SUBSIDIARY COMPONENTS OF THE FLAGELLA OF CHLAMYDOMONAS REINHARDII

J. M. HOPKINS
Department of Biophysics, University of London Kings College, 26-29 Drury Lane, London, W. C. 2, England

SUMMARY

Some subsidiary components of flagella from the alga Chlamydomonas reinhardii have been studied in the electron microscope using frayed and partially dispersed material, negatively stained. In all, 5 distinct subsidiary structures have been observed, 3 of which are associated with the 9 pairs of outer tubules and 2 with the central pair of tubules.

1. Radial spokes, about 33 nm long and 5 nm in diameter, are attached at right angles to the A tubule of each outer pair and extend into the lumen of the flagellum in the direction of the central pair of tubules, but do not reach them. The spokes usually occur in pairs along the length of each A tubule. The interval between pairs is about 70 nm and that between the 2 members of each pair about 30 nm.

2. 'Secondary fibres'. The distal end of each spoke terminates in a hammerhead-like attachment some 10-20 nm by 5 nm with its axis parallel to the long axis of the flagellum. These hammerhead attachments are now identified with the so-called 'secondary fibres' previously deduced from micrographs of embedded and sectioned material. There is no evidence from the present work of a continuous secondary fibre throughout the length of the flagellum.

3. Side arms are found attached to the A tubule of each outer pair. These arms, which occur in pairs, are roughly at right angles to the radial spokes which are also attached to the A tubules. The side arm material is distributed along the tubule at regular intervals of about 14 nm.

4. The chemically more stable centre tubule has 2 longitudinal rows of projections, each projection being about 18 nm long with a repeat distance of about 16 nm.

5. Occasionally, on the chemically less stable centre tubule, there is observed one row of projections which are somewhat similar to those on the other tubule.

New information has made it possible to reinterpret earlier work and to present a 3-dimensional picture of the external flagellum and its parts.

INTRODUCTION

The more obvious features of the fine structure of cilia, flagella and sperm tails are now well known from the work of, for example, Fawcett & Porter (1954), Afzelius (1959), Gibbons & Grimstone (1960), Ringo (1967) and many others. In addition various other components have been described, such as the spokes and so-called secondary fibres, together with still other structures associated with the central pair of tubules. These subsidiary—but perhaps functionally important—structural features have been less easy to observe and characterize and precise details are unknown.

The object of this paper is to remedy this defect as far as possible for the flagella of Chlamydomonas reinhardii, as a step towards understanding what part (if any) each component plays in the overall function of the organelle.

Fig. 1 summarizes diagrammatically the most commonly observed results of many
workers on the main and subsidiary structural components as they would appear in transverse section. This diagram is for guidance only as it is impossible to include all items or variants that have been reported in the literature. So far as is known, all these structures are to be found along the greater part of the length of the external flagellum, extremities excepted. Most of the information contained in Fig. 1 has been derived from sectioned material. From such observations it has been suggested that

attached to tubule A of each outer doublet (AB) there are 2 types of structure: (i) hooked side arms (sa) lying in the circumferential gap between peripheral pairs (Allen, 1968), and (ii) radial spokes (rs), placed roughly at right angles to the side arms and which connect the 9 peripheral doublet tubules to the 2 central tubules (Afzelius, 1959; Gibbons & Grimstone, 1960; Gibbons, 1961; Birge & Doolin, 1969). At a point about mid-way along the radial spoke, as seen in transverse section, there is a concentration of material termed by Gibbons & Grimstone (1960) a secondary fibre (sf). They suggested that it was a slender continuous fibre running longitudinally along the length of the flagellum, though some doubt has been placed on this interpretation by Birge & Doolin (1969). Fawcett (1961), Birge & Doolin (1969) and Allen (1968) have reviewed and supplemented information on some of these subsidiary structures, again from information based mainly on sectioned material. A number of minor structures believed to be associated with the central pair of tubules have also been reported from the examination of sectioned material. These are more difficult to
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summarize in a single diagram and have been indicated by dotted lines in Fig. 1. The sheath described by Gibbons & Grimstone (1960) for *Trichonympha* is represented by ss, while the twin projections α, β have been observed by Allen (1968), Williams & Luft (1968) and J. M. Hopkins & Sir John Randall (unpublished) in *Tetrahymena* and identified by Chasey (1969) as 2 longitudinal rows of projections. These have been termed 'rungs'. The additional projections (γ) have been identified in the present study on *Chlamydomonas*.

