The Cytoplasmic Structures of *Spirostomum ambiguum* (Ehrenberg).

By

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With Plates 17 and 18 and 3 Text-figures.

**MATERIAL.**

The Spirostoma used in this study were obtained from test-tube cultures made with boiled wheat-grains and boiled aquarium- or boiled pond-water, or with boiled leaves collected from ponds to which boiled aquarium- or pond-water was added. Details of these culture methods were given in an earlier paper (1, p. 402). Occasionally 'wild' Spirostoma, collected from a pond on Coe Fen, Cambridge, or from one in Cheshire, were used. The study was made almost entirely upon the large variety of *Spirostomum* known as *Spirostomum ambiguum Major* (21), but *Spirostomum ambiguum Minor* was studied sufficiently to show that the structures are similar in the two varieties.

**METHODS.**

A number of different fixatives were tried, including Schaudinn's solution with varying amounts of glacial acetic acid below 5 per cent.; the alcoholic modification of Bouin's fluid; strong Flemming's fluid; 1 per cent. osmic acid; the picro-mercuric fixative used by Yocum (31) having the formula—

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
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<tr>
<td>Mercuric bichloride</td>
<td>2 grm.</td>
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<tr>
<td>Picric acid</td>
<td>1 grm.</td>
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<tr>
<td>95 per cent. alcohol</td>
<td>110 c.c.</td>
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<tr>
<td>Ether</td>
<td>20 c.c.</td>
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<tr>
<td>Acetic acid</td>
<td>20 c.c.</td>
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<tr>
<td>40 per cent. formol</td>
<td>50 c.c.</td>
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The fluid used by Neresheimer (17), i.e.—

8 per cent. formol . . . . . 20 c.c.
2 per cent. calcium bichromate . . . . 20 c.c.
Acetic acid . . . . . . . . . . . . . . . . . 1 c.c.

to which a drop of osmic acid is added before using, was also used.

Schaudinn’s solution, Bouin’s solution, and the picro-mercuric solution were all used hot. The best preparations of sections were from material fixed by Bouin’s solution, Schaudinn’s solution, or the picro-mercuric solution. Of the whole mounts, whether stained with haematoxylin or Mallory’s triple stain, the best preparations were of material fixed with Schaudinn’s solution, though those fixed with Bouin’s solution were quite satisfactory.

To facilitate orientation during embedding the fixed animals were tinged faintly with borax carmine. The animals were cleared in xylol, rapidly put through two changes of wax by means of a warmed pipette, and were transferred in the same way to a glycerined slide where they were embedded in a drop of wax, one individual to each drop. The sections were cut in thicknesses varying from 2 to 10μ.

Aqueous and alcoholic iron haematoxylin and alcoholic iron haematein were all used for staining sections. The best results were obtained with aqueous iron haematoxylin. Mallory’s triple stain was used according to the method described by Sharp (25). The Spirostoma were stained in 0.5 per cent. fuchsin S for forty-five seconds to one minute, washed in distilled water, mordanted in 1 per cent. phosphomolybdic acid for one minute, and stained in a mixture containing 0.5 grm. aniline blue, 2 grm. orange G, 2 grm. oxalic acid, 100 c.c. water, for one to two minutes.

The Cytoplasmic Structures.

Spirostomum ambiguum is a large elongated ciliate belonging to the order Heterotricha. The peristomial band of membranellae runs from the extreme anterior tip of the animal
to the middle or some point posterior to the middle of the animal's length. The oral aperture, or cytostome, is situated at the posterior end of the peristomial band of membranellae.

In a completely expanded animal it can be seen that the band of peristomial membranellae does not run in a straight longitudinal line down the animal's body, but is deflected so that it forms part of a sinistral spiral. This sinistral spiral becomes much more pronounced when the body contracts, and it may then completely encircle the circumference of the body.

Occasionally when animals contract, the peristomial band of membranellae does not contract to the same extent as the rest of the body. It then has a wavy appearance. Pätter (18) figures examples of a contracted Spirostomum in which the peristomial band of membranellae is (fig. 10) contracted and (fig. 11) uncontracted. Blättner (2) reproduces these figures. These figures tend to give a wrong idea of the shape and position of the mouth in relation to the peristomial band. I presume that in these figures the peristomial band of membranellae are on the side of the animal farthest away from the reader, and are therefore seen through the body of the animal; in such a case the position of the mouth is correct.

The whole surface of the body is covered with narrow stripes which run from the anterior to the posterior end. Like the band of peristomial membranellae, these stripes follow a sinistral spiral course which is much more marked in the contracted than in the expanded animal. Although they, too, form a sinistral spiral, the angle at which they turn is slightly different from that of the peristomial membranellae, so that the course of the body stripes actually crosses that of the band of peristomial membranellae (fig. 4, Pl. 17).

The striped appearance of the animal is due to the presence of ridges and furrows in the ectoplasm. The ridges (or 'Rippenstreifen' as the German authors call them) are granular and stain fairly deeply, whilst the furrows (or 'Zwischenstreifen') are formed of clear protoplasm and do not stain. The ridges and furrows lie parallel to one another for the greater part of their spiral course. They radiate out from the anterior end of the...
body (fig. 1, Pl. 17) and converge at the posterior end round the anus. At the extreme anterior and posterior ends the ridge stripes and furrows are very narrow, but as they pass in their spiral course towards the middle of the animal both become wider. The approximate width of a ridge stripe and furrow together at any point near the middle of the animal is 3.4μ.

