasm of *Nosema frenzelinae* (LÉGER and DUBOSCQ, 1909), *N. apis* (FANTHAM and PORTER, 1912; MAASSEN, 1912; TRAPPMANN, 1926), *N. bombi* (FANTHAM and PORTER, 1914), *N. bryozoides* (SCHRÖDER, 1914), *N. bombycis* (KUDO, 1916), *N. binucleatum* (WEISSENBERG, 1926), *N. nepae* (POISSON, 1928), *N. nonagriae* (SCHWARZ, 1929), *Plistophora macrospora* (LÉGER and HESSE, 1916 a), *P. (Glugea) danilewskyi* (GUYÉNOT and NAVILLE, 1922), *Thelohania ephestiae* (MATTES, 1928), *Mrazekia mrazeki* (HESSE, 1905), *M. argoisi*, *M. brevicauda*, *M. caudata* (LÉGER and HESSE, 1916), *M. piscicola* (CÉPÈDE, 1924), *M. niphargi* (POISSON, 1924), *Octosporea simulii* (DEBAISIEUX, 1928), *Telomyxa glugeiformis* (LÉGER and HESSE, 1910).

The data here summarized, do not seem to support DEBAISIEUX's generalization contained in his excellent paper (1928), that the spores of the species which possess unisporoblastic sporonts, contained a binucleate sporoplasm, while those of the species which possess polysporoblastic sporonts, a uninucleate sporoplasm.

The observation on the schizogonic changes of the present species as stated above, is highly fragmentary as a single host larva in an advanced state of infection was only available. But the changes are unique for the genus Nosema in which no autogamy in the schizont has been reported up to date, although the latter

[1]) In a former paper (KUDO, 1924 b), I quoted LÉGER and HESSE's classification (1922) of Microsporidia. It was later found that the three genera which LÉGER and HESSE created were preoccupied. I proposed, therefore, family Coccosporidae, genera *Coccospora*, *Spirospora* and *Toxospora* for family Cocconemidae, genera *Cocconema*, *Spironema* and *Toxonema* respectively (KUDO, 1925 b). Shortly after the appearance of the latter notice, it was found, through the kindness of Dr. C. W. STILES of Washington, that LÉGER and HESSE (1924) had substituted already *Spiroglugea* and *Toxoglugea* for their *Spironema* und *Toxonema*. Therefore, *Spirospora* and *Toxospora* become synonymous with *Spiroglugea* and *Toxoglugea*.

process has been recognized in several species of the genus *Thelohania* by DEBAISIEUX (1919, 1928), DEBAISIEUX and GASTALDI (1919), KUDO (1924, 1924 a, 1925) and MATTES (1928). In *Nosema bombycis, N. apis, N. bombi, N. anophelis, N. frenzelinae* and other species of the genus, the schizonts are uninucleate. Binucleate forms were frequently found in them, but they were considered as division stages.

On the other hand in *Nosema binucleatum* (WEISSENBERG, 1926) and *N. nonagriae* (SCHWARZ, 1929), both the schizonts and sporonts are distinctly binucleate throughout and there is no nuclear fusion in any stage. In *Nosema aedis*, the nuclear division appears to be amitotic during the schizogony and at present, I am unable to make out the significance of the binucleate form which becomes a sporont upon the fusion of its two nuclei.

During the transformation of the sporont (in *Nosema*) or the sporoblast (in other genera) into a spore, several authors noted the appearance of five nuclei, of which two form the spore membrane, one the polar capsule and the filament, and the other two the sporoplasm. Recently Poisson further observed this type of sporulation in *Toxoglugea mercieri* (1924) and *Nosema nepae* (1928). In *Thelohania legeri* and *T. indica* (KUDO, 1929), I saw two nuclei in each sporoblast. One remained as the sporoplasm nucleus, while the other formed the polar capsule and the filament. In the present species, the nucleus of the sporont remained single, although its membrane became less conspicuous and some of the peripheral chromatin granules disappeared from it as the spore formation approached its completion. Thus, a single nucleus seems to control the sporulation in *Nosema aedis*.

Condensed description of *Nosema aedis* nov. spec.

Habitat. In the adipose tissue of a larva of *Aëdes aegypti*. San Juan, Porto Rico. April, 1929.

