THE AXOSTYLE OF SACCINOBACULUS

I. Structure of the Organism and Its Microtubule Bundle

J. RICHARD McINTOSH, EDWARD S. OGATA, and STORY C. LANDIS

From the Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309. Dr. Ogata's present address is the Department of Pediatrics, San Francisco Medical Center, University of California, San Francisco, California 94132. Mrs. Landis's present address is the Department of Neuropathology, Harvard Medical School, Boston, Massachusetts 02115.

ABSTRACT

The axostyle of the flagellate Saccinobaculus is a motile ribbon composed of microtubules, cross-bridged to form interconnected rows. We find a centriole-related row of dark-staining tubules near the nucleus at the anterior end of the axostyle. Other tubule rows bind parallel to this primary row, acquire ordered relationships, and become the tubules of the axostyle proper. The number of tubule rows is constant in Saccinobaculus lata from the region near the nucleus to within a few micrometers of the posterior tip of the cell. In Saccinobaculus ambloaxostylus a few tubule rows are added to the axostyle posterior to the nucleus, giving this axostyle a leaf spring construction. The tubules of S. lata are held in rows by links with a 140 Å periodicity along the tubule axis; bridges between rows of tubules are also seen but are not apparently periodic. Each tubule in S. ambloaxostylus shows an axial periodicity of 150 Å due to pairs of arms, one of which is always part of the intrarow link. Interrow bridges in this species run either from tubule to tubule or from tubule to the free arm, but as in S. lata they do not display an obvious axial periodicity. An average unit cell is presented for the axostyle of each species, and the relation of the intertubule links to the microtubule substructure is discussed.

INTRODUCTION

Microtubules are widely distributed as components of biological motile systems, but it is not yet known whether they are simply passive, cytoskeletal elements, whether they serve as a framework for the organization of mechanochemical enzymes, or whether they participate directly in the production of mechanical work. To discriminate between these possibilities, one needs a system which is functionally and biochemically simple and in which structure studies can reveal the time-dependent events at high space resolution. An impressive example of tubule-associated cell motion is the whiplash action of the axostyle in Saccinobaculus (Grimstone and Cleveland, 1965), a genus of anaerobic flagellates which helps in cellulose digestion for its host, the wood-feeding roach Cryptocercus punctulatus (Cleveland et al., 1934). The axostyle is an intracellular bundle of cross-bridged microtubules extending the length of the cell, a distance of more than 100 μm. In healthy specimens the axostyle is repeatedly deformed by waves of bending which propagate from the anterior to the posterior end of the ribbon. Other flagellates have motile axostyles (Grassé,
FIGURE 1 A drawing showing the relationship between the cell, its axostyle, and the axostyle tubules and bridges. The circular inserts show the axostyle viewed from different directions. The arrows labeled A and B on the left-most insert show the lines of sight for the other two inserts.

1956), but we have selected *Saccinobaculus* as a system for morphological investigation of microtubule function because of the large size of its axostyle, the relative simplicity of the rest of the cell, and the frequency with which it is found in the extruded contents of *Cryptocercus* gut.

The biology of *Saccinobaculus* and its host has been investigated in detail by Cleveland and his co-workers (Cleveland et al., 1934; and Cleveland, 1950). The fine structure of *Saccinobaculus* and several related flagellates as seen after osmium fixation has been described by Grimstone and Cleveland (1965). The following description draws heavily upon the work of these investigators.

The axostyle begins in a specialized cytoplasmic region at the anterior end of the cell near the centrioles of the previous mitotic division. It passes over the nucleus, adhering to it tightly, and runs a helical course to the posterior extremity of the cell where it terminates in a differentiated region at the cell membrane (Fig. 1, and see also Figs. 2 and 10). The axostyle is constructed solely of cross-bridged microtubules arranged in approximately parallel rows. It stains as if composed of protein. One set of cross-bridges defines rows of tubules by linking neighbors in a line. The tubule rows are also interconnected, but by bridges which are less well defined: intrarow links are periodic along the tubule axis whereas interrow bridges occur only occasionally. The axostyle bends within a plane perpendicular to the tubule rows (Fig. 1).

Before cell division, the entire axostyle is cast loose from the anterior zone, and degenerates in the cytoplasm. During early nuclear division, a new axostyle begins to grow from each mitotic spindle pole. A description of division and of the sexual cycle in this organism at the light microscope level is given in Cleveland's papers (Cleveland et al., 1934; and Cleveland, 1950).

Axostyle bend formation and propagation is an attractive subject for morphological analysis of microtubule function for several reasons. Since the axostyle is composed of cross-bridged microtubules and contains no observable subsidiary components such as those found in cilia and flagella (Gibbons and Grimstone, 1960), it may represent one of the simplest biological engines composed of tubules. The almost crystalline order within the axostyle makes possible analysis by structure-averaging techniques, such as optical diffraction, which are an invaluable aid in objective structure studies (Grimstone and Klug, 1966; Reedy, 1968; Dekker and Klug, 1960). Finally, the wave of bending is a traveling wave; the position-distribution and the time-distribution of a traveling wave are related by the velocity of wave propagation. Thus *Saccinobaculus* may allow high space- and time-resolution observations of active microtubules to be made with the electron microscope.

