

Observations on the Microanatomy of the Spermatozoid of the Bracken Fern (*Pteridium aquilinum*)

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PLATES 196 TO 207

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ABSTRACT

Salient features of structure of the spermatozoid of a fern (*Pteridium aquilinum*) have been determined by a combination of visual and ultraviolet microscopy, with electron microscopy of shadow-cast whole mounts and thin sections, using magnifications up to but not exceeding 50,000 diameters. Attention has been concentrated on the arrangement rather than on the internal details of the various parts. The most important component, apart from the spirally wound nucleus, numerous (about 40) cilia, and mitochondria, is a sheet of parallel fibres spirally wound near the surface of a cone of cytoplasm to which all the other major components are in various ways attached. The diameter of the individual fibres is of the order of 200 A. A few details are given of other minor cell constituents including additional mechanical materials, starch-containing leucoplasts, and the smaller cytoplasmic inclusions.

INTRODUCTION

The spermatozoid of a fern is one of the most elaborate types of ciliated cell known in plants, and for this reason it would have been useless to attempt to investigate its structure before a good deal of knowledge of less complicated cells had been obtained. Even with this knowledge the conical shape and spiral configuration make randomly cut sections at first sight uninterpretable, unless constant reference can be made to the appearance of intact cells as seen by other forms of microscopy. It is also essential to select a fern with relatively small cells, since even these are enormous compared with other plant gametes studied with the electron microscope (*e.g.*, *Fucus*, *Dictyota*, *Sphagnum*, *Prasiola*), and mere size can at first greatly impede obtaining and recognizing the few critically significant planes of section, notably the median longitudinal section (LS), which alone provides an interpretable key to the structure.

In selecting the Bracken (*Pteridium aquilinum*) for the present study an additional reason has been the fact that prothalli of this fern are much used for experimental work in several laboratories and the methods for culturing them in optimum conditions on agar are so well known that it has been

found practicable on this occasion to use agar cultures sent by post from the Botany Department at Glasgow University. Fixation obtained with these has not been perfect in every respect but is adequate for a first elucidation of salient features of structure which is all that is attempted here.

Methods

The method of raising agar cultures of Bracken prothalli in bulk will be found in various publications from the Glasgow department; *e.g.* Conway, 1949; Hutchinson and Fahim, 1957. It is sufficient to say here that cultures of any age from 6 weeks to 6 months are suitable provided that fertilizations have not occurred on such a scale as to crowd out the young prothalli by growth of sporophytes. On receipt of such a culture by post it is necessary to illuminate for some hours before use; this was done by standing the Petri dishes a few inches away from a 40 watt fluorescent tube in the laboratory for 12 to 48 hours. To induce liberation of gametes the culture is then flooded with warm distilled water (the temperature is not critical but should not exceed those likely to be encountered in nature). The presence of gametes can be detected almost immediately after flooding, though at first they are motionless, each being coiled within a containing membrane at the time of liberation. The number of free gametes increases continuously, to a maximum after

about 20 minutes, at which time fixation can best be carried out. After a longer time the spermatozooids become exhausted and finally only dead ones remain; these may be very informative for studying stages of dismemberment in whole mounts or by light microscopy but are unsuitable for embedding. A culture from which spermatozooids have been taken can be used again after a period of growth. The minimum time required for this has not been ascertained but 2 weeks is ample. One of our cultures was used three times, with this interval between each, without any sign of diminution of vigour in spite of numerous fertilizations, and it could probably have been used yet again had this been needed. In the whole investigation two cultures only have been used, one received in December 1957 and the other in December 1958.

When most fully active, the spermatozooids are indifferent to centrifugation and can usefully be concentrated by it after which they quickly resuspend without ceasing to swim. Such a resuspended concentrate can then be squirted into an equal volume of 2 per cent osmium tetroxide. Fixation for 1 hour at pH 7 has been preferred. The embedding procedures used are standard.

For light microscopy, preparations were made on slides by killing with osmic vapour before adding a drop of iodine in potassium iodide to prevent bacterial growth. Since ultraviolet microscopy was intended, these preparations were made on quartz and covered with a quartz coverslip sealed with wax. Such preparations can either be used directly or stored for a year or more without apparent injury.

Shadow-cast material for the electron microscope was prepared in the usual way by osmic vapour killing.

The microscopy has all been carried out in Leeds using the Cooke, Troughton, and Simms UV microscope with Zeiss 2 mm. glycerin immersion lens working with the 2750 Å cadmium line for the ultraviolet microscopy, and a glycerin immersion glass lens for the one control picture taken with visual light, which is reproduced. The electron microscopy was done on a Siemens Elmiskop I.

OBSERVATIONS ON GENERAL PRINCIPLES OF CONSTRUCTION

A rapid preliminary scrutiny of the whole mounts reproduced in Figs. 1 to 9 together with the median LS reproduced as Fig. 24 will be the quickest way of comprehending the general principles of construction of the cell. From such a scrutiny the following facts emerge:

1. The over-all shape is roughly that of a truncate cone in which the centre (see especially Fig. 24) is occupied by cytoplasm which is, however, so translucent to normal and ultraviolet light that it could escape detection unless inclusions had

been rendered conspicuous by staining, as with the starch grains stained with iodine in Fig. 1.

2. Within the cytoplasm a very elongated nucleus, pointed at both ends and narrowly attenuated forwards (see especially Figs. 3 *a* and *b*), is wound in a spiral of about $2\frac{1}{2}$ turns and is therefore transected 5 times in a median LS of the cell as a whole (Fig. 24). The direction of coiling is left-handed (Figs. 2 *a* to *d*).

3. The spiral configuration is very readily lost as a postmortem artefact if the nucleus bursts out of its subtending membranes as in Figs. 3 and 4. It can then be seen that the spiral is, in fact, an attitude imposed on the nucleus by certain structures in the cytoplasm which also carry the cilia and other relatively opaque materials. These structures can retain their spiral shape even after the cell has burst and it can then be seen that the nucleus in fact ends about half a gyre below the actual tip of the cell (Figs. 3 *a* and *b*).

4. The opacity of the cilia-bearing band to ultraviolet light, which in the intact cell is almost as great as that of the nucleus (Figs. 2 *a* to *e*), is partly due to a layer of mitochondria lining it on the inner side. These are at once revealed as such by sections (for further details see p. 415), but they are also detectable directly as strongly ultraviolet-absorbent small bodies in a cell such as that of Fig. 4 in which the inner side of the cilia-bearing membrane is exposed, or under similar conditions by their opacity to the electron beam in Figs. 5 and 10. They can also be seen if an intact cell is examined with ultraviolet light at exactly the right focal level (Figs. 2 *d* and *e*) as a zigzag array of small dark bodies within the substance of the cilia-bearing band above the level of the nucleus.

5. The total number of cilia is of the order of 40 (see especially Figs. 6 and 9). They are most numerous at the front end of the cell where they are distributed in two characteristic ways: (*a*) as a close-set fringe arising near the upper edge of the cilia-bearing band (Fig. 2 *b*), and (*b*) scattered, in varying density according to position on its outer surface. All cilia are initially directed backwards relative to the coiled nucleus.

6. An additional observation, easily made in life but recorded here incidentally in one section (Fig. 13), is that when first liberated from the antheridium the spermatozooids are motionless, each being completely enveloped in a covering membrane which has first to dissolve before swimming is possible. All the comments made above

about shape refer to cells in the fully functional state after this membrane has been lost.

OBSERVATIONS OF DETAIL IN SECTIONS

The Nucleus.—Apart from the gross morphology, sections have added nothing significant to our knowledge of the nucleus in these cells, and nuclear fixation, though adequate for topographical purposes, is by no means perfect. It will, therefore, be sufficient here to draw attention to the low-power views of the hind end shown in Fig. 14 in which the plane of section in two cases coincides with a complete gyre of the nucleus in its widest part. These views can usefully supplement the numerous transverse sections of the nucleus seen at varying degrees of obliquity in later plates.

Plastids.—The starch-containing plastids are well seen in the central cytoplasm of Fig. 14 and more magnified views such as those of Figs. 24 and 25 add little except to emphasise the general absence of other internal constituents. There is still a distinct bounding membrane (Fig. 14) but only traces of internal lamellae and other opaque materials, notably granules. This is not the structure of a normal green plastid but it compares closely with the vestigial plastids of a moss spermatozoid (*Sphagnum* (Manton, 1957)). It seems probable, therefore, that these are leucoplasts which have been virtually used up in forming the gamete and that they have little, if any, further function.