The ridges are always considerably broader than the furrows (fig. 2, Pl. 17). The width of the furrow at the above-measured point is approximately 0.87μ.

In *Stentor coerulescens* and *Stentor roselii* many workers, Stein (26, p. 227, Pl. viii, fig. 16), Schuberg (24), Johnson (13), and Schroder (23), have described branchings in the stripe system, Schuberg being the first to describe definite ramifying zones ("Verästelung"). Wetzel (30) describes these not as branching zones but as zones of convergence. No such ramifying zones are to be found in the stripe system of *Spirostomum ambiguum*, and I have never found any isolated cases of branching in any individual.

The outer surface of the ectoplasm, and therefore of the stripes, is covered by a very delicate cuticle. Immediately below the surface of the furrows lie the thread-like myonemes. The course of the myonemes can be traced in preparations stained with Mallory's triple stain, by which they are stained purple or purplish-red. They stain more distinctly with this stain than with iron haematoxylin. Good preparations can be obtained by staining with fuchsins S alone. In the living expanded animal the myonemes are very fine, but they thicken upon contraction. Since *Spirostomum* always contracts when fixed, all permanent preparations show the myonemes in a contracted condition. In such preparations (fig. 4, Pl. 17) the myonemes do not appear as perfectly taut threads but are slightly sinuous.

The myonemes are circular in transverse section. Bütschli (3, p. 1298), Neresheimer (17), Schröder (23), and Wetzel (30) found that the myonemes of *Stentor* were oval in shape, whereas Johnson (13) and Maier (14) say that they are circular. In *Spirostomum ambiguum* the myonemes are embedded in the ectoplasm. In *Stentor* a different condition
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has been described by many workers. Bütschli (3), Schröder (23), and Roskine (20) described the myonemes of Stentor as lying in a definite canal, but Johnson (13), Maier (14), and Neresheimer (17) failed to find these canals. Of these canals Wetzel says (30, p. 236), ‘... ich Andeutungen eines Kanales immer nur bei weniger gut fixierten Objekten gefunden habe’. Schröder’s detailed figures of the ectoplasmic structure of Stentor do not suggest badly fixed material.

The myonemes can be studied best in detail in tangential longitudinal sections which just slice off the ectoplasm. Bütschli (3) and Schröder (23) described light and dark alternating bands in the myonemes of Stentor, but Johnson (13) and Roskine (20) both failed to find these bands. I have not found any light and dark alternating bands in the myonemes of Spirostomum ambiguum, but in some preparations I have found a somewhat beaded appearance of the myoneme.

Like the stripe system the myonemes radiate out from the anterior end and converge about the anus. They follow exactly the course of the furrows beneath which they lie. They do not fuse at either end of the body nor are they attached to any structure. They taper gradually as they approach the anterior and posterior ends of the body and finally disappear at the anterior and posterior tips.

Johnson (13), Schröder (23), and Wetzel (30) state that in Stentor where the stripe system branches the myonemes branch also. But the myonemes of Spirostomum ambiguum do not branch at any point.

On the anterior side of each myoneme, at the point where the granular ridge stripe meets the clear furrow, lie the rows of basal granules from which the body cilia spring (fig. 2, Pl. 17). In a contracted animal each basal granule lies about 0.75μm apart from its neighbour. The rows of basal granules are parallel to the myonemes. Maier (14) figured the basal granules as lying below the myonemes both in Spirostomum ambiguum and Stentor. In my preparations of Spirostomum the basal granules lie slightly above the myonemes. Schröder (23) figured the basal granules of Stentor above the level of the
myonemes. I have found no ciliary rootlets in my preparations, nor any connexion between the basal granules and myonemes.

In his study of the Heterotrich ciliates, Stentor coerulescens and Spirostomum ambiguous, Neresheimer (17) described, in addition to the myonemes, a second set of fibrils which run parallel to but above the myonemes. According to his description this second set of fibrils in Stentor, where he studied them most thoroughly, began at the aboral pole of the body, and, unlike the myonemes which run straight to the frontal field, ended at some point about the middle of the body. They branched irregularly and independently of the myonemes. This second set of fibrils he called Neurophanes. He called the myonemes Myophanes. He had only a limited number of Spirostomum at his disposal, but he says that in several individuals he found, by means of Mallory's triple stain, the two fibrils in each furrow or ‘ Zwischenstreif’. He found, however, that the fibrils were so fine that the study of them was difficult. He did not find the double set of fibrils in Carchesium nor in Epistylis. He attributed a nervous function to the neurophanes. In preparations stained with Mallory’s triple stain he found that the nucleus stained red to orange or blue, the endoplasm dark blue, the myonemes red, and the neurophanes dark violet. If these staining reactions are compared with those obtained by Sharp (25), Yocum (31), Rees (19), and McDonald (16), for structures termed by them neuromotor, a difference, as Yocum pointed out, will be found. In the work upon the neuromotor apparatus all the fibrils of this system stain red with acid fuchsin, whereas in Neresheimer’s work it is the myonemes which give this reaction whilst the neurophanes stain dark violet. In Calosecolecamelinus Dogiel (8) discovered an apparatus which he considers to be homologous with the motorium described by Sharp in Diplodinium. In preparations stained with Mallory he found that the micronucleus, the macronucleus, and the motorium all stained orange, whilst the surrounding protoplasm stained dark violet. The reactions with this stain, therefore, do not appear to be of value in determining the function of the structures.
Maier (14), whose account of the ciliary apparatus of Infusoria appeared in the same volume of the Archiv für Protistenkunde as Neresheimer's description of Spirostomum and Stentor, did not find this second set of fibrils in the Stentors nor in Spirostomum. Schröder (23), in his detailed study of Stentor rosellii and coeruleus, did not find any trace of neurophanes, though his description and figures are much more detailed than are those of Neresheimer. Wetzel (30) made a study of the cytoplasm and ciliary apparatus of a number of ciliates amongst which were Stentor and, in less detail, Spirostomum; and he also found only the myonemes. Schröder suggested that Neresheimer's results were due to poor fixation, which caused Neresheimer to mistake the Zwi- schenstreifen for special fibrils.

