On Flagellar Structure in Certain Flagellates*

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ABSTRACT

This paper describes the structure of the flagella, basal bodies, and some of the associated fibre systems in three genera of complex flagellates, *Trichonympha*, *Pseudotrichonympha*, and *Holomastigotoides*.

Three groups of longitudinal fibres occur in a flagellum: two central and nine outer fibres such as have been repeatedly described in other material, and an additional set of nine smaller secondary fibres not previously identified as such. Each central fibre shows a helical substructure; the pair of them are enveloped in a common sheath. Each outer fibre is a doublet with one subfibre bearing projections—called arms—that extend toward the adjacent outer fibre.

The basal body is formed by a cylinder of nine triplet outer fibres. Two subfibres of each triplet continue into the flagellum and constitute the doublets. The third subfibre terminates at the transition of basal body to flagellum, possibly giving rise to the nine radial transitional fibres that seem to attach the end of the basal body to the surface of the organism. The central and secondary flagellar fibres are not present in the lumen of the basal body, but other complex structures occur there. The form of these intraluminal structures differs from genus to genus.

The flagellar unit is highly asymmetrical. All the flagella examined have possessed the same one of the two possible enantiomorphic forms.

At least two systems of fibres are associated with the basal bodies of all three genera.

This paper is an attempt to describe the fine structure of the flagella, basal bodies, and some of the associated fibre systems in three genera of complex flagellates, *Trichonympha*, *Pseudotrichonympha*, and *Holomastigotoides*. These protozoa, as will be apparent, constitute exceptionally favourable material for the analysis of flagellar structure. By embedding in epoxy-resin and staining with heavy metals it has been possible to obtain excellent preservation of structural detail, combined with electron micrographs of unusually high contrast. As a result, it is possible to give rather precise accounts of the structure of the flagellar systems in these organisms and to describe certain features—notably the basal bodies—in greater detail than has hitherto been feasible. We are able to confirm, to a large extent, the recent findings of Afzelius (1) on sea urchin sperm tails.

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Materials and Methods

The flagellates studied all live in the gut of termites. *Trichonympha* was obtained from the termite, *Zootermopsis angusticollis*, which contains three members of the genus, *T. campanula*, *T. collaris*, and *T. sphaerica* (29, 30). The other genera, *Pseudotrichonympha* and *Holomastigotoides*, came from *Prorhinotermes simplex*. The species and races of *Holomastigotoides* in this termite have been considered by Cleveland (11), to whose paper reference should be made. The species of *Pseudotrichonympha* in *Prorhinotermes* has apparently

not yet been described, but it is similar to the unnamed species from *Coptotermes travians* figured by Cleveland *et al.* (12). Within each genus the fine structure of the flagellar apparatus appears to be essentially uniform, and for the most part no attempt will be made to distinguish between species in the following descriptions.

Termites were fed on moist wood and paper towelling. Flagellates were obtained from *Zootermopsis* by gently squeezing the abdomen of the termite and from *Prorhinotermes* by removing and slitting open the hind gut. The living organisms were studied by phase-contrast microscopy in undiluted gut fluid, and other material, fixed and stained by conventional techniques, was examined in the light microscope.

For electron microscopy, organisms were usually fixed for 45 to 60 minutes at room temperature in a solution of 1 per cent osmium tetroxide buffered to pH 7.9. The composition of the fixative was: 0.6 ml. 0.1 N hydrochloric acid, 1 ml. of a solution containing 0.14 M sodium acetate and 0.14 M sodium veronal, 1 ml. 1 per cent calcium chloride (anhydrous), 1 ml. 10 per cent sodium chloride, 22 ml. distilled water, 25 ml. 2 per cent osmium tetroxide. The flagellates were then taken through 50, 70, 85, and 95 per cent acetone (about 10 minutes in each) to pure acetone, where they were allowed to remain for one to several hours. Some were stained at this point by soaking for several hours in 1 per cent phosphotungstic acid in acetone, excess stain being removed by a brief wash in acetone. The organisms were sedimented by gentle centrifugation prior to each change of reagent. All material was then embedded in araldite epoxy-resin (Ciba (A.R.L.) Ltd., Duxford, Cambridge, England), essentially according to the instructions of Glauert and Glauert (17).

Other fixatives used to a limited extent were 40 per cent (*w/v*) osmium tetroxide in carbon tetrachloride (1), and 5 per cent formaldehyde in 0.028 M veronal-acetate buffer, pH 7.9.

A preliminary study was made of the structure of glycerol-extracted flagella. Specimens of *Trichonympha* were soaked for 24 hours at 0°C. in a 50 per cent solution of glycerol (5 ml. glycerol, 1 ml. 0.1 M phosphate buffer pH 7.0, 1 ml. 1 M potassium chloride, 3 ml. distilled water) (27), and then washed for 1 hour with cold buffer of the same ionic strength. After this they were fixed in 1 per cent osmium tetroxide and processed in the standard way.

Sections were cut on a Porter-Blum ultramicrotome fitted with a glass knife and a trough of distilled water. After being flattened with xylene vapour (41), the sections were picked up by applying a carbon-filmed grid to them from above. A few sections were examined without further treatment; the majority were stained by floating the grids, section-side downwards, on a fresh saturated solution of uranyl acetate in 50 per cent ethanol for 90 minutes or more, after which they were rinsed thoroughly. (To retard its decomposition, this staining solution should be kept in the dark.)

The electron microscope used was an RCA EMU-3D operating at 100 kv. with a 50 μ objective aperture and a 125 μ condenser aperture. Photographs were made at initial magnifications of 16,000 or 23,000. The microscope was calibrated with a carbon replica-grating obtained from E. F. Fullam Corporation, Schenectady, New York. All measurements were made directly on the photographic plates with a binocular microscope fitted with a calibrated eyepiece.

RESULTS

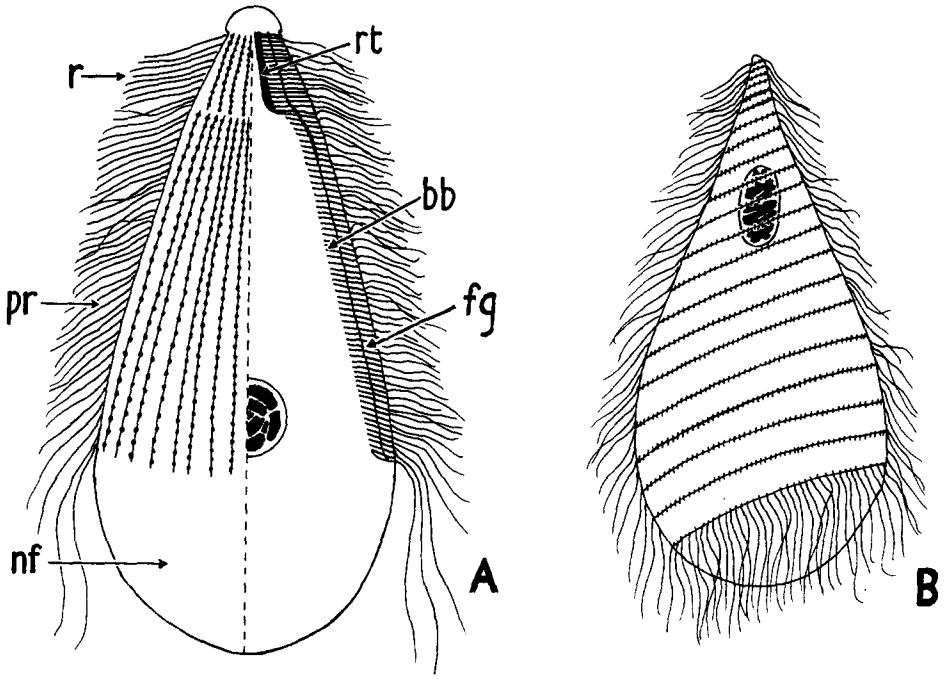
1. Trichonympha and Pseudotrichonympha:

The organisation of the flagellar apparatus is very similar in these genera and they will be treated together.

The papers of Kirby (29, 30) provide detailed descriptions of *Trichonympha*, based on light microscopy, and many features of the fine structure of the flagellar apparatus in this genus have already been described by Pitelka and Schooley (36). However, although it involves repetition, in order to present an intelligible account of our new observations it is necessary to begin with a brief description of the whole organism and the gross organisation of its flagellar system.

The species of *Trichonympha* studied here range from about 150 to 300 μ in length. The body is divided into an anterior part, bearing flagella, and a posterior, non-flagellated region (Text-fig. 1 A). At the anterior end of the flagellated region is the rostrum, which contains a rigid cylindrical structure, the rostral tube. The part of the body behind the rostrum is called the post-rostrum. The flagella are arranged in longitudinal rows of which there are about fifty in the rostrum and rather more than twice that number in the post-rostrum. The flagella are packed closely together and the total number is always very large: *T. campanula*, for example, has between 12,000 and 14,000. This great abundance of flagella is extremely helpful in studying their fine structure.

The outer layer of cytoplasm in the flagellated region constitutes a thick, well defined ectoplasm which contains the long basal bodies of the flagella. Corresponding to each row of flagella is a deep, narrow, longitudinal groove in the cell surface, at the bottom of which the flagella emerge from the general cytoplasm (Text-fig. 1 A; Fig. 2). Since the width of the grooves (which will be called flagellar grooves) is not much greater than the diameter of the flagella, the bases of the latter are rather closely constrained. The flagella are extremely long (up to 150 μ) and extend obliquely backwards: the angle which they make with the cell surface varies in different regions of the body. The basal bodies are up to 5 μ long and usually run more or less transversely in the ectoplasm. There is sometimes a rather sharp bend at the junction of the basal body and flagellum.

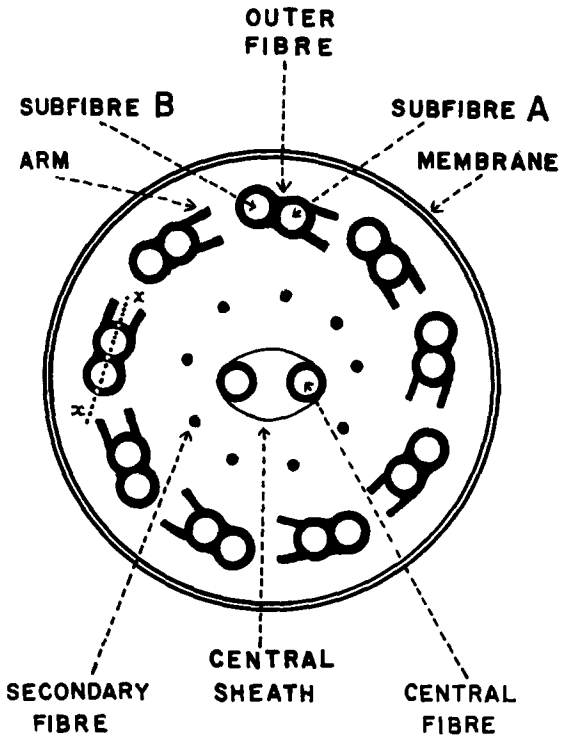


TEXT-FIG. 1. *A. Trichonympha*, showing the arrangement of the flagella in surface view (left) and in section (right). *r*, rostrum; *pr*, post-rostrum; *nf*, non-flagellated region; *rt*, rostral tube; *bb*, basal bodies; *fg*, flagellar groove. *B. Holomastigotoides*, with spiral flagellar bands.

The flagellar apparatus of *Pseudotrichonympha* is organized on essentially the same plan as that of *Trichonympha*, the chief differences being that the flagellated region extends almost to the hind end of the body, leaving only a tiny non-flagellated region, and that the rostrum bears the same number of rows of flagella as the post-rostrum. In some individuals, and particularly near the posterior ends of the flagellar grooves, the bases of the flagella pass through shallow cylindrical pits before entering the groove proper. Further posterior there is usually no common groove at all and each flagellum emerges through a deep cylindrical pit (Fig. 15). (This may also occur in *Trichonympha*, but it is infrequent.)

In both genera the great number and length of the flagella, combined with their vigorous activity, make it extremely difficult to observe the movements of individual flagella. It is fairly clear that undulatory waves are transmitted along the flagella from base to tip, but it is not certain whether these are two- or three-dimensional waves, and we are unable to distinguish a particular plane of beat. The movements and behavior of the whole organism lie, for the most part, outside the scope of the present paper, but some mention must be made of the bending movements performed by *Trichonympha*. These were described in detail by Kirby (29). In its simplest form bending consists of a slight inclination of the rostrum with respect to the rest of the body. In more extreme form it involves a bending of the rostrum and anterior part of the post-rostrum through as much as 180°, so that the tip of the rostrum may be directed posteriorly. Both the degree and direction of the bending may be changed rapidly and organisms are sometimes seen in which the rostrum is repeatedly swung round, tracing out a circle around the body. According to Kirby, these movements are effected solely by the flagella, bending resulting from the activity of the flagella on only one side of the rostrum at a time. The evidence for this view is not entirely conclusive, but it is noteworthy that the electron microscope has so far failed to reveal myonemes in *Trichonympha* (22, 36). If the flagella are indeed responsible for these movements a considerable degree of coordination between them is implied.

In describing detailed fine structure it will be convenient to consider in order the flagellum, the basal body, the special structures at the transition between basal body and flagellum, and the fibres, etc., associated with the basal bodies. A few points of terminology must be noted. A flagellum and its basal body is a long cylindrical structure. In the descriptions which follow the terms *inner* and *outer* will be used in the sense of radially inner or outer, and *proximal* and *distal* will be used to refer to position along the length of the cylinder, with



TEXT-FIG. 2. Diagrammatic transverse section of a flagellum. The line *xx* indicates the axial plane of the outer fibre.

reference to the end of the basal body that lies deepest in the cytoplasm. In the region of transition between flagellum and basal body the structures in the former will be described as *above* those of the latter. *Anterior* and *posterior* will be used to refer to position on the whole organism. Unless otherwise stated, descriptions apply equally to both *Trichonympha* and *Pseudotrichonympha*.

(*A*) *Flagellum.*—The flagella are typical cylindrical structures, with tapering tips. Text-fig. 2 illustrates our concept of the structures to be seen in transverse section; in many respects, this diagram is similar to that of the sea urchin sperm tail given by Afzelius (1).

Three groups of fibres can be distinguished in the flagellum: the customary 9 *outer* and 2 *central* fibres, such as have been repeatedly described in other material, and an additional set of 9 smaller, *secondary fibres*, which have not previously been identified as such.