In this paper we describe the axostyle and associated structures in two species of *Saccinobaculus*. The anterior and posterior ends of the axostyle are investigated, and the morphological uniformity of this motile organelle is examined. We describe
new features of the links which connect adjacent tubules and treat the axostyle as a quasi-crystalline system. For each species, a unit cell is presented which suggests certain inferences about microtubule substructure. In the subsequent paper we build upon this information to study the formation and propagation of the axostyle bend (McIntosh, J. Cell Biol., 56:324).

MATERIALS AND METHODS

Cryptocercus was collected at the Mountain Lake Biological Station of the University of Virginia. For observations in the light microscope, insects were killed and their alimentary tracts emptied onto slides with Trager’s culture solution CX6Z or solution D (Trager, personal communication; Trager, 1954). The protozoa of Cryptocercus are unusually sensitive to changes in the environment: atmospheric oxygen or small variations in the ionic composition of the medium are toxic, but we were able to maintain the symbionts in a healthy state for at least 3 h. The protozoa were observed with a Zeiss phase-contrast microscope equipped for 35 mm photography.

For electron microscopy, intestines of Cryptocercus were teased apart in a solution of 3% glutaraldehyde and 3% acrolein in 0.025 M phosphate buffer at pH 7 and fixed for 1-6 h. We have determined that this fixative stops axostyle beating within about 2 s (Fitzharris et al., 1972). The gut contents were then centrifuged into a pellet and washed with buffer for 24 h. After a 15-30 min postfixation with 1% OsO4 in phosphate buffer at pH 7.0 and a 2 h wash with six changes of buffer, the cells were dehydrated by dropwise addition of 100% ethanol, washed with propylene oxide, and embedded in Epon (Luft, 1961). The plastic, containing fixed gut exudate, was polymerized in a thin film on glass slides coated with carbon or Drislip spray lubricant (3M Co., 3M Center, St. Paul, Minn.). Individual cells were identified in the light microscope, circled with an objective slide marker, cut out, and remounted for sectioning.

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Electron microscopy was used to study the structure of the protozoa. Observations were made on sections cut with a diamond knife on a Sorvall MT-1 or MT-2 ultramicrotome (Ivan Sorvall, Inc., Norwalk, Conn.), picked up on Formvar-coated slot grids (Ladd Research Industries, Inc., Burlington, Vt.), stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined on a Philips 300 electron microscope equipped with a goniometer stage allowing 45° of tilt in any direction.

Saccinobaculus fixed with glutaraldehyde-acrolein precipitates OsO4, particularly in its axostyle. Most of our preparations contained a speckling of osmium granules which interfered with some aspects of the analysis (see, for example, Fig. 3). The grains could be removed from the sections by floating the grids with ribbons of sections on 0.2 M ethylenediaminetetraacetic acid at pH 8.0 for several hours before staining for electron microscopy.

Direct measurements were made on plates with a Nikon Shadowgraph 6C. The absolute accuracy of our measurements is probably no better than ± 8%, but the regularity of the axostyle permits structural comparisons at far higher precision, using the axostyle itself as an internal standard. Such comparisons reflect the variability within the axostyle, not the experimental error.

OBSERVATIONS

There are at least two large, morphologically distinguishable cells which fulfill Cleveland’s definition of Saccinobaculus. We identify them as Saccinobaculus lata (S.1) and Saccinobaculus ambloaxostylys (S.a).

General Considerations

A prominent feature of both species is the axostyle, which runs the length of the organism (see A in Fig. 2 b for S.1 and in Fig. 10 for S.a). The axostyle (A) runs almost the length of the cell. Large dark-staining granules are common (arrows), and a finer granularity (G) is characteristic of regions near the axostyle and the nucleus. The dark line shows the approximate location and plane of section for Fig. 3 c. The almost vertical lines are knife marks. 

Figure 2. Fig. 2 a is a differential interference contrast micrograph of a living specimen of S. lata (S.1). X 320. Fig. 2 b shows the EM image of an S.1 cell. The nucleus (N) contains a prominent nucleolus. The axostyle (A) runs almost the length of the cell. Large dark-staining granules are common (arrows), and a finer granularity (G) is characteristic of regions near the axostyle and the nucleus. The dark line shows the approximate location and plane of section for Fig. 3 c. X 8000. Fig. 2 c is a cross section showing the crescent shape of the unbent axostyle (A) in transverse view. The almost vertical lines are knife marks. X 10,000.