Dierks (7) describes fibrils in Stentor coeruleus which lie above and somewhat lateral to the myonemes. He called these fibrils 'neuroids'. They differ from the myonemes in that they are much more slender and that they do not thicken when the animal contracts. Dierks comments upon the similarity in appearance of the neurophanes and myonemes in Neresheimer's figures. The 'neuroids' described by Dierks are so different in appearance, though similar in position, to the 'neurophanes' described by Neresheimer that Dierks cannot be certain that the 'neuroids' and the neurophanes are identical structures. Dierks describes branches of the neuroids which connect with the myonemes. Neresheimer did not find any connexion between the neurophanes and the myonemes.

Preparations of Spirostomum which I fixed with the fluid used by Neresheimer were all poor, the endoplasm having shrunk away from the ectoplasm. The whole fixation was unsatisfactory. Neither in sections nor in whole mounts, with any of the fixatives and stains given above (pp. 147, 148), have I found any trace of the neurophanes in Spirostomum ambiguum. I have only found one set of fibrils, the myonemes. Between the ectoplasm and the endoplasm Maier (14) describes a sheet of fine, circular myonemes. In a transverse section of the animal these circular myonemes appear as
delicate broken threads. In my preparations these threads were much less marked than they appear in Maier's figures. In many preparations they were very difficult to find, and in some I could find no trace of them.

The endoplasm is very vacuolated. The vacuoles are large, and are easily seen in the living animal.

**The Anus.**

The anus lies at the posterior end of the animal at the base of a shallow depression in which the myonemes taper away and end. The lumen of the anus is capable of great expansion since the faecal balls are often large. I have failed to find any circular myonemes encircling the anus which could be responsible for its contraction and expansion.

**The Peristome, Cytostome, and Pharynx.**

The band of peristomial membranellae begins at the extreme anterior end of the animal at the point from which the stripes and myonemes radiate out. Its course down the body forms part of a sinistral spiral. The cytostome, or mouth, is situated at the extreme posterior end of this band of peristomial membranellae. The distance of the cytostome, and therefore of the posterior end of the peristome, from the anterior end of the animal depends upon the time which has passed since the individual last divided. Immediately after division has occurred the mouth is near the posterior end of the daughter individual, level with the dilated portion of the contractile vacuole. As this daughter individual grows the portion of the animal's body behind the mouth grows more rapidly than the part in front, with the result that the mouth appears to move forward. At the time when another division is about to occur the mouth is situated somewhere about the middle of the long axis of the body.

As described above, the myonemes and body stripes are not quite parallel to the band of peristomial membranellae. Their spiral path cuts across that of the peristomial membranellae.
(fig. 4, Pl. 17). A short distance from either side of the band of peristomial membranellae both the stripe system and the myonemes cease. There is therefore a narrow tract on either side of the band of membranellae in which there is neither stripe system nor myonemes (fig. 1, Pl. 17). The width of each of these plain bands in the contracted animal is approximately $7\mu$ the lower and $5-25\mu$ the upper.

At the outer edges of these tracts the stripe system ends abruptly. The myonemes, on the contrary, do not end so abruptly but taper before disappearing at the edge of the tract.

If the living animal is suspended in a hanging drop over a chamber containing a little chloroform vapour, it can be studied in the contracted condition. It is then seen that this tract on either side of the peristomial band of membranellae from which both stripe system and myonemes are absent, is occupied by rectangular areas raised above the surface and separated from each other by narrow furrows (fig. 4, Pl. 17). Each of the rectangular blocks is set obliquely to the direction of the peristomial band. One of its shorter sides is parallel to or in contact with the peristomial band, but its longer sides are not at right angles to this; the whole block is therefore directed obliquely away from the band of peristomial membranellae to end where the stripe system begins.

All these rectangular blocks are wider than the ridge stripes. Those on the anterior side of the peristomial band are never quite so marked as those on the posterior side. There are no myonemes in the furrows between the blocks.