The Small Cytoplasmic Inclusions.—"Golgi bodies" (dictyosomes) are absent as such though the cytoplasm when well fixed as in Fig. 25 is crowded with objects, some of which could represent the Golgi material in a transformed and unidentifiable condition. The ground substance of the cytoplasm appears faintly grey. In addition to the larger organelles it contains a few vesicles (*v* in Fig. 25) of sufficient size to have been derived perhaps from vacuoles, and vast numbers of smaller vesicles, some with thick walls and some thin, some with traces of contents and others without. Sinuous tubes, identifiable as such in LS (Fig. 25) but indistinguishable from vesicles in other planes of section, are also present, and there is an occasional very opaque fat body (Fig. 25).

The Mitochondria.—Although a few unattached mitochondria can be found scattered in the cytoplasm of the centre and hind end of the cell the majority are accumulated at the front end of the cell where they are firmly attached to the inner side of the cilia-bearing surface. This may be seen to advantage in Fig. 27, which is cut tangentially through the mitochondrial region. Individual mitochondria of various sizes and shapes are arranged in short diagonal rows in the centre of the field in a manner already indicated by the whole mount of Figs. 2 *d* and *e*. The top of the mitochondrial field is, however, delimited by a continuous band of mitochondrial material (*mr* in Fig. 27) not obviously divisible into separate organelles. This band, seen in trans-

verse view at the top of Fig. 36 (*mr*) and elsewhere, is covered on its upper side by the body membrane and it forms a conspicuous raised rim to the ciliated band at the level sectioned. This is doubtless a major component of the "border brim" described in several ferns by Yuasa, 1932-6 (= "*Randsaum*" of Dracinschi, 1930): it is, however, not the only component, as will be shown below.

That the mitochondria lining the ciliated surface do so in a single layer is demonstrated by transverse views of the front part of the cell cut at two levels in Figs. 28 and 32. Fig. 33 adds some details regarding the mode of attachment of the mitochondria to the surface on which they are held. This is by means of intercalary material of a chemically unknown kind which is mechanically of sufficient strength to retain these organelles in place even in a burst and damaged cell such as those of Figs. 4 and 5.

The internal structure of the mitochondria themselves is demonstrated in a general way in Figs. 32 and 33. They appear as if gorged with metabolites of various kinds, some of which are transparent and others opaque or represented by large granules. It is difficult to assess the degree of distortion which may perhaps have been introduced by fixation, but it is inherently probable that the mitochondria of these short-lived but active gametes are in a rather special state and that some of their peculiar appearance is genuine.

Attachment of the Cilia.—Some general information on the pattern of attachment of the cilia can be obtained from the low-power tangential views of Figs. 11 and 12. Both show that while the direction of emergence of the cilia near the bottom edge of the band is almost parallel to the adjacent edge of the nucleus, the cilia of the fringe are uptilted at an angle of about 35°. Apart from the fringe, the arrangement of ciliary bases on the surface is in short oblique lines forming an almost tessellated pattern in the most crowded portion, but spaced out in a manner which has not been traced in detail on the less crowded lower parts of the cell surface.

Each individual cilium (see especially Figs. 29 *a* to *c*, 30, and 33), is apparently impaled on a peg of dense material which enters the ciliary base from the bottom and penetrates through half to two-thirds of its length. There is also dense material investing the outside of each basal body in varying amounts according to the position on the cell. This can be studied to advantage in the sections cut in various planes reproduced in Figs. 28 to 33 and elsewhere. In occasional areas where cilia are sparse their place is occupied by a spongework of the dark material, so arranged as to display a double layer of internal cavities of very similar appearance in all planes of section (compare, for example, Fig. 32 top centre with *d* in Fig. 37).

The structure of the cilia themselves has only been illustrated incidentally. It is, however, perhaps worth drawing attention to Fig. 31 which transects the cilia of the fringe at a range of levels. On the left of the sec-

tion are four ciliary bases, two including the central peg and two above it. The remaining profiles are all of the free parts of the cilia at levels increasingly far removed from the base as the section passes from left to right. The plane of internal symmetry is clearly discernible in almost all and has been indicated in ink on the print as a precaution against loss of clarity in reproduction. The extreme regularity of the whole row is its most striking feature together with the clear confirmation of the presence of a spiral twist in each cilium as the distance from its base increases. Both the regularity and the spiral twist are features to be expected from previous knowledge of ciliary structure in ferns (Manton and Clarke, 1951) and other plants, but they have not previously been demonstrated in fern cilia *in situ*.

Structure of the Front End of the Ciliated Band.—The tip of the cell is illustrated in Fig. 7 and in Figs. 15 to 17. The ciliated band itself ends in a thickened ridge when dried (Fig. 7), this ridge corresponding to a cushion of dense material visible centrally in Figs. 15 *a* to *d* and, more highly magnified, in Fig. 16. Whether this dense material is the same as that surrounding the ciliary bases or is only similar in opacity is uncertain though there is some evidence to suggest the former. It is doubtless the component referred to as "lateral bar" by Yuasa (1933, 1936, etc.).

Behind the tip (Figs. 15 *a* and 17) there is a crest-like ridge of dense material which is also similar to and perhaps in continuity with the terminal cushion. This crest-like ridge runs along the outer side of the mitochondrial rim already described (*mr* in Figs. 17 and 27) at a level just above the attachment of the cilia of the fringe. It may be seen in TS in Fig. 26 at one selected level (arrows), or again in a series of levels at lower magnifications in Figs. 15 to 17; at levels below the topmost gyre it decreases rapidly until it ceases to be visible. When present, the dense material of the ridge is separated from the outer cell surface by several membranes and a layer of fibres so that its mechanical significance is probably appreciable. It may, indeed, be suggested that the extra degree of rigidity encountered in the extreme tip of a damaged cell such as that of Fig. 3 is likely to derive primarily from these extra ridges and crests, though a limited amount of less opaque capping material (*c* in Fig. 18) may perhaps also be involved.

Fibres of the Ciliated Band.—The most mechanically important components of the cell as a whole are undoubtedly the central fibres of the ciliated band since these not only hold the cilia and mitochondria in place but are the foundation of the spiral path of the nucleus. They may be seen exposed in a tangential section at the level of Fig. 34 from which there can be no doubt that the main direction of the fibres is parallel to that of the nucleus. Fig. 35 represents a surface view of similar fibres from another section at a higher magnification to show that in the region overlying the mitochondria the

fibres are apparently cross-linked at regular intervals to give a fine meshwork. This character is not detectable in the system of fibres covering the outer face of the nucleus which will be described below.

Separating the fibres from the mitochondria is a thin layer of dense material considerably more opaque than the fibres themselves and, therefore, appearing as a dark line resembling a membrane in Figs. 28, 32, 33, and elsewhere.

The fibres holding the nucleus in position are easier to study, being less obscured by ancillary materials. They may be examined to advantage in Figs. 36 and 38. The latter is in an exceptionally favourable plane of section, being exactly transverse to the fibres over a considerable area. They can be seen individually as little circular profiles about 200 Å in diameter arranged in a single layer just beneath the cell membrane at a distance apart which varies somewhat according to position, but is commonly rather less than this.

The fibres covering the nucleus never do so completely but extend over the outer and lower sides to give a more or less U-shaped sling. They are always separated from the nuclear surface by membranes (Figs. 36 to 38) which doubtless include the double nuclear membrane, and they are covered externally by the body membrane. Their position relative to the cell surface is, therefore, rather different from that of the more deeply seated fibres underlying the ciliated zone, though in transverse diameter the individual fibres of both regions are similar (Fig. 38). Superficial fibres covered only by the body membrane are present again over the mitochondrial rim and crest-like ridge at the forward end of the cell described above, where in favourable sections they can be shown to overlap the top edge of the fibrous band of ciliated region. It is, therefore, possible that more than one and perhaps as many as three separate sheets of fibres are involved which are in close proximity at their edges without being entirely continuous. Further clarification of this, however, requires either better material or a study of developmental stages.

DISCUSSION

Though many matters of detail could be further pursued with profit, the general picture which emerges from the above description is of a rather more simply constructed cell than could have been foreseen at the outset. Once the position and mechanical importance of the cytoplasmic fibres has been ascertained from a few critically informative planes of section (notably the median LS), interpretation of the gross morphology of the whole cell is not merely relatively easy, no matter what plane of random section may be presented to the viewer, but it is seen to be fundamentally far less different from the structure of the superficially

very dissimilar spermatozoid of a moss (*e.g. Sphagnum* (Manton, 1957)) than might have been expected.