The two *central fibres* are approximately circular in cross-section, 240 A in diameter and about 300 A apart (centre to centre). In transverse sections they have a dense annular outer region (about 45 A thick) and a less dense core (Figs. 1, 3, 21). Favourable areas of longitudinal sections (Figs. 27 to 29) show further evidence of substructure. Here, the boundaries of a fibre appear as slightly scalloped dense lines, and from each cusp on these a line of slightly increased density runs across the fibre to a cusp on the opposite boundary. This gives the central region of the fibre a cross-striated appearance. These apparent cross-striations often seem to run slightly obliquely, making an angle of 15° to 25° with the transverse axis; this is seen particularly clearly where a fibre is passing from the plane of the section. The periodicity of the apparent cross-striations, in the regions where they are most regular, is about 130 A.

This evidence suggests that the central fibres consist, at least in part, of coiled filaments forming a helix. Both the measured inclination of the apparent cross-striations and direct inspection of the micrographs favour a two-strand helix; however, the possibility of a three-strand helix cannot be completely excluded. (The pitch angle of a two-strand helix with the observed periodicity and over-all diameter is 21°, of a one-strand 10°, and of a three-strand 33°.) In the few areas where the coiled filaments appear with sufficient clarity

to be measured they have a thickness of about 45 A. This is about the same as the dense annular region seen in transverse sections, so that the annular appearance can be adequately explained as the end-on view of the helix. The central open core appears structureless and of low density in our micrographs.

There are indications that some form of *central sheath* envelops the two central fibres. In transverse section this appears as a moderately dense line running out from one of the fibres and curving round to join the other (Fig. 1); often there are two such lines, one on each side of the fibres (Fig. 3). The apparently corresponding structure in longitudinal sections seems to take the form of occasional slanting lines running across from one fibre to the other (Figs. 27, 29). It is entirely possible that the central sheath consists of one or more filaments coiled around the pair of central fibres, though this interpretation is as yet far from certain.

In addition to this possible sheath there is usually a region of increased density directly between the two central fibres.

Each of the nine *outer fibres* appears as a dense figure-of-eight in cross-section (Figs. 1, 3, 21) and measures 370 by 250 A over-all. For reasons which will be explained in the Discussion, we shall describe them as if composed of two subfibres. The longitudinal axes of the two subfibres define a plane which we will call the axial plane of the fibre (Text-fig. 2). This plane is inclined at 5 to 10° to the tangent to the flagellum at the centre of the fibre, so that one subfibre is slightly closer to the centre of the flagellum than the other. Like the central fibres, the two subfibres of the doublet structure have a dense periphery and a less dense core. In transverse section the thickness (about 45 A) and density of the central partition between the two subfibres appears the same as that of the outer wall. In longitudinal sections, however, it sometimes appears considerably more dense (Figs. 30, 31).

We have found indications of some form of helical substructure in the outer fibres but are not yet able to describe it in detail.

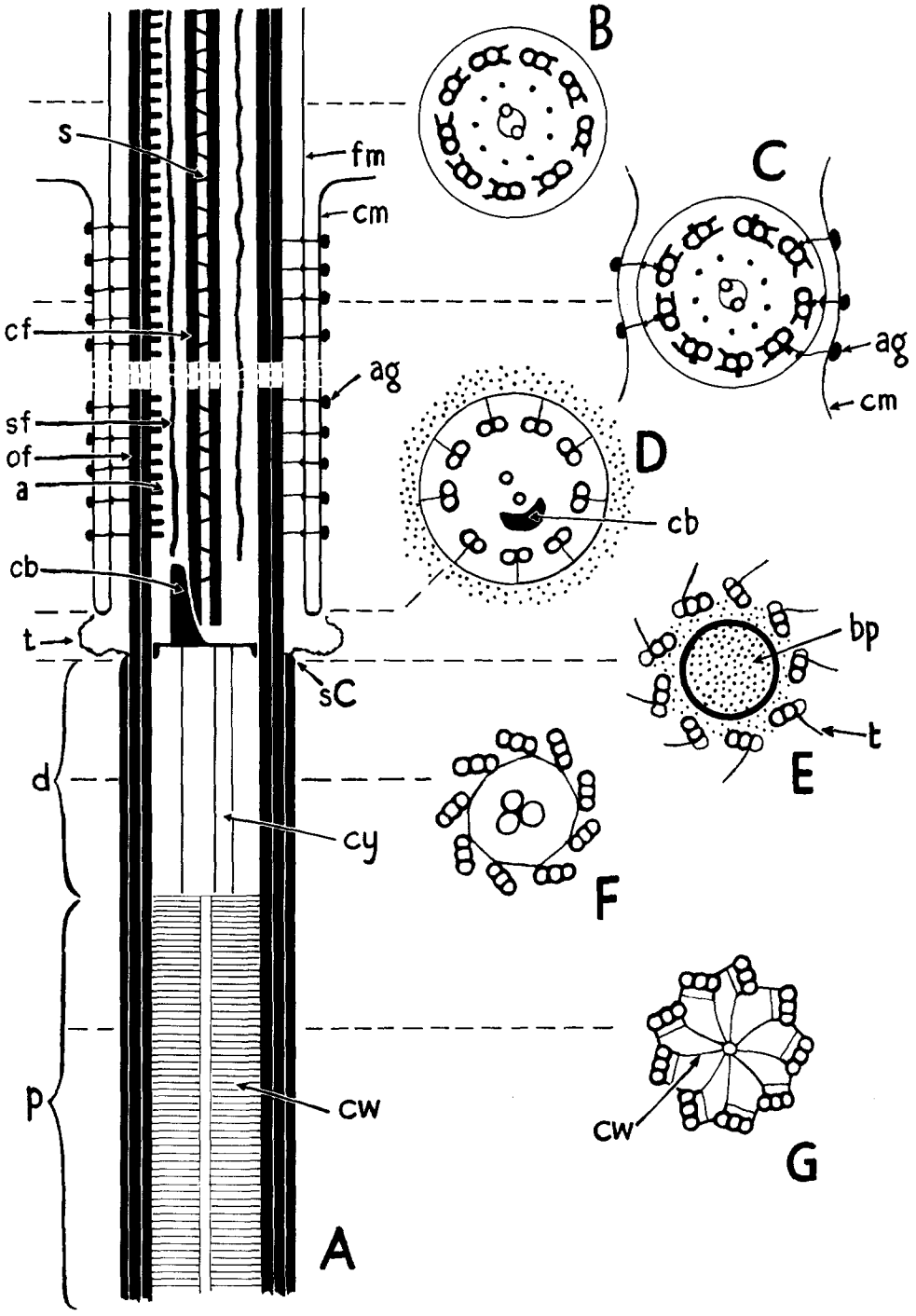
From one of the subfibres of each outer fibre there arise short projections which (following Afzelius) we shall call *arms*. In transverse sections two of these are usually visible on each outer fibre and their typical disposition is shown in Figs. 1, 3, 21, and Text-figs. 2 and 3. They are about 50 A thick and 150 A long and always point in the same direction on all the fibres in a flagellum. This gives the flagellum an asymmetry, and we shall define this as *clockwise* if, to an observer looking along the flagellum from base to tip, the arms point in a clockwise direction. So far as can be determined, all the flagella on a given organism have the same asymmetry. Furthermore, the six individuals of *Trichonympha* in which the point has been studied (by cutting sections of known orientation) have all been found to have flagella of the clockwise form.

The subfibre bearing arms will be called A, and the other B. Subfibre A occasionally has a denser core than does B, and sometimes appears slightly smaller in diameter (Fig. 21). It is subfibre A that lies closer to the centre of the flagellum (see above).

The arms sometimes appear to connect adjacent outer fibres (Fig. 1). In any given cross-section of a flagellum there is usually only one pair of fibres which appears to be connected in this way. However, it is not at all clear that these pictures show real linkages, rather than mere proximity. The latter is perhaps the more probable. Only a few micrographs appear to show the situation that Afzelius describes, in which one of the B subfibres also bears arms, which connect with those of the preceding A subfibre, and we have found no instance in which this interpretation is unequivocally correct. Even in stained material the arms are of low density and their boundaries are by no means easy to trace.

The arms can be identified in longitudinal sections (Figs. 30, 31) as approximately rectangular structures, about 150 A long by 50 A thick and spaced about 130 A apart (centre to centre). Their description as "battlements" (1, 33), seems appropriate. They are usually difficult to see in longitudinal sections, since their density is low and the flagellum must be cut in exactly the right plane.

The question of numbering the outer fibres may appropriately be considered at this point. In many cases it appears that, providing the flagellum is not unduly distorted in sectioning, a line drawn from the centre of the flagellum perpendicular to the line joining the two central fibres passes through one of the outer fibres. This may be called number 1. Afzelius has pointed out that the presence of the arms then allows the remainder of the fibres to be identified, and his procedure is to number them in sequence, starting from 1 and passing round the flagellum in the direction in which the arms point. However, a careful study



TEXT-FIG. 3. Diagrammatic reconstruction of a flagellum and basal body in the anterior body-region of *Pseudotrichonympha*. *A* combines features seen in median and tangential longitudinal sections. *B* to *G* show transverse sections at the levels indicated. *a*, arms; *ag*, anchor granule; *bp*, basal plate; *cf*, central fibre; *cb*, crescentic body; *cm*, cell membrane; *cw*, cartwheel structure; *cy*, cylinders; *d*, distal region of basal body; *fm*, flagellar membrane; *of*, outer fibre; *p*, proximal region of basal body; *s*, central sheath; *sC*, distal end of subfibre C; *sf*, secondary fibre; *t*, transitional fibre.

of our sections has suggested that considerable caution is necessary in accepting that the outer fibres can be unequivocally identified in this way. The difficulty is that it is not always easy to decide which fibre should be called number 1. In a micrograph such as Fig. 1 it appears that although the flagella are almost all oriented in approximately the same direction the fibres cannot be numbered in the same manner in all cases; sometimes fibre 1 lies towards the top of the picture, sometimes towards the bottom. The same point is more rigorously demonstrated when the bases of the flagella lying in the grooves are examined (*e.g.* Fig. 2). Here there can be no reasonable doubt about the uniformity in orientation; yet if a large number of sections is examined and the fibres numbered it is found that 3 and 8 lie with equal frequency on the side of the flagellum facing the bottom of the grooves. It, therefore, seems reasonable to refrain from numbering the fibres for the time being.

Further aspects of the orientation and spacing of the outer fibres are considered in the Discussion.

The *secondary fibres* appear in transverse sections as a set of 9 dots or lines, each situated very precisely between the central fibres and one of the outer fibres, and usually closer to subfibre A than B (Figs. 1, 3; text-fig. 2). The interpretation of these structures as fibres is perhaps open to question, but is adopted for two reasons: (1) in transverse sections dots are more common than lines, and (2) in favourably oriented longitudinal sections it is possible to see sinous lines of slightly increased density, about 50 A wide, running between the central and outer fibres (Figs. 27, 32). If, as seems to be the case, the secondary fibres bend a good deal, rather than running straight up and down the flagellum, it is to be expected that the transverse sections will sometimes show apparent spoke-like structures. It is also significant that the fibres are most dense when they appear as dots, and least so when they form radial lines: in the former case they presumably occupy the whole thickness of the section, in the latter (being much thinner than the section) only a fraction of it. However, while the micrographs strongly suggest the presence of fibres, they do not preclude the possibility of other or additional structures. Sometimes when the fibres appear as dense dots (and are presumably running perpendicular to the plane of the section) there seem to be fine lines extending from them to the outer and central fibres (*e.g.* Fig. 3), and it is entirely possible that these represent radial connections between the different sets of fibres. It cannot yet be determined from the longitudinal sections whether the secondary fibres are continuous through the whole length of the flagellum.

It is most likely that all sets of fibres (central, outer, and secondary) run straight down the flagellum. Certainly no single set of fibres can spiral by itself, for if it did the sets would not appear in a constant spatial relation to each other in transverse section. Furthermore, examination of any row of transversely sectioned flagella lying in a groove shows that all the component fibres are oriented in precisely the same direction in all the flagella (Fig. 2). This is particularly obvious in the case of the central fibres, the line joining which almost invariably makes an angle of some 5° to 10° with the plane of the groove. The constancy of this orientation which, with few exceptions, seems to hold for all the flagella of both *Trichonympha* and *Pseudotrichonympha*, is very striking; its importance here lies in the fact that since these flagella are cut at different distances from their bases it is clear that in the proximal regions, at least, the fibres do not spiral. It is more difficult to be sure that this is the case in the distal parts, since longitudinal sections contain only rather short lengths of flagellum (up to 5 μ) within the plane of the section and we, therefore, cannot exclude the possibility that the fibre complex as a whole follows a loose spiral. However, there is no evidence in favour of this as a uniform feature, and measurements show that if it does occur then the pitch of the spiral must be of the order of at least 50 μ.

In transverse section the over-all outline of the axial fibre complex usually appears elliptical with a ratio of minor to major axes of 0.7 to 0.95. The orientation of the axes of the ellipse bears no constant relationship to the orientation of the central fibres: on the contrary, the axes always lie in the same direction in all the flagella in a given field of view, irrespective of whether the central fibres are aligned or not. For this reason we believe that the ellipticity is an artefact caused by one-dimensional compression of the section. Peachey (35) has shown that 30 to 50 per cent compression usually occurs during the preparation of thin sections, and that 10 to 25 per cent remains even after flattening as we did here.

The membrane bounding the flagellum is continuous with the plasma membrane of the general cell surface (Fig. 32). It is about 90 A thick and clearly triple (Fig. 21) with an outer dense 20 A

layer, a less dense middle layer, about 30 A thick, and an inner, dense 40 A layer. (The general cell membrane is only about 70 A thick, its inner dense layer being only half as thick as that of the flagellar membrane.) The membrane is frequently lifted up in either small blisters or over the whole surface of the flagellum (Figs. 1, 3, 22). Such irregularities are presumably artefacts. Sometimes the membranes are distorted in such a way as to give the flagella a rather characteristic quadrilateral appearance in transverse section (Figs. 4, 22). This is particularly the case in the grooves, where it is possibly accounted for by the close packing of the flagella and their lateral constraint by the walls of the grooves.

It is remarkable that however badly the membrane may be distorted, the fibres (central, outer, and secondary) retain their relative positions very consistently. They do this even in an occasional abnormal flagellum in which the membrane appears to be partly lacking (Fig. 45). Presumably the fibres are bound together in some way, either by direct links between them or by being surrounded by a stable matrix. The former explanation is suggested by an examinination of the tips of the flagella, the structure of which must now be considered.