When a living contracted animal expands the blocks flatten out and disappear. I conclude that they are caused by the creasing of the area on either side of the band of peristomial membranellae when the animal contracts. They were present in all individuals which I examined for them and were regular in size and shape. I have not found any mention of these blocks in the literature upon Spirostomum nor do they appear in Stein's (26) figures of this species. Whether they are present in related genera having a somewhat similar body-form, for example in Blepharisma, I have not been able to ascertain
for myself. Stolte (28) does not mention nor figure them in his description of Blepharisma.

Maier (14) describes and figures (Taf. 4, figs. 11 a and 11 b) a longitudinal myoneme on either side of and running parallel to the band of peristomial membranellae. I have found these myonemes in sections through the membranellae (fig. 6, Pl. 17), but not in relation to those membranellae near the cytostome. I have not found them to be so large as Maier figures.

Early workers upon ciliates thought that the membranellae which form the peristomial band were strong cilia or cirri: thus Dujardin (9), in describing the genus Spirostomum, speaks of 'une rangée de cils plus forts', whilst in their description of the same genus Claparède and Lachmann (6) said, 'Une rangée de cirrhes assez forts conduit de l'extrémité antérieure jusqu'à la bouche.' In 1878 Sterki (27) recognized that the adoral cilia of Oxytricha were really a kind of membranella formation.

In Spirostomum ambiguum the membranellae are arranged in a series one behind the other; those at the anterior end of the animal are narrower than those at the oral end of the peristome.

Each membranella is a plate-like structure which is, when viewed from in front or from behind, more or less triangular in outline. Each is attached by a broad base set at right angles to the longitudinal axis of the band of peristomial membranellae. Each membranella tapers towards its narrower free edge.

Schuberg (24), Bütschli (3), and Maier (14) have shown in a number of Heterotricha and Hypotricha that each membranella is composed of two parallel rows of cilia, the two rows being separated slightly at their bases but fused at their tips. A similar structure can be seen in the membranellae of Spirostomum. Sections which pass transversely across the membranella show it very well (fig. 3, Pl. 17), and the presence of a double row of basal granules beneath the membranella decides the matter. Some fixatives, such as Schaudinn's solution, will break up the membranella into its constituent cilia, but others, such as 1 per cent. osmic acid and strong Flemming solution,
will not. Chambers and Dawson (4 and 5) have shown by micro-
dissection methods that a touch of the micro-dissection needle
breaks up the undulating membrane of Blepharisma into its
constituent cilia, which beat separately but which reunite when
the needle is withdrawn. They suggest that the cilia are united
by a secretion of slime. Taylor (29) has found that the mem-
branellae and cirri of Euplo
tes patella are formed of a
number of cilia ' embedded in a gelatinous matrix that is highly
viscous '. If the membranella is injured it splits up into separate
cilia along which the matrix appears as minute coagulated
globules. I have not been able to perform any such experi-
ments upon the membranellae of Spirostomum am-
biguum, but from their normal appearance and the action of
fixatives upon them I conclude that they must be similar in
structure.

The details of the structure of membranellae have been
studied by Büt
tschi (3), Schuberg (24), Johnson (13), Maier (14),
and Schröder (23) in Stentor, and Maier (14) has studied
them in other Heterotricha and in Hypotricha also. These
authors are agreed that a membranella in Stentor or
Spirostomum consists of the following parts (Text-fig. 1):

(a) The membranella proper, external to the body and formed
from a double row of cilia.
(b) The basal plate (' Basalsaum ' or basal rim), an oblong
plate level with the surface of the ectoplasm. Em-
bedded in this are the two rows of basal granules of
the cilia composing the membranella. This basal plate
forms also the outer edge of the basal lamella.
(c) The basal lamella, a triangular plate, much thinner than
the basal plate, whose apex is directed inwards and
whose base is the basal plate.
(d) The end-thread. This is a delicate fibril hanging inwards
from the apex of the basal lamella.

In my preparations of sections through Spirostomum
a typical membranella agrees with the above general descrip-
tion, but I have found that the basal granules which lie in a
double row beneath each membranella are so close together in *Spirostomum* that they seem to have fused so that each row appears as a line. Since the basal plate itself stains darkly it is difficult to make out the basal granules embedded in it. It has been questioned whether the basal lamella is formed from

**Text-fig. 1.**

Diagram of the membranellae and their intracytoplasmic structures in *S. ambiguum*.

double row beneath each membranella are so close together in *Spirostomum* that they seem to have fused so that each row appears as a line. Since the basal plate itself stains darkly it is difficult to make out the basal granules embedded in it. It has been questioned whether the basal lamella is formed from the ciliary rootlets of the cilia that fuse to form the membranella and whether the end-thread is a further prolongation of these ciliary rootlets. I have never found a fibrillar appearance of the basal lamella, nor do those fixatives which break up the membranella into its component cilia produce any appearance of fibrils in it. Its flat surface always appears homogeneous, with darkly stained edges.
In *Stentor* Schuberg (24), Johnson (13), and Maier (14)
each described a thread which ran below the membranella and
into which the end-threads joined. This they termed the basal
fibril. Neresheimer (17) denied the presence of the basal fibril.
He said that what had been mistaken for it was really the end-
threads which made right-angled bends, but these bent portions
never really joined. In addition he described and figured
(Taf. 7, fig. 11 d) complicated branchings of the end-threads
which formed an arcade-like structure. Schuberg (24) later
concluded that the end-threads were really bound together by
a kind of membrane. Schröder agreed with this and called the
membrane the basal band. He found that in each portion of the
basal band bordered by two neighbouring end-threads there oc-
curred two rows of alveoli. The under-border of the basal band
was thickened in most cases, and Schröder thought that this thick-
ened edge was what Schuberg had first taken for the basal fibril.