The salient feature which both moss and fern spermatozoids share is the fibrous band. In the moss this is only a narrow ribbon of 4 to 5 fibres accompanying the nucleus throughout its length and carrying the two cilia and some mitochondria in a peculiar state near the tip of the cell. That this is a true homologue of the cilia-bearing band with its lining of mitochondria, mitochondrial rim, and covering of cilia can scarcely be doubted. Other points of resemblance are the peculiar appearance of the mitochondria themselves, the absence of dictyosomes, and the presence of vestigial starch-containing leucoplasts, singly in the moss and multiply in the fern. The general resemblance of the curved nucleus has, of course, long been familiar from the light microscope.

The metabolic significance of mitochondria for ciliary activity is better shown here than in any other plant owing to the large number of cilia involved and the consequent accumulation of many mitochondria attached in an orderly manner to the physically nearest surface. That there is probably also a physical basis for co-ordinated movement is suggested by the way in which the cilia are attached to the cell, which is far more elaborate here than is usual in plants, a difference the significance of which seems unlikely to be entirely mechanical.

Finally, the orientation of the cilia, most fully studied in those of the ciliary fringe, is a clear reminder that in plants no less than in some animals (*cf.* Fawcett and Porter, 1954) the orientation of the plane of bilateral symmetry relative to a surface or, more probably, relative to a particular type of movement is significant. Until more is known about the actual movements carried out by the cilia of ferns it is not profitable to pursue this matter in greater detail, but it is perhaps helpful to draw explicit attention to one of many observations which require closer study.

I have to thank my former technical assistant, Mr. B. Clarke, for making the preparations used for light microscopy and shadow-cast whole mounts. I am also greatly indebted for photographic and other technical help to Mr. K. Oates and Miss Sheila Wright. Very special thanks are due to Mrs. Elsie Conway of Glasgow for supplying cultures on two occasions, without which the work might not yet have started.

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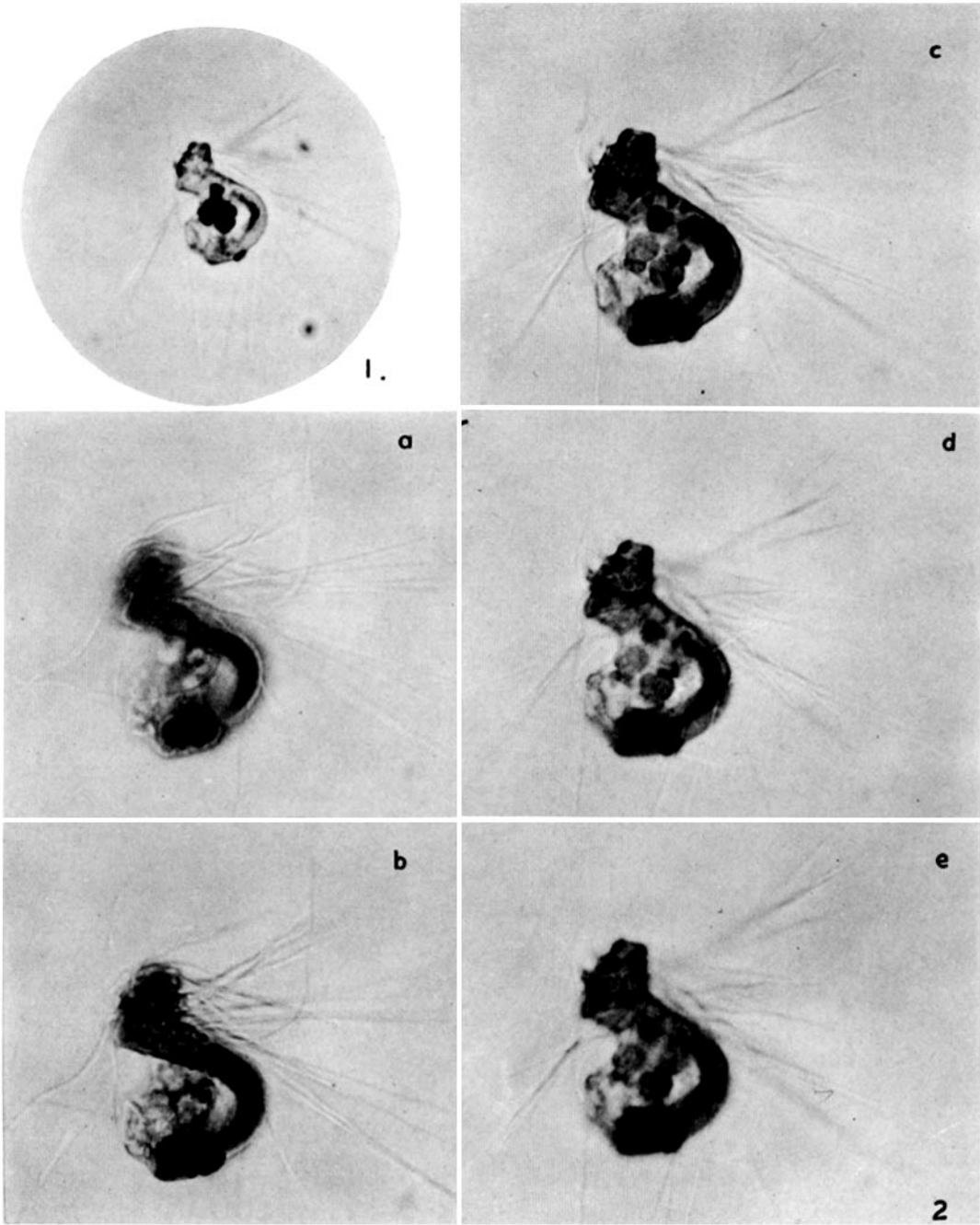
EXPLANATION OF PLATES

PLATE 196

Spermatozoid of the fern *Pteridium aquilinum* after killing by 30 seconds' exposure to osmic vapour; mounted in water to which a drop of iodine in potassium iodide has been added, preparation on quartz.

FIG. 1. The cell, photographed with visual light to show the group of starch-containing plastids in the central cytoplasm. $\times 1,000$.

FIGS. 2 *a* to *e*. Serially arranged optical sections from above downwards of the cell of Fig. 1 taken with ultraviolet light (wavelength 2750 Å) by means of a 2 mm. Zeiss quartz glycerin immersion lens and $\times 5$ quartz eyepiece on a Cooke, Troughton, & Simms horizontal ultraviolet microscope. The coiled nucleus and ciliary band attached to it are strongly UV-absorbent and appear dark; the residual cytoplasm including the starch-containing plastids relatively transparent. The nucleus is coiled in a left-handed spiral of about $2\frac{1}{2}$ turns. $\times 2,000$.