Approaching the tip, the first detectable changes seem to be that the arms and secondary fibres both stop and the outer fibres approach each other more closely (Figs. 34, 35). This results in the cylinder of outer fibres becoming slightly smaller in diameter. At about the same level the central fibres tend to lose their central position and the symmetrical arrangement and even spacing of the outer fibres is partly lost. Some of the outer double fibres then become single; this seems to result from the rather abrupt termination of one subfibre. The flagella continue to decrease in over-all diameter and the outer fibres, having become single, eventually end. The length of the singlet portion varies from about 0.2 to 0.5 μ. Both the change from doublet to singlet and the ending of the singlets occur at different levels in different fibres, so that transverse sections show variable numbers of mixed doublets and singlets up to the maximum of eleven (nine outer plus two centrals) (Figs. 36 to 44). The central fibres are not easily identified after they have lost their central position, but they seem to end in the same manner as the outer fibres. Just before a fibre ends it takes on a characteristic rectilinear appearance in place of its hitherto circular section. Its extreme end appears entirely dense (*i.e.* with no light centre), which presumably indicates that the end is closed (Figs. 41 to 44).

The flagellar tips strongly suggest an important role for the arms (and possibly for the secondary fibres too) in maintaining the spacing and orientation of the central and outer fibres. They also raise interesting morphogenetic problems, which will be discussed later.

Finally, the proximal parts of the flagella (*i.e.* the parts which lie in the grooves) must be described. These contain some specialized structures which are not found elsewhere.

Firstly, in this region, running along the middle of the outermost surface of each outer fibre there is a low ridge which, on some of the outer fibres, gives rise to remarkable linkages between the flagella and the walls of the grooves (Text-fig. 3). The structure of these is as follows. The grooves are extremely narrow, so that the cell membrane lining them is in close proximity to the flagellar membranes. On the inner surface of the cell membrane, and precisely opposite the outer fibres of the adjacent flagella, there occur small irregularly shaped, dense granules, about 180 A in size (Figs. 4, 22, 48). In transverse section there are usually four or five of these to each flagellum, two on one side, two or three on the other. On the inner surface of the flagellar membrane, immediately opposite these granules, there are usually small, irregular dense masses which in micrographs of particularly well preserved flagella, can be seen to be connected to the ridges on the adjacent outer fibres (Fig. 4). They are also connected—and this is the important point—to the granules under the cell membrane. These linkages are effected by short fibrils which run across the (extracellular) gap between the flagellar and cell membranes. (These fibrils probably penetrate the two membranes to establish the connections, though it is difficult to be sure about this point.) Thus the flagella appear to be anchored, *via* their outer fibres, to the walls of the grooves. In the special case mentioned earlier, where in *Pseudotrichonympha* the posterior flagella lie in pits rather than grooves, there are similar linkages, usually occurring in relation to six of the outer fibres (Fig. 15).

In longitudinal sections the granules under the cell membrane occur in parallel rows (Fig. 48). They are particularly prominent after fixation in formaldehyde and staining with uranyl acetate (Fig. 46). (Small irregular condensations of dense

material may also occur under the flagellar membrane, though not necessarily opposite the outer fibres, in the more distal parts of the flagella (Fig. 1.))

The second feature of the flagella in the grooves is, in many respects, similar to the linkages just described and takes the form of connections between adjacent flagella of a row. These have so far been seen only in *Trichonympha* and there only in rare micrographs: either they are only occasionally preserved, or they are only rarely present. They take the form of fine fibrils which link two of the outer fibres of adjacent flagella (Fig. 47). If we adopt Afzelius' numbering system for the outer fibres (see above) the links run between 3 and 8. (This remains true whichever fibre we identify as 1.) The fibrils run out from the peripheral surface of subfibre B and make contact with the flagellar membranes at points directly opposite one another. There are sometimes small granules where the fibrils meet the membranes. A short connecting link runs across the extracellular gap between the ends of the two fibrils and joins the two flagella together. As with the other linkages, it seems probable that the flagellar membranes are actually penetrated by this fibril. An interesting feature of these links is that they do not connect the two most closely adjacent outer fibres. Fibre 8 would be nearest to 3, however, if the flagellum were twisted slightly to align the central fibres to the flagellar groove.

(*B*) *Basal Body.*—The cylinder formed by the outer fibres of the flagellum continues deeply into the cell and forms the basal body. The central fibres and secondary fibres terminate at the transition to the basal body. (In this paper the whole intracytoplasmic part of the flagellar unit is called the basal body. In Kirby's papers most of this is called the flagellar root and only the innermost part is referred to as the basal granule or rodlet, but this terminology does not correspond to current usage.)

In the basal body each outer fibre appears *triple*, instead of double as in the flagellum (Figs. 2, 25, 26; Text-fig. 3). It will be described as if composed of three subfibres. As in the flagellum, the longitudinal axes of the subfibres define the axial plane of the fibre. This plane is inclined at an angle of 30° to 50° to the tangent through the centre of the middle subfibre. Thus the triplet fibres are "tilted inwards." In the next section, which describes the transition between basal body and flagellum, it will be shown that the innermost subfibre is a continuation of subfibre A, that the middle one is B, and that the outermost one, which will be called C, terminates at the transition.

The axial planes of the nine triplet fibres are all inclined in the same direction. This gives the basal body an asymmetry corresponding to that given the flagellum by the arms on the outer doublets. To agree with our earlier definition for the flagellum (see above), we will call *clockwise* the condition in which, to an observer looking along the basal body from the proximal end, the subfibres are encountered in the sequence C, B, A when moving clockwise. So far as we have been able to determine, all the basal bodies in any one organism have the same direction of asymmetry. Furthermore, just as for the flagella, the six individuals of *Trichonympha* in which we have studied the point have all had clockwise basal bodies.

Along most of their length adjacent outer fibres in the basal body are joined together by a series of links between subfibres A and C (Figs. 11, 26). In transverse section these *A-C connections* appear as moderately dense lines about 45 A thick. They sometimes have a distinct kink and a small region of higher density near their middle which suggests that they may be composed of two portions: one derived from subfibre A, the other from C. The study of longitudinal sections has not yet revealed unequivocally whether these links are continuous ridges or rows of parallel fibrils, though the former seems the more probable.

The A-C links are not present in the most distal region of the basal body, just beneath the transition to the flagellum. In transverse sections of this region, however, there are frequently tenuous connections between adjacent A subfibres, running around the inside of the basal body (Figs. 10, 25). These *A-A connections* are themselves joined together in a continuous ring, which is possibly related to the annular rim on the basal plate, described in the next section.

The triplet outer fibres measure about 535 by 260 A in over-all cross-section. The dense outer wall and the inner partitions are each about 45 A thick. In the region where no A-C connections are present the three subfibres seem to be of roughly the same diameter and density, and their longitudinal axes are co-planar. In the presence of the A-C connections, however, the fibres are characteristically distorted: subfibre C is pulled

radially inwards towards subfibre A of the adjacent outer fibre, so that the longitudinal axes of the subfibres are no longer co-planar (Fig. 26). In this distorted form C usually appears somewhat larger and less dense than the others. The whole appearance suggests that the A-C connections are under tension and are pulling the subfibres from the positions that they would otherwise occupy. This, if true, could perhaps be caused by contraction of the A-C connection, induced by fixation.

Approaching the proximal end of the basal bodies in *Pseudotrichonympha* some, though not all, of the triplet fibres revert to doublets (Figs. 2, 13): subfibre C appears to end before the others or continues only as a narrow ridge or flange from which the A-C connections still originate. This reversion usually occurs in five or six of the nine fibres and usually in the same fibres of basal bodies in adjacent flagellar rows, which suggests that it may prove to have some significance. All the other subfibres of the basal body seem to terminate at exactly the same level—unlike the situation in flagellar tips described earlier.

We have, so far, been concerned only with the cylinder formed by the outer fibres. The contents of the cylinder must now be considered. These are differentiated into a distal region about 0.6 μ long, and a proximal region ranging up to 5 μ.

Extending the whole length of the proximal region is a remarkable structure that, in transverse sections, resembles the hub and spokes of a cartwheel (Figs. 11, 18, 26). The "hub" is a small open circle about 250 A in diameter, from which nine "spokes" radiate out as rather delicate lines about 45 A thick. Each of these connects with a small structure (a ridge, or perhaps a series of short fibrils) on one of the lateral surfaces of subfibre A (Fig. 26; Text-fig. 3). This ridge (if such it is) closely resembles the similar structure on subfibre C (*i.e.* one part of the A-C connection), and it points in the same direction. Their ends are often connected transversely by a thin line, which probably itself represents a series of transverse fibrils. In most micrographs the spokes are not perfectly radial but appear somewhat curved. This is, in part, an optical illusion, caused by their running to the ridge rather than to the subfibre itself, but after allowing for this the spokes still show a slight curvature. The "cartwheel" structure is shown in longitudinal section in Fig. 33. The transversely striated material with periodicity of about 130 A presumably represents the "spokes." It is not clear whether they should be regarded as true spokes— that is, as rods— or whether they are actually the cut ends of radiating cross-striated lamellae. The "cartwheel" structure appears essentially the same in both *Pseudotrichonympha* and *Trichonympha*.

In the distal region of the basal body there are rather substantial differences between the two genera which necessitate separate descriptions.

The distal region in *Trichonympha* apparently contains no well defined structures (Fig. 25).

In *Pseudotrichonympha* two completely different structures have been found—apparently associated with different parts of the organism. In the rostrum and the anterior part of the post-rostrum, the distal region of the basal bodies contains a structure that, in transverse section, usually appears as three circles each about 400 A in diameter, with a dense periphery 130 A thick and a less dense inner zone (Fig. 10). These circles are most frequently grouped in a "clover-leaf" arrangement, although they are sometimes less regularly arranged and occasionally only two are present. The corresponding longitudinal sections show a central strip about 700 A wide running through the whole distal region of the basal body. Two moderately dense lines form the boundaries of the strip and a third dense line runs up its centre (Fig. 14). The most likely interpretation of the whole structure is as a group of three rather irregular cylinders running from the transition between flagellum and basal body to the beginning of the "cartwheel" structure.

In the posterior part of the organism, where the flagella emerge from the body in pits rather than grooves (see above), instead of these cylinders there are many dense granules that fill the basal body down to the "cartwheel" structure. The granules have polymorphic profiles and vary in size from 200 to 300 A. Sometimes elongated profiles that suggest filaments are visible, but these are probably caused by overlapping granules. Despite their irregular shape, many of the granules seem regularly disposed with respect to the outer fibres. Longitudinal sections (Figs. 19, 20) show that each of the latter has a single row of granules disposed along it, with a rather regular spacing of about 250 A (centre to centre). This arrangement is confirmed by transverse sections (Figs. 16, 17), which show a granule positioned next to each A subfibre. In many cases there is a tenuous link running between granule and subfibre. The nine

rows of granules do not themselves completely fill the lumen of the basal body; more granules similar, but randomly arranged, fill the centre. The "granule-containing" distal region is frequently much longer than the "cylinder-containing" distal region of the other basal bodies. The A-C links between outer fibres are lacking throughout the "granule-containing" region.

The significance of these features is not known.

(*C*) *Transition from Basal Body to Flagellum.*—The arrangement of the flagella in longitudinal rows, combined with their great number, permits a fairly complete reconstruction to be made of the special structures which occur at the junction of basal body and flagellum. An oblique section through the flagellated region of the organism cuts the flagella and basal bodies at progressively lower levels as it penetrates from the cell surface into the body, so that if the orientation is suitable it may provide a number of correctly ordered sequences of sections—one sequence from each longitudinal row of flagella—passing from the more distal parts of the flagellum to the bottom of the basal body (Fig. 2). In other words, a single micrograph can provide "serial" sections. Of course, since the sections come from different flagella or basal bodies they are not contiguous, so that regions may be duplicated or omitted in some rows—indeed this is usually the case. This necessitates the examination of many rows in order to make a complete reconstruction.

The outer fibres are the only structures common to both basal body and flagellum, and even they do not continue unchanged. As already noted, the outermost subfibre (C) terminates so that the triplets of the basal body become doublets in the flagellum. The termination of C appears rather abrupt; nothing suggests that it fuses with B (Figs. 8, 31). It is subfibre A (the innermost subfibre) which gains arms just after the outer fibres leave the body (see Fig. 2 and the sequence in Figs. 3 to 12): therefore, the arms are not a derivative of C (as was tentatively suggested by Afzelius (1)). On passing from basal body to flagellum, the inclination of the axial plane of the fibres (as defined previously) decreases from about 45° to 5°, so that the fibres lose most, though not all, of their inward tilt. This change of inclination occurs as a gradual twisting of the fibre over about 0.5 μ on each side of the transition. The external diameter of the cylinder formed by the triplet fibres (0.20 μ) is the same as that of the cylinder of doublet fibres in the flagellum. Therefore, the change of inclination is a result of the A subfibres moving radially outwards in the latter rather than the other subfibres moving inward.

In *Trichonympha* the cylinder formed by the outer fibres often narrows down from its usual 0.20 μ to a diameter of 0.18 μ for a distance of about 0.15 μ on each side of the transition. This gives the cylinder of fibres a more compact appearance than elsewhere, in transverse section. No such constriction has yet been observed in *Pseudotrichonympha*. For a short distance above the transition all nine outer doublet fibres are linked by short flanges to the flagellar membrane (Figs. 6, 7, 23; Text-fig. 3 *D*).

The central fibres begin about 250 A above the level at which the C subfibres end. They seem to commence rather abruptly on or close to a transversely running *basal plate*. In most micrographs this is not particularly well defined; in transverse sections it appears as moderately dense material extending most of the way across the lumen of the basal body (Figs. 8, 24), while in longitudinal sections it seems to be a lamina about 250 A thick (Fig. 32). Above the basal plate and embracing the end of one of the central fibres is a dense, eccentrically placed crescentic structure (Figs. 5 to 7, 23). This always occurs around the central fibre which appears nearer the bottom of the groove—or, since the grooves run longitudinally on the organism, on the central fibre which is directed inwards towards the general cytoplasm. In longitudinal section its lower end seems to merge with the basal plate; in transverse sections short connections extend from it to the four nearest outer fibres. This structure presumably anchors the end of one central fibre (and subsequently the other fibre also, by way of the sheath surrounding the two). By its presence around only one of the central fibres the crescentic body emphasises still further the fundamental asymmetry of the flagellum.