Figure 3. A segment of the primary row (PR) from the S.1 cell shown in Figs. 2 b and 4-9. The dark speckling is an osmium precipitate. One and possibly a second centriole (C) are near one end of the PR along with some amorphous, dark-staining material. Segments of rows similar to the PR are evident. (The cell nucleus is off the picture to the lower left.) The granules at G are characteristic of the zone marked G in Fig. 2 b. X 34,000.
FIGURES 4–9 a This set of nonadjacent serial sections from the cell shown in Figs. 2 b and 3 covers a net distance of about 3 μm. Short rows of dark-staining, thick-walled microtubules cluster up against the primary row (PR), take on ordered relationships, and become normal-looking axostyle tubules. The PR is seen to have a centriole-like structure (C) at each end. The cluster of tubule rows is surrounded by a "fence" of cross-bridged tubules called the thin lamella (TL). A structure like the PR but unattached to the axostyle is found in Fig. 8. We call it the old centriole (OC). Granular material (G) characteristic of the region labeled G in Fig. 2 b is plentiful in Figs. 8 and 9. Fig. 9 corresponds to the part of the axostyle near the nucleus and labeled A on Fig. 2 b, but the section for Fig. 9 is about ½ μm farther into the cell. × 14,000. Fig. 9 a is a diagram depicting the anterior end of the axostyle. Flagella (F), thin lamella (TL), primary row (PR), nucleus (N), axostyle (A), and old centriole (OC) are labeled. The insert shows the relationship between the PR and the other rows of the forming axostyle.
surface of both species is covered with characteristic invaginations which are probably related to pinocytosis (Figs. 2 b for S.1 and 11 for S.a). The cytoplasm contains large, membrane bounded, dark-staining granules (arrows in Figs. 2 b for S.1 and 11 for S.a). The granules in S.1 stain more darkly than those in S.a. Both free ribosomes and rough endoplasmic reticulum are plentiful, and there is a plethora of dark-staining structures slightly larger than ribosomes (G in Figs. 2 b, 3, 8, and 9 for S.1 and in Figs. 11, 20 a, and 27 a for S.a). They appear as dense 250-Å spheres bound in a sleeve of less dense matrix. There is no evidence for any kind of mitochondrion or mitochrondrial equivalent in either species, an almost unique feature among eucaryotic cells, though fine structure is clearly not the critical assay for this property.

Two cytoplasmic zones can be distinguished in each species. Immediately toward the cell axis from the axostyle in S.1 there is a region containing many small, dark-staining granules but devoid of all other organelles (G in Fig. 2 b). Elsewhere in the cytoplasm, all organelles and inclusions coexist. In S.a, there is no zone where the dark-staining granules congregate, but one can define a zone near the nucleus which is free from dark-staining particles of all kinds (Z in Fig. 11).

The Anterior End of the Axostyle

At the anterior end of the axostyle there is a structure which has been variously called the "ruban axial" (Grassé, 1956) and the "curved rod" (Grinstein and Cleveland, 1965). With serial sections of aldehyde-fixed specimens of S.1, we have been able to clarify the relationship of the curved rod to the axostyle proper (Figs. 3-9). Spanning the 3.5 μm between two centriole-like structures which are thickly coated with amorphous, dark-staining material (C in Figs. 3, 4, and 5), there is a row of about 125 thick-walled, dark-staining, 250-Å diameter microtubules whose surfaces almost touch each other (see Fig. 9 a, especially the insert). The center-to-center periodicity in the row is 280 Å. The surface of the row facing away from the nucleus is coated with a cross-hatched web of 40-Å diameter fibrils which measure about 600 Å in length. The surface of the web facing away from the tubules is attached to a row of fibers which lie approximately parallel to the row of tubules (Fig. 3). When traced toward the posterior end of the cell through about 1 μm of sections, the tubules of this row are seen to lose their dark-staining character and become the outermost row of tubules in the axostyle. We will refer to this outermost row over the entire length of the axostyle as the "primary row" (PR). On the side of the PR facing the nucleus there is a cluster of similar but shorter rows of tubules (fewer tubules per row) (Figs. 3, 4, and 5). Traced through serial sections toward the posterior end of the cell, these rows bind parallel to the PR, acquire well-defined positions relative to each other, and ultimately continue as normal-looking axostyle tubules (Figs. 4-9 and 9 a). Some regions in the cluster of short rows show the web of cross-hatched fibers but others do not. All tubules in these forming rows have dark-staining walls and

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**Figure 10** An S. ambloaxostylus (S.a) cell in rigor. The helical course of the axostyle (A) is evident. The approximate levels of sectioning for Figs. 11 and 13-16 are indicated. X 800.

**Figure 11** Cross section of S.a near the anterior end, at the level marked on Fig. 10. A small portion of the nucleus (N) is visible near the axostyle (A). A short segment of the thin lamella (TL) is seen in the zone (Z) which is devoid of granular material, including ribosomes and the slightly larger particles marked G. The membrane-bounded granules (arrows) are also absent from Z. Away from the nucleus the axostyle enters the section a second time. The orientation of the rows has shifted, due to the helical twisting, but the PR is still turned toward the cell cortex. At this point the axostyle contains more than 8000 microtubules arranged in about 60 rows. The obvious crescent shape of the rows is clearly not symmetric; from serial sections we know that the blunt end (B) is continuous with the part of the axostyle connected to the dark-staining strand (D). The center of crescent curvature (CC) is connected by an inked line (R°) to the PR. X 8000.

