A Study of the Micronuclei of Spirostomum ambiguum major during division.

By

Ann Bishop, M.Sc.,

The Zoological Department, Cambridge.

With Plates 51 and 52 and 1 Text-figure.

1. Materials.

The Spirostoma for this study were obtained from ponds in north Cheshire or from Coe Fen, Cambridge. The animals were grown in cultures, some of which were made of boiled pond-water to which boiled wheat-grains were added whole (roughly two wheat-grains to each test-tube), and others of boiled leaves and boiled pond-water. A detailed account of these culture methods is given in an earlier paper (1). With a little practice no difficulty was experienced in detecting individuals about to divide. Such Spirostoma are longer and do not seem so contractile as ordinary individuals.

2. Methods.

(*a*) Fixation.—Numerous fixatives were tried but the best results were obtained with Woodcock's modification of Schaudinn's solution. The individuals to be fixed were placed in a watch-glass with as little culture fluid as possible. The fixative was then warmed and poured on to them. Bouin's fixative and Flemming's strong solution were used for some preparations.

(*b*) Embedding.—Whole mounts were not used because in them the thickness of the cytoplasm renders it difficult or impossible to study the micronuclei. To prevent misunder-

standing of the order of succession of the various stages of the mitosis single individuals were embedded. For this the method described in an earlier paper (1) was used. Additional sections of masses of individuals were useful for the study of single stages. For such mass sections the method used by Caullery and Chapellier and described by Langeron (3) was employed.

(*c*) Staining.—All the sections were mordanted in 3 per cent. aqueous iron alum solution for at least twelve hours, well washed in distilled water, and stained for twelve to eighteen hours in iron haematoxylin three weeks to two months old.

Differentiation was carried out in a 1 per cent. solution of aqueous iron alum. The sections were cleared in xylol.

3. The Division of the Micronuclei.

An account of the general morphology of S. ambiguum major, the position of the micronuclei and the movements of the meganucleus during division, has been given in an earlier paper, so that no more than a brief recapitulation is necessary here.

Each micronucleus is a small globular body and is surrounded by a pale halo, which is extra nuclear. In its resting phase the micronucleus appears to be quite homogeneous in structure without any well-defined lumps or grains of chromatin. It has a diameter of approximately 2μ. The micronuclei are scattered irregularly along the edges of the lobes of the moniliform meganucleus ; but they are not attached to them in any way. They are seldom so numerous as the lobes. It is a striking fact that different individuals contain different numbers of micronuclei, and the study of the division of the animal suggests a simple explanation of this inconstancy in their number.

The first change to occur in the meganucleus during division is the loss of its lobation. This is followed by its gradual contraction to form an oval mass in the anterior part of the body. The micronuclei all migrate forward with the meganucleus.

During their journey forward they often become separated from its edge by a short distance. Both the micronucleus and the halo now increase a little in size. These micronuclei stain feebly. A similar swelling of the micronuclei has been described in the division of other Ciliata, and it can be regarded as the first stage in the division of the micronuclei.

The meganucleus now migrates back again comparatively rapidly to the middle of the body of the animal, the micronuclei accompanying it. The meganucleus remains stationary in this situation. It is during this migration backwards of the meganucleus or immediately after it has taken up its stationary position at the middle of the body that the division of the micronuclei is continued and completed. It should be realized clearly that the division of the micronuclei is complete and that the daughter micronuclei return to their resting phase before the actual division of the meganucleus into two parts begins.

In well-differentiated sections of individuals in which the meganucleus is stationary at the middle of the body, micronuclei can be found in which the chromatin now stains deeply and can be readily distinguished from the unstained framework of the micronucleus upon which it is carried. The chromatin is now beginning to show signs of a definite arrangement. In some micronuclei, which are frequently seen, the chromatin is arranged in bands which form three sides of a rough square (fig. 6, Pl. 52), the whole micronucleus being still surrounded by a rather indistinct halo. In others the chromatin bands form all four sides of a square. Yet others are similar to these, but chromatin is also present along a line joining two opposite corners of the square (fig. 5, Pl. 52). Careful focusing shows, however, that two sides of this square and the line of chromatin joining the corners also are at a different focus from the other side. This suggests that the chromatin is at this stage (figs. 5 and 6, Pl. 52) really present in the form of a continuous band, but it is difficult to be sure that the band is continuous. In forms like fig. 5, Pl. 52, the halo is very indistinct, or cannot be seen at all. I consider stages like those figured in figs. 5 and 6,

Pl. 52, to be the second stage in mitosis and to be roughly comparable with the spireme stage in the dividing metazoan nucleus.