Schizogony. Early development is unknown. The youngest schizonts founds in the host cell, measure about 4 μ in the largest diameter. They undergo multiplication through binary and multiple fission, which seems to occur repeatedly. The nucleus of these uninucleate schizonts divides without being followed by a cytoplasmic division, thus producing binucleate schizonts. These binucleate forms seem also to multiply by binary and multiple fission, which results in production of a number of binucleate schizonts. The two nuclei finally fuse to form a uninucleate sporont.

Sporogony. Each uninucleate sporont transforms itself into a single spore. The nucleus becomes situated at one of the extremities, where the protoplasm becomes differentiated, while the greater space near the other extremity, develops the polar capsule containing a polar filament.

Spore. Pyriform. At the broad posterior end, there is a comparatively large vesicular nucleus, containing an eccentric endosome. Surrounding the nucleus and extending towards the anterior end close to the spore membrane, is seen the deeply staining cytoplasm of the sporoplasm. The polar capsule is an elongated sac and extends from the narrow anterior end to the posterior end of the spore It varies in size. It is, therefore, assumed that the length of polar filament, if extruded, may differ greatly. The spores fixed in BOUIN's fluid and observed in water, measure 7,5 to 9 μ long by 4 to 5 μ broad; those stained and mounted in sections, 6,5 to 8 μ long by 3,5 to 5 μ broad.

Summary.

1. Microsporidian parasites of the mosquitoes of the genus *Aëdes* are chronologically reviewed.

2. A new microsporidian, *Nosema aedis*, parasitic in a larva of *Aëdes aegypti* of Porto Rico, is described.

3. At the end of schizogony, autogamous union of two nuclei in a schizont results in formation of a uninucleate sporont.

4. The nucleus of the sporont remains undivided during the spore-formation.

5. The structure of the spore is described and compared with that of several known microsporidian spores.

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Explanation of figures.

Plate 4—5.

All the figures except Fig. 58, relate to *Nosema aedis*.

Figs. 1—8 from hanging drop preparations; Figs. 9—58 from section preparations. Figs. 9—11, 19—23, 35, 45—47, 51, 52 and 54, stained with Dobell's alcoholic iron haematein; Figs. 12—18, 24—34, 36—44, 48—50, 53, 57 and 58, stained with Giemsa's solution and mounted in cedar oil; Figs. 55 and 56, stained with Heidenhain's iron haematoxylin and eosin. The magnification is uniformly 2300 : 1.

Plate 4.

Figs. 1—5. Preserved spores as seen in water.

Figs. 6—8. Preserved spores stained temporarily with methylene blue M. P.

Fig. 9. A schizont.

Figs. 10—19. Stages in schizogony.

Figs. 20—23. Formation of binucleate stages.

Figs. 24—29. Multiplication of binucleate schizonts.

Figs. 30—32. Stages in the nuclear fusion of the binucleate schizonts.

Figs. 33, 34. Sporonts.

Figs. 35—44. Transformation of the sporont into a spore. Fig. 44 shows two independent sporoblasts and partial sections of two mature spores.

Figs. 45—50. Mature spores.

Figs. 51 and 52. Two views of a single muture spore. Fig. 51 an upper surface view; Fig. 52 a view at a lower focal plane.

Figs. 53, 54. Abnormal spores.

Figs. 55, 56. Spores found scattered in the body cavity of the host larva.

Fig. 57. A hypertrophied nucleus of a heavily infected adipose tissue cell.

Fig. 58. A normal nucleus of an uninfected adipose tissue cell.

Plate 5.

Photomicrographs. All from sections stained with DOBELL'S alcoholic iron haematein, except Fig. 61 which was taken from a section stained with HEIDENHAIN'S and eosin. Fig. 59, 90 : 1; Fig. 60, 200 : 1; Figs. 61—67, 900 : 1.

Fig. 59. A cross-section through the posterior portion of the abdomen. D, dorsal vessel; P, three heavily infected fat bodies; N, nerve chord; S, scattered spores.

Fig. 60. Part of a section through the head, showing scattered spores (S), muscles (M) and connective tissue (C) of the host larva.

Fig. 61. Spores present in the host's body cavity. The spore (A) shows its vesicular nucleus; the spore (B) shows faintly the polar capsule and the sporoplasm with its nucleus; the spore (C) shows the spore membrane.