**Figure 12** A short segment of a PR with a few associated dark-staining tubules in S.a. The dark-staining amorphous bodies (AB) are similar to, though smaller than, those seen at the row ends farther down the axostyle (Fig. 13). A second structure like the PR is not attached to the axostyle. This picture of S.a is analogous to Fig. 8 for S.I. X 16,500.
almost touch their neighbors in the row. Apparently the cross-hatched web is not necessary for the addition of all axostyle rows, but in three cells, it has been characteristic of the PR and a few of the nearest row segments. Following the rows down the axostyle, the cross-hatched web disappears before the disappearance of the dark-staining character of the tubule walls (Figs. 4–9).

In two S.1 cells, a second structure like the anterior part of the PR was seen near the anterior end of the axostyle, but even with serial sections we could not observe a connection of this row to the axostyle (Fig. 8). In two cells a pair of flagella emerged from basal bodies associated with the structure. Cleveland has reported that the “new centriole” at each spindle pole is associated with axostyle initiation, and we infer that the second primary row may correspond to the structure Cleveland saw in the light microscope as the “old centriole” (OC) (Cleveland, 1950). Surrounding the anterior end of the PR and the associated cluster of microtubule rows is a fence of parallel, cross-bridged 250-Å diameter tubules spaced at about 1000 Å. This fence corresponds to the “thin lamella” (TL) described by Grimstone and Cleveland (1965) (Figs. 4 and 11). In sections which contain the nucleus, the TL enlarges to pass around the nucleus as well as the axostyle. The TL ends just posterior to the nucleus. These observations are diagrammed in Fig. 9a.

The axostyle-associated structures at the anterior end of S.a seem similar to those of S.1, but we have not studied them in detail. Fig. 12 shows a portion of a primary row and an old centriole from S.a. The PR is again connected to the outermost row of the axostyle which runs from one end of the ribbon to the other. Like S.1, S.a contains a thin lamella of cross-bridged tubules which surrounds the anterior portion of the axostyle and the nucleus (TL in Fig. 11).

The Midregion of the Axostyle

The cross section of an unbent axostyle shows that each tubule row is curved, concave toward the center of the cell, giving the whole axostyle a crescent shape in transverse view (Fig. 2 c for S.1 and Figs. 11 and 13 for S.a). The axostyle twists through the cell, tracing out a left-handed helix of varying radius. The primary row in both species is always turned so as to be the row nearest to the cell membrane, and the crescent is always concave in. The helix pitch is a function of the bending state of the axostyle, and thus is not constant, but when cells stop wriggling, they often assume an elongate state which we call rigor (Fig. 10). For S.a cells in rigor, axostyle pitch in the midregion of the cell is about 100 μm; for S.1, it is even greater.

The curvature of the cross-section crescent seen...
in unbent portions of the axostyle of either species is approximately constant over most of the length of a cell. The minimum radius from the center of crescent curvature (CC) to the PR ($R_c$ min—see Fig. 11) does not vary appreciably along the length of the unbent axostyle, except near the axostyle ends. In the midregion of S.1, $R_c$ min = 9 μm ± 15% (one organism), and in S.a $R_c$ min = 7 μm ± 10% (two organisms). In regions where the axostyle is bent (as in Figs. 2 c for S.1 and 10 for S.a) there are large changes in the curvature of the cross-section crescent. These are discussed in the following paper.

The Posterior End of the Axostyle

The posterior end of the Saccinobaculus axostyle is wrapped in a sheath (Cleveland et al., 1934; Grimstone and Cleveland, 1965). The sheath swathes the posterior $\frac{3}{4}$ of the length of the axostyle down to its end at the caudal extremity of the cell (see Figs. 14–17 for S.a and Fig. 18 a for S.1). The sheath is composed of cross-bridged 250-Å diameter tubules with a center-to-center spacing of 1000 Å (S in Figs. 14 and 17 for S.a and in Fig. 18 for S.1). The structure is therefore similar in its design to the thin lamella of tubules surrounding the anterior portion of the axostyle in both species (Figs. 4 and 11). Thus, circumferentially oriented tubules are present at the axostyle ends both in S.1 and in S.a. These sheaths may play a role in attaching the axostyle to the rest of the cell (Grimstone and Cleveland, 1965).

At the level where the sheath appears, the crescent curvature, as seen in cross section, increases markedly (Figs. 13–16). With the curvature increase, both the number of tubule rows and the length of the rows decrease sharply. In this region cross sections show row ends within the axostyle, giving rise to dislocations (Fig. 18 b, arrows). Only about 15 rows run all the way to the posterior extremity of the cell, where the axostyle terminates at a dark-staining cup next to the plasma membrane (Figs. 17 for S.a and 18 a for S.1).