The next stage in the division that has been definitely recognized is one in which the micronucleus is still globular in shape, but from it two conical structures project approximately at right angles to one another (figs. 7 and 8, Pl. 52). These cones do not stain with iron haematoxylin. Figures of structures very similar to them are found in papers upon Gregarines. Léger and Duboscq (**4**) (figs. 15, 16, and 17, Pl. ii) in their study of the division of Nina gracilis in the cyst show nuclei with a conical projection surmounted by a centriole, the division of this centriole and cone into two, and the subsequent formation of a spindle between the daughter products. Hoffmann (**2**) (figs. 4–14, Pl. ix) figures a similar phenomenon. He calls the projections 'Polkegel'. Léger (**5**) (figs. 20–3, Pl. xiv) figures projections carrying centrosomes in the nucleus of Stylorhynchus; but in these figures definite rays arise from the centrosome.

I shall call the conical projections from the micronuclei of S. ambiguum 'polar cones'. No centrosome grain is found in association with them, nor is a centriole found at any stage in their mitosis. No stage is found earlier than that at which the polar cones are at right angles to one another; but a number of preparations made show all stages between that at which the cones are at right angles and the stage at which the spindle is completely formed.

In deeply stained preparations micronuclei with polar cones at right angles appear as in fig. 1, Pl. 51; but in well-differentiated preparations the chromatin is arranged either (1) in the form of concave bands crossing the bases of the two polar cones (fig. 7, Pl. 52) with another band traversing half the remaining circumference of the optical section of the sphere, or (2) the concave bands crossing the bases of the polar cones only (fig. 8, Pl. 52). Occasionally isolated clumps of chromatin are found on the side of the sphere opposite to either of the

Micronuclei at this stage measure $3\frac{1}{2} \times 3\frac{1}{2}\mu$. As the polar cones gradually separate and move to opposite poles of an ordinary spindle (figs. 10, 11, Pl. 52), the whole structure elongates a little and the chromatin forms a broad, darkly staining mass across the middle of the spindle (figs. 9, 10, and 11, Pl. 52). This mass, which corresponds to the equatorial plate of other mitoses, is composed of granules so densely packed that it is not possible to count them. The completed spindle (fig. 11, Pl. 52), at this stage with its equatorial plate, measures $5–6\mu$ from pole to pole and about 3μ across its equator. The difference in size between micronuclear spindles formed in dividing Spirostoma and those formed in conjugants can be seen by comparing figs. 11 and 15, Pl. 52. The spindles lie in an elongated clear area, which is presumably the original halo greatly swollen. This halo measures approximately $4 \times 7\mu$.

Preparations of a stage immediately after the division of the equatorial plate are rare. But stages are found in which the chromatin forms two plates which are passing towards the poles of the spindles (figs. 12 and 13). In these also the granules are so closely packed together that it is not possible to count them.

The chromatin next forms two irregularly shaped caps at the poles of the elongating spindle. This telophase appears to last a long time. It is found in many preparations. During it the chromatin at the poles of the spindles becomes closely aggregated into two small irregularly shaped clumps (fig. 14, Pl. 52). The clumps measure about 2μ in diameter. They are each surrounded by a small and rather indistinct halo. The length of the 'separation spindle', and therefore the distance apart of the two daughter micronuclei varies considerably (figs. 14, 16, and 17, Pl. 52). Some 'separation spindles' in the telophase measure only 8μ whilst others measure 25μ. The shorter 'separation spindles' are wide and do not stain, but as they elongate they grow narrow and thread-like and often are constricted in the middle. The elongated 'separation spindles' stain quite deeply with iron haema-

toxylin (fig. 17, Pl. 52). Finally they break through the middle and are gradually absorbed, leaving the daughter micronuclei as compact, darkly staining little spheres about 1·5 μ in diameter (fig. 4, Pl. 51).

During the division of S. ambiguum all the micronuclei must undergo division, because no unchanged micronuclei are found in dividing individuals. They are all undergoing mitosis. Further, in the early stages at least, all the micronuclei of any particular individual seem to divide together; as a rule sections of individuals in such early phases rarely contain more than one stage. Sections of individuals at a later phase may, however, show spindles with 'polar cones' (fig. 7, Pl. 52), and all intermediate stages up to the complete spindle (fig. 11, Pl. 52) and the late anaphase all occurring in the same individual at the same time.

