STRUCTURE, PROTEIN COMPOSITION AND BIREFRINGENCE OF THE COSTA: A MOTILE FLAGELLAR ROOT FIBRE IN THE FLAGELLATE TRICHOMONAS

W. B. AMOS, A. V. GRIMSTONE AND L. J. ROTHSCILD*  
Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, England

AND R. D. ALLEN
Department of Biology, Dartmouth College, Hanover, New Hampshire 02755, U.S.A.

SUMMARY

The costa is a rod-shaped intracellular organelle lying in the cytoplasm immediately below the undulating membrane. In certain large species of Trichomonas (T. gigantea, T. termopsidis and an undescribed species from the termite Porotermes adamsoni) this organelle is motile. Bending waves are transmitted along the length of the costa, in either direction. It is shown that the bending is actively produced by the costa itself. The form of the movements is described in detail. The costa is birefringent. At the point at which bending occurs there is a sharply localized reduction in birefringence. Electron microscopy shows that the costa is composed of longitudinally running lamellae, 2-3 nm thick and spaced 12 nm apart. These are connected to transversely running elements spaced at intervals of about 37 nm. The lamellae occur in two alternative configurations: straight and zig-zag. Bending probably results from a local, transient change from the straight to the zig-zag configuration. This would account for the local change in birefringence which accompanies bending. Polyacrylamide gel electrophoresis of isolated costas shows them to contain a number of protein components, of which the principal one has a molecular weight of about 90000. Preliminary cytochemical evidence is presented for ATPase activity in the costa. The costa is a type of motile system distinct from any hitherto described.

INTRODUCTION

This paper is concerned with an organelle – the costa – which is found in the flagellate Trichomonas and which, in at least some members of the genus, is motile. In many of its properties, and in particular its fine structure and protein composition, this organelle is unlike any other motile system so far studied. It appears not to contain actin, myosin or tubulin. It is structurally quite different from muscle, cilia and flagella, and other microtubular organelles, and does not resemble any of the filamentous structures which have been described in cells showing amoeboid movement or cytoplasmic streaming. Movements of the organelle are accompanied by marked localized changes in birefringence. We present in this paper a description of the main features of its movements, structure, composition and optical properties.

The discovery that the costa of Trichomonas gigantea is motile was made by the late

* Present address: Department of Biology, Jordan Hall, Bloomington, Indiana 47401, U.S.A.
L. R. Cleveland, though his observations were not published. Professor Cleveland kindly made available to us some of his ciné films of this species and we have used them in the analysis of movements reported in this paper.

**MATERIALS AND METHODS**

**The flagellates**

Three species of *Trichomonas* were used in this study, all from the gut of termites, in which they live as symbionts. *Trichomonas termopsidis* was obtained from *Zootermopsis angusticollis*, *T. gigantea* and an unidentified species similar to *T. termopsidis* from *Porotermes adamsoni*. Colonies of both species of termite were maintained in the laboratory and fed on damp wood and paper towelling. The flagellates were obtained either by squeezing them out of the hind gut of the termite or by removing and opening the gut.

For examination of living organisms, preparations were made in undiluted gut fluid, or occasionally in 0.6% KCl. The coverglasses were ringed with Vaseline or oil in order to prevent evaporation and, as far as possible, to exclude oxygen, which is toxic to the flagellates. *Trichomonas* remained alive for a few hours in such preparations, but with steadily declining activity; the very rapid movements seen on initial isolation were lost within a few minutes. No methods are known for maintaining the termite flagellates alive for long periods in optimal condition for observation.

**Light microscopy**

Observation of living organisms or costs obtained from lysed cells were made by phase-contrast, and the Nomarski differential interference-contrast method. For study of birefringence properties a polarizing microscope was used equipped with a Brace-Köhler compensator. Measurements of dry mass were made with a Jamin-Lebedeff interference microscope. In all cases Zeiss (Oberkochen) instruments were used. Ciné records were made on Kodak Plus-X or Pan F film with a 16-mm camera, using an HBO 200 mercury lamp, or, for certain films taken with polarizing optics, solar illumination.

**Transmission electron microscopy**

Cells were usually fixed in 2.5% glutaraldehyde in 0.03 M cacodylate buffer at pH 7.4. After washing in buffer they were then postfixed in 1% osmium tetroxide in the same buffer, dehydrated in ethanol and embedded in Araldite epoxy resin. Some organisms were fixed in 1% osmium tetroxide alone. Thin sections were double stained with uranyl acetate and lead citrate. Electron micrographs were taken with a Philips EM 200 electron microscope operated at 60 kV.

**Scanning electron microscopy**

Gut fluid containing the flagellates was mixed with a solution containing 1% osmium tetroxide and 20 mM cacodylate buffer, pH 7, and allowed to fix for 1 h at room temperature. By centrifuging and resuspending, the specimens were taken through several washes of buffer and dehydrated progressively in ethanol. When in absolute ethanol they were transferred to a specimen stub in the form of a metal container with a perforated lid. A description of this device, which is a modification of that described by Taylor (1975), is in preparation. The whole apparatus was immersed in amyl acetate and the specimens were then subjected to critical-point drying as described by Anderson (1951). They were then sputter coated with a 50-nm layer of gold/palladium and examined in a Cambridge S 4 scanning electron microscope operated at 19-20 kV with a beam current of 130-150 µA.
Costa of Trichomonas

Cytochemistry

Cytochemical observations were made only on *Trichomonas gigantea* and at the level of light microscopy. Unless otherwise stated the methods used were those described in Pearse (1968). Tests were made for adenosine triphosphatase (ATPase) activity by the lead method (Wachstein & Meisel, 1957). This was done after brief fixation in 2.5% formaldehyde or glutaraldehyde in 0.03 M cacodylate buffer, pH 7.4. Isolated costas were prepared by suspending the cells in 0.5% KC1 and then squirting them through a Pasteur pipette, the tip of which was pressed against the bottom of a centrifuge tube, or by treatment with 0.005% digitonin in distilled water. The digitonin did not appear to affect the tests for ATPase.