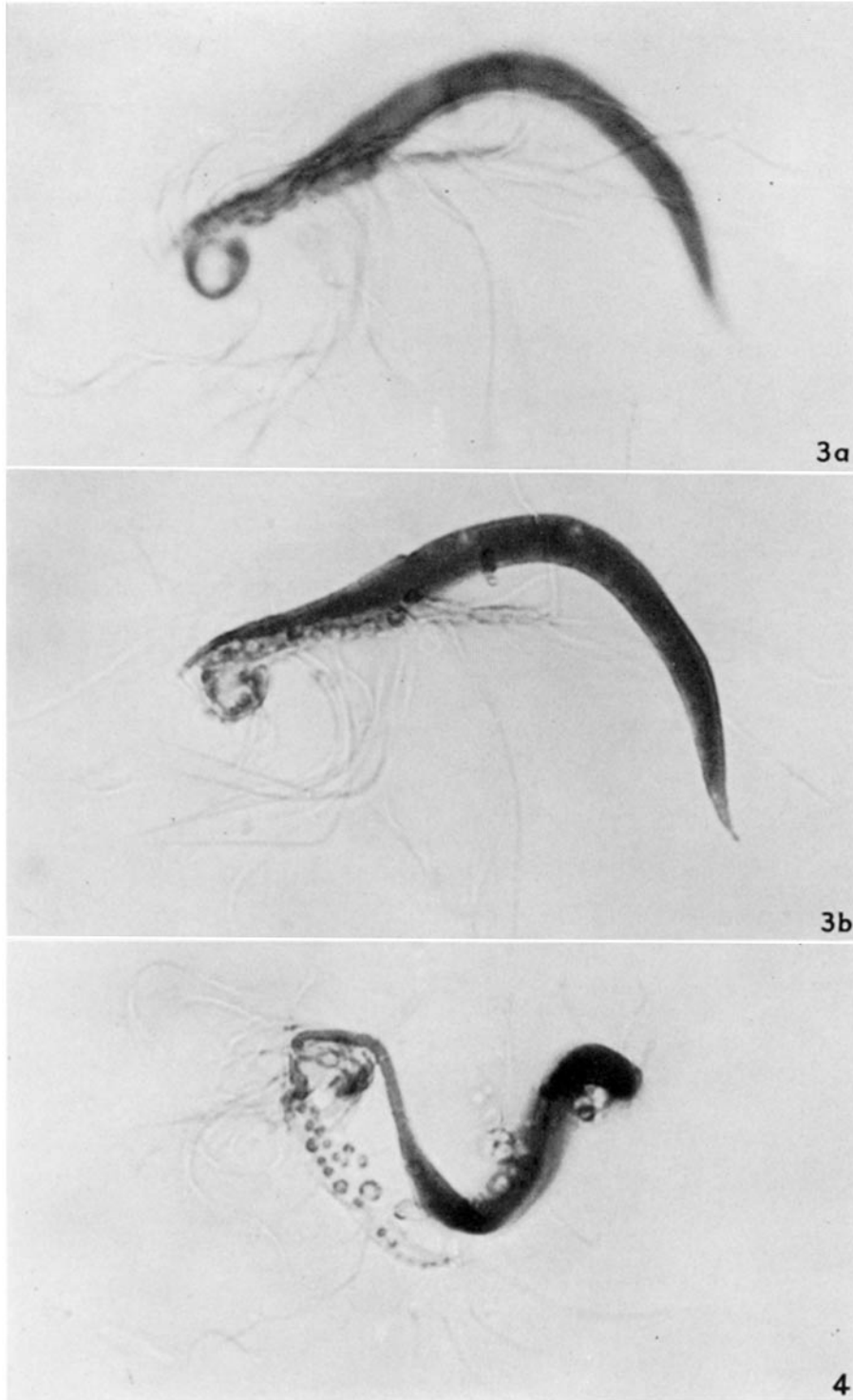
(Manton: Microanatomy of spermatozoid)

PLATE 197

Spermatozoids of the fern *Pteridium aquilinum* showing various degrees of dismemberment after killing with osmic vapour (other details as Figs. 1 and 2); photographed with ultraviolet light.

FIGS. 3 *a* and *b*. Two focal levels of one cell to show the shape of the nucleus which has become uncoiled; the ciliary band can be seen separately at the front end of the cell where it extends for about half a gyre beyond the forward termination of the nucleus; other cytoplasmic components, including the group of plastids seen in Figs. 1 and 2, have fallen away. $\times 3,000$.

FIG. 4. Another cell to show the basement membrane of the ciliated band with dark bodies on its inner side identifiable as mitochondria on comparison with sections. $\times 3,000$.



(Manton: Microanatomy of spermatozoid)

PLATE 198

Shadow-cast whole mounts of spermatozoids of the fern *Pteridium aquilinum* killed directly onto carbon films with osmic vapour before drying. Micrographs taken on 35 mm. film, at 60 kv. except Fig. 10 which is at 80 kv.

FIG. 5. A cell with the ciliary band splitting away from the nucleus but with a row of mitochondria (arrows) still attached to it; other cytoplasm washed away. Exposure S660.7; magnification, *ca.* 3,000.

FIG. 6. A fairly complete cell with the nucleus and ciliated band still cohering and collectively appearing dark; traces of other cytoplasm spread on the field, together with the complete array of about 40 cilia. Exposure S652.8; magnification, *ca.* 3,000.

FIG. 7. The tip of the specimen of Fig. 6 more highly magnified to show the thickened front edge of the ciliated band. Exposure S652.21; magnification, 12,000.

FIG. 8. A similar cell to Fig. 6 more highly magnified to show specially the shape of the ciliated band (partly transparent) in the middle region. Exposure S652.10; magnification, *ca.* 5,500.

FIG. 9. Reversed print of a complete cell with attached cilia. Exposure S652.6; magnification, *ca.* 3,000.

FIG. 10. The front end of a specimen in an attitude similar to that of Fig. 9 but more dismembered and showing spherical mitochondria (arrows) and traces of the fibrous band along the concave side of the curved nucleus; cilia, cytoplasmic remains, and a dividing bacterial cell also in the field. Exposure S672.14; magnification, *ca.* 12,000.



(Manton: Microanatomy of spermatozoid)

PLATE 199

Low-power sections taken on 35 mm. film

FIG. 11. A tangential section near the surface of the cilia-bearing region to show the angle of divergence (about 35°) of cilia near the top and bottom border; for further details of the fringe of cilia near the top border see Figs. 30 and 31. The arrangement of other cilia is in short diagonal lines (arrows) on the surface above the nucleus. Exposure S651.2; magnification, *ca.* 10,000.

FIG. 12. Similar to Fig. 11 but cut at a deeper level. For further details see Figs. 27 to 31; and for other tangential levels see Figs. 34 and 27. Exposure H4243; magnification, 12,000.

FIG. 13. A field containing a cell still enclosed in the membrane which covers it when first released from the antheridium; traces of another cut tangentially beside it (right); part of the back end of a cell in the fully expanded condition above it (left). Compare the latter with Fig. 25. Exposure S655.29; magnification, *ca.* 5,500.

FIG. 14. A field containing parts of the basal ends of three spermatozooids, two of them cut in planes parallel to the coiled nucleus. Note traces of the nuclear membrane on the concave side, with starch-containing plastids, a few mitochondria, and vesicles in the central cytoplasm. Exposure S655.24; magnification, 5,000.