The Uniformity of the Axostyle

Our interest in the axostyle extremities, which show structural variations, should not divert attention from the striking uniformity seen in the bulk of the axostyle. One's impression of structural constancy from a single view such as Figs. 2 b and c, 11, and 13 is borne out by more detailed study. Cross-sections of the two species show that all the tubules of a given axostyle look much alike (Figs. 19 for S.1 and 20 for S.a). In aldehyde-fixed material of both species, the tubules measure 250 Å in diameter and are spaced 425 Å center-to-center within a row. The standard error of the intrarow tubule spacing is less than 2% of the mean for any

![Figure 18](http://www.jcb.org/cgi/content/full/56/2/304/DC1/fig18a.png)

**Figure 18** Fig. 18 a shows the posterior extremity of an S.1 axostyle. A sheath (S) surrounds the axostyle, which ends on a cuplike structure next to the plasma membrane. Fig. 18 b is a cross section of a different S.1 cell at about the level of the S's in Fig. 18 a. Note the rows ending (arrows). The apparent groove in the axostyle (Fig. 18 a) is due to the crescent-shaped cross section (see Figs. 14 and 15). Fig. 18 a, × 26,000; Fig. 18 b, × 55,000.

![Figure 19](http://www.jcb.org/cgi/content/full/56/2/304/DC1/fig19a.png)

**Figure 19** Cross sections of an unbent S.1 axostyle. In Fig. 19 a the organization of the tubules into rows is evident both from the existence of links between every pair of neighbors in a row and from the frequent bends in the lattice lines that cross the rows. Interrow bridges are also numerous. They attach to the surface of the tubules at points arranged with sixfold rotational symmetry (Fig. 19 b). The arrows marked A and B show the line of sight for subsequent pictures and discussion of longitudinal sections. Fig. 19 a, × 86,000; Fig. 19 b, × 310,000.

![Figure 20](http://www.jcb.org/cgi/content/full/56/2/304/DC1/fig20a.png)

**Figure 20** Cross sections of an unbent S.a axostyle. As in Fig. 19 the arrangement of tubules in rows is clear. In this species, the tilt of adjacent rows is evident from the oblique orientation of outlying rows. The dark-staining granules in their sleeve of matrix (G) are present beside the axostyle. Fig. 20 b shows that each tubule in this species has two short arms projecting from its surface in a fixed relationship. We call these arms dog-ears. One of the arms is always connected to the neighbor tubule in the row, forming an intrarow link. The other dog-ear is always parallel to the first and it usually has one end free, but sometimes it is bound to a tubule in the adjacent row (curved arrows in Figs. 20 a and b). The arrows marked A and B show the line of sight for subsequent pictures and discussion of longitudinal sections. Fig. 20 a, × 85,000; Fig. 20 b, × 310,000.
FIGURE 21 Two views of a cross section of an S.a axostyle. Fig. 21a was tilted 17° clockwise and Fig. 21b was tilted 40° clockwise about a horizontal axis viewed from the right. Adjacent rows are therefore tilted by about 0.5° with respect to their neighbors on either side. X 85,000.

FIGURE 22 A construction defining the changes in packing of neighboring tubules. A and B are vectors connecting neighbor tubules. The angle between A and B changes because the length A is constant and consecutive A's make a small angle with one another. R_C is the radius from the crescent center (CC) to the innermost tubule row, R_O is to the outermost.

FIGURE 23 The number of axostyle rows as a function of position along the axostyle. Fig. 23a shows the data from three S.1 cells; all three cells had essentially the same curve. Fig. 23b shows data from three S.a cells. The drawings above each graph were made from light micrographs of the cells counted. Position along the cell is only approximate, since not all sections were collected.

The spacing between the rows shows more variation, ranging from 320 to 360 Å in S.1 and from 290 to 360 Å in S.a.

As reported by Grimstone and Cleveland (1965), the intrarow links connect every neighbor tubule in a line, but the bridges between rows are less common (Figs. 19 and S.1 and 20 for S.a. These figures are discussed in detail in the section on tubule bridges). In S.1 the tubules of adjacent rows
FIGURE 24 A longitudinal section of an S.1 axostyle with the plane of section perpendicular to the rows of tubules. (The line of sight for this orientation is shown by the A arrow on Fig. 19 a.) What appear to be single microtubules are superimpositions of about two tubules and the corresponding intrarow bridges. This probably accounts for the observed periodicity of 140 Å along the tubules. Viewing the picture from the side, one can see the registration of this periodicity on adjacent tubules. Some interrow bridges are visible between the tubules. Fig. 24 a, X 65,000; Fig. 24 b, X 180,000.

FIGURE 25 A longitudinal section of an S.a axostyle with the plane of section parallel to the tubule rows. (The line of sight for this orientation is shown by the B arrow on Fig. 20 a). Intrarow links with a periodicity of 150 Å are evident between the tubules. In Fig. 23 a the variation in picture darkness is a moiré pattern resulting from superimposition of differing numbers of tubule rows as their crescent shape takes them in and out of the plane of section. Fig. 25 a, X 69,000; Fig. 25 b, X 180,000.

