THE STRUCTURE OF TRICHOCYSTS IN PARAMECIUM CAUDATUM

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SUMMARY

The structure of undischarged and discharged trichocysts has been examined in Paramecium caudatum, and their light-microscopic appearance compared with their fine-structural organization. In living specimens undischarged trichocysts appear to be of a single type with a unimodal variation in length about a mean of 37 μm. When fixed for electron microscopy or compressed beneath a coverslip many of the trichocysts expand within the cell, giving rise to a variety of different forms of lower phase density.

Ultrastructurally the undischarged trichocyst consists of at least 10 different components: these include a mesh-like sheath surrounding the body of the organelle; an inner and an outer sheath enclosing the tip, the inner sheath being made up of 4 spiralling envelopes with a square net substructure, and the outer sheath being formed of a dense amorphous matrix containing longitudinal microtubules and scattered fine filaments; a boundary surface to the outer sheath; a membranous trichocyst sac the apical region of which is surrounded by a cylinder of microtubules joined to each other with dense material; and lastly, the crystalline matrix of the trichocyst body and tip. This crystalline appearance is apparently related to the presence of a loosely interwoven complex of fine filaments which form a highly regular pattern of unit structures repeating at 16-nm intervals.

In extended trichocysts the 60-nm banding pattern of the body is also composed of fine filaments arranged in a different, elongated manner in 2 distinct and alternating patterns which are taken to be 2 views of the same structure. Measurements indicate that when trichocysts extend they elongate by a factor of from 6 to 8. It is proposed that the crystalline pattern of the unextended trichocyst body transforms into the extended form by a simple rearrangement of the constituent filaments accompanied by their elongation. Possible models of the undischarged and discharged states of organization are suggested.

INTRODUCTION

The trichocysts of Paramecium have been the subject of many fine-structural studies since the work of Jakus, Hall & Schmitt in 1942 (see the reviews of this topic by Grimstone, 1961; Pitelka, 1963; Hovasse, 1965; see also the papers of Sedar & Porter, 1955; Yusa, 1962, 1963; Stewart & Muir, 1963; Ehret & de Haller, 1963; Jurand & Selman, 1969; Selman & Jurand, 1970; Stockem & Wohlfarth-Botterman, 1970). Similar structures are also found in other genera of holotrich ciliates such as Frontonia (Beyersdorfer & Dragesco, 1953; Rouiller & Fauré-Fremiet, 1957; Yusa, 1965), Nasula and Dissematostoma (Dragesco, 1952) and Neobursaridium (Dragesco, 1968). Recently the problem of the chemical composition of trichocysts has also been reopened (Stears, Beisson & Marchesi, 1969). However, a number of problems concerning their ultrastructure and the changes accompanying extension still remain to be settled.
The body of the discharged trichocyst possesses a characteristic transverse banding with a periodicity of about 60 nm. Undischarged trichocysts, in contrast, have been described as showing an amorphous internal structure, although transverse banding with a spacing of 6–10 nm has been described in some specimens (Potts, 1955; Sedar & Porter, 1955). It was considered by Yusa (1962, 1963), Jurand & Selman (1969) and Selman & Jurand (1970) that this striated appearance characterizes an early stage in the maturation of trichocysts, whereas the 'amorphous' structure typifies the fully mature state immediately prior to discharge. Rouiller & Fauré-Fremiet (1957) reported that in Frontonia many trichocysts elongated inside the organism when fixed for electron microscopy, and these authors showed micrographs of trichocysts apparently caught in the process of transforming from an amorphous state through a transitional zone of 10- and 25-nm cross-banding to the fully extended pattern denoted by 50-nm cross-banding. This transition from a structureless to a highly ordered state which has been thought to accompany trichocyst extension is made even more remarkable by the finding of a highly complex substructure within the 50–60 nm period banding of the discharged forms (Nemetschek, Hofmann & Wohlfarth-Bottermann, 1953; Jurand & Selman, 1969; Steers et al. 1969; Stockem & Wohlfarth-Bottermann, 1970).

The present paper examines this problem in the light of some new findings on the morphology of undischarged and discharged trichocysts, and proposes a possible model for the transition from one state to the other.

MATERIALS AND METHODS

 Cultures of Paramecium caudatum were grown in a modified Peters' medium (de Terra, 1966), and for measurements of trichocyst lengths by light and electron microscopy, a single clone was isolated and cultured to eliminate variation due to genetic differences.