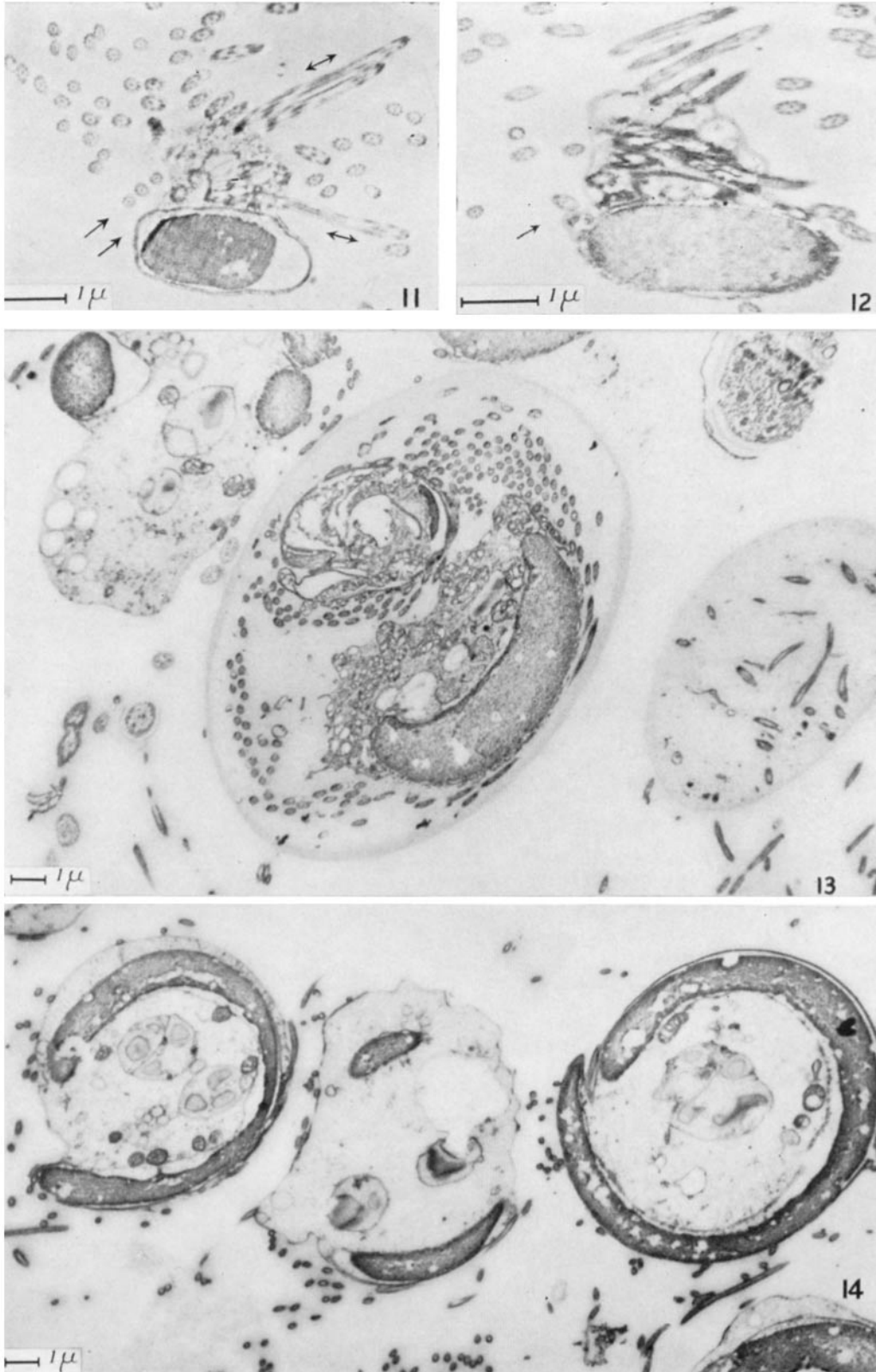
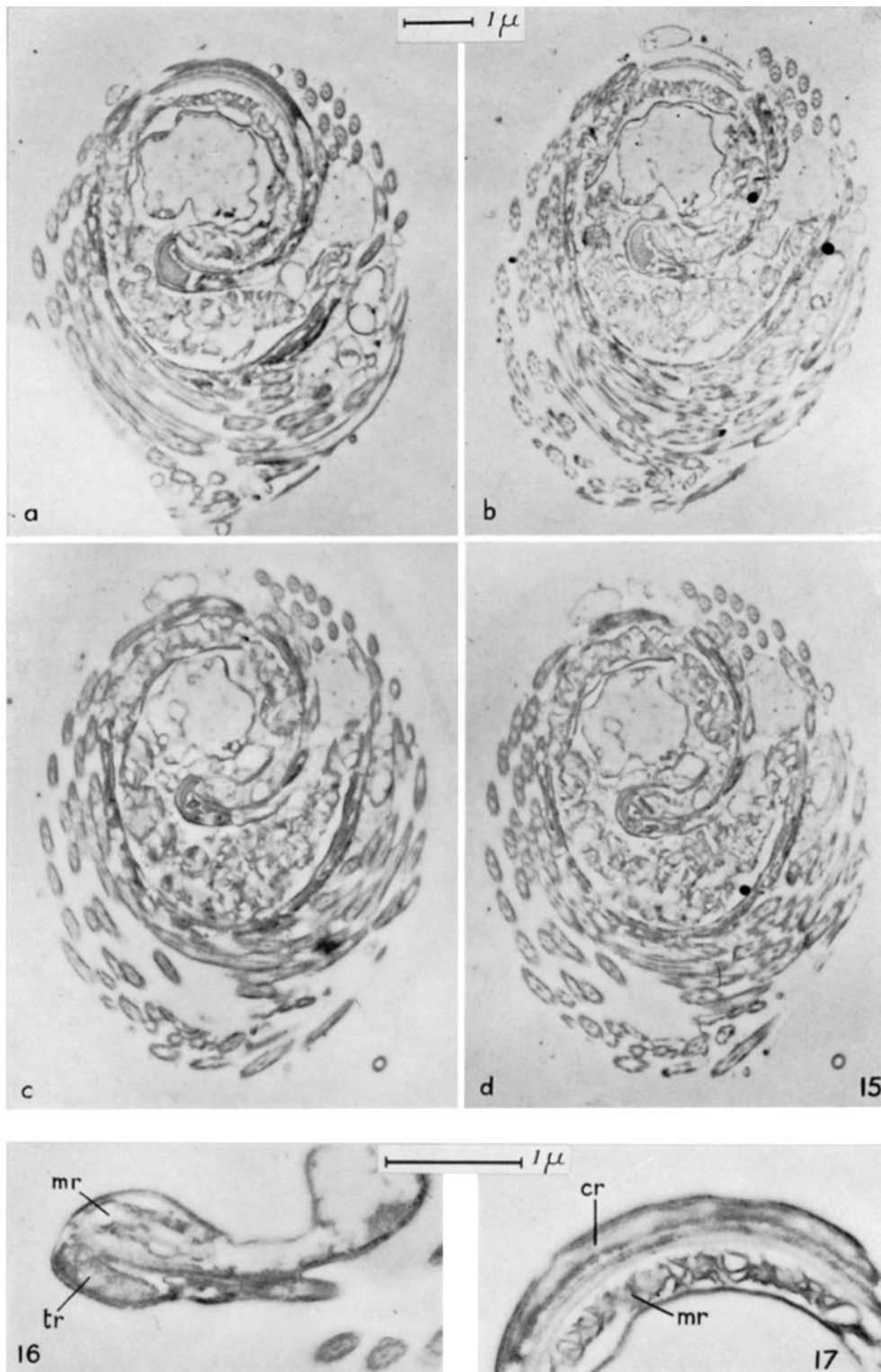


PLATE 200

FIGS. 15 *a* to *d*. Four successive sections through the front end of a closely coiled spermatozoid which has not yet spread out to swim, but on which the enveloping membrane is almost dissolved and is detectable only as a faint diffuse line here and there (see especially Fig. 15 *c* top right). The level of section is through the ciliated band only, being too near the cell surface to include the nucleus. Note in particular the shape of the thickened distal extremity of the band (central in all the sections) and compare with the whole mount (Fig. 7) and with Fig. 16 below. Exposures H4493, H4492, H4489, H4481; magnification, 10,000.

FIG. 16. The swollen tip of the ciliated band from an expanded cell in the swimming state; *mr*, the mitochondrial ridge (see Fig. 27 and p. 415); *tr*, the terminal cushion (see p. 416); compare with Fig. 15 and with Fig. 7. Exposure H3221; magnification, 20,000.

FIG. 17. Part of the ciliated band just behind its swollen tip at the level of the mitochondrial border (*mr*) and outer dark ridge (*cr*); compare with the upper part of Fig. 15 *a* and with the sections in other planes reproduced as Figs. 18 to 23 and 26. Exposure H3677; magnification, *ca.* 20,000.



(Manton: Microanatomy of spermatozoid)

PLATE 201

An array of low-power views of longitudinal sections through the front ends of spermatozooids to show some of the variations in appearance according to the degree of tangentialness in the level of cutting. The principal components visible, and where necessary labelled, are the nucleus (*N*), the mitochondrial border (*mr*), the ridge (*cr*), capping material (*c*), together with cilia, ciliary bases, etc.

FIGS. 18 and 19. Two sections of one cell, Fig. 18 nearer the surface than Fig. 19. For a higher magnification of part of Fig. 18 see Fig. 26. Micrographs H4504 and H4449; magnification, 10,000.

FIG. 20. Another cell including (top left) part of the terminal gyre beyond the end of the nucleus. For higher magnifications of other sections comparable with this see Figs. 36 and 37. Exposure H3700; magnification, 10,000.

FIG. 21. Another cell nearer to the surface than any others on this plate. Exposure M4519; magnification, 10,000.

FIG. 22. Another cell with unusually well preserved cytoplasm cut at a level near to that of Fig. 20 but more tangential in the distal gyre. (For a more highly magnified view of the lower part of this cell see Fig. 25.) Exposure H3688; magnification, 10,000.

FIG. 23. Another cell comparable to those of Figs. 20 and 22, but showing the distal tip separated (top left). Exposure H3730; magnification, 10,000.