FIGURE 26 A longitudinal section of an S.a axostyle with the line of sight down the A arrow on Fig. 20 a. The section is thin enough to allow the intrarow links to dominate some regions in which the tubules lie just above and below the plane of section. X 65,000.
are approximately parallel to one another, but in S.a they are tilted, conferring an apparent dissimilarity upon adjacent regions of the axostyle (Fig. 20 a). With the goniometer stage one can bring different areas of a given cross section into true transverse orientation and show that an entire section does contain similar tubules and bridges (Fig. 21 a and b). Each tubule row is tipped relative to its neighbors by about 0.5° about an axis which is perpendicular to the plane of the row at that point. (This is just about the right tilt angle to insure that adjacent rows can all be packed into one helix with a pitch of about 100 μm and radius varying from about 5 μm to 7 μm.)

The only apparent structural variation within the axostyle of each species is the distribution of the interrow bridges and the packing arrangement of adjacent tubules, as noted by Grimstone and Cleveland (1965) (Figs. 15 b, 19, and 20). Some portions of an axostyle cross section show hexagonal packing while others are in a square array; many intermediates are seen. This variation can be attributed in part to the curvature of the tubule rows. Since the intertubule distance within a row is well defined and since the radius from CC to different tubule rows is necessarily different, the angular relationships between the tubules must change (Fig. 22). As expected from this reasoning, the angle between the vectors A and B connecting a tubule to its neighbors varies with respect to position on a given row at a rate which decreases with decreasing row curvature. (See Fig. 22 and then compare the variations in packing along the rows on the inside and outside of the crescent in Fig. 13).

We have also looked for possible variations in the axostyle along its length. The images seen in Figs. 19 and 20 are representative of unbent S.1 and S.a axostyles at any point posterior to the region of the dark-walled tubules at the nuclear end and anterior to the sheath. The number of tubule rows shows a striking constancy in S.1. The three specimens counted had 25 ± 1 rows over the majority of their length (Fig. 23 a). S.a contains as many as 66 rows, and there is more variability in row number (Fig. 23 b). Rows in S.a are always added onto the axostyle surface away from the PR. The tubules of these rows are shorter than the tubules near the PR, giving the axostyle a leaf spring construction. Associated with the ends of many axostyle rows is an amorphous ball (AB) of dark-staining material similar in texture to the pericentriolar material associated with the PR (compare Fig. 13 with Figs. 3 and 4). In both species, the rapid decrease in row number at the posterior end occurs just as the sheath appears. There is more variability in the number of tubules per row than in the number of rows, but since the axostyle bends in a plane perpendicular to the rows, variation in row length is probably not mechanistically important.

**Bridges between the Tubules**

**AXOSTYLE CROSS SECTIONS:** Cross sections of S.1 and S.a axostyles show that although the packing of adjacent tubules varies from a four- to sixfold arrangement, the symmetry of the points of attachment of the bridges to the tubule's surface is always approximately sixfold (Figs. 19 for S.1 and 20 for S.a). In S.1 the two sites of attachment for intrarow links are always occupied, but the interrow bridge binding sites are sometimes occupied, sometimes vacant. In S.1 we have counted 1740 interrow bridges for 3760 intrarow links in unbent portions of the axostyle. (For a comparison of straight and bent axostyles, see the following paper.)

In S.a there are several morphologically distinguishable structures attached to the axostyle tubules. Each tubule in Fig. 20 b has two arms which we call “dog-ears” projecting from its sur-

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**Figure 27** A longitudinal section of an S.a axostyle with the line of sight down the A arrow on Fig. 20 a. There is a 150 Å periodicity along the tubules, and some interrow bridges can be seen. The dark-staining granules in their sleeve of matrix (G) are evident in Fig. 27 a. In Fig. 27 b it is clear that each interrow bridge binds to a tubule at a place where an intrarow link is seen projected onto the tubule's surface. Fig. 27 a, X 65,000; Fig. 27 b, X 180,000.

**Figure 28** Two pictures of the same region of an S.a axostyle. Fig. 28 a is tilted by 90° from Fig. 28 b about a line parallel to the tubule axis. The arrows mark the same piece of stain precipitate. Periodicity along the tubules (view A, in Fig. 28 a) is converted to periodicity between the tubules (view B, in Fig. 28 b) by the tilting. X 72,000.
The dog-ears are about 100 Å long and 50 Å wide; they are separated by about 100 Å center-to-center and lie almost parallel to one another (see diagram, Fig. 29). One dog-ear, generally the one nearer the central axis of the cell, is connected to the nearest side of the next tubule in the row by a bridge about 100 Å long, forming the intrarow link. The other dog-ear is generally unattached to neighboring tubules. The dog-ears cannot be resolved on obliquely oriented tubules (Figs. 20 a and 21 a and b), but with the goniometer stage we have seen that all tubules and associated links or arms in a given S.a axostyle cross section have the same EM image. Further, from complete serial sections of three specimens of S.a, we know that the structure of the tubule appendages does not change until the sheath is seen. At that level the dog-ears are no longer visible, and the tubules are simply linked together in curved rows (Fig. 15 h).