 Observations on living specimens were made with phase-contrast and polarizing light microscopy. Measurements of trichocysts were obtained by means of a micrometer eyepiece, undischarged trichocysts being held at right angles to the direction of view by light compression of the organism with a thin coverslip; other undischarged trichocysts were prepared by mechanical disruption of cells in a 20 % sucrose solution which was found to inhibit discharge in the same manner as MgCl₂ (Steers et al. 1969). Discharged trichocysts were prepared by light mechanical shock applied to organisms previously irrigated with 0·01 % acetic acid or 10 % ethanol. With undischarged trichocysts, measurements were made to the outer rim of the bright halo surrounding these highly refractile bodies so that the sizes quoted represent a maximum value. The minimum width of such halos was about 0·3 μm. Likewise it should be noted that in discharged trichocyst bodies, electron micrographs show that the central end of the body tapers below the limit of resolution of the light microscope so that these values represent a minimum length.

 To investigate the effects of fixatives on the light-microscope appearance of trichocysts, suspensions of Paramecium were irrigated with various fixing solutions such as the glutaraldehyde fixative used in electron microscopy (see below), phosphate- or veronal-buffered 1 % osmium tetroxide (pH 7·3) at 4–15 °C, and the same solution at 37 °C. Other specimens were lightly centrifuged, fixed for a few seconds or a few minutes in these various fixatives, and observed by light microscopy.

 For electron microscopy, sectioned and disrupted negatively stained material was prepared. For sectioning, specimens of Paramecium were lightly pelleted and resuspended in 3 % glutaraldehyde buffered with 0·2 M phosphate at pH 7·3. After 2 h fixation in this solution, the organisms were again centrifuged into a pellet which was washed for 2 days in several changes of phosphate buffer, then treated with 1 % osmium tetroxide solution buffered at pH 7·3 with
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Fig. 1. Histograms of the length distributions of trichocyst bodies (see also Table 1). A, undischarged trichocysts; electron-micrographic data are expressed in white columns superimposed on the light-microscope measurements (black). B, discharged bodies. White columns represent electron-microscope data, superimposed on light-microscope measurements (black).

0.2 M phosphate (containing 45 mg/ml sucrose), dehydrated in ethanol and embedded in Araldite. Sections were stained with uranyl acetate and lead citrate. Other specimens were fixed in 1% osmium tetroxide as used in the glutaraldehyde procedure described above. For negative staining, suspensions of Paramecium were pelleted by light centrifugation, resuspended in 2–3 drops of potassium phosphotungstic acid at pH 7.0 and then passed 3 times through a hypodermic needle to disrupt the cells. Drops of the resulting suspension were allowed to settle for up to 4 min on carbon-Formvar coated grids which were then drained and examined in the electron microscope.

Measurements of electron micrographs were made from prints at a plate magnification of X 4000 and a final magnification of X 10000. Densitometric measurements were made directly from negative plates taken at a magnification of 50000. Magnifications were calibrated with a carbon replica of a grating ruled at 32700 lines to the inch. All fine-structural observations were made with an RCA EMU-4A electron microscope operating at 50 or 100 kV. A specially constructed specimen holder allowed tilting of the specimen up to 30° for stereoscopic analysis. Some of the negatively stained or unstained whole trichocysts were also shadowed at low angles with gold-palladium prior to stereoscopic examination.

RESULTS

Light-microscope observations

In living organisms the undischarged trichocysts are situated immediately below and perpendicular to the pellicle where they form a palisade-like layer of refractile rods up to 6 μm deep. When organisms were compressed lightly beneath a coverslip and viewed with phase optics the undischarged trichocysts appeared as dense carrot-shaped bodies surmounted by a tapering tip (Fig. 8). The bodies varied in length unimodally about a mean of 3.7 μm, with a width of about 1 μm (Table 1, Fig. 1). When suitably stimulated (see Materials and Methods) by a sharp mechanical stimulus, the undischarged trichocysts discharged immediately into the surrounding medium as typical elongated spindle-shaped structures, still surmounted by conical tips (Fig. 9).
Table 1. Length measurements of undischarged and discharged trichocysts