The difficulty of repeating observations on all minor structures in sectioned material has led a number of workers to study frayed flagella and sperm tails negatively stained or metal shadowed for corresponding evidence (see Manton & Clarke, 1952; André & Thiéry, 1963; Pease, 1963; Grimstone & Klug, 1966; Hookes, Randall & Hopkins, 1968; Chasey, 1969). It now appears that the use of phosphotungstic acid (PTA) destroys or disrupts many of the minor components previously observed in sectioned material. Chasey (1969) has also suggested that negatively staining with PTA has an adverse effect on preservation of some of the more delicate structures found in the cilia of *Tetrahymena pyriformis*. Some investigators using this method were chiefly concerned with the apparent subunit structure of the axoneme tubule, and little definitive information was obtained relating to the minor structures with which this paper is chiefly concerned. Below we give further details of the structure and arrangement of subsidiary components found in the flagella of *Chlamydomonas reinhardii* using fragmented material stained with uranyl acetate.

MATERIALS AND METHODS

The biflagellate green alga *Chlamydomonas reinhardii* was allowed to grow for 3 days in a simple defined inorganic salt medium in continuous light and then washed 3 times in a solution containing 1 mM ethylenediaminetetra-acetic acid (EDTA), 10 mM tris buffer, 5 mM KCl and 0.01% mercaptoethanol at pH 7.7 kept at 4 °C (T/EDTA). After washing, the cells were subjected for several seconds to ultrasonic vibrations. The fixed average output of the apparatus (manufactured by Headland Engineering Development Ltd.) was 60 W. The specimens were contained in pyrex centrifuge tubes and after ultrasonic treatment were deposited on carbon-coated electron-microscope specimen grids. The excess liquid was then drained away. A drop of 0.5% uranyl acetate in distilled water at pH 4.0 was then put on the grid and after a few seconds the excess was absorbed with filter paper. Finally the grid was allowed to dry in a dust-free atmosphere. The specimens were examined in either an AEI 6 B or a Philips EM 300 electron microscope using a 30-μm objective aperture, the instruments operating at either 60 or 80 kV.

OBSERVATIONS

After treatment with the T/EDTA solution and ultrasonics, varying degrees of breakdown of the flagella occurred, ranging from little or no fraying to the complete disruption of the flagella. Normally most of the flagella became detached from the cell during treatment, but some remained attached (Fig. 5), even though the flagellar membrane was removed and the axoneme frayed open. This type of specimen was often the most informative, in that after the fraying treatment most of the subsidiary components so far observed were present still attached to the tubules (Fig. 6).
Outer tubules

Radial spokes and 'secondary fibres'. Thin filaments attached to the A tubule of each peripheral pair have been observed. These structures are about 5 nm wide and 33 nm long; they project inwardly and approximately at right angles to the long axis of the tubule but do not reach the centre pair. The term A tubule as used here and as defined by Afzelius (1959) and Gibbons & Grimstone (1960) applies to the one from which the side arms arise (Fig. 1). The spokes lie in pairs at regular intervals along the length of the tubule. The interval between pairs is about 70 nm and that between members of each pair about 30 nm (Figs. 7, 9). The sites of attachment of the filaments to the tubule lie in a straight line parallel to the axis of the tubule. The radial spokes of any one peripheral pair can best be observed, and are at their longest, when only one tubule of the pair rests on the supporting film, i.e. when one tubule is obscured by the other (Fig. 2B). It is not uncommon to see several of the peripheral pairs of one flagellum each balanced on one tubule (Fig. 7), although the more stable arrangement would be expected when both tubules are in contact with the substrate (Fig. 2A). Presumably the uranyl acetate is acting as an effective support for the tubules. A foreshortened view of the spokes is obtained when both tubules rest on the supporting film (Fig. 8).