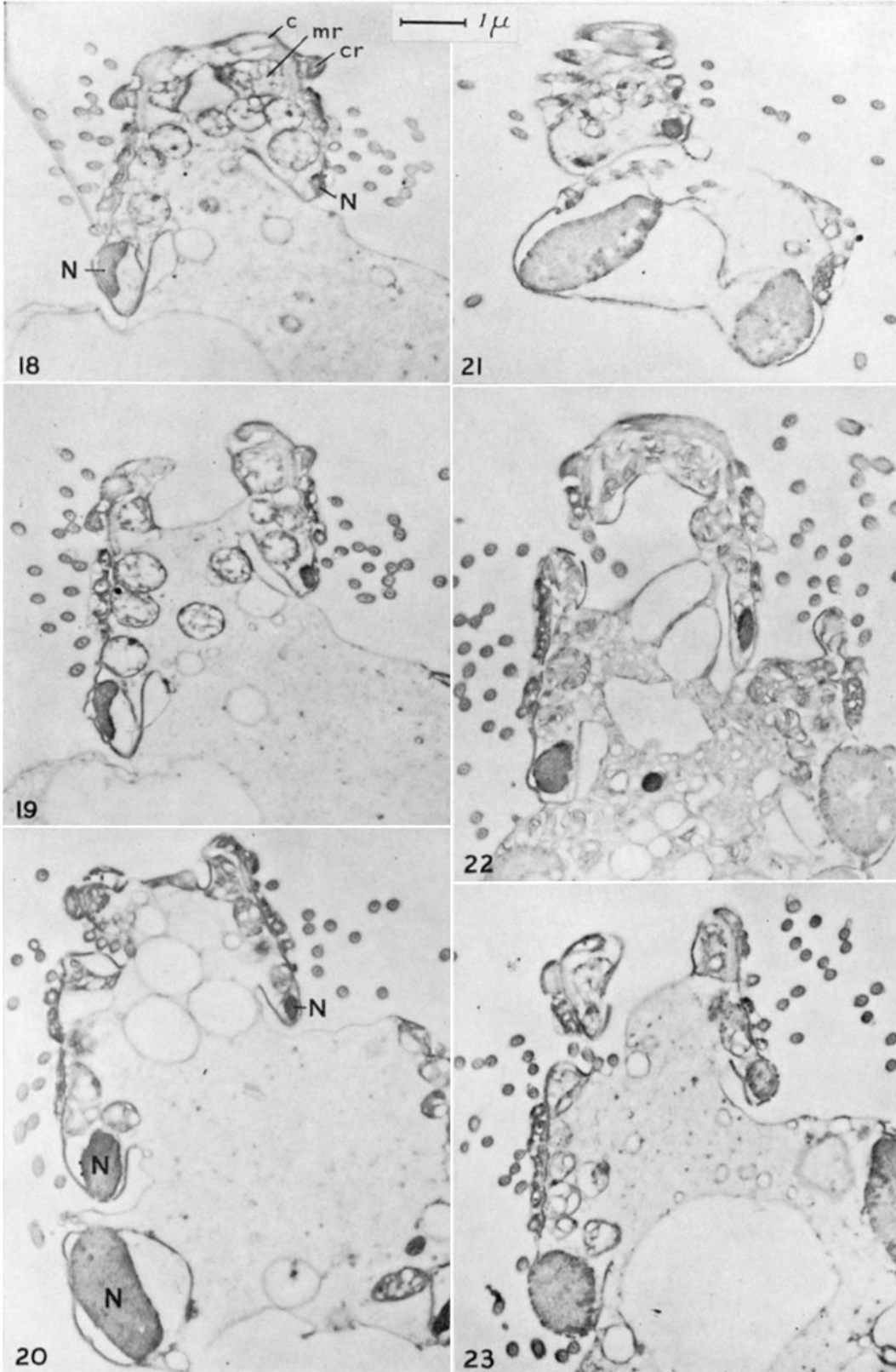
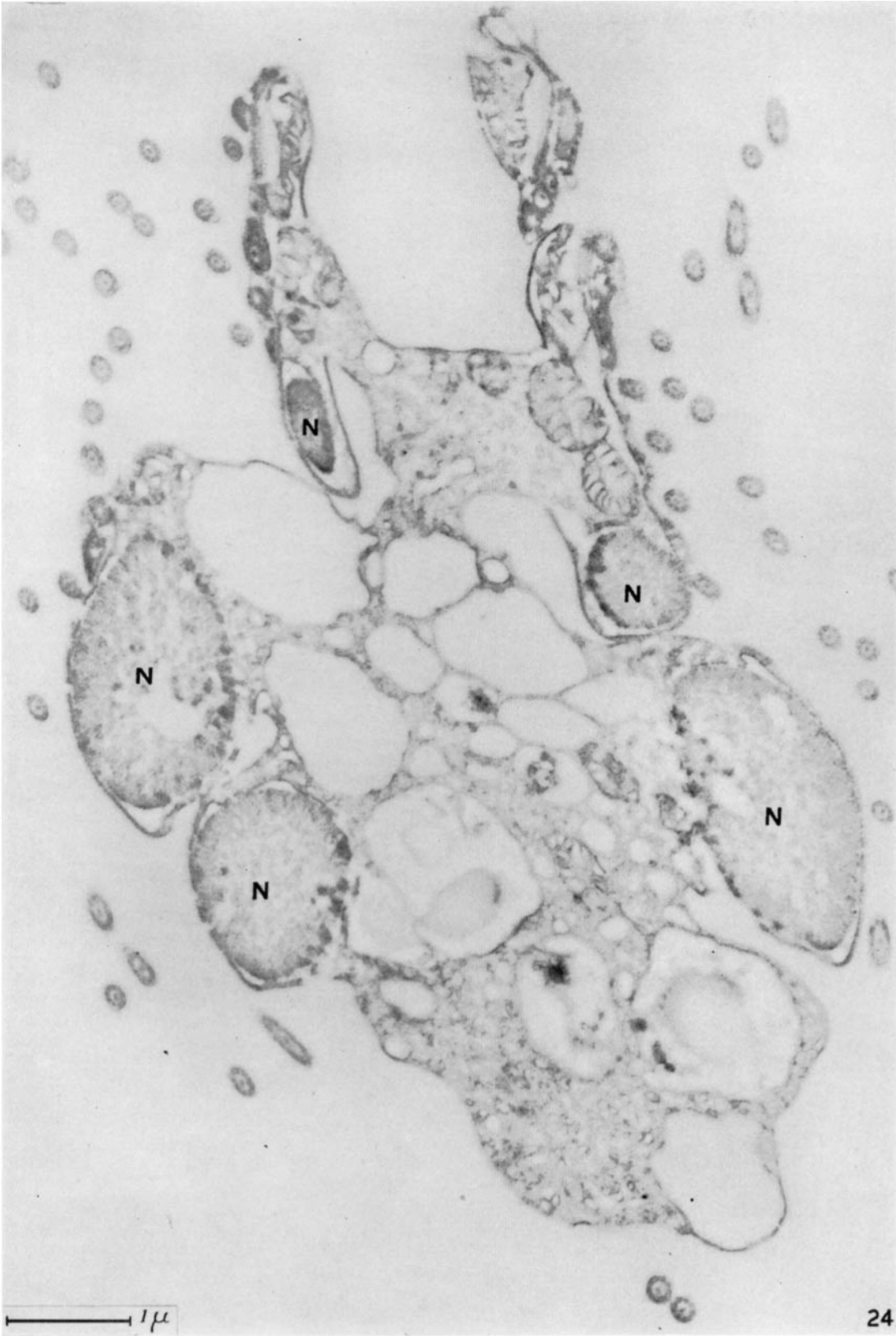


PLATE 202

FIG. 24. Median longitudinal section through a spermatozoid of the fern *Pteridium aquilinum* to show the general topography. Note that the nucleus (*N*), coiled through $2\frac{1}{2}$ turns of a spiral, is transected five times though it does not extend quite to the tip of the cell, so that the detached section (top right) representing the uppermost half gyre contains fibrous and mitochondrial material and ciliary bases only. For further details of the more important components revealed by this plane of section see especially Fig. 25, and Figs. 34 to 38. Exposure H4103; magnification, 20,000.