In *Pseudotrichonympha* a raised annular rim runs around the under side of the basal plate (Fig. 9). Also below the basal plate in *Pseudotrichonympha* are the ends of the three large hollow cylinders described in the previous section.

Finally certain structures external to the basal body must be described; these are the nine *transitional fibres*. In longitudinal sections (Fig. 31), each of these appears as a rather poorly defined dense line extending from an outer fibre at about the level where subfibre C ends, toward the cell membrane at the point where it turns

to become the flagellar membrane. It is not yet certain whether the fibre actually ends on the membrane. In suitable transverse sections (where subfibre C ends within the plane of the section) the nine fibres are seen extending outwards from the outer fibres (Figs. 8, 24). They sometimes appear radial, but more often are inclined to the radius at an angle up to 45° in the same direction as the normal to the axial plane of the outer fibre. The density of the background surrounding the fibres in transverse sections results largely from the bend of the cell membrane overlapping the fibres within the plane of the section. In our best micrographs, the appearance of some of the transitional fibres suggests that they are composed of two fine filaments, each about 50 A thick, which twist loosely once or twice around each other and then diverge and end separately in association with the membrane (Fig. 8).

It remains only to be pointed out that there is no obvious plane which can be singled out as the junction of flagellum and basal body. The change from triplets to doublets, the transitional fibres, the basal plate, and the start of the central fibres, however, all occur within a region about 500 A long, in which the junction can be considered to lie. The flagellar membrane usually begins 300 to 500 A above the basal plate.

(*D*) *Fibres Associated with the Basal Bodies.*—Two systems of fibres can be identified connecting, or associated with, the basal bodies. The first, composed of relatively large fibres, occurs just beneath the proximal ends of the basal bodies; the second is extremely delicate and complex and extends over the proximal region of the basal body.

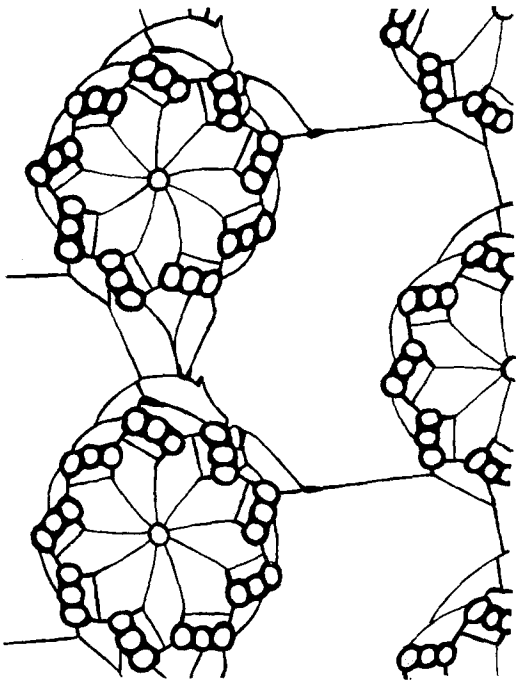
The large fibres will not be described here in any detail. In *Trichonympha* they form a system of some complexity, possibly continuous with the parabasal filaments (see references 23, 36), which will be dealt with elsewhere in conjunction with the centrioles and the detailed structure of the rostrum. All that need be said for the present is that in the post-rostrum there is a system of cross-striated, collagen-like fibres underlying and probably linking the proximal ends of the basal bodies (Fig. 51), and that in the rostrum the rostral tube, which lies directly under the basal bodies and may connect with them, is composed of a series of complex, rib-like structures, also cross-striated. In *Pseudotrichonympha* there is a similar rostral tube but the post-rostral system is different. Instead of cross-striated fibres, fibrous ribbons underlie the basal bodies. In the anterior part of the post-rostrum they are composed of flat ribbons, with transverse fibres running across them at intervals. In the posterior part of the body, the ribbons are much narrower and follow an extremely sinuous course (Fig. 50). The detailed morphology of this system remains to be elucidated and it is mentioned here only in a preliminary way. It is not yet certain how closely the basal bodies and the ribbons are associated.

The second, more delicate system of fibres is apparently quite unlike anything which has been previously described. It appears to be preserved only in rare preparations, so that it is not yet possible to describe it at all completely. However, it does seem important to present such information as is available, as an indication of the sort of complexity which may be expected in this kind of system.

In *Pseudotrichonympha* all that has so far been seen is a series of fine fibres running around the outside of the proximal portion of the basal bodies (Fig. 12). These apparently do not connect adjacent basal bodies, but rather adjacent C subfibres.

A more complex arrangment has been found in the rostrum of *Trichonympha* (Fig. 49). Here there are apparently the same fibres linking adjacent C subfibres, but, in addition, there are numerous other fibres running more peripherally and linked up in very complicated ways with each other and with the different subfibres of the various outer fibres. There are also long fibres running both longitudinally and transversely to connect these networks of fibres to those of adjacent basal bodies. The arrangement of these fibres around and between the basal bodies is highly asymmetrical. Text-fig. 4 shows our interpretation of these fibres, as far as they have been traced at present. Further work will almost certainly add new details and also, no doubt, clarify the picture. It must be stressed that the structures shown in this diagram occur identically in several flagella and, therefore, cannot be regarded as artifacts. Longitudinal sections confirm that these connections are in the form of fibres (rather than laminae) and that they are restricted to the proximal portion of the basal body.

It may well be the presence of these numerous fine fibres which makes the proximal portion of the basal body appear denser than the distal region when seen with the light microscope. This



TEXT-FIG. 4. Reconstruction of the fine fibre system of the proximal region of the basal bodies of *Trichonympha*. The reconstruction is a tentative one, and includes only the more commonly occurring fibres. The flagellar rows run vertically in the figure, and sections at the top are distal to those at the bottom.

higher density is very noticeable when fixed and embedded organisms are examined with the phase-contrast microscope. It also shows as a region of higher intensity in stained preparations, corresponding to the "inner ectoplasm" of Kirby (29).

(*E*) *A Note on Glycerinated Flagella.*—Electron micrographs of glycerinated *Trichonympha* have shown that most of the structures in both flagella and basal bodies are retained after 24 hours extraction. However, all the structures seemed to show somewhat lessened density.

2. Holomastigotoides:

Only a brief description of this organism will be presented, since the detailed fine structure of its flagella and basal bodies appears to be largely the same as in *Pseudotrichonympha* and *Trichonympha*.

The flagella here are disposed not in longitudinal rows but in spiral bands, two or more in number, each of a single row of flagella. These start at the anterior tip of the organism and run in rather shallow spirals almost to the posterior end of the body (see Cleveland (11) and Text-fig. 1 *B*).

The detailed structure of the flagella appears to be identical to that in the other two genera (Fig. 52) and need not be further considered here. The only point worthy of mention is that the proximal 0.8 μ of the flagellum usually appears to be closely applied to the body surface. Just inside the cell membrane and exactly opposite each flagellum there is a thin dense ribbon 50 A thick, 600 A wide, and 0.5 μ long (Fig. 56). From its position it seems possible that this ribbon serves to anchor this part of the flagellum to the body surface, and that it corresponds to the granules which probably anchor the flagella in the body grooves of *Trichonympha* and *Pseudotrichonympha*. Connections between ribbon and flagellum have not, however, been seen so far.

It is in the basal bodies that most differences from the other two genera occur. The general organisation is very similar (Figs. 53, 54, 56): the outer fibres are inward-tilted triplets; they have A-C connections along most of their length, and A-A connections in the most distal portion. The major difference is that the whole lumen of the basal body appears empty. There is no differentiation into proximal and distal regions and the cartwheel structure is definitely absent.

The transition between basal body and flagellum seems substantially similar to that in the other genera, though it has not been worked out in such detail. The basal plate and the transitional fibres are certainly present, but so far there is only slight evidence for the existence of the asymmetrical crescentic structure surrounding the end of one central fibre.

At least two kinds of connecting structures appear to be associated with the basal bodies:

(1) A well defined fibrous band runs along the outermost side (that is, the side nearest the body surface) of the basal bodies, connecting them together (Figs. 54, 55). This band is extremely well defined and has a uniform and characteristic structure. It appears closely connected with only two of the outer fibres of each flagellum, at the point of contact with which it narrows and becomes much denser. There may be, in addition, rather more tenuous connections between it and the two adjacent outer fibres. Between the basal bodies the band widens out and forms a moderately dense ribbon, across which, exactly half-way between adjacent basal bodies, runs a narrow, very dense, transverse band. In specially thin

sections, the ribbon appears composed of numerous very fine longitudinal fibres, and it is perhaps legitimate to compare it with the system of delicate fibres connecting the basal bodies in *Trichonympha*. In both genera there are fine fibres, restricted to the proximal region of the basal bodies, associated with particular outer fibres and linking adjacent basal bodies. However, it must be noted that there are also indications of other fine fibres in *Holomastigotoides*, which, although at present not satisfactorily preserved, appear more closely comparable to those in *Trichonympha* than the fibrous ribbon.

(2) In the anterior part of the organism the basal bodies are associated with a thick, moderately dense band that lies interior to the row of basal bodies (Fig. 57). Along one margin there are well defined fibres, which appear similar to (and probably connect with) the ribbons of axostylar filaments that run through the interior of the cell. This band appears associated in a general way with the basal bodies but there is no evidence of connections to specific triplet fibres. In this respect it is similar to the cross-striated fibres that underlie the basal bodies in *Trichonympha* and to the corresponding fibrous ribbons in *Pseudotrichonympha*.

From its position relative to the basal bodies it seems that the fibrous ribbon described first above corresponds to the densely staining "flagella-bearing" portion of the flagellar band of Cleveland (11), while the larger structure presumably corresponds to the lightly staining "supporting" portion of Cleveland's description.

DISCUSSION

In describing structures from electron micrographs it is always necessary to consider how far they correspond to the pre-existing structure of the living organism and how far they derive from the preparative techniques employed. In the present study we can only apply the criteria of orderliness and reproducibility to validate our findings: it is unlikely that preparative techniques alone will produce asymmetrical yet highly ordered and reproducible structures—such as we have described here—where none existed previously, and we, therefore, believe that they must derive from some comparable structure in the living cell. How much this has been altered in preparation it is impossible to say with certainty, but the fact that two fixatives (osmium tetroxide and formaldehyde) and two stains (phosphotungstic acid and uranyl acetate) produce similar results suggests that the changes are probably of a limited character. It should be borne in mind, however, that even if this is so, we have at best described here only the macromolecular skeleton of the flagellar apparatus.

In discussing our findings we shall be chiefly concerned with structure; functional and morphogenetic aspects will be only briefly touched upon.

Structure:

The widespread uniformity of the basic "9 + 2" organization of flagella and cilia is already so well known as to require no detailed comment. All motile flagella and cilia appear to contain two central and nine outer fibres similar to those described here.

It is not yet known whether the other components which we have described are of such general occurrence. The *arms* on the outer doublets have now been observed in several groups of animals and plants. They were first shown (although incorrectly interpreted) in fragmented spermatozoids of the moss, *Sphagnum*, by Manton and Clarke (33), in a remarkable paper which anticipated many more recent observations on sections. More recently, arms have also been demonstrated in sea urchin sperm tails (1), and in tracheal cilia of the rat (Gibbons, unpublished observations), so that they may well be of widespread, if not universal, occurrence.

The *secondary fibres* have not previously been described as such. In sea urchin sperm, Afzelius has described "spokes" (*i.e.*, radial connections between the central region and subfibre A of each outer fibre) in the approximate position of our secondary fibres, and there have been other reports of vague radial connections in sperm tails (2, 10). It is possible that there is a real difference here between the flagellar structure of flagellates and that of some spermatozoa. However, it must be noted that Afzelius found the thickest and most distinct part of his spokes in the same region as our secondary fibres, while we have found occasional apparent connections between the outer and secondary fibres and between the central and secondary fibres. The difference may not be as great as it appears at first sight.

Several previous reports have mentioned occasional periodic beading or cross-banding in some of the flagellar fibres (*e.g.*, 14, 21, 39), and it

seems likely that these were incompletely resolved traces of helical substructure, similar to that which we have described in the central fibres.

The structure which we have described as a *sheath* enveloping the two central fibres is probably similar to that found in *Sphagnum* by Manton and Clarke (32, 33).

We have not yet been able to determine the detailed substructure of the outer fibres and so elucidate their basically single or multiple nature. However, the fact that they change from triplets to doublets and from doublets to singlets by an apparently abrupt termination of one of the subfibres indicates that the latter have some degree of individuality. It, therefore, seems justified at present to describe the fibres, as we have, in terms of component subfibres, though it must be admitted that further knowledge may point to a different terminology. The subfibres are evidently tightly bound together, for there is no good evidence that they become separated by the techniques used to fragment flagella (reviewed in reference 14). As was first pointed out by Rhodin and Dalhamn (38), the fibres cannot consist of subfibres simply juxtaposed together because the central partition has the same thickness as the outer wall. On the basis of their study of fragmented flagella, Manton and Clarke (33) suggested that the fibres consist of subfibres bound together by a sheath. This remains a possibility.

In cross-section the ratio of width to height of a doublet outer fibre is 370 to 250 A, or approximately 3 to 2. For a triplet fibre it is 535 to 260 A or approximately 2 to 1. Such ratios are unlikely to be given by simply juxtaposed subfibres. They do, however correspond to the numbers of dense zones in the fibres. Thus a triplet fibre has four dense zones in its width (two outer walls and two partitions) and two dense zones in its height (two outer walls), which gives a ratio of 2 to 1. A doublet fibre has only three dense zones in its width and so gives a ratio of 3 to 2. The significance of these relationships is obscure. We can only suggest that the dense zones, which are possibly larger when hydrated than after fixation and dehydration, may in some way determine the over-all size of the fibres.

Cleland and Rothschild (10) have recently described an uneven spacing of the outer fibres in the tail of the bandicoot spermatozoon. We have tried to ascertain whether this arrangement occurs consistently in our material and conclude that it does not. While some of the micrographs do indeed show uneven spacing, there appears to be no uniformity in the fibres which are grouped together (we can assert this in spite of the difficulties in numbering them), and there are many instances of uniform spacing. This variation is inconsistent with the concept of uneven spacing as a fixed structural feature, and while it does not exclude the occurrence of slight relative movement between outer fibres as a feature of flagellar bending, it seems more likely that the apparently uneven spacing in our micrographs was introduced by the preparative procedures. A uniform one-dimensional compression of about 18 per cent (which is to be expected in the preparation of thin sections (35)) would change even spacing into an uneven spacing similar to that described by Cleland and Rothschild.