Cross sections of an S.a axostyle also show distinct bridges between tubule rows, but as in S.1, these bridges are less common than the intrarow links. We have counted 757 interrow bridges for 3960 intrarow links in unbent S.a axostyles. Sometimes they bridge the rows by running from tubule to tubule, but they are also seen bridging a tubule to the free end of that dog-ear which is not linked to its intrarow neighbor tubule (Figs. 20 a and b, curved arrows). The often-observed splitting of the axostyle along the surface between tubule rows (Fig. 13, curved arrow, and also Figs. 11 and 15 a) indicates that the bridging between rows is weaker than the linking within a row, consistent with the difference in the number of bridges seen.

Longitudinal sections: In longitudinal section, the axostyle tubules may be viewed with the line of sight either parallel to or perpendicular to the tubule rows (the arrows labeled A and B on Figs. 19 a and 20 a). Looking down the A line of sight, we will see tubules connected by interrow bridges; looking down the B line of sight, we will see tubules connected by intrarow links. Figs. 24 a and b show a longitudinal view of S.1 down the A line of sight. There is a 140 Å periodicity along the tubules, and Fig. 24 b shows some bridges between the tubules. Figs. 25 a and b show a longitudinal view of S.a down the B line of sight. The tubules are connected by interrow links with a periodicity of 150 Å. Figs. 27 a and b show an S.a axostyle viewed along the A line of sight. Fig. 27 b reveals interrow bridges and a periodicity along the tubules of 150 Å.

We suggest that the periodicity seen along the tubules viewed down the A line of sight results from a projection of the intrarow links onto the tubules' surface. When very thin longitudinal sections of an S.a axostyle are viewed along the A line of sight, certain of the tubules show little periodicity (Fig. 26). In such micrographs rows of dots with a periodicity of 150 Å can be seen between many of the tubules. We interpret the dots as a series of interrow links sliced off from the tubules to which they are normally bound. Thus, in our micrographs of fixed, thin-sectioned material the tubules themselves do not appear to possess an axial periodicity, suggesting that any periodicity is due to bridges and arms.

With the goniometer stage, we can obtain further evidence that the periodicity seen along the tubules when viewed along the A line of sight is due to the intrarow links. Fig. 28 a shows a longitudinal section of S.a axostyle tubules viewed from the A direction with the section tilted about the axostyle axis by 45°. Fig. 28 b is the same field (note the granules of stain precipitate at the arrow tips which serve as markers) tilted about the same axis 45° the other way, enabling us to view the structure from the B direction. The plane of section for Fig. 28 is the bisector of the angle between A and B. The tilted pair demonstrates that the periodicity along the tubule in one view is equal to the periodicity between the tubules when seen at right angles. Proper orientation of the bridges is seen in only a limited area of the two pictures because of variation in the packing of the tubules.

The view of the S.a axostyle showing intrarow links between the tubules (Fig. 25) demonstrates that the sites which bind the intrarow links to opposite sides of a given tubule lie on a line perpendicular to the tubule axis. Moreover, when an interrow bridge is seen, it generally binds to the tubule at a place where an intrarow link is seen projected onto the tubule's surface (Figs. 24 b for S.1 and 27 b for S.a). We conclude that the binding sites for tubule bridges and arms lie in a plane perpendicular to the tubule axis.

The slant of the imaginary lines connecting the tubule periodicities in Fig. 27 a (view the picture from the side almost perpendicular to the tubules' axes) shows that the registration between adjacent rows is slightly oblique, placing equivalent points on a line making about 8° with the plane perpendicular to the tubules' axes. The orientation of this line varies in different pictures from 0° to...
12°, its theoretical maximum (one-half the angle between the lines connecting an intrarow link seen in longitudinal section with its nearest neighbors in an adjacent row). This variation indicates that the relative position of adjacent rows is not tightly set.

DISCUSSION

The axostyle is so highly ordered that we may profitably consider it as a crystal and discuss its structure in terms of an average unit cell and the common variations in such a cell. The virtue of the unit cell approach is that it allows us to organize all the structural information into a single, simple unit.

Figs 29 a, b, and c show an average unit cell for S.a viewed down each of the lattice vectors, and Figs. 30 a, b, and c are the same for S.l. Common structural variations found in the unit cells of both species are the angles between the A and B and the B and C lattice vectors, the position and orientation of the interrow bridges, and

![Diagram](Image)

**FIGURE 29** Three views of an average unit cell from an S.a axostyle. The edges of the unit cell are defined by the arrows. Dark stippling represents a bridge or arm which is always seen; light stippling represents bridges which are occasionally present.