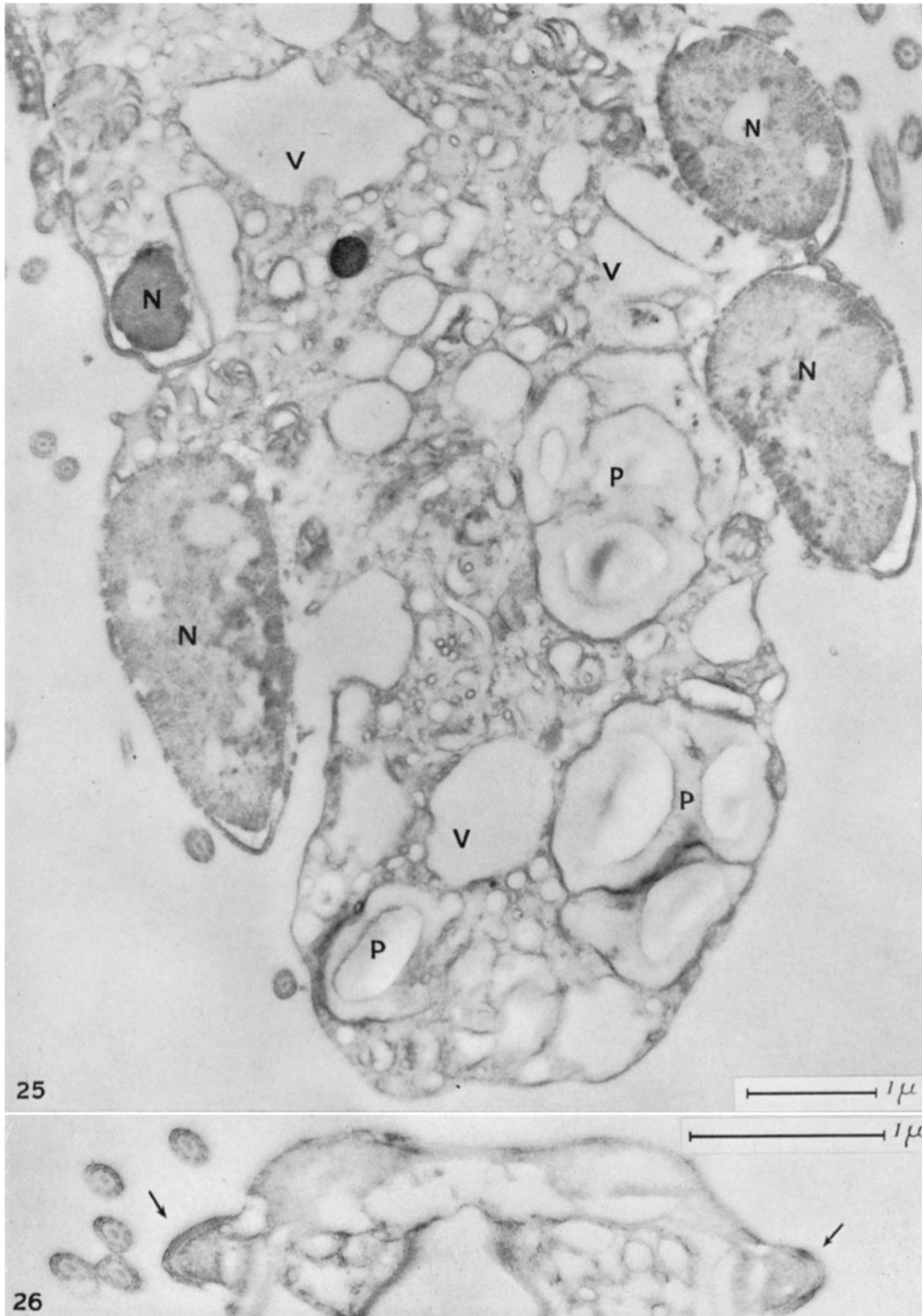


(Manton: Microanatomy of spermatozoid)

PLATE 203

FIG. 25. The hind end of the cell of Fig. 22 to show details of the central cytoplasm. Parts of three starch-containing plastids (*P*), several large vesicles (*V*), tubes, granular cytoplasm, one fat body, small vesicles, traces of mitochondria cut tangentially, and 4 sections of the nucleus (*N*). Exposure H3687; magnification, 20,000.

FIG. 26. The tip of the cell of Fig. 18 more highly magnified (arrows point to the crest-like ridge (*cr* of Fig. 18)); compare also with Figs. 35 and 36. Exposure H4505; magnification, 30,000.



(Manton: Microanatomy of spermatozoid)

PLATE 204

FIG. 27. Tangential longitudinal section near the inner surface of the ciliated band in its widest part (compare right-hand side of Fig. 24) showing the terminal mitochondrial ridge (*mr*) and some of the diagonal rows of separate mitochondria lining the inner face; some ciliary bases and the fibrous band itself cut obliquely on the two sides. Exposure H4098; magnification, 20,000.



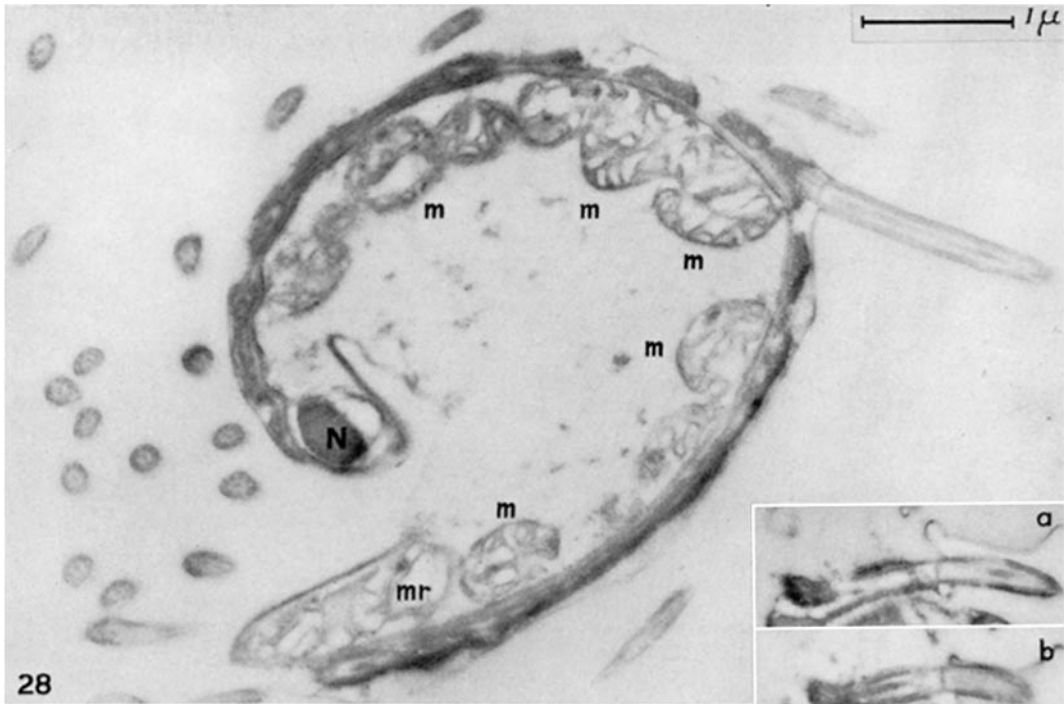
PLATE 205

FIG. 28. Transverse section of a cell through one of its upper gyres showing the nucleus (*N*), the lining of mitochondria (*m*) on the inner side of the fibrous band, with various obliquely cut cilia embedded in dark material on the outer side. Exposure S655.32; magnification, 20,000.

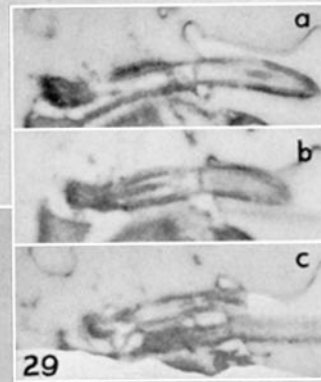
FIGS. 29 *a* to *c*. Three serial sections through the base of a cilium (a second one appearing in Fig. 29 *c*) to show the internal column of dark material which enters the basal body through the bottom but which does not extend as far as the diaphragm separating the basal body from the free part of the cilium. Exposures H4532, H4538, H4528; magnification, 20,000.

FIG. 30. Oblique section passing through the fringe of cilia near the top edge of the ciliated band (compare with Figs. 2 *b*, 11, and 12) to show the close spacing of basal bodies partly embedded in dark material and each with a central column of dark material coming up through the bottom as in Fig. 29. For other views see Figs. 31 to 33. Exposure H3674; magnification, 20,000.