Dierks (7) denies the presence of the basal fibril or basal band
described by Schuberg and Schröder. He figures (Text-fig. x,
p. 58) the intracytoplasmic portion of the membranella as a
broad basal lamella. According to Dierks, the end-threads and
basal fibril described by earlier authors are optical illusions due
to the torsion of the broad basal lamellae.

In *Spirostomum ambiguum* the end-threads of the
membranellae are bent slightly and curved. In longitudinal
section a basal fibril can be seen lying below the end-threads
(fig. 11, Pl. 17, and fig. 16, Pl. 18). The basal fibril is not
straight but curves upward and downward. In many prepara-
tions (fig. 16, Pl. 18) it curves slightly upward at the point
where each end-thread meets it. But there are larger upward
and downward curves in addition to these. The apices of the
upward curves are sharp whilst the downward curves are much
flatter and broader. These sharp upward curves occur at fairly
regular intervals. The end-thread which joins into the apex
of each of these upward curves seems a little stronger than the
other end-threads. In preparations stained with Mallory's
triple stain this basal fibril stains red. With iron haematoxylin
it stains intensely.
I conclude, therefore, that in *Spirostomum ambiguum* the 'basal fibril' is a simple fibril and not a basal band such as Schuberg found in *Stentor*.

**THE CYTOPHARYNX AND PERISTOMIAL DEPRESSION.**

The cytopharynx (Text-fig. 2) of *Spirostomum* is a straight funnel-shaped tube, narrowest at its deeper end where it ends blindly in the naked endoplasm, and broadening out to its other end at which it opens into a depression in the body-

![Diagram of the cytopharynx and peristomial depression in S. ambiguum. c = cilia on granular band. d = depression. bp = basal plate. l = lip.](image)

...surface, roughly flash-shaped in outline, which I shall call the Peristomial Depression. This opening of the cytopharynx into the peristomial depression is the cytostome. It is situated at one side of the peristomial depression, and for convenience in the description of the whole peristomial apparatus I shall call this side the right side of the peristome. At its anterior end the peristomial depression is bounded by a gradual slope which leads into a shallow groove lying on the right side of the band of membranellae. This groove does not extend far along the body. It quickly flattens out and disappears, and the 'blocks' described above make their appearance in the contracted animal, immediately on either side of the band of membranellae.
The left side of the peristomial depression is concave and its wall slopes upward and inward, so that the surface of the body overhangs the left side of the depression as a kind of lip. Towards the anterior end of the depression this lip is scarcely noticeable, but it broadens as it approaches the posterior end of the depression, where it is widest, and it overhangs the lower end of the depression as a kind of hood. In the right corner of the posterior end of the peristomial depression is the opening which leads into the cytopharynx. Anterior to this opening the right edge of the peristomial depression is convex, projecting slightly into the cavity of the depression. This edge rises gradually like a mound and has no overhanging lip.

The concave shape of the left side of the peristomial depression is much more noticeable in contracted than in extended animals.

At the anterior end of the peristomial depression the membranellae become broader. The membranellae lie along the left side of the peristomial depression. They are slightly tilted, since each basal plate is embedded partly in the floor of the depression and partly up the sloping left wall. At the posterior end of the depression the basal plates are embedded only in the floor of the depression. The basal plates can be focused in the living animal through the lip on the left side, and the tips of the beating membranellae are to be seen projecting from under the lip. The course of the membranellae follows the curve of the peristomial depression. At the posterior end of the depression, therefore, where they curve round towards the cytopharynx, their basal plates radiate in a spoke-like fashion. The membranellae are continued down the cytopharynx, and in the living animal they can be focused through the body-wall and their beat watched inside the cytopharyngeal tube.

In fully expanded animals the membranellae can be seen extending down the cytopharynx, but in contracted individuals, and sometimes in individuals only slightly contracted, the membranellae in the cytopharynx seem to be pulled upwards to the posterior end of the depression (figs. 13 and 19, Pl. 18). This is the condition found in most fixed animals.
On the right side of the peristomial depression, at the base of the mound-like wall which limits it on this side, lies a row of large closely set cilia. This row of cilia extends from a point near the anterior end of the depression almost to the posterior end of the depression. The cytoplasm below them appears to be very dense.

In fixed and stained preparations there exists on the right side of the depression, for a short distance, at the base of the mound-like wall a darkly staining line (fig. 10, Pl. 17, and fig. 12, Pl. 18). It is densely granular or almost homogeneous. It lies immediately below the ectoplasm at a much higher level than the fibrils from the membranellae with which it has no connexion. Its position corresponds with that of the row of closely set cilia at the base of the right wall of the depression, and it underlies these cilia.