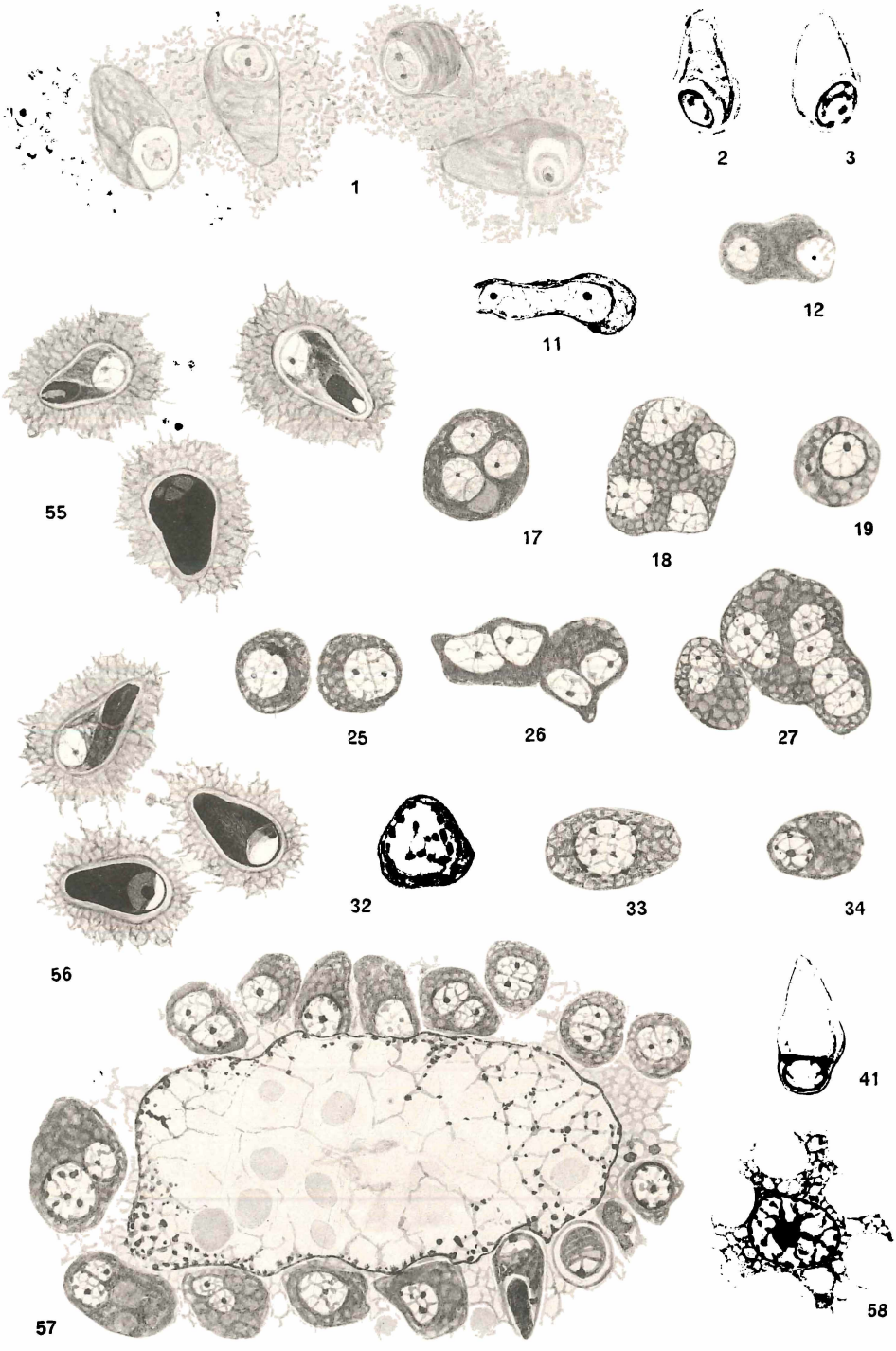
Fig. 62. Part of a section through an infected fat body, showing chiefly spores of the microsporidian. In a number of spores, particularly in the spore (S) is seen the polar capsule.

Fig. 63. One (S) of the spores shows the deeply stained polar capsule.