The periodic acid-Schiff method for carbohydrate was applied to smears containing *T. gigantea* that had been fixed with 2% osmium tetroxide for several minutes. Controls in which oxidation with periodic acid was omitted were performed.

Polyacrylamide gel electrophoresis

In order to obtain costas for electrophoresis a suspension of gut fluid from *Porotermes* containing both *Trichomonas gigantea* and the undescribed smaller trichomonad was mixed with approximately 10 times its volume of a solution containing 0.1% sodium azide and 5 mM p-tosyl arginine methyl ester (TAME) in 20 mM cacodylate buffer, pH 7.0. These inhibitors were added in an attempt to prevent bacterial activity and proteolysis during preparation. The cells were lysed by pipetting in this solution, as described above. The freed costas were clearly visible under a low-power binocular microscope. They were transferred individually to a clean solution of the same composition but containing 30% (v/v) glycerol, using fine tungsten needles to pick them up. This was repeated several times to free them of wood particles from the gut fluid and cytoplasmic debris. More than a thousand costas were then transferred to a small volume of 30% (v/v) glycerol solution to remove the cacodylate buffer, and finally transferred to a solution containing 2% sodium dodecyl sulphate (SDS), 0.5% mercaptoethanol, 0.1% sodium azide, 2 mM TAME, 0.04%, fluorescein, 30% (v/v) glycerol and 0.125 M Tris buffer, pH 6.8. The sample was run on a 7.5% SDS polyacrylamide gel and stained according to the formulae of Laemmli & Favre (1973) in a micro-slab gel apparatus (Amos, 1976).

RESULTS

General structure

The morphology of the genus *Trichomonas*, as it can be seen in the light microscope, is well known (see, e.g. Grassé, 1952) and need not be described here. The essential features are shown in Fig. 1. The structure of *T. termopsidis* has been described by Andrews (1925), Kirby (1931) and Hollande & Valentin (1968), that of *T. gigantea* (formerly termed *Pseudotrypanosoma giganteum*) by Grassi (1917), Kirby (1931) and Cleveland (1961). These two species differ greatly in size: *T. termopsidis* is 70–100 μm long (Fig. 3), *T. gigantea* up to 230 μm (Fig. 4). The latter is by far the largest member of the genus known.

These large species of *Trichomonas* have three potential means of progression. There is a group of 4 flagella at the anterior end of the cell; there is the undulating membrane, which is a recurrent flagellum that arises anteriorly with the others but is reflected backwards and extends to the posterior end of the cell, attached to a fold in the body surface; and there is the costa, which lies within the cytoplasm immediately below and closely associated with the undulating membrane (Fig. 1). The costa is a long rod, roughly elliptical in cross-section, tapering gradually at each end and attached anteriorly to the centriolar apparatus, in which are located the basal bodies of the
flagella. Being curved, the costa is slightly longer than the cell itself. In *Trichomonas termopsidis* its maximum diameter is 1.3 μm at its widest part, in *T. gigantea* about 6 μm. Particularly in the latter species, therefore, it is a substantial structure and can be seen and studied readily in living organisms by phase-contrast microscopy (Fig. 2).

The different ways in which costa, flagella and undulating membrane are used to produce and control movements in the two species are described in the next section.

The other organelles of *Trichomonas*, as far as we can determine, play no immediate part in the production of movements and can be disregarded here. In particular, the axostyle, which in some other polymastigote flagellates is a motile structure (Grimstone...
Costa of Trichomonas

& Cleveland, 1965; Mooseker & Tilney, 1973) is in Trichomonas a seemingly inactive structure in the form of a hollow rod, the wall being formed of one or more layers of microtubules. It appears at most to undergo passive bending movements and is certainly not involved in major or rapid changes in cell shape.

Locomotion of the flagellates

The locomotion of the two small species, Trichomonas termopsidis and the undescribed species from Porotermes, will be described first, since it is more similar to that of other trichomonads. When freshly isolated from the termite gut these organisms swim rapidly and unceasingly. The body is elongated, pointed at the anterior end, with the free flagella directed laterally or anteriorly (Fig. 1). The costa is curved, following a long-pitch helix from the anterior to the posterior end of the body, and since the undulating membrane is firmly attached to it (the structure of the undulating membrane and its connexion to the costa will be described elsewhere), this too has a helical form. The undulating membrane is in these species the principal means of locomotion: rapid bending waves pass along the recurrent flagellum and serve to drive the cell along. Presumably because the undulating membrane is helically twisted, the cell rotates around an axis parallel to the direction of movement as it moves forward. The 4 free flagella are commonly inactive, but may give occasional rapid, concerted strokes which serve as a means of quickly changing the over-all direction of movement, for example, when obstacles are encountered. Apart from this the free flagella do not seem to be important in locomotion.

As the cells become less vigorously active, or if they become compressed between slide and coverglass, the elongate, pointed form of the body is often lost and the organisms become flattened and more or less diskoidal. The costa then loses its spiral form and runs around the edge of the disk in a flat curve, with the undulating membrane following it (Figs. 3, 5). The undulating membrane remains active and the cell now rotates, in a direction opposite to that of the waves passing along the recurrent flagellum, without any over-all progression taking place. The assumption of this diskoidal shape is a reversible process. Organisms may change back to the elongated form, and they may alternate between the two forms (cf. Figs. 5 and 6). It seems that the flattened shape is a normal one which the cell can take up, but it is certainly more common in old preparations than in fresh ones, and it seems to be an almost invariable response to compression. The essential factor producing these changes in body shape seems to be an active alteration in the over-all form of the costa. The reasons for this assertion will become apparent below.

Apart from these large changes in the over-all form of the body, T. termopsidis may also display rapid, small-scale changes in shape. These chiefly take the form of bending or twisting movements, or local deformations of the cell surface. Elongated individuals, in particular, commonly bend and writhe actively (Fig. 6). Again, for reasons which will become apparent when the movements of T. gigantea are described, it is believed that all such changes in body form are the result of active changes in the configuration of the costa. Since the undulating membrane is bound to the costa all along its length,
the shape of the costa determines the over-all direction of the undulating membrane, and hence whether rotation or progression results.