<table>
<thead>
<tr>
<th></th>
<th>Length, (\mu m)</th>
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<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
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<tr>
<td><strong>Light microscope</strong></td>
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<tr>
<td>undischarged body</td>
<td>3.7</td>
<td>2.7-5.0</td>
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<td>discharged body</td>
<td>23.6</td>
<td>13-37</td>
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<tr>
<td><strong>Electron microscope</strong></td>
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<tr>
<td>undischarged body</td>
<td>2.87</td>
<td>1.33-5.50</td>
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<tr>
<td>discharged body</td>
<td>21.6</td>
<td>9.8-35.6</td>
</tr>
<tr>
<td>tip</td>
<td>1.91</td>
<td>1.27-2.87</td>
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Fig. 2. The relationship between body and tip length in undischarged trichocysts (electron-microscopic data). Regression coefficient, 0.395.

a mean of 23.6 \(\mu m\) (Table 1, Fig. 2). Some of these trichocysts were allowed to dry overnight, but showed no detectable change of length on desiccation.

If, instead of a sharp stimulus, prolonged, steady pressure was applied to the coverslip, undischarged trichocysts transformed in a number of different ways. Some discharged externally in the normal manner but others extended externally as partially elongated forms of much greater width than is usual (2-3 \(\mu m\) compared with the normal widest diameter of about 0.5 \(\mu m\)). Others remained within the cell, suddenly swelling into spherical bodies with a diameter of up to 4 \(\mu m\), or into structures with the same general shape as that of undischarged bodies but of greater dimensions (up to 8 \(\mu m\) long by 3 \(\mu m\) wide). In all of these aberrant transformations the trichocyst bodies showed reduced phase density and refractility. An attempt was
made to estimate any change in birefringence with polarizing optics, but both undis-
charged and extended trichocysts showed only slight birefringence so that it was not
possible with the apparatus available to be certain that any change had occurred (see
also Discussion). Undischarged trichocysts isolated in 20% sucrose or in 1% MgCl₂
were identical in size distribution and phase density to those of intact organisms, but
when perfused with distilled water they extended partially into coiled or straight
spindle-shaped forms without attaining the full length of the normally discharged
trichocysts. When living specimens of Paramecium were irrigated with various fixatives,
a range of different forms also resulted; with cold osmium fixation, many of the
trichocysts discharged in the normal manner to the outside, whereas others remained
in situ to expand partially within the cell. With warm osmium, no external discharge
took place, but some of the trichocysts expanded internally to lengths of up to 10 μm.
With glutaraldehyde fixation more frequent ‘internal’ expansion of trichocysts
occurred than with warm osmium. These partially extended intracellular trichocysts
corresponded to the pale elongated forms visible in electron-microscope sections and
in 1-μm Araldite sections prepared for light microscopy by staining with toluidine
blue (see also Selman & Jurand, 1970). They contrast with the more compact, densely
staining trichocysts which correspond to the undischarged forms visible in living
organisms.

Ultrastructural observations

Undischarged trichocysts lie in the cytoplasm with their tips situated immediately
beneath the pellicle in the inter-alveolar ridges (Fig. 11). Each trichocyst is contained
within a membrane-lined sac (Fig. 21). Within this, the trichocyst is divisible into a
broad body and a narrower conical tip which is ensheathed by a number of investing
structures, collectively termed here the trichocyst tip sheath (corresponding to the
‘operculum’ or ‘cap’ of other authors; see Hovasse, 1965). In negatively stained,
disrupted organisms undischarged trichocysts were often found to have remained
intact, with the entire trichocysts tip sheath still in position (Figs. 10, 17). Fine-
structural details of the sheath, investigated in sections, are shown diagrammatically
in Figs. 3 and 4 (see also Figs. 11-15, 21). Two distinct layers are present, comprising
a relatively thick outer sheath and a thinner covering to the tip matrix itself, the inner
sheath.