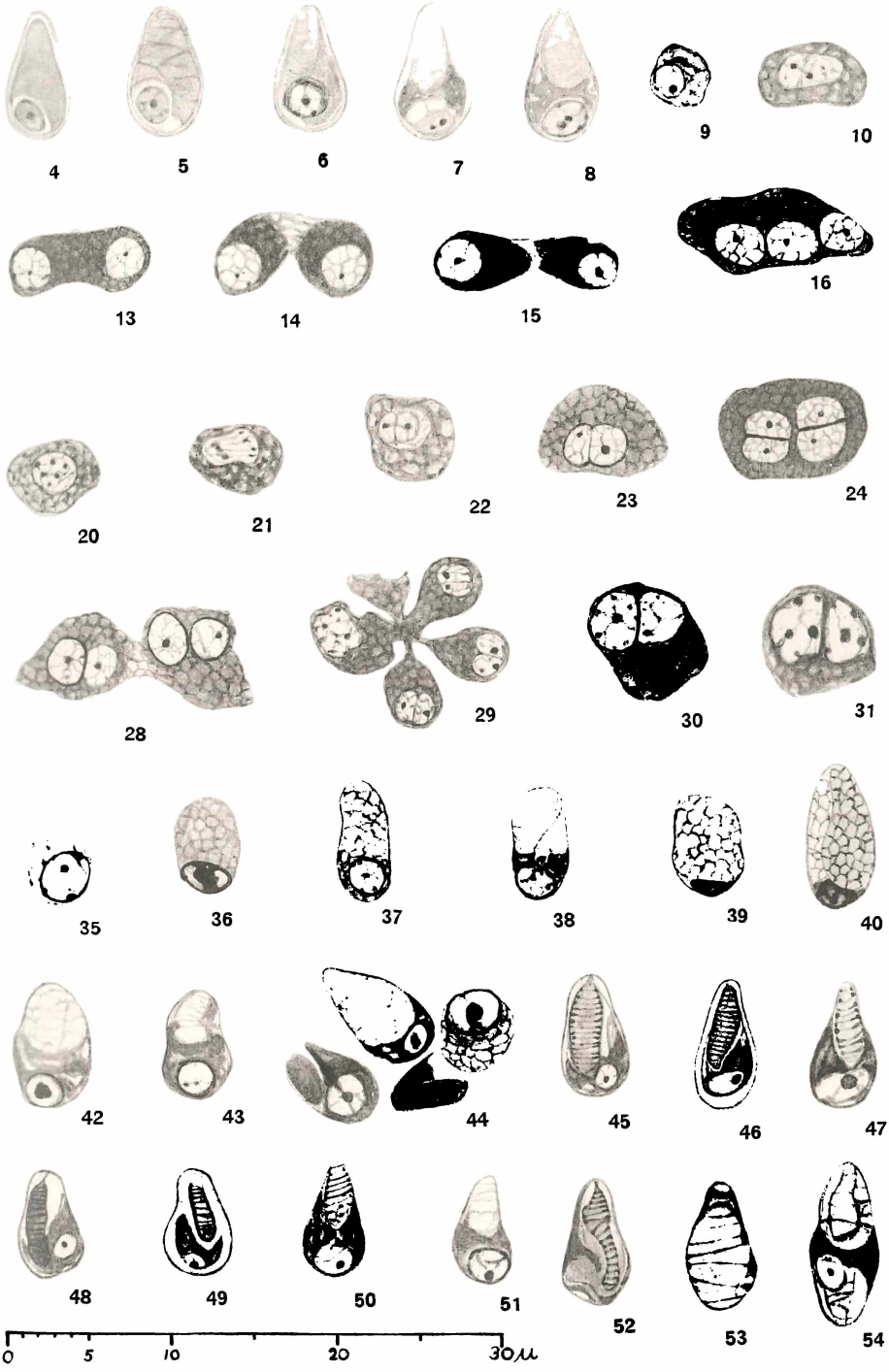
Fig. 64. A number of spores, especially two marked with S, showing their polar capsules.

Figs. 65, 66. Two views of a single spore. Fig. 65 is an upper surface view showing the sporoplasmic nucleus, while Fig. 66 a view at slightly lower focal plane, showing the polar capsule and the sporoplasm.