![Diagram](Image)

**FIGURE 30** Three views of an average unit cell for the S.l axostyle. Dark and light stippling is as in Fig. 29.
small differences in the interrow distances. The major difference between the two unit cells is the presence of the dog-ears on all tubules of S.a. Since both species are motile, it is unlikely that this difference is of great functional significance.

The placement of projections from the tubule’s surface suggests certain properties of tubule structure, subject to the assumption that binding sites for similar surface projections are markers for similar places on the tubule. The view down C in Figs. 29 a and 30 a depicts our observation that bridges can attach to the tubule at points showing approximately sixfold symmetry, suggesting that the tubule subunits are arranged in a pattern which is a multiple of sixfold. While the curvature of the rows in the unbent axostyle might suggest a departure from an even-numbered rotational symmetry, the curvature is not large enough for an odd number near to 12, the approximate number of subunits found in other studies (e.g., Cohen et al., 1971). We think it likely that the crescent is a result of structural properties of the bridges.

The view down B portrays our observation that the attachment sites for intrarow links lie on a line perpendicular to the C axis (Figs. 29 c and 30 c). The view down A reminds us that the binding sites for interrow bridges lie in the same plane, perpendicular to the tubule axis, as the sites for the intrarow links (Figs. 29 b and 30 b). We infer that the six bridge-binding sites show cylindrical symmetry, and that if the surface lattice of the tubule is helical, it must be constructed from an integral multiple of six helices. This model is consistent with the X-ray data available on sperm tail flagellar tubules (Cohen et al., 1971).

In both species of Saccinobaculus the tubules have the ability to bind by bridges to six near-neighbor tubules, but fourfold packing is often seen; we infer that interactions within a row are strong enough to negate the simple principle that the tubules should pack so as to maximize the number of intertubule bonds. The occasional peeling off of one row from another cited above is further evidence for the row-forming tendency. The dog-ears on the tubules of S.a provide a structure with which to rationalize row formation, but in S.1 we must postulate a “sticky” portion of the tubule’s surface which has no manifestation at the EM level. This evidence for both obvious and subtle departures from cylindrical symmetry of an order around 6 suggests that there may be at least two distinguishable sides to each axostyle tubule. The possibility thus exists for force-producing, two-fiber type interactions between equivalent, parallel microtubules: the A side of tubule 1 could interact with the B side of tubule 2 to produce a net force. This possibility is of obvious mechanistic significance, since the distribution of the number of tubule rows along the axostyle provides no evidence that the axostyle is constructed from two antiparallel, interdigitating families of tubules (Fig. 23). The formation of doublet microtubules in cilia and flagella and the binding of arms at two sites on one flagellar subtubule may be other manifestations of a departure from higher order cylindrical symmetry (Gibbons and Grimstone, 1960). The evidence for several polypeptides within the wall of a single flagellar microtubule provides a conceptual framework within which to understand this structural complexity (Witman et al., 1972), but further work will be necessary to clarify the situation.

The cross-sectional size and shape of the axostyle are defined by factors which we do not entirely understand. The number of tubules in the PR in S.1 contributes to the definition of the breadth of the axostyle, but the mechanism for specifying the number of rows is unclear. The presence of balls of amorphous, dark-staining material at the ends of some axostyle rows in S.a suggests that microtubules can be added during interphase to an apparently complete and certainly functional axostyle. The distribution of microtubule subunits between elongation of existing tubules and initiation of new tubules is presumably under the control of the undefined system which initiates microtubules.

The primary row and the structures associated with the anterior end of the axostyle may be a delightfully complex example of a “microtubule-organizing center” (Porter, 1966; McIntosh and Porter, 1967; Gibbons et al., 1969; Pickett-Heaps, 1971; Tucker, 1971). In spite of the apparently extended nature of this assumed organizing center, we believe that its essential properties are embodied in a functional unit cell composed of two dark-walled tubules and probably some cross-hatched fibers and the adjacent fiber row. Once the position, orientation, and timing of this control unit can be understood, the remainder of axostyle development will probably be comprehensible in terms of short-range forces and the principles of self-assembly. We think it likely that the formation of the control unit involves no processes which
are intrinsically different from the processes of tubule control in other, less complicated systems, though, as Grimstone and Gibbons have pointed out, the symbionts of wood-feeding insects have enjoyed a privileged environment for so long that they may well have evolved under unusual selection pressures (Grimstone and Gibbons, 1966).

Since the axostyle appears to be a lattice-like arrangement of microtubule subunits and intertubule bridges, and given evidence from other ordered biological systems, it is an obvious conjecture that the geometry of the array is defined by the geometry of both the intra- and intertubule bonds. The spatial periodicity of the axostyle makes it possible to treat bend formation and propagation as a set of systematic perturbations in these local interactions which results in a deformation of the lattice. The perturbations are then reiterated both in space and in time. It is the primary purpose of our study of bend morphology and behavior presented in the following paper to determine the nature of these lattice perturbations.

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