Each radial spoke terminates in a hammerhead attachment some 10-20 nm long with its axis parallel to the long axis of the flagellum. The structure is about 5 nm wide at its extremities and slightly larger where attached to the radial spoke (Fig. 9). These longitudinally aligned hammerhead structures are now identified with the so-called
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'secondary fibres' previously deduced by Gibbons & Grimstone (1960) from micrographs of embedded and sectioned material. Both the radial spokes and 'secondary fibres' appear to be fairly rigid structures and not capable of bending, for they are invariably observed at right angles to the long axis of the A tubule and occasionally, when seen detached from the tubule, they still retain their basic structure (Fig. 7, ii). Our preparations have so far failed to reveal any continuation of the radial spokes beyond the secondary fibre, although Afzelius (1959) and Gibbons & Grimstone (1960) from sectioned material reported that a direct connexion exists between the outer and inner pair of tubules by means of the radial spokes.

Side arms. Grimstone & Klug (1966) observe that side arms are readily detached in making negatively stained preparations, whereas in sectioned material they are normally seen attached to the A tubules. There have been similar difficulties in retaining the side arms in situ in Chlamydomonas and in many instances when the A and B tubules are seen together (see Fig. 2a) the A tubules are free of any external component (Fig. 13, od). When side-arm material is present, as in Figs. 6 and 11, it is found distributed along the length of the A tubule at regular intervals of about 14 nm and placed roughly at right angles to the radial spokes, which are also attached to tubule A (see Fig. 1). Measurements were made directly on the photographic plates with a travelling microscope and since great difficulty was experienced in detecting a regular repeat by eye, a wide range of measurements was obtained. The fact that the side-arm material at its best was not well preserved and was usually seen as vesiculated clumps probably accounted for the wide range of measurements. In many instances the side-arm material had become detached from the A tubule and deposited on the substrate as small vesicles about 13 nm in diameter (Fig. 12). Optical diffraction methods of measurement were applied to the micrographs of side-arm material and a value for the repeat distance of about 12 nm was obtained, which is nearer the value obtained from observations on Tetrahymena pyriformis. (D. Chasey, unpublished). Whatever the correct repeat distance for side arms may eventually prove to be, preliminary indications are that it is different from the spacings obtained from spoke material.

Side-arm material is more readily observed when tubules A and B both lie on the supporting surface, as shown diagrammatically in Fig. 2a; and in the micrographs Figs. 11 and 12. Fig. 10 shows an outer doublet which has been twisted during staining and drying. At the top of this micrograph only one tubule is visible, with pairs of spokes (rs) attached at right angles (compare Fig. 2b). Lower down, twisting of the tubules has occurred in the direction of the arrow. Both tubules A and B are now visible, with side-arm material (sa) attached to the A tubule (compare Fig. 2a). In passing from top to bottom the spokes have been rotated by about 90° and so are obscured by tubule A. Fig. 8 also shows a twisted outer pair of tubules, but in this instance twisting of the tubules is from left to right. At the top of the micrograph pairs of radial spokes attached to tubule A are visible. Twisting has occurred so that the spokes now lie across the B tubule and the side arms are exposed. From examination of Figs. 8 and 10 and many other similar micrographs it is concluded that both side arms and radial spokes originate from the same tubule i.e. tubule A. On occasions
during specimen preparation the side-arm material appears to clump around the pairs of radial spokes, giving the A-fibre attachments an undulating profile with centre-to-centre peak distances of about 100 nm (Fig. 6).

Central tubules

Jacobs, Hopkins & Randall (1969) have found one of the two central tubules, C₁, of the flagella of C. reinhardii to be chemically more stable than the other, C₂ (Figs 1, 3). It will be convenient to use this nomenclature to distinguish between their structural attachments.