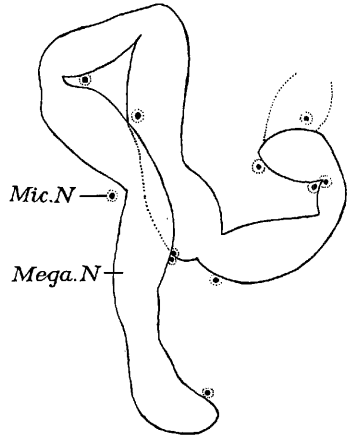
The axis of the spindle during the telophase does not, as one might expect, coincide with the long axis of the individual. The spindles elongate at any angle to the long axis of the animal, and whilst one daughter micronucleus remains close to the meganucleus the other may be pushed out for a considerable distance through the cytoplasm (fig. 4, Pl. 51). After the division of all the micronuclei is completed, the daughter micronuclei are therefore scattered irregularly throughout the cytoplasm.

It should be noted, also, that it is not until the division of all the micronuclei is complete that the meganucleus, hitherto stationary and contracted at the middle of the body, begins to elongate prior to its own division into two parts. The micronuclei, which are, as a result of their own division, scattered irregularly throughout the cytoplasm, now become arranged again about the periphery of the elongating meganucleus (fig. 3, Pl. 51). But they do not take up any definite position there. At a much later stage, when the meganucleus has elongated too and is about to separate into two halves, and when also the whole animal is constricted at the middle and about to divide into two, the micronuclei are still scattered quite irregularly along the border of the meganucleus. Even

at a still later stage, when the meganucleus and the animal itself have actually divided into two and the daughter meganucleus is resuming its lobation in the daughter animal, the micronuclei do not bear any definite relation to the formation of its lobes and commissures (Text-fig. 1).

It is obvious, therefore, that when the meganucleus divides

TEXT-FIG. 1.



there can be no equal division of the micronuclei between the two daughter individuals. It seems to be a fact that there is no regulation of this distribution of the micronuclei between the daughter animals. If more of them happen to have arranged themselves alongside one-half of the meganucleus than alongside the other half, then more will go to one daughter than to the other. This is doubtless the reason why the number of micronuclei is so extremely variable in different individuals of S. ambiguum.

The division of the meganucleus, the division of the cyto-

plasm, and the formation of the two complete daughter individuals are described in my earlier paper (1).

I should like to thank Dr. Keilin for the advice he has given me upon questions of technique, and Dr. Borradaile, under whose supervision this study has been made. I am grateful to Professor Hickson, F.R.S., in whose laboratory this paper was completed, for the stimulating interest he has shown, and to Dr. G. Lapage for all the helpful criticism and advice he has given me on many occasions.

Literature.

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2. Hoffmann, R.—" Über Fortpflanzungserscheinungen von Monocystideen des Lumbricus agricola ", ' Archiv für Protistenkunde ', Bd. XIII, 1909.
3. Langeron, M.—' Précis de Microscopie. Collection de Précis Médicaux ', Masson et Cie, Éditeurs, Paris, 1921.
4. Léger, L., et Duboscq, O.—" Études sur la sexualité chez les Grégarines ", ' Archiv für Protistenkunde ', Bd. XVII, 1909.
5. Léger, L.—" La reproduction sexuée chez les Stylorhynchus ", ibid., Bd. III, 1904.

DESCRIPTION OF PLATES 51 AND 52.

All the figures, with the exception of 3 and 4, were drawn with the camera lucida from preparations under a Zeiss 2 mm. apochromatic objective and a Zeiss No. 18 compensating ocular.

Plate 51.

Fig. 1.—Section of S. ambiguum showing a part of the contracted meganucleus (*M.N.*) and two micronuclei (*Mic.N.*). From the micronucleui project the polar cones (*c.*). The halo (*H.*) is seen surrounding the micronuclei.

Fig. 2.—Section through the meganucleus (*M.N.*) showing micronuclei (*Mic.N.*) at the telophase. The middle part of the separation spindle (*s.s.*) is stained darkly.

Fig. 3.—The meganucleus (*M.N.*) elongating after division with the daughter micronuclei (*Mic.N.*) round its edge (× approx. 475).

Fig. 4.—Transverse section through a dividing S. ambiguum showing the contracted meganucleus (*M.N.*) with the daughter micronuclei (*Mic.N.*) scattered through the cytoplasm. To some of the micronuclei part of the separation spindle (*s.s.*) is still attached (×approx. 475).

PLATE 52.

Figs. 5 and 6.—Early phase in the mitosis of the micronuclei.