*Trichomonas gigantea*. Although this species possesses the same 3 types of motile organelle as the smaller species, its method of locomotion is fundamentally different. The principal means of progression here is quite clearly not by means of the undulating membrane but by active changes in body shape. Movement has never been observed in the absence of these changes and, conversely, cells may remain immobile even though the undulating membrane is vigorously active. The difference between the two species in this respect probably results from the difference in size, the undulating membrane being too small to move a cell as large as *T. gigantea*.

Active organisms are elongated, as in *T. termopsidis*, and the whole cell bends actively and rhythmically (Figs. 4, 7A). Bending waves pass backward from the anterior end of the cell at the average rate of about 2 per s, the waves moving at about 100 μm per s. The amplitude of the waves increases somewhat posteriorly, and often there appears to be a sharp and rapid flexure of the end of the cell, with a corresponding acceleration of the body forwards. The movement produced by this method is like that of a fish, thrust being developed at the backwardly moving, inclined surface of the body (Fig. 7A). The maximum observed rate of progression by this method is about 100 μm per s.

As in *T. termopsidis*, the cell may lose its elongate shape and become flattened. Such cells tend to remain stationary. Minor shape changes of various kinds continue and may be easily observed, and in such organisms the active role of the costa becomes particularly obvious. Fig. 7B shows a tracing made from a ciné film of such a flattened organism. In Fig. 7B a two-dimensional bending wave is shown passing along the costa. There is a corresponding change in the over-all shape of the body.

Before describing in detail the changes in form of the costa it is necessary to consider the evidence that these changes are active, and not the result of passive bending of the costa by the rest of the cytoplasm. The most convincing evidence for this comes simply

Fig. 2 *Trichomonas gigantea* lysed by compression between slide and coverslip. The nucleus (n) is surrounded by the remains of the axostyle. The costa (c) is conspicuous. Phase contrast.

Fig. 3. *Trichomonas termopsidis* in the gut fluid of the termite Zootermopsis angusticollis. The two specimens shown have assumed a diskoidal form, with the costa and undulating membrane at the periphery of the disk. These specimens were photographed by electronic flash while rotating rapidly in a direction opposite to that of the waves passing along the undulating membrane. Other protozoa and spirochaetes are also visible. Phase contrast.

Fig. 4. *Trichomonas gigantea* in the gut fluid of Porotermes adamsoni. This photograph is printed on the same scale as Fig. 3, to show the size of this species. Phase contrast.

Fig. 5. Scanning electron micrograph of the smaller trichomonad flagellate from *Porotermes adamsoni*, fixed in the rounded form. The anterior flagella (f) and undulating membrane (UM) are conspicuous.

Fig. 6. Two individuals of the same species as shown in Fig. 5. Their shape appears to be that adopted during normal writhing movements. Another flagellate (probably *Trichonympha* sp.) is partly visible. Scanning micrograph.
from direct examination of living organisms, or of ciné films of them, which give an extremely strong impression that the costa is actively motile and drags the cytoplasm with it. The correctness of this visual impression is supported by a number of more objective lines of evidence. First, the movement of the costa is independent of the activity of the undulating membrane; the costa continues to bend in individuals in which the undulating membrane has become motionless. Secondly, disintegrating but still living organisms have been seen in which much of the cytoplasm has been cast off, leaving the bulk of the costa free or surrounded by only a thin cytoplasmic layer.

Fig. 7: A, tracings from a ciné film of forward swimming in *Trichomonas gigantea*. The interval between tracings is $\frac{1}{3}$ s. B, tracings of movement of a specimen of *T. gigantea* compressed between slide and coverslip. The ellipse is the outline of the nucleus and the heavy line to the right of each tracing represents the costa. The interval is $\frac{1}{6}$ s.

The costa may continue to bend or undulate in this condition. Thirdly, displacement of particles in any given region of the cytoplasm is roughly proportional to the amplitude of movement of the costa; in highly compressed individuals the bulk of the cytoplasm may be almost static, while the costa continues to carry minute bending waves of low amplitude. Fourthly, the form of the bending waves and the way they are propagated along the costa strongly suggest active movement. The bending regions are not smooth sinusoidal curves but are more or less sharply angled (see Fig. 7B), and they maintain this form as they pass backwards. Waves of bending follow each other rhythmically. It is difficult to explain how this could be achieved if the bending were the result of forces applied externally to a passive costa, since it would require the operation of highly localized and precisely coordinated activity of the general cytoplasm. It is far more probable that the observed movements are the result of propagated waves of contraction or expansion passing down the costa itself. Finally, in organisms preparing for division a second costa is produced long before visible signs of mitosis (Cleveland, 1961), so that cells are commonly observed with two costas. In such cases the second costa is never active, while the original one is. The reason for the inactivity of the new costa is not known, but the fact that of two organelles lying in a common cytoplasm one may be bending vigorously while the other is motionless strongly suggests that the bending must be a process intrinsic to the organelle and not
something imposed upon it by the rest of the cytoplasm. The final proof that the costa is inherently motile will perhaps be forthcoming only when it can be reactivated after isolation, and this has not yet been achieved (see below). However, the weight of evidence already available is so strong that we have no doubt that the costa is capable of active bending.

The free flagella may be involved in changes in direction of the cell, as in the two smaller species, though we have no evidence on this point. As already noted, the undulating membrane in *T. gigantea* appears to be ineffective in locomotion, its activity being over-ridden by that of the costa. Its functions, if any, are not known.

It should be noted that, although the costa and undulating membrane are closely associated, they move independently of each other. The waves of bending in the two structures are propagated at different speeds, their wavelengths are very different (about 5 μm in the undulating membrane against 100–200 μm in the costa of a normally active organism), and the frequency of initiation of bending waves in the two structures must therefore be very different. Further, as mentioned above, either organelle may be active independently of the other.