When frayed flagella are observed in the electron microscope the central pair of tubules often lies some distance from the rest of the axomere in the form of an arc and is easily recognized, as in Fig. 13 where projections on C₁ are clearly visible. In Fig. 14 tubules C₁ and C₂ are so close together that it is impossible to distinguish between the two and interpretation is difficult, although it is clear that there are cross-striations with a spacing of about 16 nm. In Figs. 15 and 16 the 2 tubules are reasonably well separated and the projections, particularly on C₁, are clearly visible. They lie in these views on opposite sides of C₁. No differences in overall dimensions of the 2 sets of projections have been detected. The projections in each set are about 18 nm long and have a regular repeat distance of about 16 nm. Since we do not know the precise points of attachment to C₁ of the two sets of projections, or the mean value of the angle subtended between them, the ‘length’ of 18 nm must be regarded as provisional. The two sets of projections are staggered with respect to each other and not in register. The micrographs also suggest that the projections are slightly kinked about their mid points, with a slight thickening or blob at their distal ends (arrows 1 and 2, Fig. 15). In Fig. 15 there is an apparent helical arrangement of material attached to C₁ (hm); we have no evidence that this is a fibrous attachment. In many micrographs projections are confined to tubule C₁; occasionally C₂ may bear a single set. In the examples so far examined the C₂ projections appear shorter (about 14.5 nm) than those of C₁ and often not as heavily stained (Fig. 16). The repeat distance of the C₂ projections is the same as those of C₁, that is about 16 nm. Occasionally when projections have separated from C₁ they do so as a group and not individually and appear to be joined to one another, perhaps by material stripped from the tubule.

Our present information from transverse sections of Chlamydomonas flagella is not in disagreement with the above evidence from negative staining on tubule C₁ and indicates that the sets of projections may subtend an angle of about 120° to each other.

DISCUSSION

It is convenient to deal first with the outer 9 pairs of tubules. Like others, we have observed 3 kinds of attachment: radial spokes, secondary fibres and—less satisfactorily preserved—side arms. These structures have been previously deduced either from the examination of metal-shadowed preparations (Manton & Clarke, 1952); or from thin sections (Alfzelius, 1959; Gibbons & Grimstone, 1960; Allen, 1968); or from the use of frayed material together with either sodium phosphotungstate or sodium
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tungstate stain (Grimstone & Klug, 1966; Behnke & Forer, 1967). The use of uranyl acetate as a negative stain in the present work at an optimal pH of about 4 appears to have led to better preservation of both microtubules and of the above associated structures. Chasey (1969) has reached similar conclusions in his experiments with *Tetrahymena*. The improved preservation of structure, together with the fortuitous observation of tubules in different orientations relative to the supporting surface, have enabled us to build up a more satisfactory 3-dimensional picture of these fragile components than hitherto. To obtain this information, disruption of the flagellar membrane has been necessary and this in itself amounts to rather harsh treatment. It is possible, therefore, that some structures may have been either largely destroyed, dispersed or otherwise distorted. For example, the fibrous connexions between the outer 9 doublets and the membrane inferred by Allen (1968); those between each set of doublets, together with the continuation of the radial spokes beyond the secondary fibres (Gibbons & Grimstone, 1960) may all have been lost in preparation. The existence of these additional structures has been inferred as a result of the examination of sectioned material, but they have not yet been seen in negatively stained material. Attempts to use fixatives prior to negative staining have been made with a view to stabilizing the flagellum components, but it was found that fraying did not occur after such treatment. There is evidence from previous papers already cited, as well as from the present work, that the use of uranyl acetate at pH 4 leads to better preservation of the subsidiary flagellar components.