The outer sheath encloses the tip completely except at the base, and is separated
from the inner sheath by a narrow space. It is bounded externally by an electron-dense
layer which may represent a cytomembrane, peripheral to which is the cavity of the
trichocyst sac. The matrix of the outer sheath is irregularly granular, and embedded
in it are scattered fine (4-nm) filaments, together with a cylindrical array of micro-
tubules positioned on its outer aspect (Figs. 3, 4, 13-15). Longitudinal sections show
these microtubules to extend from the tip of the sheath to its base, being tilted slightly
at about 5° to its long axis, so describing a helical course. Near the apex of the tip,
transverse sections show about 30 microtubules, each 24 nm in diameter, to be present
in this complex (Fig. 14). In collapsed negatively stained tips of undischarged tricho-
cysts, longitudinal striations corresponding to these microtubules are visible (Fig. 17).
Fig. 3. Diagrams showing the morphology of the apical regions of the undischarged trichocyst. A, longitudinal section, showing the various sheathing structures surrounding the crystalline matrix of the trichocyst. B–D, transverse sections of the trichocyst tip taken at the levels indicated on A. Note that the form of the transverse section B is found at 2 levels in the trichocyst, on either side of section at C. Sections B, C and D are comparable with the electron micrographs shown on Figs. 13, 14 and 15, respectively. For labelling, see Abbreviations on figures, p. 917.

Within the outer sheath there are cylindrical areas of low density, termed here 'lacunae' (Figs. 3, 4, 13, 14) which correspond in position and number to the more peripheral ring of microtubules (see also Dragesco, 1968). These are prominent at the apex of the tip but became indistinct towards the base, or disappear.

The inner sheath is seen in longitudinal section to be double (Fig. 12), consisting of 2 concentric sheets each showing subunits with a longitudinal periodicity of about
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Fig. 4. A schematic 3-dimensional reconstruction of the detailed structure of part of the trichocyst tip and sheathing structures, cut away to expose the different layers.

4.5 nm. These sheets appear to be interrupted at points in longitudinal sections into a number of overlapping segments; these can be seen in Fig. 12 if the page is tilted to cause a foreshortening of the tip. In transverse sections (Figs. 3, 13–15) the concentric sheets are seen to be joined together in places to form 4 crescentic profiles, each arranged so that it is overlapped at one side by its neighbour (Fig. 13). In discharged trichocysts where the outer sheath is lost, the 2 laminae of the inner sheath are seen where they are folded over at the lateral edge of the tip in flattened negatively stained specimens (Figs. 18, 28, 32–34); each lamina is then seen to be composed of globular subunits of about 4 nm diameter, arranged at 4.5-nm intervals. In tangentially sectioned tips and in those negatively stained specimens in which the inner crystalline material of the tip had disintegrated to show the flattened pattern of the inner sheath, these subunits appear to be arranged in a square net tilted slightly (10–20°) with respect to the longitudinal and transverse axes of the trichocyst. In tips which had fractured during specimen preparation the inner sheath was often intact, although the enclosed crystalline material of the trichocyst had broken completely (Fig. 20).

The inner sheath of the trichocyst tip extends from the apex to the base of that
structure, giving way to a different type of sheath which invests the expanded body of
the trichocyst, the trichocyst body sheath (Figs. 3, 21, 22). This is closely applied to
the surface of the crystalline interior, and consists of a loosely arranged irregular
network of fine (3-nm) fibrils, enlarged into conspicuous beads of dense material (Fig.
22) at the points of intersection.

The whole trichocyst is enclosed, as already stated, in a membranous trichocyst
sac; in the apical regions of the tip, this is further enclosed in a cylinder of about 35
microtubules which appear to be attached to the outer surface of the investing mem-
brane (Figs. 3, 4, 13). These microtubules, each about 24 nm in diameter, are helically
oriented in the same direction and at the same angle as those of the outer sheath of the
trichocyst tip, and have a centre-to-centre spacing of about 30 nm. This cylinder
extends for about 0.8 µm in length; the individual microtubules are connected together
by a dense material, bars of which extend on both ends of the cylinder complex to
give the appearance shown in Figs. 3B and 14. Fine (3-8 nm) microfilaments are
present in the cytoplasm surrounding the cylinder of microtubules, to which some of
them are apparently attached (Fig. 14).

When trichocysts discharge, the membranous sac and its outer ring of microtubules
are left intact within the cell (Fig. 16). Once outside the cell the outer sheath disinte-
grates but the inner sheath remains intact.