The structure from which flagella and cilia arise has been given different names in different groups of organisms: blepharoplast, centriole, centroblepharoplast or basal granule in flagellates; kinetosome in ciliates; basal granule or basal corpuscle in cilia of metazoa; proximal centriole in metazoan sperm tails. Most of these are simply the inner ends of the cilia or flagella (to which, presumably, they give rise), and since, for the most part, they appear to be morphologically similar, it seems desirable to call them by one name. We propose the term *basal body*. (In some cases, and especially in complex flagellates such as *Trichomonas*, the basal bodies appear to be associated with various accessory structures which presumably have other morphogenetic functions (3; Grimstone, unpublished observations). It seems undesirable at present to include these other structures under the term basal body, which we feel should be restricted to the structure from which the cilium or flagellum actually arises.)

Most basal bodies possess a dense cylindrical structure with a less dense core (*e.g.*, 14, 39). In the flagellates we have examined, the cylinder is composed of nine triplet fibres, and it has the same composition in tracheal cilia (38), sea urchin sperm tails (1), the ciliate, *Stentor polymorphus* (Fig. 19 of reference 37), and the phytoflagellate, *Chlamydomonas* (Gibbons, unpublished observations). In view of the widespread distribution already found, it is reasonable to assume that most basal bodies have a cylinder of nine triplet fibres. The striking resemblance of a basal body to a centriole (4, 26) has been widely commented upon and taken as evidence for the (phylogenetic) derivation of one from the other.

In most cases that have been examined, the doublet fibres of the flagella and cilia are continuous with the triplets of the basal body (14, 38, 39), and it seems likely that the transition from triplet to doublet in these will be similar to that in flagellates. Amphibian and molluscan cilia, however, appear to have a different form of association between cilium and basal body, for here the doublet fibres end without entering the basal body (5, 14).

Transitional fibres extending outwards from the approximate level at which subfibre C terminates have been observed in the "cilia" of retinal rod cells (44) and in tracheal cilia of rat (Gibbons, unpublished observations), as well as in the flagellates described here. This widespread distribution suggests that they have some general significance, and it may be tentatively suggested that they are modified extensions of subfibre C which attach the end of the basal body to the cell membrane.

There is much less uniformity in the structures found within the lumen of basal bodies—as is typified by the difference in the three genera described here. In *Chlamydomonas* the central fibres end in a small intraluminal cylinder (16), while in most ciliates they end in association with a granule or basal plate (*e.g.*, 37, 42). In *Euplotes*, however, they continue, in a modified form, into the lumen of the basal body (39). In addition to these differences between groups, differences are also encountered among basal bodies of a single organism. In *Pseudotrichonympha* we have found some basal bodies to be filled with granules, and Randall and Fitton-Jackson (37) have reported a similar situation in *Stentor*. Some basal bodies of rat tracheal cilia contain a single large granule (38). There is no evidence yet concerning the significance of these "granule-containing" basal bodies.

We can say little regarding the function of the intraluminal structures in the flagellates we have studied. The basal bodies are atypical in being much longer than the usual 0.3 to 0.4 μ of other organisms. This is particularly true of *Pseudotrichonympha* and *Trichonympha*, in which the basal bodies are up to 5 μ long. The differentiation into proximal and distal segments is possibly correlated with this greater length, and the cartwheel structure may well act simply as a stiffener. In *Holomastigotoides*, in which the basal body is shorter, there is no such differentiation and no cartwheel structure.

The links which appear to join together the triplet fibres are of comparatively widespread distribution, but they do not seem to be essential for maintaining the integrity of the cylinder or for the inward tilt of the fibres, because they are lacking in the "granule-containing" basal bodies in *Pseudotrichonympha*. The inward tilt of the triplet fibres seems a uniform feature of all basal bodies and also of centrioles, but its significance is almost completely obscure. Possibly related to it are the other skewed structures of the flagellate basal bodies: the transitional fibres and the spokes of the cartwheel structure. If one postulates a mechanical, semi-rigid basal body that is free to rotate except for attachment by the transitional fibres to the cell surface, then the bending of the spokes of the cartwheel, the tilt of the triplet fibres, and the inclination of the transitional fibres are all such as would be given by the application of an anti-clockwise twisting couple to the hub of the cartwheel. However, there is no reason at present to regard this as more than a mnemonic.

As already mentioned, the tilt of the outer fibres in the basal body and their arms in the flagellum render the flagellar unit asymmetrical, and it could theoretically exist in two enantiomorphic forms—clockwise and anti-clockwise in the convention defined previously. Our observations on *Trichonympha* suggest that all basal bodies and flagella in this genus have the clockwise orientation. In that the flagella are presumably morphogenetic derivatives of the basal bodies, the asymmetry of the latter is the more fundamental. One implication of a uniform orientation is that the flagellum always arises from the same end of a basal body. The "rule of desmodexy" (8), according to which the kinetodesma in ciliates always lies to the (ciliate's) right of the basal body, provides another example of asymmetry related to basal bodies. It seems likely that all motile flagella and cilia will prove to have the same, clockwise, orientation. However, if both enantiomorphs should exist, then a study of their distribution in a range of organisms and tissues might prove to be of some genetic or phylogenetic interest, though caution would be necessary in making such interpretations (24).

We have found a longitudinal periodicity of about 130 A associated with the central fibres, the arms on the outer fibres, and the "spokes" of the cartwheel structure in the basal body. The repeated occurrence of this periodicity suggests

that it has some fundamental significance in the structure of the flagellum.

The functional need for the two distinct systems of fibres associated with the basal bodies in *Trichonympha* and *Pseudotrichonympha* is not obvious. One system is presumably concerned with coordinating the beat of the flagella, while the other might serve as a series of mechanical links to hold the basal bodies together. However, other possibilities cannot be excluded. Of the two systems in *Trichonympha*, the complex system of fine fibres seems the most likely candidate for coordination by reason of its regular linking of specific triplet fibres in adjacent basal bodies, though the manner in which such a system might function is at present totally obscure.

The links between the flagellar outer fibres and the membrane of the body groove in *Trichonympha* and *Pseudotrichonympha* might, perhaps, serve to prevent bending or twisting in the groove but it seems more likely that their primary function is to provide further mechanical support for the flagella and so maintain the integrity of the flagellar rows. The corresponding anchor-ribbon in *Holomastigotoides* may serve similarly.

Mechanism of Flagellar Movement:

It may be assumed that the bending waves of a flagellum are the result of the localized shortening of longitudinal contractile elements (31). So it is of interest to examine the extent of the similarities between flagella and muscle. Structurally, there are rather striking resemblances between flagella and vertebrate striated muscle (28): both consist of an array of large and small fibres and in both the large fibres bear projections and show evidence of helical substructure. However, there are also important differences. The large fibres of flagella are of two kinds (central and outer), both complex; they are continuous; and their projections point towards adjacent large fibres. In muscle the large fibres (the primary filaments) seem simpler, are discontinuous, and their projections point towards adjacent small fibres (the secondary filaments). There are evidently important physiological and biochemical differences as well. The observed undulations of flagella can be produced by localised contractions of about 5 per cent of the resting length, whereas muscle may contract by as much as 50 per cent. Furthermore, glycerinated flagellar models undergo repeated undulations in the presence of adenosine triphosphate (27), in contrast to muscle models which give only a single irreversible contraction (43). The results of biochemical analyses of flagella are not yet as clear cut as those of muscle but it seems that the major component of flagella is similar to myosin and, like it, possesses adenosine triphosphatase activity. No component similar to the actin of muscle has been found in either the cilia of *Tetrahymena* or bull sperm tails (7, 9).

In considering possible mechanisms by which local contractions might occur along the length of the flagellum a sliding fibril mechanism, similar to that proposed by Hanson and Huxley (25) for striated muscle, is an obvious possibility. In particular, the apparently unequal length of the outer fibres and subfibres in a flagellar tip suggests that some might slide relative to others; however, a serious objection to any such hypothesis is the fact that flagella often assume the form of one complete wave length or more, at a given instant (19, 20). Hence, since the fibres apparently all run straight, there must occur, simultaneously, limited regions of relative movement in both directions at different positions along a single fibre, which could only happen if the fibres were also elastically extensible. This seems improbable.

Two other mechanisms can be envisaged. First, a fibre might wrinkle. There is no evidence for this. Second, contraction might occur by actual shortening of the fibres, through some form of molecular rearrangement. Present evidence favours this hypothesis in default of the other two. It has the advantage of being able to account for the comparatively large peripheral fibres found in those flagella where bending entails bending of accessory structures as well as the axial filament itself: for example, in the mid-piece of mammalian sperm (13); in some invertebrates (*e.g.* locust (15)) in which mitochondrial cylinders run along much of the sperm tail; and in the flagellate, *Pyrsonympha vertens*, in which the flagella are attached along their length to the body surface and cause it to move as a whole (18).

Tubule-like fibres, similar to those of flagella, occur in a variety of contractile organelles in protozoa. The axostyle of *Pyrsonympha* is of particular interest for it undulates in a way somewhat similar to a flagellum. Grassé (18) has shown that it is composed of stacks of lamellae made up of numerous tubular fibres, each morphologically similar to an outer subfibre. In the contractile ciliate, *Stentor*, the kinetodesmata are composed of layered tubular fibres (37), while in non-contractile *Paramecium* (42) or *Tetrahymena* (34) the

kinetodesmata, although otherwise similar, are composed of comparatively large cross-striated fibres. Roth (40) has noted other examples of tubular fibres in the cytoplasm of Protozoa. In view of the possibility that a helical substructure will be found generally in such tubular fibres, it is worth noting that, in principle, helical structures can undergo contraction by such minor changes as tightening of the coils. The only case known at present of a helical structure of this order of size which is contractile under physiological conditions seems to be the tail sheath of bacteriophage T2 (6).

If contractility resides in the individual fibres, it may be suggested that much of the additional complexity of the flagellum is concerned with the coordination of contractions to yield undulatory waves. Bradfield (5) has discussed possible mechanisms by which this might come about.

CONCLUSION

Considering the whole organisation of the flagellar apparatus in these organisms one cannot fail to be impressed by its extraordinary complexity. The relative simplicity of what might be called the "nine-plus-two" concept of the flagellum has given way to a far more sophisticated picture, in which the number of different components and the complexity of their structure and arrangement have both greatly increased. Quite probably complexity of this order will be found generally at this level of organisation: we have a comparable example in the exquisite morphology of the T2 bacteriophage (6). It raises, clearly, daunting problems, not only of functional interpretation, but of morphogenesis and evolution. What processes were and are responsible for the generation of these structures? At the simplest level, if a flagellum is an "outgrowth" from a previously formed basal body, how are we to account for the structure of the tips of the outer fibres, and where do the secondary and central fibres and the arms of the outer fibres come from? What is the origin of the complex, asymmetrical, yet highly constant arrays of delicate fibres which link together specific subfibres of the basal bodies? We can, of course, offer no answers to these questions. It is already clear, however, that no simple explanation in terms of "crystallisation" or orientation under mechanical stress is likely to be adequate.

One of us (A. V. G.) is much indebted to Professor L. R. Cleveland for an invitation to work at the Biological Laboratories, Harvard University. We are grateful to Dr. E. M. Miller, University of Miami, for assistance in obtaining *Prorhinotermes*, and to Mr. R. P. Ambler and Mr. R. J. Skaer for reading and criticising the manuscript.

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Explanation of Plates

Except where otherwise noted, all figures show preparations fixed with 1 per cent osmium tetroxide and section-stained with uranyl acetate.

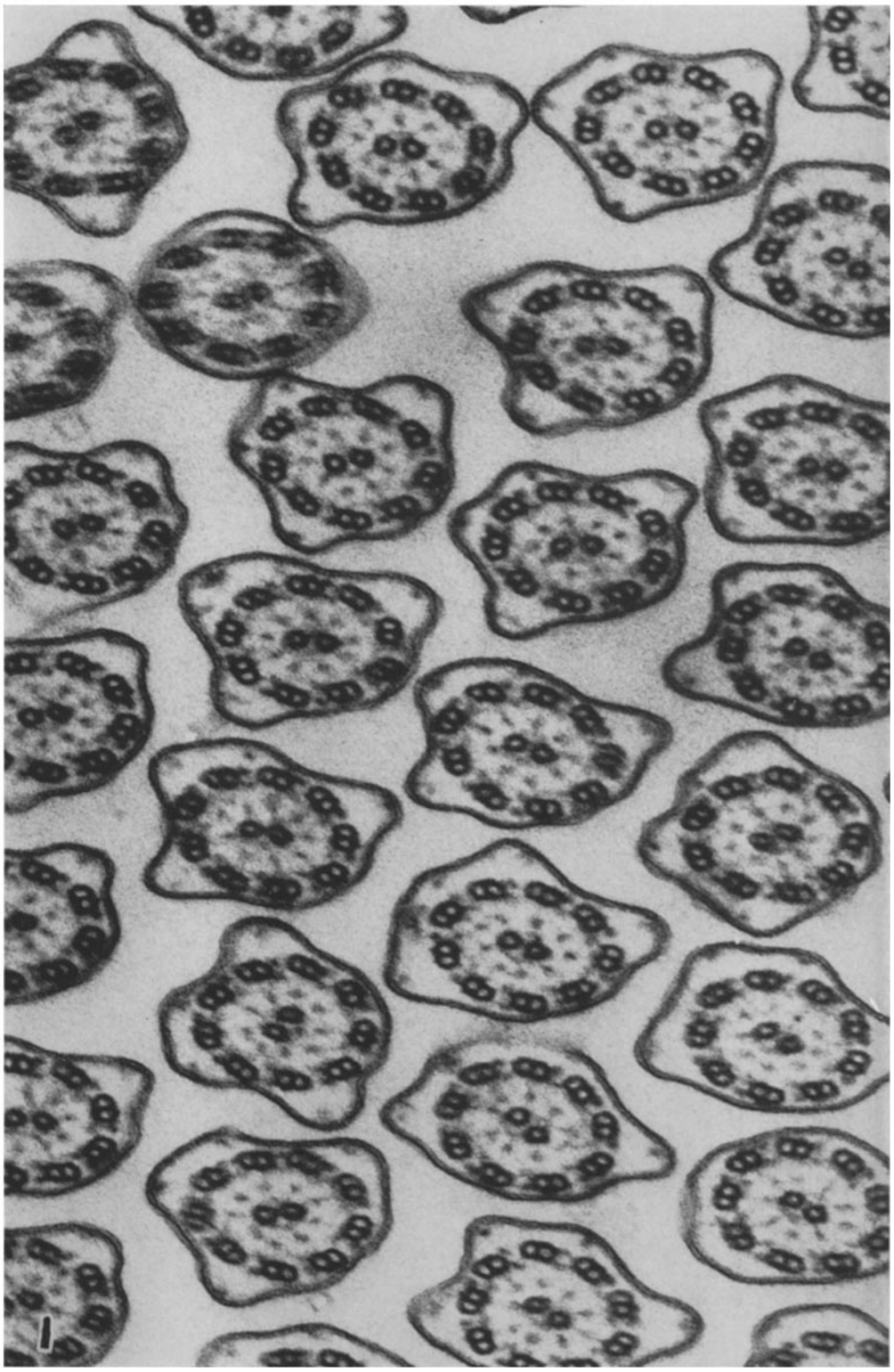
All transverse sections are shown with clockwise orientation. This corresponds to the view seen by an observer looking outwards from the proximal end of the basal body towards the tip of the flagellum.