Of all the structures found in cilia, flagella and sperm tails from examination of sectioned and frayed material, radial spokes and secondary fibres have caused the most confusion. Birge & Doolin (1969) and Gibbons (1961) both report the possible absence of some radial spokes as seen in transverse sections of cilia, i.e. not every peripheral doublet has a spoke associated with it. Gaps were also observed in an otherwise regular spacing of spokes in longitudinal section. The missing spokes have been attributed to imperfect preservation. In our frayed preparations of flagella from *C. reinhardii* the radial spokes are about 30 nm apart and occur in pairs, the distance between each group being about 70 nm. Since there is no reason to suppose that the sets of radial spokes from the 9 outer pairs are in transverse register, it cannot be expected or assumed that any arbitrary thin section (about 50–60 nm thick) would necessarily contain the full number. Nor is it to be expected that our measurements on frayed material would be in exact agreement with those obtained from sectioned material. This discrepancy could be attributed to the known and substantial shrinkage caused by dehydration and embedding in sectioned material. Owing to the fact that both radial spokes and side arms occur on the A tubule of the outer doublets (Afzelius, 1939; Gibbons & Grimstone, 1960; Allen, 1968; and the present paper) a certain amount of confusion has existed as to the identification of these components when axoneme tubules have been frayed open and their orientation with respect to the rest of the axoneme disturbed. The 'battlements' observed by Manton & Clarke (1952) in frayed plant sperm tails were regularly spaced structures attached to the outer doublets on the side remote from the membrane and were regarded by the authors as part of a coiled fibre wrapping itself around the central pair of fibres. On the other hand,
Afzelius (1959) considered these 'battlements' to be side arms. Behnke & Forer (1967) observed pairs of structures distributed along the length of the A tubule at regular intervals in negatively stained preparations of crane fly spermastsids. The two members of each pair were about 30 nm apart and the pairs themselves separated by about 60 nm. These lateral projections were said to be either arms or spokes, although the measurements are not in disagreement with those obtained for radial spokes in *C. reinhardii*. Burton (1966) also observed similar lateral projections on the outer A tubules of lung fluke sperm and likewise interpreted them as being either radial spokes or side arms. It is hoped that Fig. 10 clarifies this confusion. This micrograph shows a peripheral doublet, one end of which has been twisted through about 90° relative to the other. The extremes of this rotational variation thus correspond with the views of the doublet presented in Fig. 2A, B. As a consequence Fig. 10 shows on one and the same tubule both radial spokes and side-arm material. It therefore seems incontrovertible that both these subsidiary structures are attached to the A tubule. The radial spokes occur in pairs. Each spoke is terminated distally by a longitudinal hammerhead. This information refers strictly only to *C. reinhardii*. Nevertheless, the structures reported previously (and cited above) from a variety of cilia, flagella and sperm tails strongly suggest essential similarities to those just described. Granted the basic equivalence of the 9 + 2 structure in these instances, we conclude that the 'battlements' of Manton & Clarke (1953), and the structures observed by Lewin & Meinhart (1963), Grigg & Hodge (1949), Burton (1966), and Behnke & Forer (1967) are radial spokes. It is interesting to note that in *Chlamydomonas* the radial spokes occur in groups of 2 whereas in *T. pyriformis* the spokes occur in groups of 3, with the spacing between each member occurring at intervals of about 24 nm (D. Chasey, unpublished). We further identify the secondary fibres of Gibbons & Grimstone (1960) with the hammerhead spoke attachments described here. There are therefore no grounds for supposing that the so-called secondary fibres are continuous—at least in *Chlamydomonas*—since they are in fact short, orthogonal attachments to the radial spokes sited parallel to the longitudinal axis of the flagellum. In embedded and sectioned material compression could possibly give rise to the appearance of a continuous fibre.

According to Williams & Luft (1968) and Allen (1968) who used the rotation technique of image clarification (Markham, Frey & Hills, 1963), the paired side arms associated with the outer tubule A are of 2 distinct types. They both lie at right angles to the short axis of the A tubule and both have hook-like structures at their distal ends. From other worker's sectioned material and our own negatively stained preparations, side arms occur at regular intervals along the length of the A tubule, the longitudinal repeat being about 14 nm and their length about 15 nm. To summarize the differences between spokes and side arms as visualized from both sectioned and negatively stained frayed material: (a) both types of structure occur on the outer A tubule but are positioned at about 90° to each other (Fig. 1); (b) spokes are 33 nm long, occurring in pairs along the length of the tube. The interval between pairs is about 70 nm and that between the two members of each pair about 30 nm; (c) spokes have hammerheads at their distal ends. Side arms have hooks (Allen, 1968); (d) side arms also occur in pairs but each pair lies in the same transverse plane. The longitudinal spacing of the
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Pairs is regular and about 14 nm, and their length is considerably shorter than that of a spoke, i.e. 15 nm; (e) side arms are more easily detached from the A tubule than spokes and are more difficult to characterize.