The crystalline matrix of undischarged trichocyst tips and bodies presents a variety
of different appearances. In general the detailed structure is most clearly seen in
trichocyst tips in negatively stained preparations. Since the structure of the tip
remains unaltered after discharge, much of the finer detail was investigated in dis-
charged forms where the outer tip sheath was lost, revealing the inner matrix clearly.
As described by other authors (see Selman & Jurand, 1970; Stockem & Wohlfarth-
Botterman, 1970) trichocyst tips show regular transverse dense lines in longitudinal
sections, each about 5 nm wide and spaced at 15-17 nm intervals (Figs. 25, 26);
these will be referred to as the 16-nm period lines. Between these lie zones of low density,
interrupted by another, fainter dense line, termed here the intraperiod line, which
often appears as a broken transverse band of short segments. Some sections also show
faint obliquely longitudinal striations which break up the low-density region between
the 16-nm period lines into tilted rhomboidal units, thus giving a crystalline appear-
ance to sectioned tips (Fig. 25). Other sections lack these diagonal lines; their presence
or absence seems to be a function of orientation, since in a transversely sectioned
trichocyst tip which was fractured, a crystalline pattern was visible on one side of the
fracture line but not on the other, as though rotation had occurred between the 2
pieces to change their orientation with respect to each other (Figs. 25, 26).

In negatively stained preparations the image of the tip is complex, varying from one
specimen to another. A type which was often seen (Figs. 28, 30) shows ranks of hexa-
gonal units arranged in transverse rows, the units tilted up to 30° to the left and to the
right in alternate rows (see also Jurand & Selman, 1969) to give a characteristic
‘herring bone’ pattern. Other configurations are also seen, including those shown in
Figs. 20, 29, 31-34. It seems likely that the hexagonal units are composed of fine
(3-5 nm) filaments which branch and anastomose in a regular pattern so that each
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16-nm transverse segment is a mirror image of those segments adjacent to it. When sectioned and negatively stained preparations are compared the corresponding transverse lines of various types can usually be seen (Figs. 25, 26, 28–34).

When the pattern in negatively stained tips was viewed at slightly different angles, as in fractured specimens where the pieces on either side of the break appeared to have rotated with respect to each other (Fig. 29), or in tips tilted specially to allow stereoscopic analysis (Figs. 33, 34), the alteration of the pattern consisted of a lateral shift in the position of maximum clarity of the filamentous substructure, indicating that the image is made up of more than one plane of subunits superimposed on each other. The complexity of the pattern makes 3-dimensional analysis by simple stereoscopic viewing difficult (see Discussion) although some depth of structure is detectable from Figs. 33 and 34. From these stereoscopic pairs of micrographs it was, however, seen that the inner sheath of the trichocyst tip, so prominent at the sides of the tip, contributes little or nothing to the image of the major part of the structure, and can apparently be eliminated as a possible source of visual interference in analysing the crystalline matrix organization.

Superimposed on the regular 16-nm banding of the trichocyst tip a larger wave-form is clearly visible in profile at the lateral edges. This has a repeat distance of about 32 nm, corresponding to 2 of the 16-nm transverse rows, which are staggered with respect to each other in a step-like manner. It is not clear from the micrographs whether or not the wave form is planar or helical: metal shadowing at low angles was not helpful in deciding this issue, presumably because of the presence of the inner sheath of the trichocyst tip which prevented metal from reaching the crystalline matrix within its boundary.

In transverse sections, a lattice of 8-nm squares is visible (Fig. 27). This pattern was never seen in sharp contrast, probably because it represents a perpendicular view of more than one transverse plate of substructural units tilted first to one side and then to the other in successive layers. Since each of these plates is only 16 nm thick and the section thickness probably 50 nm or more, several of such alternating plates must be superimposed on each other to give the final image.

Trichocyst bodies similar to those noted by Jurand & Selman (1969) and others (see Introduction) were observed in sectioned and in negatively stained preparations (Figs. 10, 11, 21, 23, 35, 36). These included the dense compact forms with conspicuous 16-nm cross-banding of both tip and body (Figs. 10, 21, 23, 24) and those with cross-banded tips and unbanded bodies (Fig. 35). Several other types were also seen, some intermediate in structure between these 2 extremes: these included forms with 16-nm banding on the outer regions of the body but showing amorphous interiors (Fig. 11) and other trichocyst bodies with amorphous exteriors and fully extended 60-nm banding internally. Typically those trichocysts lying deep within the cell showed the compact form of pattern more frequently than those at the surface. The trichocysts which showed the 16-nm banding pattern in all parts of the body, that is, the compact dense trichocysts, corresponded in their dimensions and appearance to the undischarged trichocysts observed in living organisms by light microscopy (see above).
Fig. 5. Densitometric tracing along the longitudinal axis of a thick portion of a negatively stained discharged trichocyst body. The major peaks in this specimen occur at 56-nm intervals and correspond to the '60-nm' period lines (arrows). Note the general symmetry of the density profiles of each 56-nm repeating unit.