Key to Symbols

TF indicates the level of the transition from basal body to flagellum.
TB indicates the level of the transition between the proximal and distal regions of basal body.
ar anchor ribbon.
cf central fibre.
cm cell membrane.
cy cylinders.
fm flagellar membrane.
of outer fibre.
sf secondary fibre.
t transitional fibre.

Plate 349

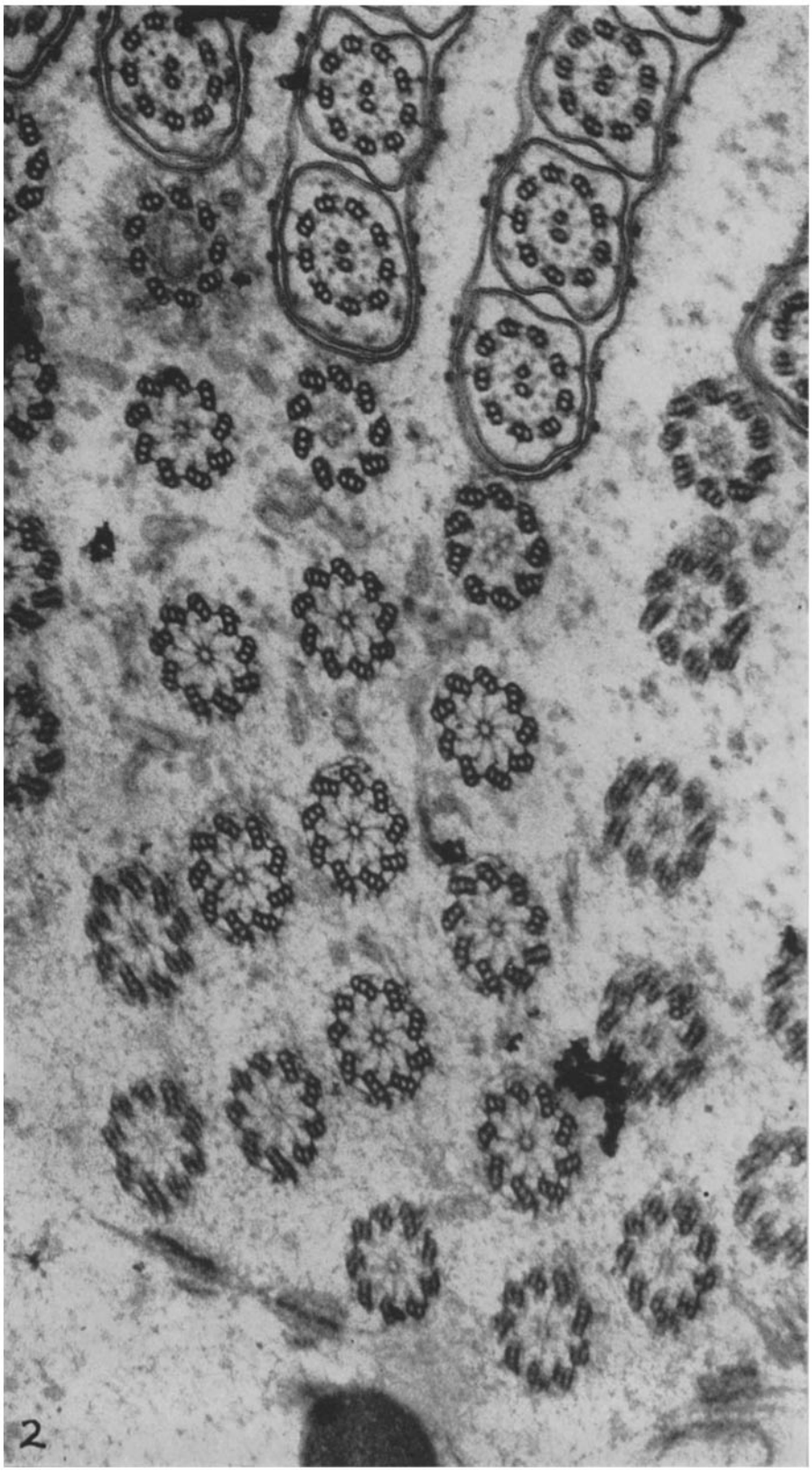
Fig. 1. Transverse sections through flagella of *Pseudotrichonympha*. The flagellar membrane shows a characteristic profile that is possibly a remnant of its quadrilateral form in the flagellar grooves (see text). In many of the sections four rather irregular condensations of dense material are present just under the membrane × 140,000.



(Gibbons and Grimstone: Flagellar structure in certain flagellates)

PLATE 350

FIG. 2. Transverse sections through basal bodies and flagella in the anterior body-region of *Pseudotrichonympha*, including the transition from the former to the latter. At the top are the flagellar grooves and at the bottom are sections through the proximal ends of the basal bodies. × 95,000.



(Gibbons and Grimstone: Flagellar structure in certain flagellates)

PLATE 351

FIGS. 3 to 13. Transverse sections cut at an ordered series of levels of flagella and basal bodies in the anterior body-region of *Pseudotrichonympha*. Figs. 6, 7, 9, 13, × 150,000; other Figs., × 130,000. Figs. 4, 8, 10 to 12 are enlargements from Fig. 2.

FIG. 3. Flagellum outside body.

FIG. 4. Three flagella in a flagellar groove.

FIG. 5. Flagellum at bottom of groove. The tip of the crescentic body is within the plane of section.

FIGS. 6 and 7. Slightly below the level of Fig. 5.

FIG. 8. Transition of basal body to flagellum. The C subfibres end within the plane of the section and so appear with lower contrast than the other subfibres. The moderately dense material in the lumen of the cylinder is the basal plate. The transitional fibres are visible and in some cases appear to consist of two fine filaments. The bend in the cell membrane, where it evaginates to become the flagellar membrane, is cut tangentially by the plane of the section, and it appears as moderately dense material overlapping the transitional fibres.

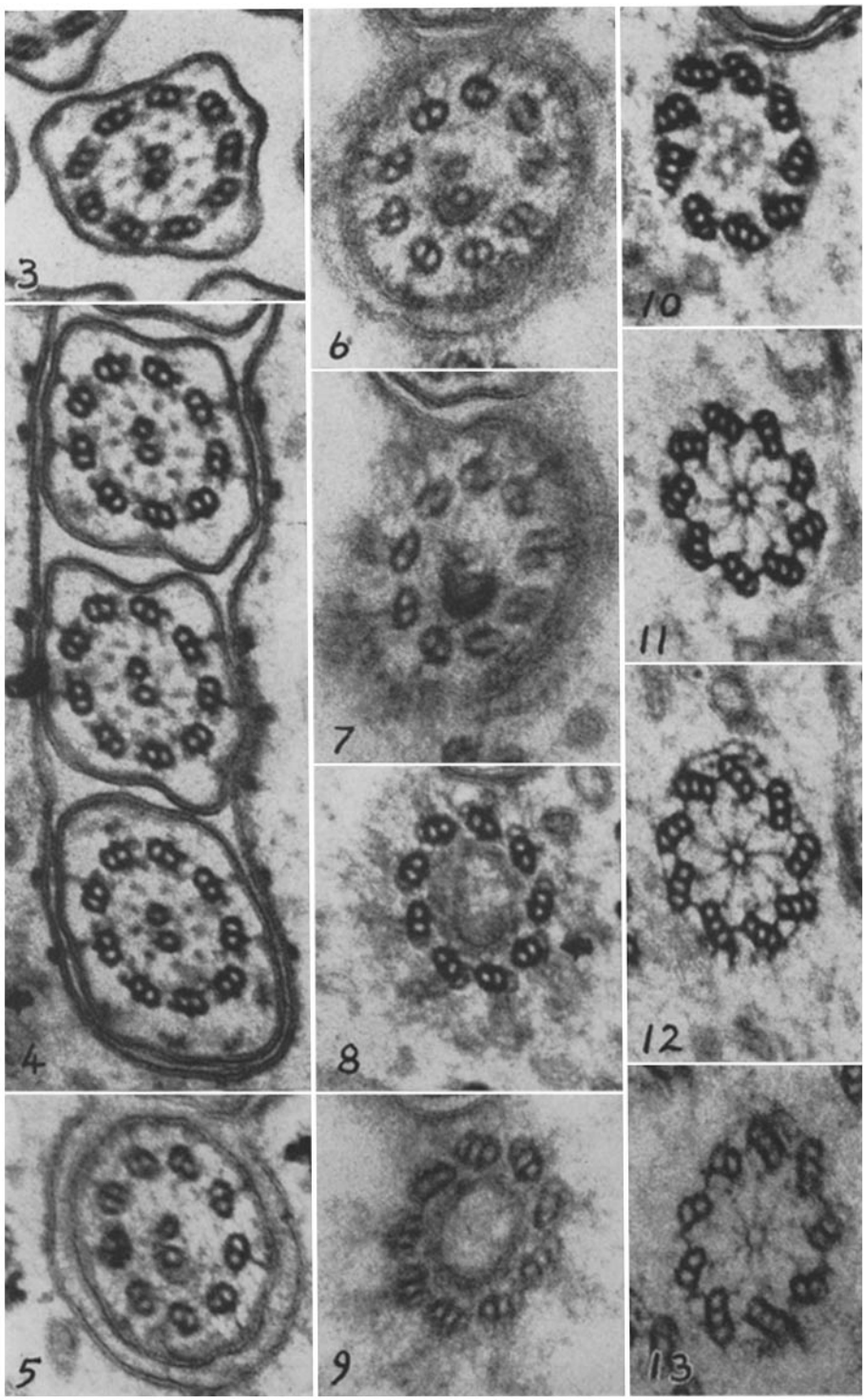
FIG. 9. Slightly below Fig. 8, showing the raised rim on the bottom of the basal plate.

FIG. 10. The distal region of basal body, with three cylinders in the lumen.

FIG. 11. The proximal region of basal body, with cartwheel structure in the lumen.

FIG. 12. Somewhat proximal to Fig. 11. Fine fibres link the C subfibres of adjacent triplets.

FIG. 13. Near the proximal end of the basal body. Some of the triplet fibres have reverted to doublets.



(Gibbons and Grimstone: Flagellar structure in certain flagellates)

PLATE 352

FIG. 14. Median longitudinal section of flagellum and basal body in the anterior body-region of *Pseudotrichonympha*. It extends from the flagellar groove through the distal region of basal body and includes part of the proximal region. × 130,000.

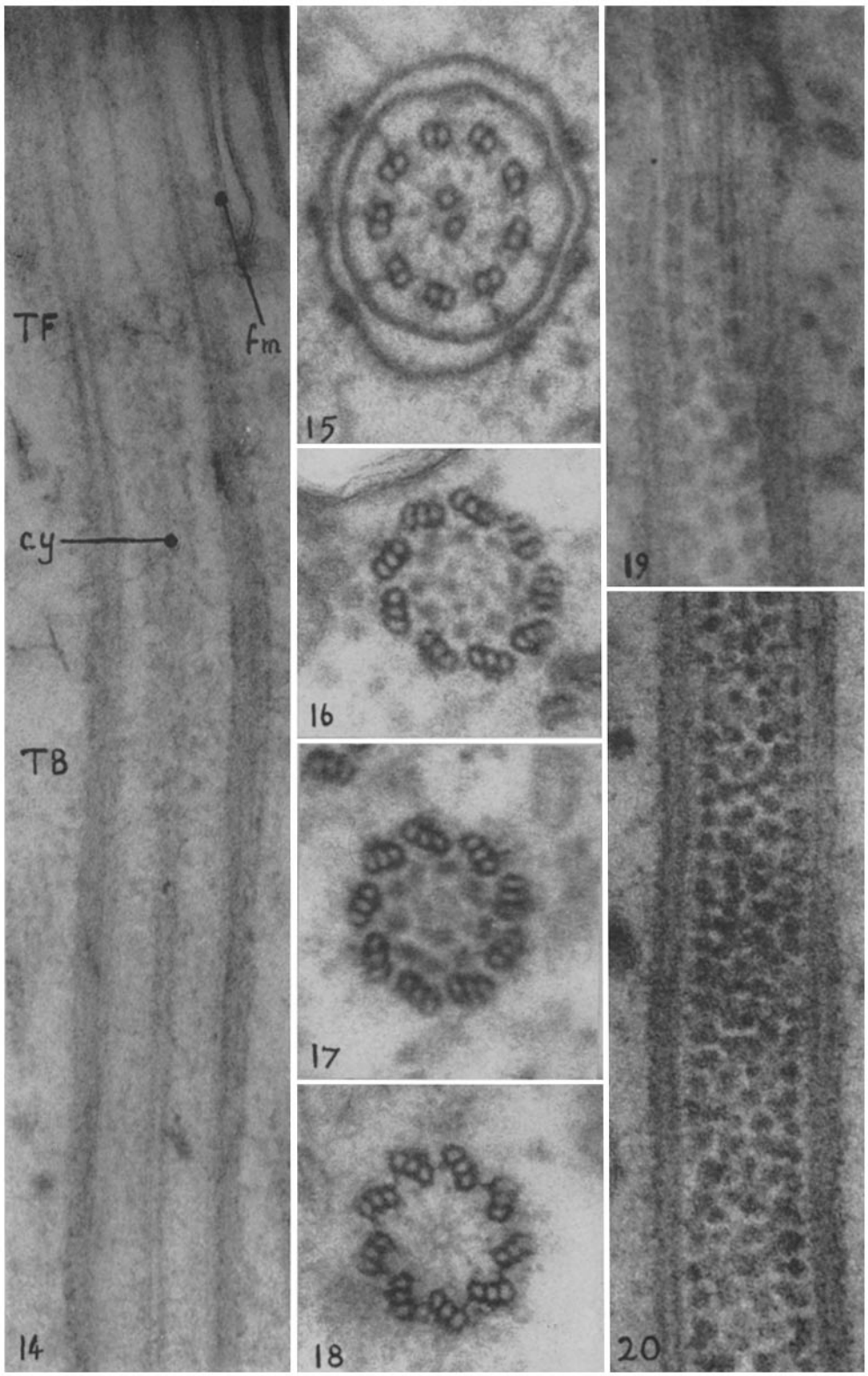
FIGS. 15 to 18. Transverse sections at an ordered series of levels of flagella and basal bodies in the posterior body-region of *Pseudotrichonympha*. × 150,000.