Although radial spokes have been reported to join outer doublet fibres to the central pair (Gibbons, 1961; Birge & Doolin, 1969), in fragmented flagella of *C. reinhardii* stained with uranyl acetate the radial spokes appear to terminate at the secondary fibre. Allen (1968) in using the Markham rotation technique on his micrographs of

Fig. 3A. Diagram showing central tubules, C₁ and C₂ with attached projections resting on a supporting surface. At the upper end of the diagram the tubules have been pulled slightly apart, while at the lower end the tubules have completely separated and curve away from each other. C₁ possesses two sets of projections (α, β), while so far only one set of projections (γ) has been observed on C₂.

Fig. 3B. Tubules C₁ and C₂ with associated projections as they might appear in transverse section. The angle subtended by the two sets of projections (α, β) on C₁ is about 120°.

*T. pyriformis* cilia does not clearly show an extension of the spokes beyond the secondary fibre. The most conspicuous portion is certainly that between the secondary fibre and outer nine. During the process of fragmentation of the flagellum and subsequent disruption of the axoneme fibres, connexions (if present) between the central pair of fibres and secondary fibres could have been broken, especially as in many instances the action of fraying causes the central fibres to be deposited some distance from the peripheral fibres. During fraying, broken residual material could also con-
ceivably contract from the central pair of fibres on to the secondary fibre or be deposited on to the supporting film.

Interpretation of the exact geometrical relationship between the comb-like fibrous projections of central pair tubules has proved difficult from the examination of negatively stained and thin-sectioned preparations alone. There is evidence that negatively stained tubules are often flattened and this could account for some obscuration. The use of tilted specimen grids and of other techniques is now in progress and should lead to a more complete understanding of the structures and their relative dispositions.

![Diagram of Chlamydomonas flagellum](image)

Fig. 4. Stereogram of a portion of a Chlamydomonas flagellum summarizing the relationship between outer and inner tubules and their associated structures from information obtained from frayed negatively stained preparations. Note outer tubules (A B), radial spokes (rs), secondary fibres (sf), central tubules (C1 C2) and central pair projections (α, β, γ).

Meanwhile our present interpretation of the central pair projections, or rungs, is summarized diagrammatically in Fig. 3 A, B and is essentially in agreement with the conclusions of Chasey (1969) for *T. pyriformis* as far as tubule *C*1 is concerned. Fig. 3 A shows a central pair, *C*1 *C*2, of a frayed flagellum. At the upper end of the diagram the tubules have been pulled slightly apart, while at the lower end the tubules have completely separated and curve away from each other. *C*1 possesses 2 sets of projections marked α and β to distinguish them. In this diagram the projections are resting on the supporting surface and not as observed in transverse section, where they subtend an angle of ~ 120° to each other. Tubule *C*2 has only one set of projections. When lying on the support these projections (γ) are shorter than α or β on *C*1. Nevertheless, when
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the tubules are a normal distance apart, the $C_2$ ($\gamma$) projections are long enough to form, accidentally or otherwise, a bridge with the $C_1$ ($\alpha$) projections. In the micrographs it is noticeable that in dispositions corresponding to the lower end of Fig. 3A, the $\alpha$ and $\gamma$ projections 'break' at points corresponding to their normal lengths, and not at random. It is of course possible that Fig. 3A represents, in regard to $C_1$ ($\alpha$) and $C_2$ ($\gamma$), an artifact of preparation; for sectioned preparations appear broadly as in Fig. 3B. If Figs. 3A and 3B both bear some relation to the living state it follows that $C_1$ ($\alpha$) and $C_2$ ($\gamma$) must be capable of movement towards each other in order to join together, as in Fig. 3A (top).

In Fig. 4 we have attempted to show an overall picture of a short piece of Chlamydomonas flagellum in which the information we have derived about the outer 9 and central pair attachments has been included. This picture presents the length of these subsidiary structures as observed in micrographs after much preparative treatment. It is quite unknown at present whether any of the projections is extensible or contractile; and in consequence whether their derived dispositions are significantly related to function. It is interesting to note that Chasey (1969) did not find in T. pyriformis the projections on the $C_2$ tubule that we have observed in Chlamydomonas. It cannot be assumed either that all existing structures have yet been observed; or that all such components are necessarily functionally significant. The role of these subsidiary structures—static supportive or dynamic—has been discussed by several authors (Birge & Doolin, 1969; Gibbons, 1965; André, 1961). The radial spokes with their hammerhead attachments bear some morphological resemblance to the well-known bridges of striated muscle. While this resemblance could turn out to have a fundamental bearing on function, it is as well to remember that no physical link between the spoke-hammerhead structure and the central pair has so far been demonstrated. Work now in progress aims to compare the structures of wild type flagella with those of paralysed flagellar mutants (Warr, McVittie, Randall & Hopkins, 1966). Such experiments may give further clues as to the nature and possible function of subsidiary flagellar structures.