At high magnifications, sections of the ‘amorphous’ interiors of the unbanded forms demonstrate fine (3-4 nm) branching filaments, and granules which may represent filaments cut in transverse section (Fig. 38).

In some negatively stained preparations a few whole organisms were seen to have escaped total disruption prior to staining, apparently having partially disintegrated on the grid during that process (Fig. 10). In such examples up to 90 undischarged trichocysts could be observed and measured from a single specimen of _Paramecium_. The results from 4 individuals were pooled, and the lengths were found to vary unimodally around a mean of about 2.9 μm. All such trichocysts had a dense, compact appearance, and when examined in detail, showed the typical 16-nm banding pattern typical of trichocyst tips and sectioned compact bodies.

_Fully extended trichocyst bodies_, seen in section and in negatively stained preparations, show the typical 50–60 nm repeat pattern described by other authors (see Introduction). In longitudinal section a densely staining transverse band, the 60-nm period line alternates with a paler broad segment which is also transversed at various positions by a symmetrical pattern of intermediate bands of lesser density than the 60-nm period line (see Stockem & Wohlfarth-Botterman, 1970), of which there are 4 in each segment (Fig. 40). Corresponding transverse bands are seen in negatively stained specimens (Fig. 41), a densitometric tracing of which is shown in Fig. 5. The details of the substructure are obscured in thick specimens, possibly by disorientation and superimposition of many planes of structure on drying, but in thin fragments the precise arrangement of subfibrils is clearly visible (Fig. 42), being similar to that shown in a micrograph of a fragment of a dialysed trichocyst by Steers _et al._ (1969) and in normal trichocysts by Stockem & Wohlfarth-Bottermann (1970). In this pattern of structure, 2 alternating sub-patterns occur (Figs. 6, 42), which will be designated patterns A (a) and B (b) for convenience of description. _Pattern A_ is formed largely by fine (3-nm) filaments linked together in hexagonal arrays; each hexagon is connected in the longitudinal direction to slightly thicker (5-nm) filaments, possibly composed of 2 filaments wound round each other. At the position of the
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Fig. 6. The organization of the discharged trichocyst body.

Above: a diagram of the 2 patterns A (a) and B (b) seen in alternate 60-nm bands (see Fig. 42).

Below: a schematic 3-dimensional model incorporating patterns A and B as different (90°) views of the same structure. A single 60-nm transverse band lying between two 60-nm period lines (dotted lines) is shown.

60-nm period line these 2 subfilaments appear to separate and rejoin in the next segment of the trichocysts where they form part of the other pattern of substructure B. Pattern B similarly is formed by thick and thin filaments, but instead of hexagons, the thin filaments are arranged in a transverse zig-zag line the angles of which connect with thick filaments. Tilting of fragments of trichocysts in which these patterns are visible indicates that they have a 3-dimensional structure, although not enough detail was seen to show its precise form.

Measurements of discharged trichocyst bodies were made from electron micrographs (Table 1, p. 902), the distribution of lengths being unimodal about a mean of 21.6 μm. Densitometric comparisons were also made between the banding patterns of undischarged and discharged trichocysts bodies; the ratios of the major periodicities of the 2 forms (i.e. the '60-nm' period to the '16-nm' period) lay between 3.4/1 and 4.0/1. Comparisons between the maximum lengths of discharged and undischarged trichocyst bodies indicate a ratio of about 6.5/1, compared with a light-microscope value of about 7.3/1 (Table 2).
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**Table 2. Trichocyst extension ratios (discharged body length/undischarged body length)**

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<tr>
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<th>Ratio of mean lengths</th>
<th>Ratio of maximum lengths</th>
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<td>Light microscope</td>
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<td>7.34</td>
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<tr>
<td>Electron microscope</td>
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**DISCUSSION**

*The state of organization in undischarged trichocyst bodies*

As stated in the Introduction, one of the greatest difficulties in understanding how the structure of the undischarged trichocyst transforms into that of the discharged form is the apparent lack of any regular organization before discharge in ‘mature’ trichocysts. However, in those trichocysts which have previously been considered as ‘juvenile’ forms, a regular 16-nm transverse banding has been shown, and it can be imagined that such a regular structure could easily give rise to the discharged pattern by a process of extension occurring between the dense transverse bands which would then come to be spaced at 50–60 nm intervals. It is therefore worth examining the basis for considering that the immediate precursor to the discharged trichocyst is the amorphous ‘mature’ undischarged trichocyst of previous authors.