The bending waves in the costa of *Trichomonas gigantea*

The exact form of the bending waves passing down the costa in actively swimming, elongated organisms has not been determined, because of the speed of movement of the cells and (more important) because the displacement of the costa is so great that it never remains wholly within the depth of focus of the microscope. It is our strong impression, however, that the waves are normally three-dimensional, the costa at all times having an approximately helical configuration, as does the entire body when viewed in the scanning electron microscope (Fig. 6). The movements of the costa can be studied in detail only in flattened, stationary organisms, and in these the costa apparently moves in only two dimensions (Figs. 3, 7B, 8). Although the activity of the costa in such individuals is much more restricted than that of freely swimming ones, there seems no reason to doubt that it is fundamentally similar, in terms of the basic mechanism, and many of our observations relate to such organisms. In these cells, while overall straightening and flexing of the costa are commonly observed, by far the commonest type of movement is the passage of bending waves, often of low amplitude (Fig. 8), and with a variable frequency in the range 1–4 Hz. The waves usually originate at the anterior end (i.e. the region of attachment to the centriolar apparatus), but this is not necessarily the case: they may start at any region on the costa. The waves usually pass backwards, though they may move in either direction. Two bending waves may originate simultaneously at different points on a costa, independently of each other, and they may move in the same or in different directions. Sometimes, in highly compressed organisms the waves are not propagated to the end of the costa but die out, often at the same point along the length of the costa in a given organism. Frequently trains of successive bending waves follow each other along the costa, separated by periods of inactivity. The amplitude of these waves may be less than 5 μm (compared with the amplitude of approximately 100 μm for the bending waves involved in normal locomotion) and they may be too small to cause any visible alter-
Fig. 8. Compressed cell of *Trichomonas gigantea*, photographed in a polarizing microscope with a $\lambda/20$ elliptical compensator. The birefringence of the costa is indicated by the reversals of contrast around the curve. The anterior tip of the costa (upper right) is carrying bending waves of low amplitude, and the zones of reduced birefringence, which appear pale, are faintly visible on the inside of each bend. Frame enlarged from a ciné film.

Figs. 9, 10. Immobile costa of *Trichomonas gigantea* showing an arrested boundary, where the birefringence changes abruptly. The boundary, which in the original photographs appears double, is viewed here at 2 different compensator positions. The undulating membrane is visible to the left of the costa.

Fig. 11. Successive views of the costa of *Trichomonas gigantea* viewed in a polarizing microscope. No compensator was present and the polarizer axis was approximately at $45^\circ$ to the vertical. A straight region (between the arrows) is shown moving up the costa. On either side of it are bent regions, where the inner half of the costa shows a reduced birefringence, indicated by a decreased brightness (cf. Fig. 3). (Extracted from a ciné film taken with solar illumination. Interval $\frac{1}{2}$ s.)
Costa of Trichomonas

amination in the shape of the cell. As already noted, the bending waves are never sinusoidal but are angular, bending often being restricted to short zones, 20–30 μm long, with relatively straight regions between them. Some further aspects of the bending waves will be dealt with in the next section.

Optical properties of the costa in Trichomonas gigantea

The costa can be seen clearly, especially under phase contrast, by virtue of its having a higher refractive index than the surrounding cytoplasm (Fig. 2). Relative to a water background, it introduces a phase difference of less than 120°. Assuming a thickness of 6 μm, the calculated dry mass concentration is 13–16%.

Fig. 12. Diagram to show the appearance of a region of the costa carrying 2 bends separated by straight regions, as viewed in the polarizing microscope. The median line between the inner and outer regions of the costa (shown dotted) is not usually visible in the polarizing microscope, but its presence is inferred from the shape of the moving zones of reduced birefringence (shown black) which are present on the inside of each bent region (cf. Fig. 11).

The organelle is clearly visible in the polarizing microscope because of its birefringence (Figs. 8–11). The magnitude of the birefringence was measured in slow-moving or immobile cells, and values in the range 2·8–3·5 × 10⁻³ were obtained, again assuming a thickness of 6 μm. The slow axis of transmission was approximately longitudinal: this point is amplified later.
The image of the costa in the polarizing microscope shows striking changes when the waves of bending pass along it. These are particularly clear when the cell is highly compressed between slide and coverslip so that large-scale movements are prevented. The costa, lying at the periphery of the then disk-shaped cell, is C-shaped (Fig. 8). Bending waves of relatively short wavelength pass round it, separated by straight regions (see Figs. 8, 11). In the straight regions the brightness of the image between crossed polaroids is almost uniform, though a median longitudinal line may sometimes be visible. In the bent regions a distinction appears between the two sides of this longitudinal line: the inner half of the costa, lying towards the concave side of the bend, shows a marked reduction in brightness, while the brightness of the outer half remains approximately the same as in the straight regions (cf. Figs. 11 and 12). The longitudinal limits of the dark zone are sharply defined. The dark zones move along the costa in exact correspondence to the regions of bending. Like them, they move in either direction and may start up or die away at any point on the costa. They seem to remain of constant length as they move along, generally about 5 \( \mu \text{m} \), though very short zones, 1–2 \( \mu \text{m} \) long, have also been observed. The simplest explanation for these changes in brightness, namely that they are due to changes in angle between the costa and the polarizing axis, can be ruled out, since only the inner half of the costa shows the changes, though both halves undergo simultaneous changes in angle. Also, the changes occur even with bending waves of low amplitude, where the angle to the polarizing axis scarcely varies. Another possibility is that the costa, which is not perfectly circular in cross-section, might twist so as to present a lesser thickness of birefringent material in the bent region, but this also can be eliminated because the median line, which by careful focussing can be seen to lie on the surface of the costa, does not depart from its median position during the passage of a bending wave. This means that there is no twisting. There is also no evidence of a change in the diameter of the costa. The most likely explanation of the changes in brightness is that the material of the costa on the inner side of the curve undergoes a reduction in birefringence as it becomes bent, and that the birefringence returns to its original level once the bend has passed.

In the Nomarski differential interference-contrast microscope, and also in bright phase contrast, the zones of reduced birefringence where the costa is bent have a greater phase-retarding effect than the adjoining material. Since, as mentioned above, there is no evidence for a change in thickness, this must be interpreted as due to a small increase in the concentration of dry mass in the bent zones.