There is no undulating membrane in Spirostomum.

If the peristomial depression of a living Spirostomum, slowed down by gum tragacanth or partially narcotized by a little chloroform vapour, is carefully studied under a \( \times 2 \) oil-immersion lens, a number of fibrils can be distinguished lying in the cytoplasm below the depression.

In describing these fibrils and their connexion with the membranellae in the peristomial depression and the cytopharynx it is simplest to begin with those membranellae which lie in the cytopharynx. The basal plates of these membranellae are long, and their basal lamellae very short and wide. Their end-threads project from under each basal plate beyond the left edge of the cytopharynx, similarly the end-threads of the membranellae anterior to the cytopharyngeal ones, i.e. the membranellae situated at the posterior end of the peristomial depression, project outwards to the left from under each basal plate. One or more thick fibrils lie on the outer side of these end-threads (fig. 9, Pl. 17), and the end-threads join into them. These thick fibrils, which I shall call the posterior basal fibrils, cross to the right below the basal plates of the membranellae situated at the posterior end of the left side of the peristomial depression. In some preparations (figs. 18 and
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19, Pl. 18) this posterior basal fibril seems to disappear deep in the cytoplasm beyond the right border of the peristomial depression. But in other cases, fewer in number (Text-fig. 3), it joins fibrils coming from the membranellae on the left of the peristomial depression.

There is a system of fibrils connected with the membranellae on the left side of the peristomial depression, but their course seems to vary in different individuals. The most usual condition is that in which the end-threads of these membranellae join into a strong fibril which may be termed the middle basal fibril. This middle basal fibril lies parallel to but a little to one side of the membranellae with which it is connected. It lies in the endoplasm well below the basal plates. In very few cases does this middle basal fibril extend in an unbroken condition to the anterior end of the peristomial

Text-fig. 3.

Diagram of a specimen in which many of the end-threads join to form one thick fibril. p.b.f. = posterior basal fibril.
depression. It is usually broken into two or, often, into many parts. The broken ends plunge more deeply into the endoplasm and run to the right below the floor of the peristomial depression, to end, like the posterior basal fibril, freely in the endoplasm on the right of the peristomial depression. Sometimes in individuals where the middle basal fibril is in a broken condition there are a number of these fibrils passing below the peristomial depression (figs. 18 and 19, PI. 18). Occasionally (Text-fig. 8) the end-threads of the membranellae lying at the posterior end of the left side of the peristomial depression all join into one thick fibril, and only the end-threads of the membranellae towards the anterior end of the depression join into a definite basal fibril.

There is always a break between the 'middle' fibrillar system and the anterior system. In the anterior system are included all the membranellae extending from the anterior end of the peristomial depression to the anterior tip of the body and the long basal fibril underlying them. This anterior basal fibril has been described earlier. The break between the anterior basal fibril and the middle fibrillar system seems to be a point of weakness, because, in strongly contracted animals the line of the membranellae is often bent sharply or is slightly distorted at the anterior end of the peristomial depression.

Maier (14) does not describe the membranellae in the cytoplastominal region of Spirostomum. Wetzel (30) figures in a series of sections (P1, Q1, R1) of Stentor, and briefly mentions in the text a fibril, which he terms the basal membrane. This fibril lies below the membranellae in the 'Mundgrube' and with it the 'basal lamellae' (end-thread?) join. Its position is similar to the basal fibril lying below the membranellae in Spirostomum.

The first sign of division in Spirostomum, as Johnson (13) found in the Stentors, is seen in the cytoplasm. As I pointed out above (see p. 154), at the time when division begins the mouth is situated at the middle of the animal. A long time before any change occurs in the nucleus the early stages of the new peristome can be seen. The new peristome begins as a
line running from the centre of the animal to a point near the posterior end. It lies at the same angle to the longitudinal axis of the body as the original peristome now in the anterior end. A more detailed study shows that it consists of a slight ridge in the ectoplasm on which stand very small stumpy membranellae. At first the ridge is straight, without any twist to the right at its posterior end to indicate the peristomial depression; nor is there yet any sign of a mouth. Sections through the young peristome at this stage (fig. 8, Pl. 17) show that the membranellae are short and rectangular, and that they do not taper towards their free ends as fully developed membranellae do. The rows of basal granules lying below the membranellae are never packed as densely together as in the mature membranellae. The basal lamellae, end-threads, and basal fibrils are all absent. The ectoplasm beneath the basal granules is finely granular.

In the living animal the young membranellae move very feebly as compared with the mature membranellae in the original peristome. The end-threads, basal lamellae, and basal fibrils are all completed before the meganucleus begins to elongate after its contraction phase. The new mouth and the structures related to it are also completed. The first sign of the formation of the new mouth is the broadening of the basal plates and membranellae at the posterior end of the band of new membranellae. These broadened membranellae become deflected towards the right.

Although the new membranellae begin quite close behind the original mouth the development of the new anterior basal fibril below the new membranellae is quite independent of the fibrils lying below the original 'mouth'.

The original peristome and cytostome undergoes no change during the division process.

Observations upon Living Spirostoma.

Ciliary Currents.