I am deeply indebted to Professor Sir John Randall, F.R.S. for his constant help during this work and in the preparation of the manuscript. My thanks are also due to Dr M. Jacobs and Mr D. Chasey for helpful discussions.

REFERENCES


J. M. Hopkins


(Received 17 February 1970)

Fig. 5. Uranyl acetate stained specimen of Chlamydomonas reinhardii showing the 2 flagella frayed open after the removal of the flagellar membrane but still attached to the cell. × 3740.

Fig. 6. A frayed flagellum showing central tubules (C1, C2) and 9 outer doublets. Note the presence of radial spokes (rs), secondary fibres (sf) and distal arm material (sa). An unfrayed flagellum (f) with attached mastigonemes (ma) appears in the lower left of the micrograph. × 69,000.
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Fig. 7. Several outer tubules of a frayed flagellum with paired groups of radial spokes (rs) and secondary fibres (sf), attached (i) and unattached (ii). $\times 79000$.

Fig. 8. Partly twisted outer tubules A and B (A and B) showing radial spokes (rs) and secondary fibres (sf) attached to the A tubule. These structures are lying across the B tubule. Note also presence of side arm material (sa) which is also attached to the A tubule. Compared with the top of the micrograph, the tubules at the bottom have been twisted in the direction of the arrow, i.e. from left to right. $\times 173000$.

Fig. 9. Radial spokes and secondary fibres attached to the peripheral tubule A. $\times 210000$.

Fig. 10. Twisted outer doublet tubules A and B showing, at the top of the micrograph, radial spokes and secondary fibres attached at right angles to the A tubule. Lower down twisting of the tubules has occurred in the direction of the arrow, i.e. from right to left. Both tubules A and B are now visible, with side-arm material (sa) attached to the A tubule. In passing from top to bottom the spokes and secondary fibres have been rotated by about 90° and so are obscured by tubule A. $\times 210000$.

Fig. 11. Peripheral doublet tubules A and B with side arm material (sa) attached to tubule A. $\times 210000$.

Fig. 12. An outer doublet tubule shedding side arm material (sa). $\times 135000$. 
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Fig. 13. A frayed flagellum with the central pair of tubules ($C_1, C_2$) lying some distance from the rest of the axoneme. Note projections ($\alpha, \beta$) attached to tubule $C_1$. For further explanation, see text. $\times 59\,000$.

Fig. 14. Central tubules lying close together. It is difficult to distinguish between the two. Note a regular periodicity along the length of the tubules. $\times 173\,000$.

Fig. 15. The 2 central tubules ($C_1, C_2$) have separated, showing 2 rows of projections ($\alpha, \beta$) attached on opposite sides to $C_1$. There is a suggestion of kinks ($\iota$) and thickenings ($\phi$) on the projections. Note an apparent helical arrangement of material attached to $C_1$ ($\kappa\iota\mu\nu$). Tubule $C_2$ bears only short projections ($\gamma$). $\times 137\,000$.

Fig. 16. Separated central tubules ($C_1, C_2$). Two sets of projections are evident on tubule $C_1$ and one set on $C_2$. The projections on tubule $C_2$ are shorter than those on $C_1$ and are seen only occasionally. Note detached projections ($p$). $\times 139\,000$. 
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13. Figure 13: Diagram showing the subsidiary components of flagella, labeled C₁, C₂, β, α, and od (A+B).

14. Figure 14: Detailed view of the flagellar structure with labeled regions.

15. Figure 15: Close-up of the flagellar components, with labels β, C₁, C₂, hm, and γ.

16. Figure 16: Further magnified view of the flagellar components.