These observations on the changes in birefringence were made by analysing ciné films. It was not possible to measure the drop in birefringence from these films. However, costas were often observed in ageing preparations in which the waves were moving extremely slowly or were totally arrested, so that measurements could be made directly with an elliptical compensator. The reduction of birefringence in the bent inner zones was found to be approximately 30\%. The arrested waves displayed the same remarkably sharp transverse boundary between regions of greater and lesser birefringence as did normal moving waves. In both arrested and moving waves, it was frequently observed that the boundary passed across the entire width of the costa.
Costa of Trichomonas

(Figs. 9, 10) rather than halfway across as described above. Costas with this appearance probably have the same structure as shown in Fig. 12 but are rotated through 90° about their longitudinal axis so that the inner and outer zones of Fig. 12 lie above one another in the optical path.

Fig. 13. Representation of the helical form which is assumed by the costa of *T. gigantea* after release from the cell by lysis. The way in which the slow axis of birefringence departs from the longitudinal geometric axis of the organelle is shown (with the angle exaggerated for the sake of clarity) by the short heavy lines.

Repeated observations on costas viewed from the side (i.e. as they normally present themselves in flattened organisms) have shown a small but curious effect: maximum extinction is obtained when the costa is oriented at an angle of about 4° to the direction of polarization. The same was found to be true in a large series of measurements on isolated costas separated from lysed cells. In other words, the morphological axis of the costa does not exactly coincide with the optical slow axis. Fig. 13 shows the relationship of the two axes. In the regions of reduced birefringence, where the costa is bent, the morphological and optical slow axes appear to coincide more closely. This observation was made on arrested waves, and high-speed filming would be necessary.
to establish whether it holds good for normal movement. It would thus appear that bending may be accompanied by two changes in the polarization properties of the costa: a decrease in birefringence and a change in orientation of the axis of birefringence. The possibility that the second of these might give rise to an apparent variation in the first can be ruled out by calculation: it is too small to cause the apparent birefringence to decrease by 30%.

The birefringence of isolated costas was preserved after fixation in 3% aqueous formaldehyde. Costas fixed in this way retained their birefringence after immersion in 100% glycerol, showing that a substantial part of the birefringence is intrinsic.

The significance of the remarkable pulses of birefringence will be considered in detail below, after the fine structure of the costa has been described. It may be noted at this point, however, that the fact that the changes occur in sharply delimited regions, with abrupt changes in birefringence at the leading and terminal edges, suggests that the material of the costa is perhaps able to exist in either of two alternative crystalline states, with no stable intermediate condition.

**Structure of the costa**

A costa which has been isolated by lysing the cell assumes a helical form with a long pitch (Figs. 13, 24). For most of its length, the costa of *T. gigantea* is roughly elliptical in cross-section, with the major diameter 6.5 μm and the minor 4 μm. In the electron microscope, transverse sections with a large notch on one side are commonly seen, so it would appear that a groove down one side is a constant feature of the costa. This may be the explanation of the median line, described above as seen on the surface of the costa in the polarizing microscope. A median line is visible with the ordinary bright-field microscope in isolated costas.

When the isolated costa is compressed between slide and coverslip, it splits readily into plates in the longitudinal direction (Fig. 22). The fact that this splitting occurs only in certain regions of the helically twisted structure suggests that there is a preferred direction of cleavage which varies along the length of the helix.

The electron microscope reveals a substructure of some complexity, which appears to be the same in *T. termopsidis* as in *T. gigantea*. The entire area of the transverse section is made up of parallel densely staining lines, 2–3 nm thick and 12 nm apart, centre to centre (Fig. 14). These lines are sometimes observed to be continuous across the entire width of the costa, as in Fig. 14. No substructure is visible within or between the lines. We follow Nielsen, Ludvik & Nielsen (1966) who described this pattern in the costa of *Trichomonas vaginalis*, in interpreting the lines as sections of longitudinal

---

**Fig. 14.** Transverse section of the costa of *Trichomonas termopsidis*, showing the lamellae, which have a spacing of 12 nm. The differentiated peripheral zone is visible. To the left of the section, the angle of stacking of the lamellae alters abruptly. This is a common feature of the costa in *T. termopsidis* and *T. gigantea*.

**Fig. 15.** Part of a transverse section of *Trichomonas gigantea*. The pattern of lamellae is traversed by indistinct darker bands, here with a spacing of approximately 120 nm. Bands of this type (also visible on the left side of the section shown in Fig. 14) are interpreted as due to an oblique sectioning of the transverse plates.
Costa of *Trichomonas*

lamellae. The lamellae presumably correspond to the direction of mechanical cleavage described above. The costa runs close beneath the plasma membrane and the direction of the lamellae, though variable, is generally parallel to the membrane.

Longitudinal sections have a complex and diverse appearance (Figs. 16–21). The most prominent features are longitudinal lines, which we interpret as sections of the lamellae seen edge-on (Figs. 16–18), and transverse bands. The lines are 2–3 nm thick and 12 nm apart, like those seen in transverse sections. The transverse bands have a spacing of 37 nm. They appear to be sections of plates, each of which transects the costa completely in a transverse plane. Alternate plates sometimes differ in density, so the true longitudinal repeat is 74 nm. The lamellae are not continuous from one transverse plate to the next along the length of the costa: they consist of many separate strips, each of which appears to pass through a transverse plate and project an unequal distance on either side of it (Figs. 16, 17). The lamellae projecting from one plate alternate with the corresponding lamellae of the next plate: they are never opposite. However, indistinct dense material may link the ends of the lamellae in adjacent plates (Fig. 17). We have observed two kinds of pattern, which may be found contiguous with one another in the same longitudinal section, as Fig. 16 shows. In one, the lamellae are perpendicular to the transverse plates (Fig. 17). In the other, found only on the inner side of the costa, the lamellae are tilted at an angle to the plates, producing a zig-zag pattern (Fig. 18). In the zig-zag type of pattern the density of the transverse plates is lower than in the perpendicular type: this can be seen clearly in Fig. 16. The total length of each lamella appears to be approximately the same in both types of pattern. It seems likely that the two types of pattern represent interconvertible states of the same material. In the Discussion, the theory will be advanced that the tilting of the lamellae is an essential part of the mechanism of movement of the costa.