Figs. 7 and 8.—Phase in mitosis of the micronuclei (*Mic.N.*) showing the two polar cones (*c.*), the arrangement of the chromatin (*ch.*) characteristic of this phase, and the surrounding halo.

Figs. 9 and 10.—Later stages in the formation of the spindle.

Fig. 11.—The fully formed micronuclear spindle.

Figs. 12 and 13.—Later stage in mitosis showing the two plates of chromatin (*CH.P.*) moving towards the poles.

Figs. 14, 16, and 17.—Three different stages in the telophase.

Fig. 15.—Fully formed spindle of conjugant.

Fig. 1.

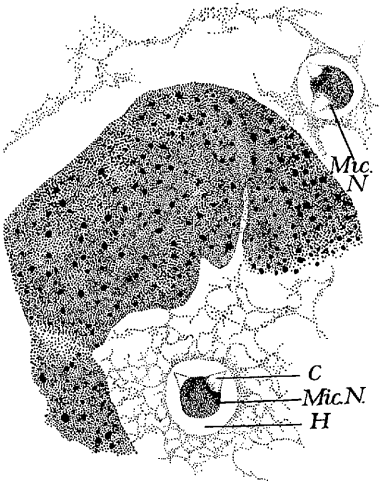

Fig. 2

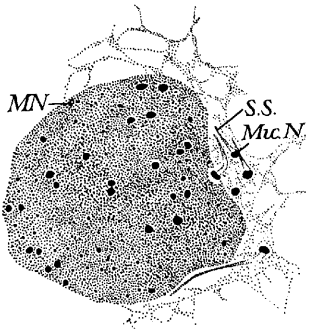

Fig. 3

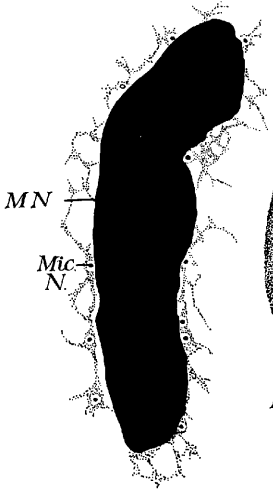

Fig 4

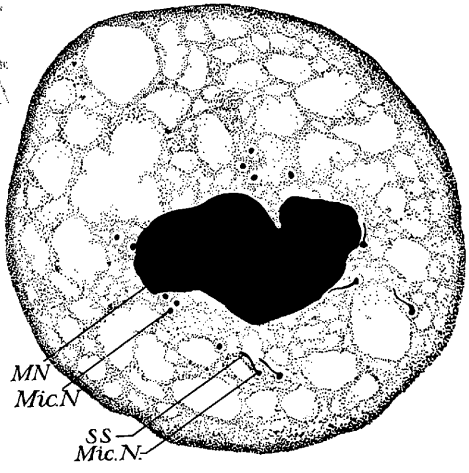

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Fig. 5.

Fig. 6

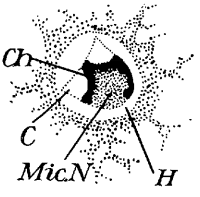

Fig. 7.

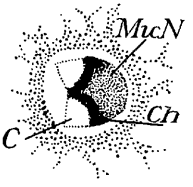

Fig. 8.

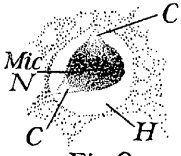

Fig. 9.

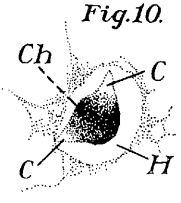

Fig. 10.

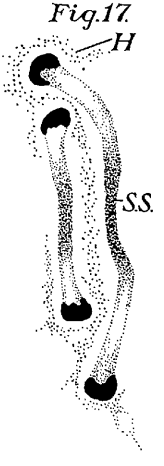

Fig. 17.

Fig. 11

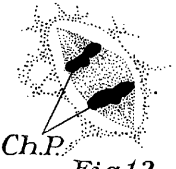

Fig. 12

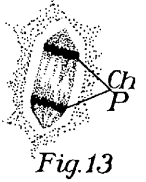

Fig. 13

Fig. 14

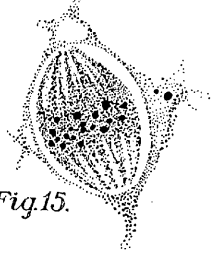

Fig. 15.

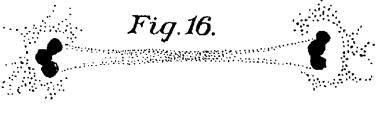

Fig. 16.

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