The argument that trichocysts lose their banded structure as they mature within the organisms rests on the interpretation of the apparent changes in structure occurring as new trichocysts are formed. Trichocyst regeneration is found after the existing trichocysts have been discharged without damaging the organism (Yusa, 1963) and also to make up the full complement of trichocysts after cell division (Selman & Jurand, 1970). It has been reported in these studies that when the trichocysts are first formed they are cross-banded, but after they have reached the pellicular surface, many of them become amorphous in organization and are seen to have expanded somewhat; thus when the lengths of trichocysts were measured and their frequency distribution plotted in sections of *P. aurelia* (Selman & Jurand, 1970) 2 peaks were found, representing 2 quite distinct populations of undischarged trichocysts. In addition, during the process of cell division Selman & Jurand (1970) found most of the undischarged trichocysts to be of the cross-banded (‘juvenile’) type, whereas at later points in the cell cycle the amorphous (‘mature’) form predominated; this was taken to indicate that before cell division occurred, all of the ‘mature’ trichocysts discharged to the exterior, to be replaced during cell division by ‘juvenile’ forms. As pointed out by these authors, this conclusion seems to be at variance with the studies of Ehret, Savage & Alblinger (1964) who, having investigated the uptake and retention of tritiated leucine in *P. bursaria*, concluded that the trichocysts are conserved by the cell throughout its generative cycle. It might also be added that such a repeated loss of trichocyst protein would be a highly uneconomic factor from the point of view of cell energetics and seems inherently unlikely on that ground alone.
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On the basis of the present results it will be argued that this loss of trichocysts and the transformation of structure from highly amorphous to highly organized are unnecessary postulates and that these can be more easily explained by interpreting the 'amorphous' or 'mature' undischarged trichocyst as an artifact of fixation for electron microscopy and by considering the cross-banded 'juvenile' trichocyst as the truly mature undischarged form. The lines of evidence supporting this view will now be discussed.

First, light-microscope observations indicate that all of the trichocysts within living paramecia possess similar optical properties and that their length distribution histograms (Fig. 1) show a predominantly unimodal pattern, certainly quite unlike the bimodal form found by Selman & Jurand (1970) in sectioned specimens. The electron-microscope appearance of such trichocysts (Figs. 23, 24) shows the highly organized cross-banding patterns which typify the 'juvenile' undischarged trichocysts of other authors. Second, many trichocysts transform within the confines of the cell to various partially expanded forms when subjected to pressure or to electron-microscope fixatives: many of such expanded trichocysts are similar to or identical with the amorphous 'mature' trichocysts of other authors. Similar forms are also found in disrupted specimens, negatively stained for electron microscopy (Figs. 35, 36). In detailed examination, the 'amorphous' trichocyst bodies appear to be made up of fine filamentous structures similar in dimensions but not in organization to the filaments of the fully extended trichocyst bodies. Third, all undischarged trichocysts in living paramecia appear to be capable of immediate extension to the fully formed discharged condition when suitably stimulated, indicating that they do not have to pass through a stage of intermediate maturation; it is true that some of the more centrally placed trichocysts may not discharge in this way, presumably because they are too deeply positioned, but all forms at the surface, regardless of their length, can so discharge.

From these avenues of investigation, it is postulated that when fixatives contact the cell surface they cause in the undischarged trichocyst bodies a partial expansion and loss of order, whilst at the same time preventing their external discharge in the normal way. This partial, confined expansion occurs in only some of the trichocysts at the surface, the proportionate numbers depending perhaps upon fluctuations in the stability of the pellicle or other cell membranes during the cell cycle. The trichocysts deeply placed within the cytoplasm are in a relatively stable environment and are therefore usually fixed with their cross-banded appearance intact. Thus the apparent maturation of trichocyst bodies from a highly organized state to an amorphous one may merely reflect an increasing predisposition to abnormal expansion when fixed for electron microscopy. It might be expected if this hypothesis is correct that a change in birefringence might occur when the trichocyst body suffers the loss of its organization; unfortunately the low level of birefringence of all trichocysts makes such measurements difficult. The unexpectedly low birefringence of these structures may be caused by the alternating sequences of structural planes seen in the 16-nm banding of undischarged trichocysts which might be mutually destructive of the birefringence given by each band separately.
The structure of the trichocyst sheaths and associated structures