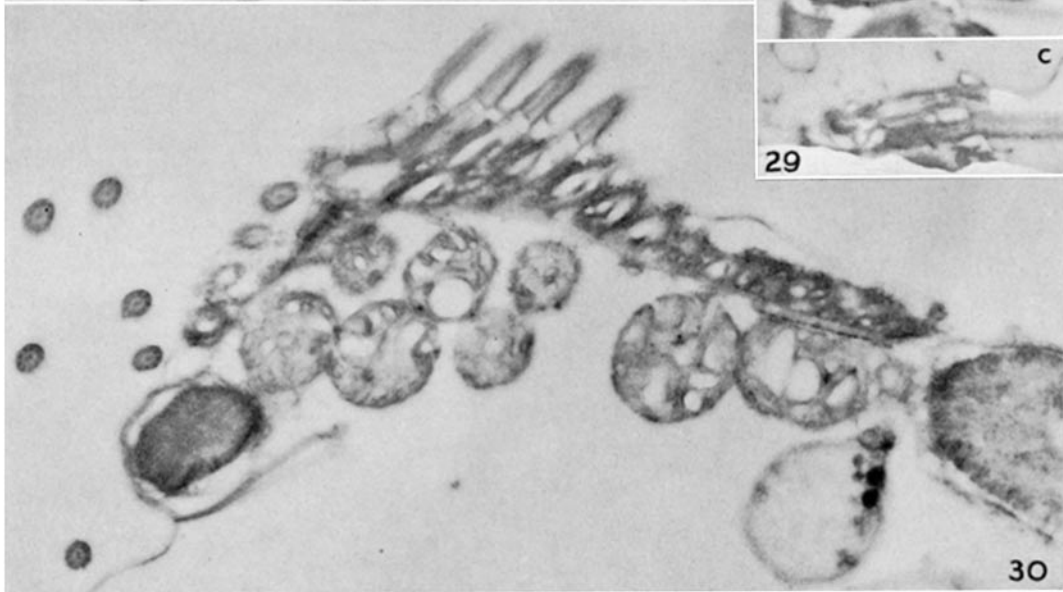
FIG. 31. Section in a plane transverse to the cilia of the fringe and perpendicular to Fig. 30 showing two basal bodies with central columns (left); beside this two basal bodies above the level of the central columns and, therefore, appearing empty; the remaining cilia cut through their free portions with the orientation of the normal internal strands indicated by lines and suggesting an essentially parallel arrangement between adjacent cilia, with a slight spiral twist in each which displaces the plane of symmetry at increasing distances from the base. Exposure H3742; magnification, 30,000.



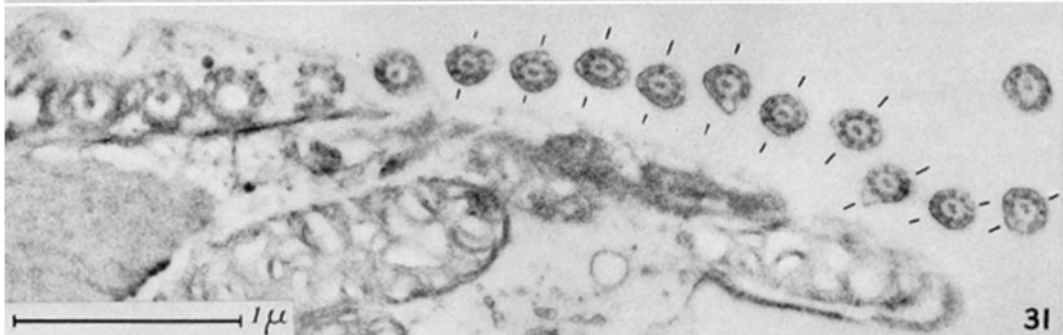
28



29



30

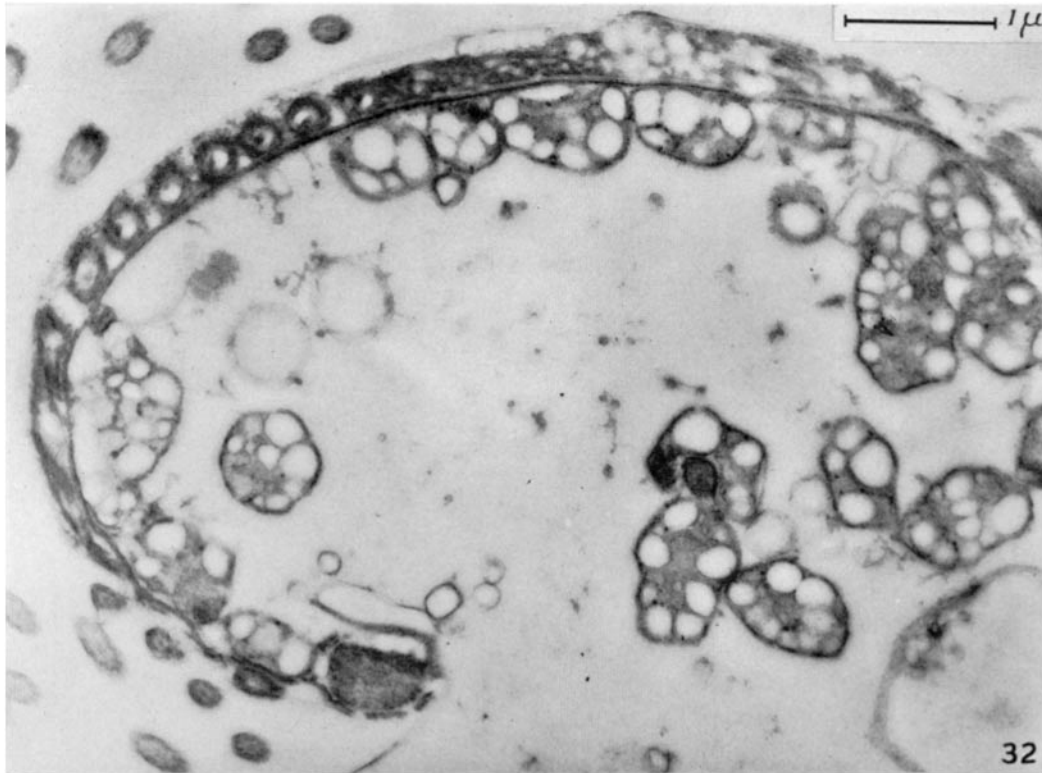


31

PLATE 206

FIG. 32. Transverse section of a cell cut in a plane comparable to that of Fig. 28 but at a lower level and, therefore, through a gyre of wider diameter. Note the obliquely cut ciliary bases (top left) with a meshwork of fibrous material (top centre) over a part of the surface not carrying cilia. Note also the details within mitochondria (right center) suggesting presence of metabolites of several kinds. Exposure H3776; magnification, 20,000.

FIG. 33. Part of a section through the ciliated band and underlying mitochondria in a plane passing longitudinally through a cilium (and tangentially grazing others) in a region where these are well spaced and showing some of the details of the dark material embedding them and connecting them; some of this dark material passes out of the field on the left as a relatively broad band, but is represented by a meshwork at the edge of the field on the right. Note that this specimen had been damaged before embedding and the concave shape of the ciliary band near the main cilium is due to this; in life the band should be smoothly convex as in other sections; apart from this the average plane of this section is parallel to that of the component fibers of the band. Note also the details of attachment of the mitochondria by intercalary material to a basement membrane underlying the ciliary band. Exposure H3708; magnification, 30,000.



(Manton: Microanatomy of spermatozoid)

PLATE 207

Details of the cilia-bearing and nuclear surfaces

FIG. 34. Tangential section cut at a level just below the ciliary bases to expose the fibres underlying them (center); similar fibres visible at the edges of the tangentially cut nucleus (*N*). Exposure H4502; magnification, 20,000.

FIG. 35. Similar to the preceding but exposing the fibres overlying parts of two mitochondria; note lateral connections between the fibres (clearest bottom center) and traces of the intercalary material attaching the mitochondria (top left). Exposure H4239; magnification, 30,000.

FIG. 36. Section of the nucleus (*N*) and ciliated surface, from one of the upper (though not uppermost) gyres; notice the lining of mitochondria (*m*), the dense material surrounding ciliary bases, and the upwardly projecting mitochondrial rim (*mr*). Exposure H4063; magnification, 30,000.

FIG. 37. Similar to Fig. 36 but a lower gyre, with the nucleus (*N*) wider in diameter, no upstanding mitochondrial rim, fewer ciliary bases (*fb fb, f*), but a reticulate pattern of dark material (*d*) replacing them centrally; the arrow (bottom left) points to the lower edge of the fibrous band suspending the nucleus. Exposure H4089; magnification, 30,000.

FIG. 38. Part of a section similar to Fig. 36 but cut normally to many of the fibres of the nuclear sling and ciliated band; arrows point to places where individual fibres can be seen in transverse section as circular profiles 200 Å in diameter; two ciliary bases and part of a mitochondrion (*m*) are visible in the upper part of the figure. Exposure H3782; magnification, 50,000.