The interpretation of the structure of the costa put forward here appears to be consistent with several other types of pattern, which we have obtained in an extensive series of sections of the costa (several hundreds). Patterns in which a longitudinal section of the costa is traversed by complex transverse bands, but without prominent longitudinal lines are interpreted as due to sectioning in a plane which lies parallel to the lamellae (Figs. 19, 20). It can be seen that when the lamellae are sectioned in the zig-zag configuration, complex transverse banding patterns could be expected to result. In some transverse sections, equidistant parallel zones of altered density run across the section (Fig. 15). The spacing of these zones is quite variable, as is their angle to the lamellae. It seems likely that this appearance is due to sectioning in an

---

Fig. 16. Longitudinal section (but not median) of the costa of *Trichomonas gigantea.* The plasma membrane is visible along the lower left margin. On the left side of the section is the perpendicular type of pattern, in which the longitudinal lamellae (here seen edge-on) are at 90° to the transverse plates. Note the increased density of the transverse plates in this region, relative to the region to the right of the section, which shows the zig-zag type of pattern. The zig-zag is interpreted as due to alternate tilting of the lamellae, possibly producing a local contraction of the right-hand side of the costa. A complex peripheral zone is visible on both sides of the section.
Costa of Trichomonas

oblique plane, each zone corresponding to a region where the section runs through one of the transverse dense plates.

Towards the surface of the costa the transverse plates appear thickened. This thickening may extend across the costa for some distance (Fig. 19). Though there is no membrane separating the costa from the surrounding cytoplasm there is a special surface layer which is structurally complex (Figs. 15, 19). We shall not describe this here.

At the anterior end of the costa (i.e. where it is attached to the centriolar apparatus) there are some modifications of the pattern described so far. Within a transverse section, there occur regions which differ in the orientation of the lamellae (Fig. 14). Also, close to the point of attachment, the transverse plates change direction in some regions as shown in Fig. 21. These disturbances of the basic pattern will be dealt with in a later paper on the centriolar region.

Composition of the costa

When preparations of flagellates in gut fluid were observed under the microscope for long periods the trichomonads often lysed, releasing their costas, apparently intact but non-motile, into the medium. This suggested that the costas might easily be isolated in quantity. Treatment with a detergent (0.005% digitonin) or mechanical shearing by pipetting the cells in a buffered medium without the addition of KCl (see Methods) were both found to be effective. Similar methods have been discovered by Goodwin & Samuels (1971) to be effective for the costa of Trichomonas augusta. The costa is relatively stable in dilute saline solutions or distilled water: it remains intact for days after isolation and can even be heated to 100 °C without disintegrating. Nevertheless, it can be disrupted by treatment with 2% sodium dodecyl sulphate (SDS) or exposure to dilute acids (10⁻³ M HCl). It stains strongly with mercuric bromophenol blue, indicating a high protein content (Pearse, 1968).

Smears of gut fluid containing T. gigantea were fixed in osmium tetroxide and examined by the periodic acid-Schiff method for carbohydrate. The major part of the cytoplasm showed a positive reaction, consisting of a homogeneous coloration which

Figs. 17, 18. Enlarged details of the longitudinal section in Fig. 16, showing, respectively, the perpendicular and zig-zag patterns. In both Figs. indistinct connexions between the lamellae of adjacent transverse plates are visible.

Figs. 19, 20. Longitudinal sections of the costa of Trichomonas gigantea, where the plane of section is not perpendicular to the lamellae. The longitudinal direction is vertical. The lamellae are not distinguishable, though the transverse plates remain clear. A complex repeating pattern of transverse lines is visible in addition to the transverse plates. These lines are interpreted as due to superimposed images of lamellae sectioned in a longitudinal plane approximately parallel to the folds in the zig-zag pattern. The complex peripheral zone of the costa is visible in Fig. 19.

Fig. 21. Region near the anterior tip of the costa in Trichomonas gigantea, where the direction of the transverse plates changes abruptly.

Fig. 22. Costa isolated from Trichomonas gigantea and crushed between slide and coverslip. The organelle has split locally into longitudinal plates, which are here seen edge-on as dark lines. Phase contrast.
W. B. Amos, A. V. Grimstone, L. J. Rothschild and R. D. Allen

Mol wt.

1000

300 HMW

220 Myosin

90 α-Actinin

55 Tubulin

41 Actin

35 Tropomyosin

Troponins & Myosin light chains
Costa of Trichomonas

was strongest in the neighbourhood of the axostyle and costa, but the costa itself was totally negative, and was thereby distinguished clearly from its surroundings. Controls in which the oxidation with periodic acid was omitted showed no colouring.

A suspension of isolated costas was prepared for SDS gel electrophoresis by mechanical lysis of the cells in cacodylate-buffered medium. Although it was easy to obtain costas in this way it proved difficult to free them of contaminating debris and to concentrate them. After unsuccessful attempts to concentrate them by centrifugation in sucrose gradients a laborious but effective method was found: the costas were washed by transfer of individual organelles with tungsten needles. The washing produced a clean preparation almost entirely free of particulate contamination (Fig. 24). The costas were transferred to 30% (v/v) glycerol without buffer to remove salts and finally dissolved in SDS/mercaptoethanol solution. There was no visible residue. Azide and the proteolysis inhibitor p-tosyl arginine methyl ester (TAME) were present in all solutions throughout the lysis, isolation, washing and preparation of the protein sample. Fig. 25 shows the result of the electrophoresis of a sample containing approximately 0.5 μg of protein (1500 costas) on a 7.5% SDS polyacrylamide microgel. The proteins of the costa were separated into three main classes: high molecular weight material, above 300,000 mol. wt., a strong band at approximately 90,000, and material of low molecular weight, less than 30,000, running with the front. Except that the 90,000 mol. wt. band ran near but very slightly in advance of α-actinin, there was no correspondence between the proteins of the costa and those of glycerinated rabbit myofibrils. In particular, actin and myosin appeared to be absent from the costa. Protein at 55,000, corresponding to tubulin, was also absent.