Jennings (10) has shown that in its forward movement Spirostomum exhibits a rotation upon its longitudinal axis
whilst swimming along a spiral course. The rotation is from left to right and the spiral is a left-handed one, but occasionally I have seen the direction reversed. Rotation on the longitudinal axis and a spiral path is also not invariable since the animal is able to swim quite considerable distances without any rotation at all.

The forward movement is brought about by the backward stroke of the body cilia and of the membranellae.

As Jennings (10) has shown, Spirostomum, if stimulated by the touch of a fine glass rod or by a vibration of the slide, or by any other suitable stimulus, will react by a rapid contraction of the body followed by a backward swimming movement. During this backward movement the body rotates on its longitudinal axis as in the normal forward movement. The backward movement—or 'flight movement'—is due to the reversal in direction of the effective beat of the body cilia, which now beat towards the anterior end of the body. If the stimulus is strong the membranellae also usually reverse their beat, but often they continue to beat towards the posterior end whilst the body cilia are beating towards the anterior.

In studying a Spirostomum swimming backwards in this manner in a fine suspension of Indian ink or dilute milk one can see that the currents carrying the suspension are passing towards the anterior end of the animal. But in addition to these anteriorly directed currents caused by the forward beat of the body cilia there is a strong posteriorly directed current due to the persistence of the backward beat of the membranellae. The globules pass rapidly along the right side of the membranellae and are swept down to the peristomial depression. Often a continual stream of these rapidly moving globules can be seen along the right side of the peristomial band of membranellae. If the animal is strongly stimulated the direction of the beat of the membranellae is reversed. Occasionally during the backward swimming movement the membranellae remain motionless for a short time.

If the course of the globules or granules down the right side of the peristomial band of membranellae is watched one sees
that the suspended particles are swept into the peristomial depression. Some of the particles are wafted down the cytopharynx and into the endoplasm. They pass quickly down the cytopharynx, borne doubtless by the currents caused by the cytopharyngeal membranellae, and are collected in a food vacuole which is formed at the blind end of the cytopharynx. When the granules are being passed frequently down the cytopharynx the food vacuole attains a large size before it separates off from the base of the cytopharynx and begins to circulate in the body.

By no means all the particles swept into the peristomial depression are passed on into the cytopharynx. The greater number of them are whisked out and carried away in the currents caused by the body cilia. Whether this refusal of food is due to a reversal of the beat of the cytopharyngeal membranellae or to a contraction of the walls of the cytopharynx itself I cannot say. But certainly whilst food is being refused there is no reversal of the beat of the membranellae down the peristome, nor of those situated on the left side of the depression. If a reversal of the beat of the membranellae occurs when food is being refused it must affect only those membranellae which lie at the posterior end of the peristomial depression and in the cytopharynx. Schaeffer (22) has shown that in Stentor selection of food is brought about by changes in the beat of the cilia of the pouch and funnel. Stentor is also able to contract and close up the pouch.

Spirostomum is able to ingest large particles of food. Thus, in addition to the bacteria which are present in wheat- and leaf-cultures and which form a large part of the diet of Spirostomum, and to small flagellates, it feeds also upon the relatively large flagellate Chilmonas paramecium, which is present occasionally in large numbers in such cultures.

Whilst studying the movements of suspended particles in the ciliary currents it was noticed that when forward movement occurred there was little disturbance of the suspended matter very far in advance of the animal. Before she was aware of Mast and Lashley's (15) work upon the ciliary currents in
Paramecium and Spirostomum the writer made a number of observations upon the reactions of Spirostomum ambiguum in a medium containing finely suspended particles, expecting to find the 'feeding cone' described by Jennings (11 and 12) in Paramecium. Indian ink was used for most of these experiments. They showed that when Spirostomum came near the edge of the cloud of ink no cone projected towards the animal, and no avoiding or flight reaction was given until the animal had pushed its anterior end into the cloud of particles. When the animal drew back the cone was formed, and appeared to be pulled out by the retreating anterior end of the animal.

These observations directly confirm those of Mast and Lashley (15), who found that the cone-shaped current was formed by Spirostomum when the body was curved during the avoiding reaction. They found that when the animal came in contact with the cloud of ink a momentary cessation of the ciliary beat occurred; this was followed by a contraction of the body which immediately elongated again taking a somewhat curved form, after which the 'feeding cone' was formed. In Paramecium they found that a cone was formed when the animal was at rest, the cone-shaped current drawing in particles from a considerable distance ahead. This cone was due to a reversal of the beat of the body cilia, whilst those of the oral groove continued to beat backwards. The cone was also formed when the animal gave the avoiding reaction. Mechanical retardation was also found to cause it. In his 1904 book Jennings (11, fig. 6) gives a diagram of the cone-like current produced by the cilia in Paramecium when the animal is nearly or quite at rest. But in his later book (12, p. 46) he describes the cone-shaped currents in swimming Paramecium. Fig. 35 shows the animal forming such a cone whilst approaching a cloud of ink. According to Jennings's account, therefore, the cone is present without the avoiding reaction occurring.
Conclusions.

No evidence has been obtained confirming the presence of neurophanes in Spirostomum ambiguum. The only fibrils found in the body-stripe system were the myonemes.