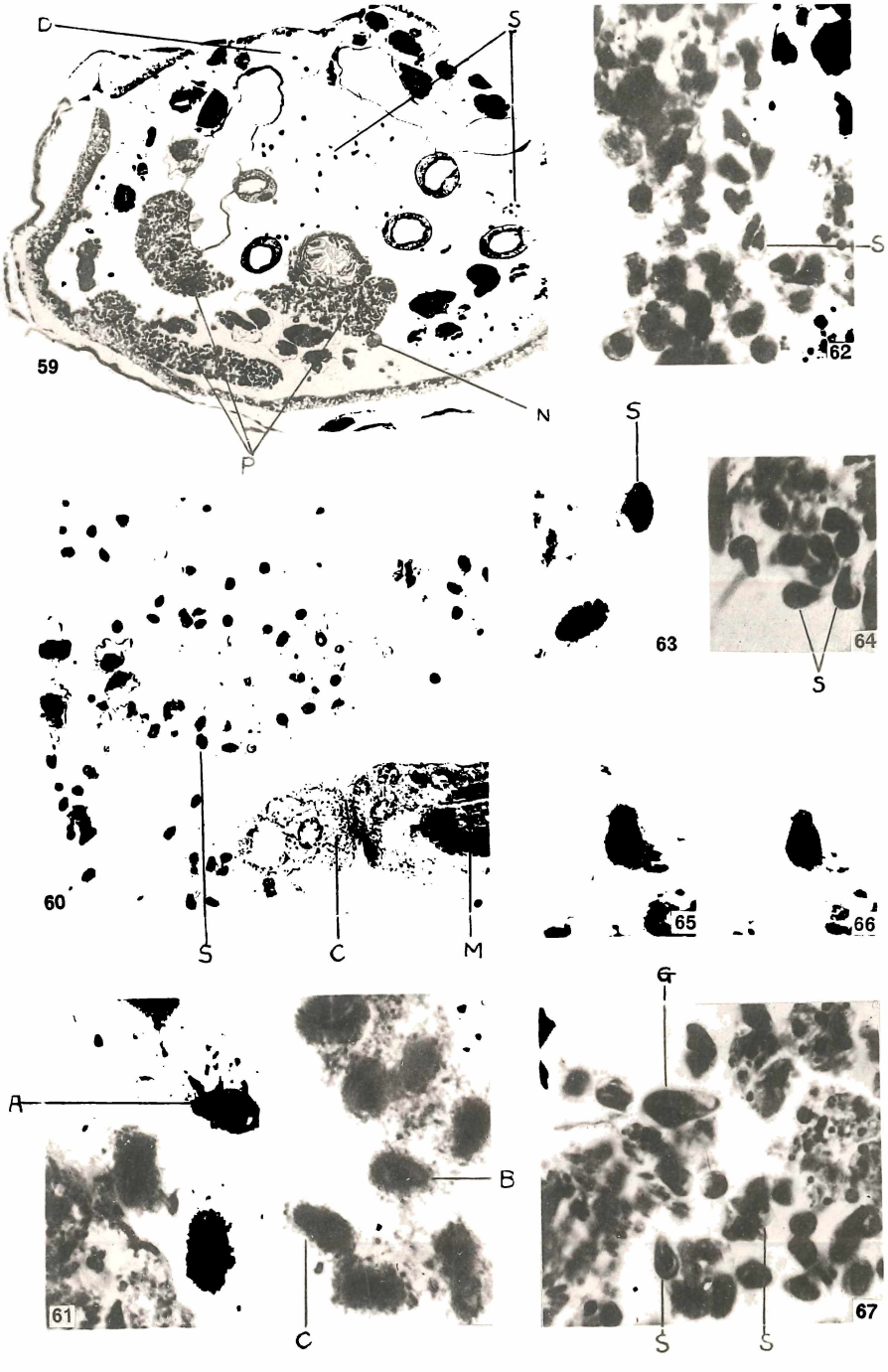
Fig. 67. Part of a cross-section through the thorax, showing normal spores (S) and an abnormal spore (G).



VERLAG VON GUS



FISCHER IN JENA.



Kudo.

VERLAG VON GUSTAV FISCHER IN JENA.