Cytochemical tests for ATPase activity were performed by the lead method, according to the procedure of Wachstein & Meisel (1957). Aldehyde fixation was found to be necessary to prevent the costas from dissolving in the ammonium sulphide solution used in the final stage of this procedure. A strong brown-coloured precipitate was

Fig. 23. Formaldehyde-fixed specimens of Trichomonas gigantea subjected to the lead method for the localization of ATPase activity, according to the method of Wachstein & Meisel (1957). A dark precipitate is visible in the somewhat swollen costas, with little staining in the rest of the cytoplasm.

Fig. 24. Costas isolated by hand-picking with tungsten needles from a suspension of lysed cells. The larger organelles are those of T. gigantea, the smaller of the undescribed trichomonad from Porotermes adamsoni. Nomarski differential interference contrast.

Fig. 25. A 7.5% SDS polyacrylamide slab microgel stained with Coomassie blue. The gel allows a comparison of the polypeptides present in 0.5 μg of costas, consisting of 1500 organelles isolated manually (left), and a number of proteins known to be important in motility (right). The latter material was obtained by adding a small quantity of microtubule protein (tubulin plus high molecular weight associated proteins), purified from bovine brain, to a solution in SDS of glycerinated rabbit myofibrils. The costa polypeptides appear to consist of 3 chief types: low molecular weight material (less than 30,000), a prominent band at approximately 90,000, running slightly ahead of α-actinin, and material with molecular weights above 300,000. Some additional high molecular weight material was present in the stacking gel, not shown in the photograph. No myosin, actin or tubulin was detected in the costa sample.
observed in the costa and not in the surrounding cytoplasm (Fig. 23). This positive result was obtained with ATP, inosine triphosphate and guanosine triphosphate as substrates, but not with β-glycerophosphate. Controls without any substrate were negative. With ATP as substrate, a divalent ion at approximately $2 \times 10^{-3}$ M appeared to be necessary for a positive reaction: Ca²⁺, Mg²⁺, Ni²⁺ and Mn²⁺ were effective but Zn²⁺ was not. Controls designed to test for the unspecific binding of lead or phosphate (Gillis & Page, 1967) gave negative results. These results favour the existence of enzyme activity. It was also observed, unexpectedly, that costas which had been boiled for 20 min after fixation gave a positive result, controls that had been boiled similarly but lacked substrate being negative. The sulphydryl inhibitor mersalyl sodium did not inhibit, even at the high concentration of $5 \times 10^{-3}$ M. The meaning of these results is not clear at present: at face value they indicate the presence of a thermostable ATPase which is not dependent on sulphydryl groups for its activity. Such an enzyme would be highly unusual.

DISCUSSION

The results presented here show that the costa is an important organelle of motility in the trichomonads which inhabit termites. The question arises of whether the costa is also motile in the trichomonads of vertebrates. Movement of the costa has not yet been recorded in these organisms. We have examined *Trichomonas foetus* and *Trichomonas vaginalis*, and have been unable to see whether their relatively minute costas (only about 1 µm in diameter) are motile or not. So far as can be judged from the limited descriptions available, their costas are similar in fine structure to those of the termite flagellates. Nielsen et al. (1966) described the lamellae and showed micrographs indicating the transverse plates in *T. vaginalis*. The perpendicular type of pattern in longitudinal sections was found in *Pentatrichomonas hominis* by Honigberg, Mattern & Daniel (1968) though not interpreted as due to the sectioning of lamellae. The latter authors later (1971) made a distinction between two types of costa (A and B) depending chiefly on whether the perpendicular pattern occurs or not, but the model presented here explains why that pattern is obtained only rarely, by fortunate planes of sectioning. Since it could so easily be missed if an insufficient number of sections is examined, there does not seem to be sufficient evidence yet for two different types of costa. This being so, all costas may perhaps prove to be motile.

It is clear from the present observations that the bending wave in the costa of the termite trichomonads is accompanied, and presumably caused, by changes in the ultrastructure of the costa. The fact that the zig-zag configuration of the lamellae is invariably observed on the inner side of the curved costa suggests that it corresponds to a longitudinally contracted state of the material with a slightly higher concentration of dry mass than the remainder of the organelle.

If the bending results from a tilting of lamellae which remain of constant length, as shown in Fig. 26, a fixed relation should exist between the angle of the lamellae and the longitudinal separation of the transverse plates. If the maximum separation (i.e. that observed in the uncontracted regions with the perpendicular pattern) is $d$, the
separation in the tilted region $d'$, and the angle between the tilted lamellae and the longitudinal axis of the costa $\theta$, then $d'/d = \cos \theta$. Although several hundred micrographs of the fine structure of the costa at high resolution were obtained, only 3, of which Fig. 16 is one, were in the correct plane for accurate measurements of $d'/d$ and $\theta$. In these 3 sections the measured values of $\theta$ were 30, 33 and 35° and the values calculated from $d'/d$ were 12.9, 22 and 17.8° respectively. This indicates that the angle of the lamellae is too great to be accounted for by simple tilting: they must also increase in length as they tilt by amounts varying, in this case, from 11 to 16%.

![Fig. 26. Simplified diagram of a longitudinal section through a bent region of the costa, showing how the local contraction might be brought about by an alternate tilting of the lamellae. Adjacent lamellae are assumed to be held together so that there is mechanical continuity along the length of the structure.](image)

The optical results suggest that one half of the costa is contractile while the other half, on the outside of the bend, is merely passive, or perhaps elastic. Possibly the lamellae in the outer half are held permanently in the perpendicular configuration. Whether the lamellae in the inner region do undergo a reversible change in angle as suggested here is not yet known with certainty. It should be possible to test this by fixing and sectioning material in such a way that the location of the bends and of the regions of reduced birefringence can be identified in the electron microscope.