The system of fibrils underlying the membranellae of Spirostomum ambiguum can be summarized as an anterior basal fibril extending from the anterior end of the body to the beginning of the peristomial depression; a middle fibrillar system which varies in its course in different individuals, but which collects the end-threads of the membranellae lying on the left side of the peristomial depression; and a posterior basal fibril into which the end-threads of the membranellae at the posterior end of the peristomial depression and in the cytopharynx join. A connexion between the posterior basal fibril and the middle fibrillar system is seldom found, and there is always a break between the middle fibrillar system and the anterior basal fibril. In no case is there a central body into which the fibrils join and which could be compared with the motorium described by Sharp (25).

Literature.

16. McDonald, D. J. (1922).—' On Balantidium coli (Malmsten) and Balantidium suis (sp. nov.), with an account of their neuromotor apparatus ', 'Univ. of California Publications in Zoology', vol. xx, no. 10.
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DESCRIPTION OF PLATES.

PLATE 17.

Fig. 1.—Anterior end of body showing ridge stripes (R) and furrows (F), myonemes (M), and basal granules of cilia (BG). Fixed Schaudinn, stained Mallory. Zeiss 2 mm. x 4 oc.

Fig. 2.—Ridge stripes (R) and furrows (F) containing the myonemes (M). The basal granules (BG) are shown along the edge where the ridges and furrows meet. Schaudinn, Fe.H. (aqueous iron haematoxylin) 2 mm. apo. x 18 oc. Zeiss.

Fig. 3.—Longitudinal section through a series of membranellae, but not showing basal fibril. Schaudinn, Fe.H. 2mm. x 12 oc. Zeiss.

Fig. 4.—A portion of the surface of the body showing the peristomial membranellae (PM). The ’ blocks ’ (B) on either side of the band of membranellae; the myonemes (M); the basal granules of the cilia (BG), (A) indicates the side towards the anterior end of the body, and (P) indicates the side nearest the posterior end. (R) indicates the right side and (L) the left side. Fixed with Schaudinn’s solution, stained Mallory. Zeiss 2 mm. apo. x 4 oc.

Fig. 5.—A transverse section at the anterior end of the peristomial depression, showing the membranella (M), the basal plate (BP), the basal lamella (BL), and the union of a number of end-threads (ET). Schaudinn, Fe.H. 2 mm. apo. x 12 oc. Zeiss.

Fig. 6.—Transverse section across a membranella showing membranella (M), basal granules (BG) forming basal plate (BP), basal lamella (BL), the end-thread (ET), the peristomial myonemes (My), the ectoplasm (E), and the vacuolated endoplasm (En). Schaudinn, Fe.H. 2 mm. apo. x 18 oc. Zeiss.
Fig. 7.—A section in the region of the peristomial depression showing a membranella (M), basal plate (BP), basal lamella (BL), end-threads (ET), basal fibrils (BF). 12 oc. x 2 mm. Zeiss.

Fig. 8.—A transverse section through a young membranella in the posterior end of a dividing Spirostomum. No basal lamella and no end-threads are developed yet. Bouin, Fe.H. 2 mm. x 18 oc. Zeiss.

Fig. 9.—Basal plates (BP) below some of the lower membranellae in the peristomial depression. The drawing shows the end-threads (ET) joining the basal fibril (BF), which passes below the plates. The basal lamellae are hidden. Schaudinn, Fe.H. 2 mm. apo. x 12 oc. Zeiss.

Fig. 10.—Section across the membranellae in the region of the peristomial depression showing the basal fibril, also the granular band (GB). Bouin, Fe.H. 2 mm. x 12 oc. Zeiss.

Fig. 11.—Section showing membranellae (M), basal plates (BP), basal lamellae (BL), end-threads (ET), basal fibril (BF), and part of one of the peristomial myonemes (My). Schaudinn, Fe.H. 2 mm. apo. x 12 oc. Zeiss.

Plate 18.

Fig. 12.—Drawing from whole mount showing the basal plates at the anterior end of the peristomial depression. These disappear from view as the floor of the depression sinks. The granular band (GB) on the right of the depression is shown.

Fig. 13.—Same as fig. 12 but at a lower focus, showing the basal plates (BP) of the membranellae (M) at the posterior end of the peristomial depression. The basal plates of the pharyngeal membranellae have been pulled upwards through contraction. The end of the granular band (BG) is shown and also the broken ends from the basal fibril (BF). Schaudinn, Mallory, 2 mm. apo. x 4 oc. Zeiss.

Fig. 14.—Lower focus of the same showing the end-threads from the membranellae at the posterior end of the depression joining their basal fibril.

Fig. 15.—Lower focus showing the basal fibril from the pharyngeal membranellae.

Fig. 16.—Longitudinal section through peristomial membranellae near the anterior end of the body, showing the basal fibril (BF). 2 mm. x 12 oc. Zeiss.

Fig. 17.—Longitudinal section through part of membranellae in the peristomial depression. Schaudinn, Fe.H. 2 mm. apo. x 12 oc. Zeiss.

Fig. 18.—Deeper section of the same. In this the basal fibril underlying the end-threads is seen.

Fig. 19.—Deeper section of the same. The threads are seen from the basal fibril crossing below the depression and disappearing in the endoplasm.