FIG. 15. Flagellum emerging through a pit.

FIGS. 16 and 17. Distal region of basal body containing granules.

FIG. 18. Proximal region of basal body containing cartwheel structure.

FIGS. 19 and 20. Longitudinal sections through distal region of basal body in the posterior body-region of *Pseudotrichonympha*. They show the granules arranged in rows along the triplet fibres. × 150,000.



(Gibbons and Grimstone: Flagellar structure in certain flagellates)

PLATE 353

FIGS. 21 to 26. Transverse sections at an ordered series of levels of flagellum and basal body of *Trichonympha* × 150,000.

FIG. 21. Two free flagella running parallel to the body surface (stained with PTA).

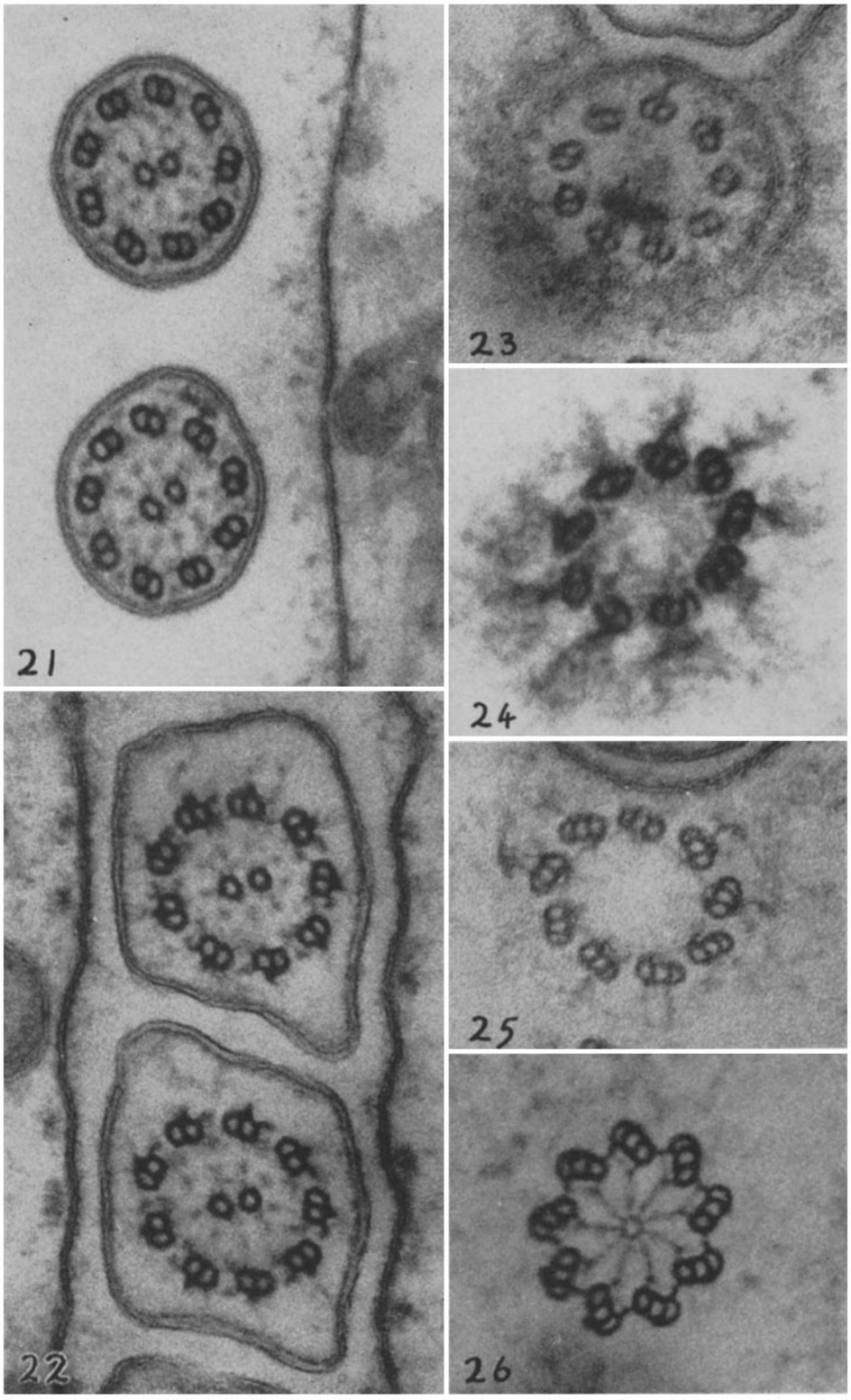
FIG. 22. Two flagella in a flagellar groove. The central fibres are inclined at a greater angle to the plane of the groove than is usually the case.

FIG. 23. Flagellum just above transition showing the crescentic body around one of the central fibres.

FIG. 24. Transition of basal body to flagellum (stained with PTA).

FIG. 25. Distal region of basal body showing structureless lumen and A-A connections between triplets.

FIG. 26. Proximal region of basal body showing cartwheel structure.



(Gibbons and Grimstone: Flagellar structure in certain flagellates)

PLATE 354

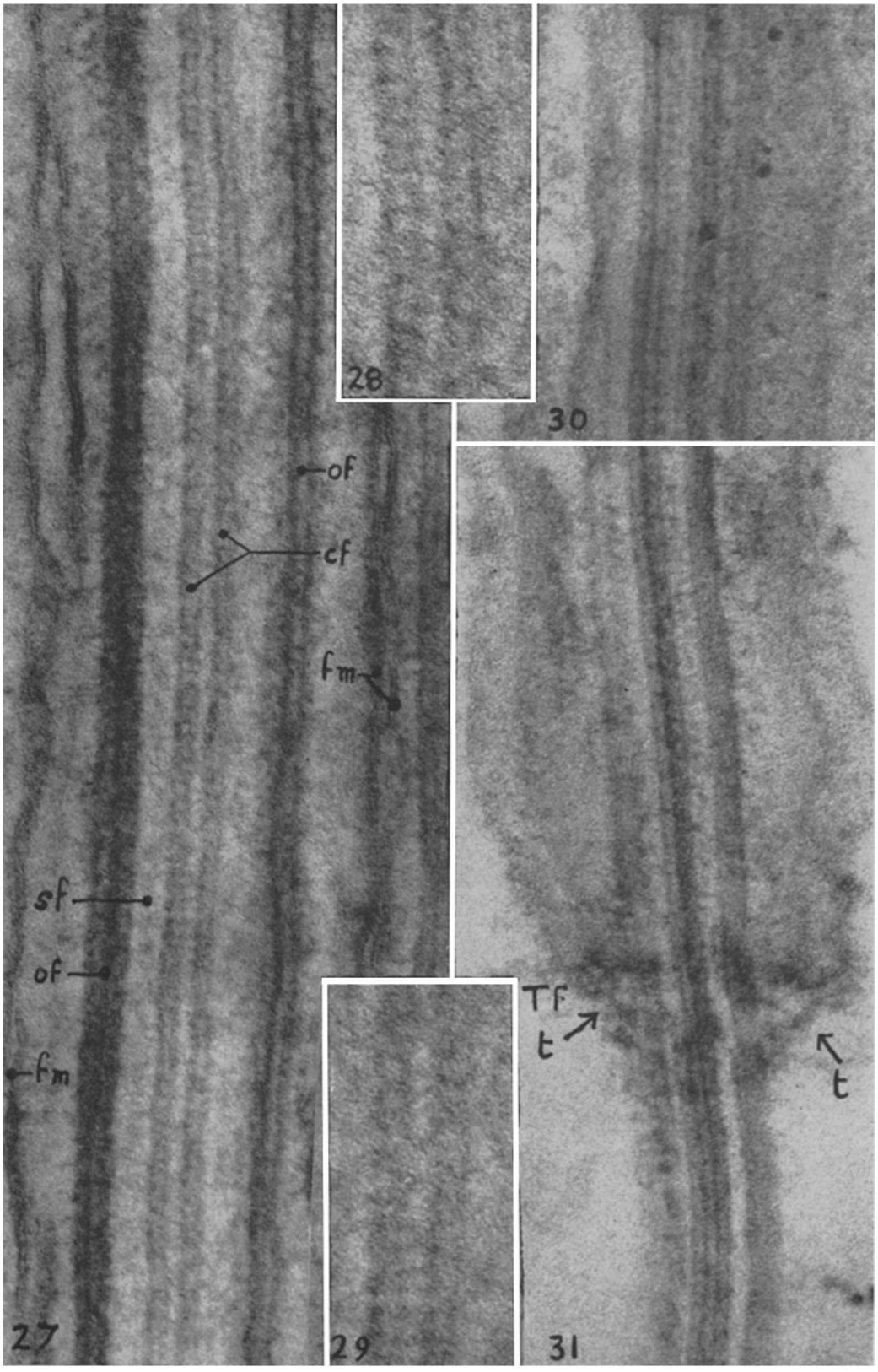
FIGS. 27 to 31. Longitudinal sections of flagella of *Trichonympha*.

FIG. 27. Median section with the two central fibres lying side by side. × 150,000.

FIGS. 28 and 29. Areas of Fig. 27 at higher magnification, showing the helical substructure of the central fibres. × 300,000.

FIG. 30. Tangential section showing three of the outer fibres. On the fibre at the left, the arms are visible as projections. × 150,000.

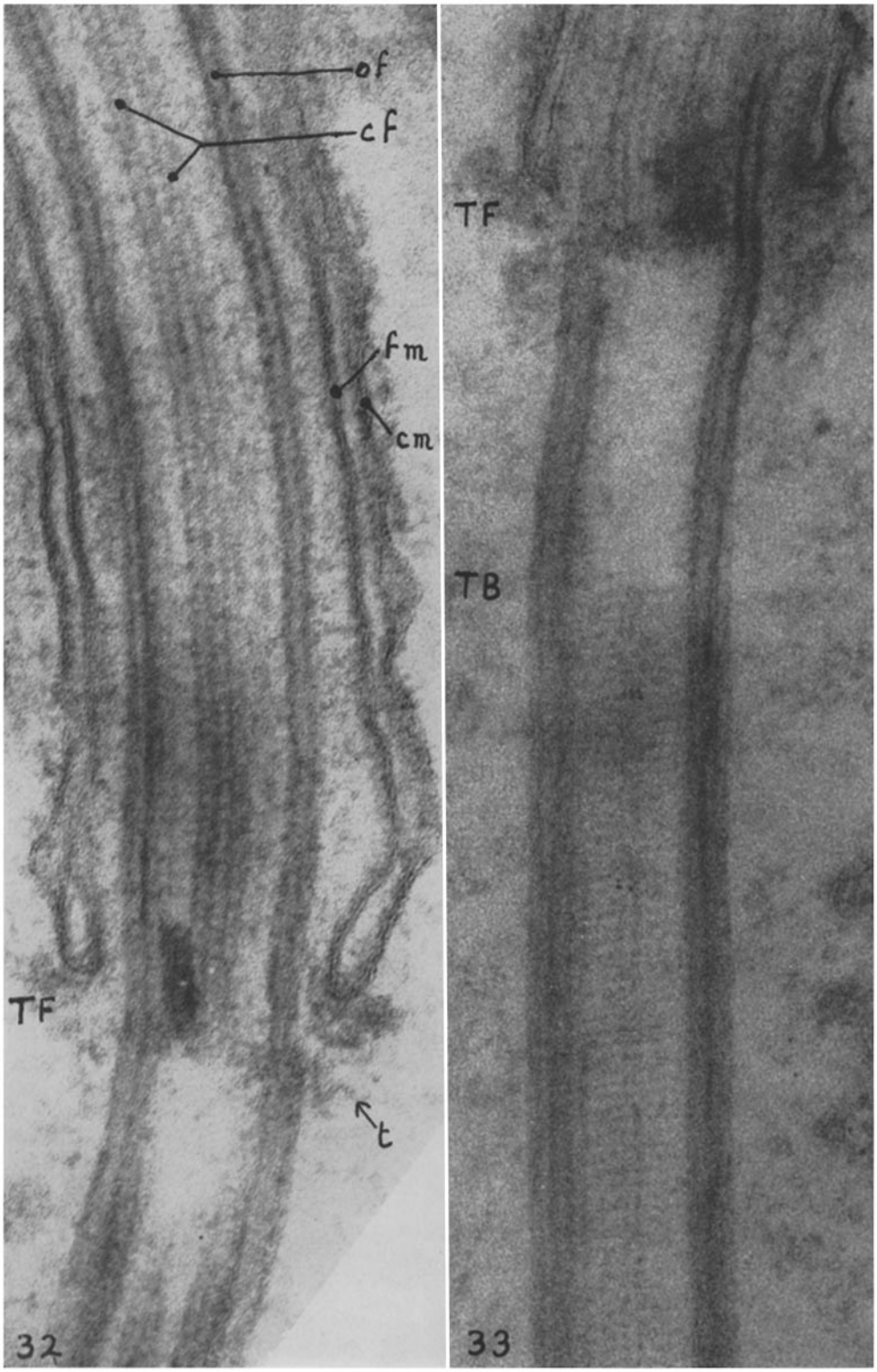
FIG. 31. Tangential section through the transition of basal body to flagellum. Three outer fibres are visible. The one in the centre shows that subfibre C terminates and subfibre A gains its arms in the transitional region. The membrane appears as a moderately dense area overlapping the outer fibres of the flagellum. × 150,000.



(Gibbons and Grimstone: Flagellar structure in certain flagellates)

PLATE 355

FIGS. 32 and 33. Median longitudinal sections through the transition of basal body to flagellum in *Trichonympf* × 150,000.



(Gibbons and Grimstone: Flagellar structure in certain flagellates)

PLATE 356

FIGS. 34 to 44. An approximately ordered series of transverse sections through the tips of flagella. Figs. 38 and 44 show *Trichonympha*; the rest show *Pseudotrichonympha*. × 100,000.