The propagated bending wave in the costa has certain features in common with the bending waves in eukaryotic flagella. It is propagated without decrement, usually from the centriolar apparatus, though any region may serve as a source of wave activity (cf. Holwill (1965) on the flagellum of Crithidia). The waves consist of straight regions alternating with curved regions as observed in some flagella (Brokaw, 1968). The mechanism by which the wave is propagated and the wavelength controlled remains an unsolved problem in both systems. It would appear from the observations on compressed cells that the costa, unlike many flagella, can bend in only one direction. The electron micrographs suggest that this direction is at right angles to the planes of the lamellae, which are generally parallel to the cell membrane. This situation is rendered more complicated by the fact that the costa is helical. The result of crushing it, as described above, suggests that the plane of the lamellae might rotate as one passes from one end to the other, i.e. there is an over-all helical organization in the fine structure which is reflected in the mechanical properties. This may be connected with the curious deviation of the optical axis from the longitudinal direction. At present
there is no clear explanation for this small but consistent deviation: it might perhaps be that the transverse plates deviate from a strictly transverse orientation in a systematic way.

Sledge, Larson & Hart (1978) have devised a procedure for isolating the costas of *Trichomonas foetus* by enzymic lysis of the cell membrane followed by enzymic digestion of the non-costa cytoplasm. They then separated the costas from the residue by differential centrifugation. The resulting preparation was examined by negative staining in the electron microscope and seemed highly pure, but on analysis its dry mass was found to be 95% carbohydrate and only 5% protein. This result conflicts with our observation that the costa contained no carbohydrate according to the periodic acid–Schiff test. With the large costa of *T. gigantea* the negative result of this test was clear, as was the very strong positive reaction of the surrounding cytoplasm. Trichomonad cytoplasm is rich in glycogen (Ryley, 1967) which is often visible as abundant granules in sections examined with the electron microscope (Nielsen et al. 1966). It therefore seems likely to us that the preparations analysed by Sledge, Larson & Hart contained glycogen from the surrounding cytoplasm. This would be consistent with their finding that the hydrolysis products consisted almost entirely of glucose.

The results of polyacrylamide gel electrophoresis of costa proteins described here are somewhat different from those obtained by Goodwin & Samuels (1971, 1972). These authors described, in brief abstracts, how they lysed *Trichomonas augusta* with cold butanol or Triton X-100 and, by sonication followed by differential centrifugation, obtained a preparation rich in costas. On electrophoresis in 7.7% gels this material was resolved into many bands, including 3 prominent ones at molecular weights of 70,000, 100,000 and 140,000. It may be that some of the additional bands observed by Goodwin & Samuels were due to contamination of the costa fraction with basal bodies and flagellar fragments: our own attempts to separate the costa of *Trichomonas foetus* by bulk methods showed that it is difficult to rid them of this material. Even with the technique of individual washing with tungsten needles, some contamination of this type was taken over, but the greatly expanded bulk of the costa in *T. gigantea* would tend to minimize the effect of contamination with fragments of the flagellar apparatus.

The fact that actin, myosin, tubulin and spasmin appear, in our results, to be absent from the costa indicates a new system of motility with a novel molecular basis. The nature of the protein with a polypeptide chain weight of 90,000 is unknown and it would be premature to identify it with α-actinin in spite of its near co-migration. It is possible, though unlikely, that the proteins responsible for motility were lost by solution or proteolysis in the course of preparation of the costas for electrophoresis, although no obvious loss of mass or structural damage took place. This objection cannot be fully answered except by reactivation of motility in the isolated costa in the same state as that in which it is prepared for electrophoresis. Several attempts were made to reactivate after brief glycerination, using media containing ATP, calcium and magnesium ions, similar to those which reactivate glycerinated muscle and flagella (Arronet, 1973) and axostyles (Mooseker & Tilney, 1973). Although reactivation of the flagella, including the undulating membrane, was achieved, no movement was observed...
in the costa. This may indicate a difference in the control mechanism or in the mechanism of motility itself. It is even possible that the costa, like the bacterial flagellum (Larsen, Adler, Gargus & Hogg, 1974), uses a fuel different from ATP.

The present description of the costa shows it to be structurally different from other types of fibre that are attached to basal bodies. In particular, lamellae appear not to have been described in other types of fibre. Simpson & Dingle (1971) have collected and compared descriptions of striated fibres from a variety of sources, including the ciliated epithelium of molluscan gills, algal zoospores, and the ciliate cortex, and find that there is an almost 3-fold variation in the spacing of the cross-striations. However, this does not in itself prove that there is no similarity in composition between the different types of fibre: the authors demonstrated in the same paper a 2-fold variation in periodicity within a single class of fibre, the rhizoplast of *Naegleria*, which they considered to be a physiological variation. Gibbons (1961) and Stephens (1975) studied the striated rootlets in the ciliated epithelium from molluscan gills and described constituent protofibrils 4–5 nm in diameter, running longitudinally. This is not totally inconsistent with the structure of the costa: the lamellae may consist of longitudinal filaments not resolved in the present study. Also, Stephens showed that the rootlets observed in the molluscan material were much more extensive when viewed in negative stain than in sections, and he concluded that conventional glutaraldehyde/osmium tetroxide fixation preserves the rootlet fibres poorly. Lamellae could therefore have been present in the molluscan rootlets but not sufficiently well preserved.

Stephens (1975) examined the proteins from a partially purified preparation of the basal apparatus from molluscan gill epithelium. Solutions which selectively extracted the proteins from the rootlet fibres contained a protein with a polypeptide chain weight of 240000, as measured by SDS polyacrylamide gel electrophoresis. This protein, which he termed ankyrin, appears to have no counterpart in the costa and there are no obvious similarities between the gel patterns obtained from the two sources. The costa is the first example of a rootlet fibre to be isolated as a pure preparation and it will be interesting to see whether, when equally pure rootlet fibre preparations from other sources are analysed, the present findings on the composition of the costa have a general relevance. The exciting possibility exists that other rootlet fibres may be motile: this has been suggested for the ciliary rootlets in retinal receptor cells (Matsusaka, 1967; Cohen, 1972).

We thank Dr A. Forer for help with the initial stages of the polarizing microscopy, and Dr K. E. Lee for sending colonies of termites from Australia.

REFERENCES


(Received 24 August 1978)