FIG. 34. Only three secondary fibres are present and four outer fibres have arms.

FIG. 35. No secondary fibres are present. One outer fibre has arms. The central fibers are displaced slightly from the centre.

FIG. 36. One doublet fibre has changed to a singlet and one subfibre ends within the plane of the section.

FIG. 37. Four doublet and seven singlet fibres.

FIG. 38. Eleven singlet fibres.

FIG. 39. One doublet and nine singlet fibres.

FIG. 40. Eight singlet fibres.

FIG. 41. Seven singlet fibres.

FIG. 42. One doublet and four singlet fibres.

FIG. 43. Four singlet fibres. Two singlet fibres.

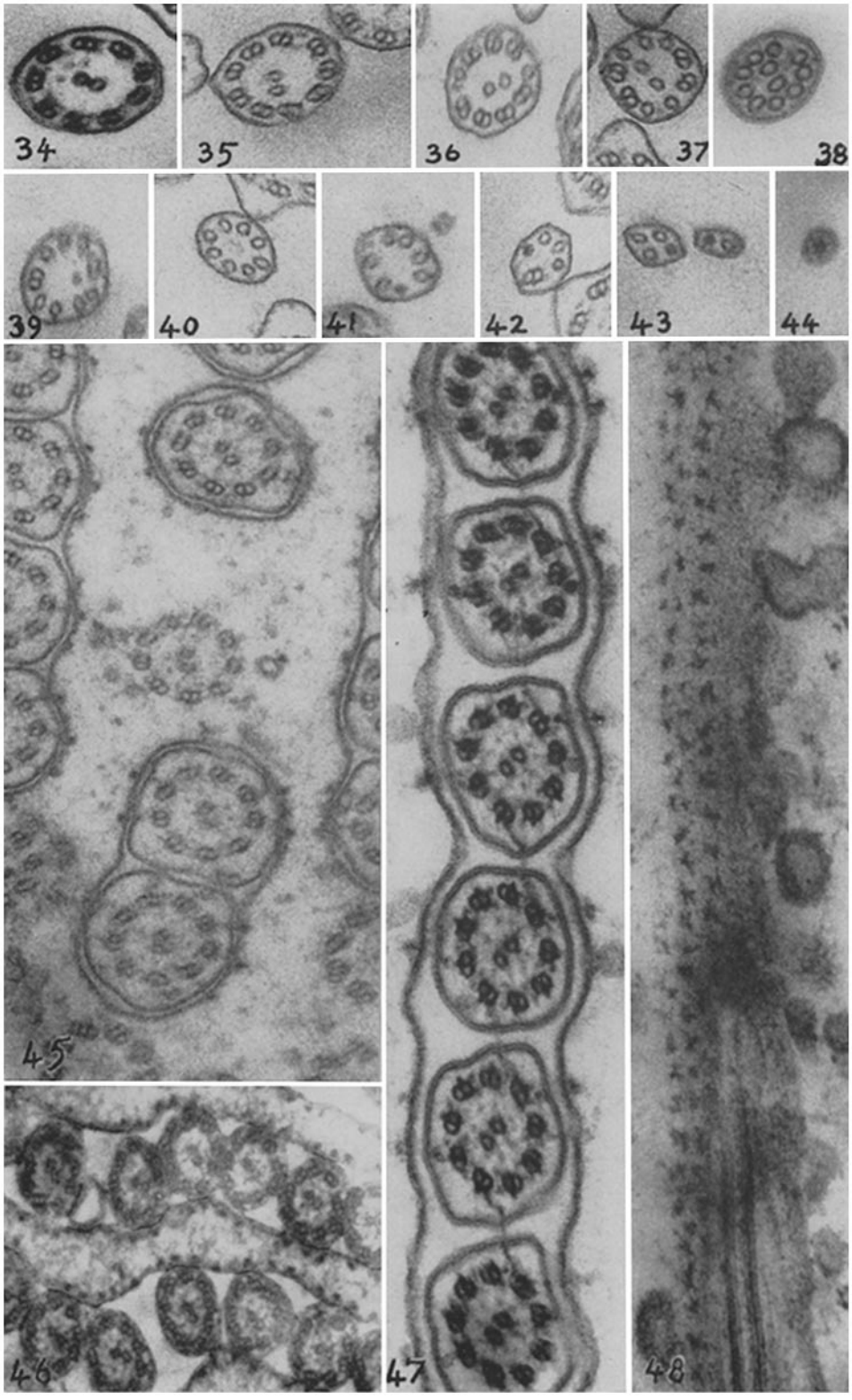
FIG. 44. One singlet fibre.

FIG. 45. Transverse section of a flagellum of *Pseudotrichonympha* which appears to lack a flagellar membrane, so that the flagellar fibres seem to lie loose in the cytoplasm. This section is cut about 2μ from the basal body. The membrane is probably present further up the flagellum. × 80,000.

FIG. 46. Transverse section of flagellar grooves in a preparation of *Pseudotrichonympha* fixed with formaldehyde. The flagella appear much smaller than after the usual fixation with osmium tetroxide. × 100,000.

FIG. 47. Flagellar groove of *Trichonympha*, with links between adjacent flagella. × 100,000.

FIG. 48. Tangential longitudinal section of flagellar groove in *Trichonympha* showing the three rows of anchor granules on the inner surface of the cell membrane. × 50,000.



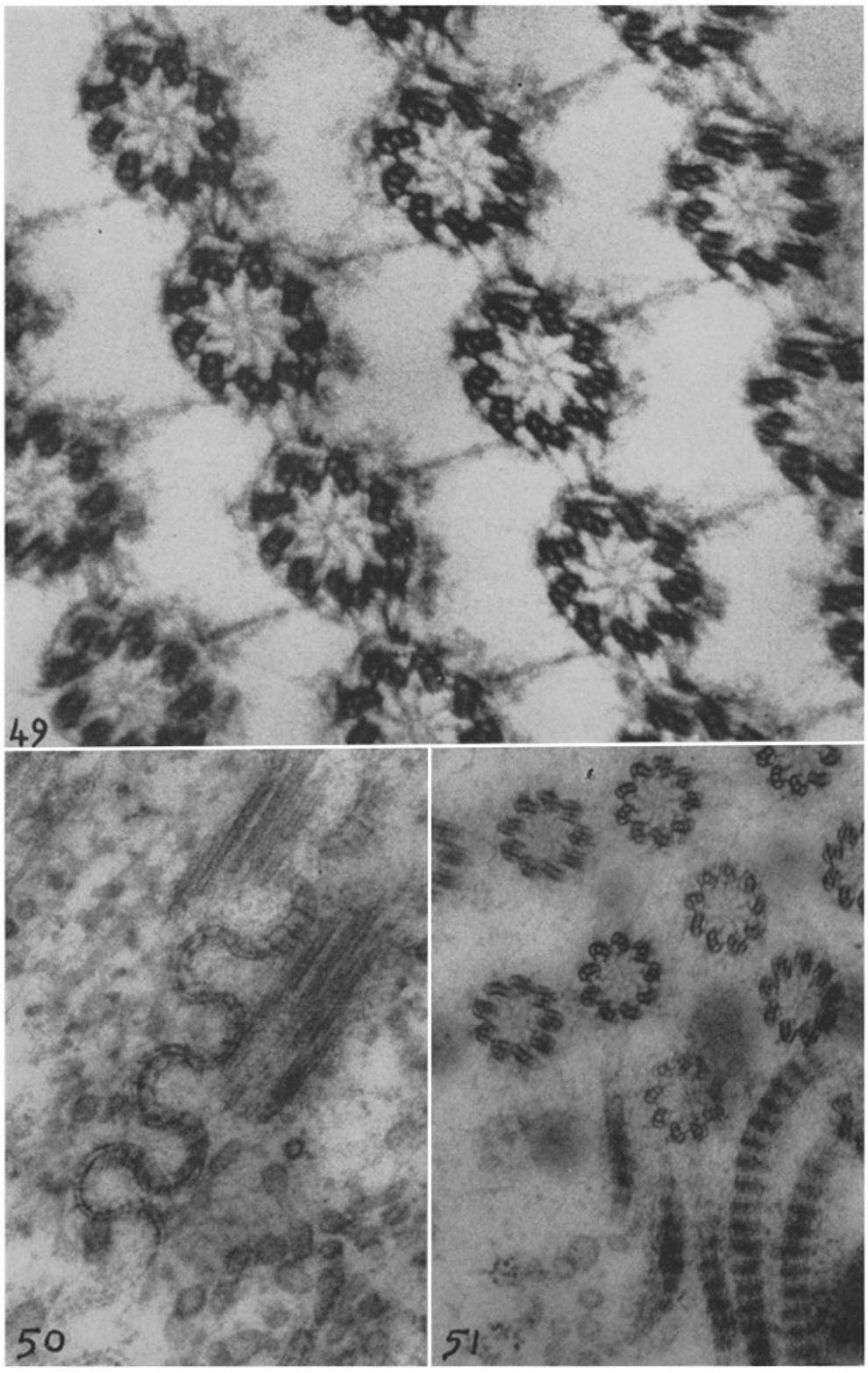
(Gibbons and Grimstone: Flagellar structure in certain flagellates)

PLATE 357

FIG. 49. Transverse section of proximal region of basal bodies in the rostrum of *Trichonympha* showing the delicate network of fine fibres linking adjacent basal bodies (*cf.* Text-fig. 4). The flagellar rows run diagonally upward to the left; sections at the top of the micrograph are distal to those at the bottom. × 125,000.

FIG. 50. Longitudinal section of the sinuous fibrous ribbon that underlies the basal bodies in the posterior body-region of *Pseudotrichonympha*. × 75,000.

FIG. 51. Longitudinal sections of the cross-striated fibres that underlie some of the post-rostral basal bodies in *Trichonympha*. × 75,000.



(Gibbons and Grimstone: Flagellar structure in certain flagellates)

PLATE 358

FIGS. 52 to 57 all show *Holomastigotoides*.

FIG. 52. Transverse section through two flagella. × 150,000.

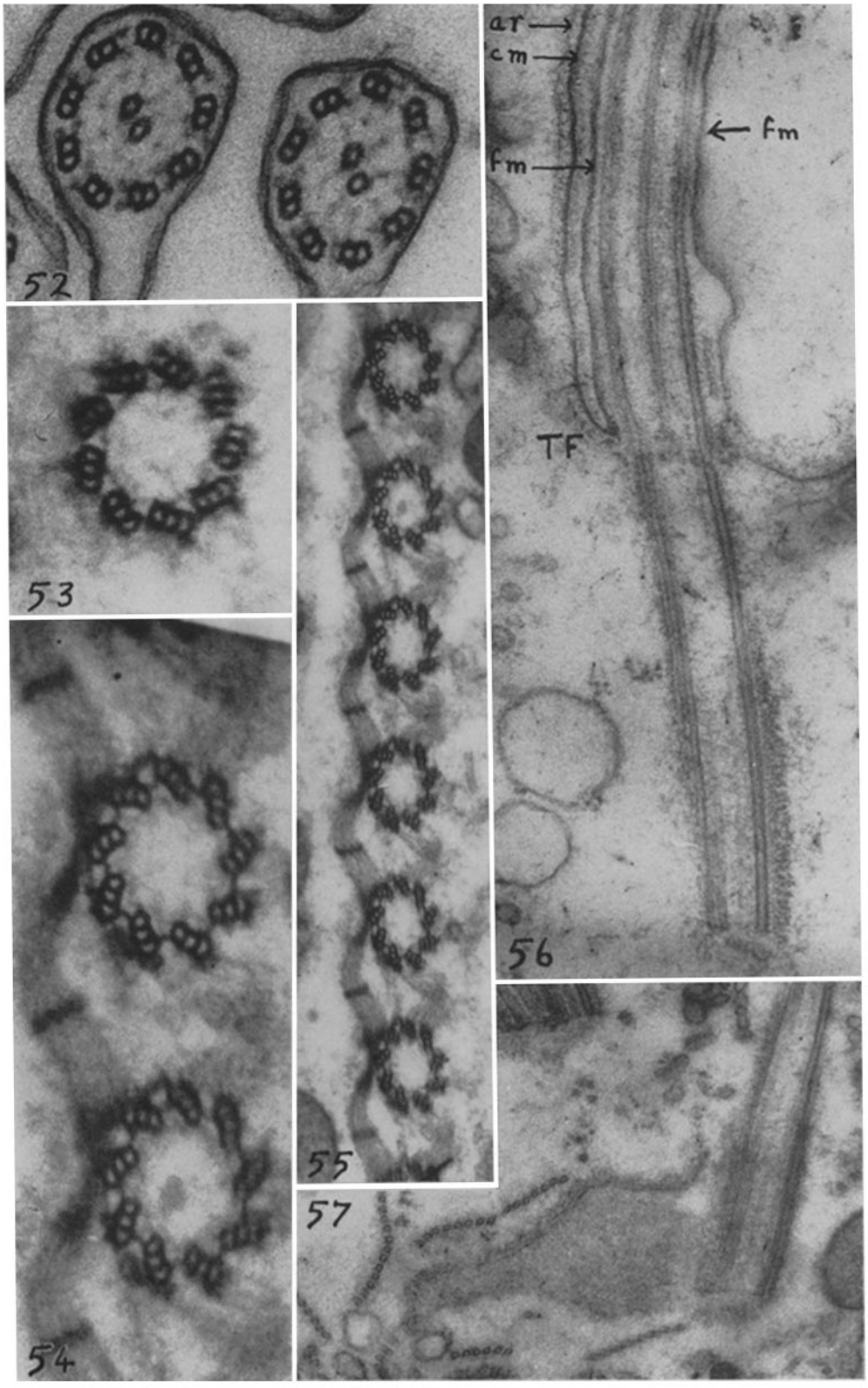
FIG. 53. Transverse section of basal body just below the transition from flagellum, showing A-A connections between triplets. × 150,000.

FIG. 54. Transverse sections of basal bodies at a level further proximal than Fig. 53, showing A-C connections between triplets and the fibrous ribbon linking adjacent basal bodies. × 150,000.

FIG. 55. Transverse section of basal bodies, showing the fibrous ribbon linking them together. × 70,000.

FIG. 56. Longitudinal section through the entire length of a basal body and the proximal part of flagellum. × 75,000.

FIG. 57. Longitudinal section of a basal body near the anterior end of organism showing the large band associated with the basal body. × 50,000.



(Gibbons and Grimstone: Flagellar structure in certain flagellates)