The outer sheath is a composite structure in which microtubules play an important structural role. Microtubules have been reported to be present in the tip region (Ehret & Powers, 1959) but their exact position has not been shown before. In the present study, these structures have been found in the outer sheath itself and in relation to the outer surface of the trichocyst sac in the tip region (Figs. 3, 4). The significance of microtubules in these positions is uncertain: the outer sheath microtubules may serve a skeletal function, whereas it is possible that the outer ring of microtubules around the apex of the trichocyst sac may act to channel the forces generated by the expansion of the trichocyst body during discharge. Additionally it might form part of the triggering mechanism for trichocyst discharge; trichocysts are extruded from the organism when it is treated with various chemicals which also cause contractions of short duration in the cytoplasm. Such contractions, perhaps associated with the layer of microfilaments in the ectoplasm (Fig. 14, see also Pitelka, 1968) could cause the pressure within the cytoplasm to rise with the consequent sliding of the trichocyst tips up the external cylinder of microtubules to rupture the overlying pellicle and allow the ingress of water to the trichocyst bodies, so initiating their elongation (see Steers et al. 1969). The role of water in trichocyst extension is further supported by the inhibition of this process in sucrose solutions of high molarity (see Materials and Methods).

The inner sheath structure is not immediately apparent from the electron micrographs in all of its details. It would seem that 2 concentric laminae are present, each composed of a square net of 4-nm subunits whose axes are slightly tilted with respect to the long axis of the whole trichocyst. These laminae are arranged in the form of 4 flattened envelopes which spiral around the tip surface, each joined to and slightly overlapping the adjacent envelope on one of its sides (Fig. 4). It is noteworthy that if any type of 2-dimensional parallel-sided net is used to cover a conical structure, there must be one or more discontinuities or seams along the longitudinal axis to allow for the changing radius of cross-section; the organization seen in the inner sheath of the trichocyst tip may be related to this geometrical requirement. The inner sheath may also be responsible for protecting the crystalline matrix of the tip against extension which occurs so rapidly in the remainder of the trichocyst, since the inner sheath apparently touches and is co-extensive with the tip matrix. It is possible that it achieves this by mechanical constraint, a function possibly related to its resistance to tearing and stretching which is apparent from electron micrographs of fractured tips (Fig. 20).

The sheath of the trichocyst body differs considerably from the inner sheath of the tip in being composed of a loosely organized network of fine filaments which must be capable of a high degree of stretching or of mechanical disruption when the body elongates by up to eight times its resting length.
The relationship between body and tip lengths in trichocysts

Another interesting relationship emerging from the measurements of trichocyst lengths is the apparent linear correlation between the length of the tip and the body. This correlation (Fig. 2) indicates that the 2 dimensions are in some way causally related. The micrographs of Yusa (1963) indicate that the tip may be completed before the full extent of the body during trichocyst synthesis, but the factors controlling the sizes of these structures are as yet unknown.

Whatever the regulatory mechanism, it appears that during the synthesis of a complete trichocyst and its surrounding structures, at least 10 distinct types of component must be coded for by the genome of the organism, as follows: (1) the sheath of the trichocyst body; (2) the inner sheath; (3-6) the components of the outer sheath of the trichocyst tip (dense boundary line externally, granular material, filaments, microtubules); (7) the membrane of the trichocyst sac; (8) the microtubules of the apical region of the trichocyst sac, and (9) the dense material connecting these microtubules; and (10) the crystalline matrix of the trichocyst tip and body. The synthesis and organization of this complex of structures could provide an interesting area of investigation which might throw light on organelle synthesis in general.

The detailed organization of discharged and undischarged trichocysts

The interpretation of the structure of the undischarged form of trichocysts depends in part on the more easily approached structure of the discharged type, so that the latter will be discussed first.

Thin negatively stained fragments of extended trichocyst bodies show a complex structure of alternating patterns A and B (Figs. 6, 42) each of which constitutes a 50-60 nm transverse band. Collapsed and thick areas of trichocyst bodies lack this alternation of substructure (Fig. 5), indicating that the 2 patterns may be differently oriented forms of the same basic structure which lose their distinctive orientations during the drying of trichocysts except when thin portions flatten down in a favourable manner. It would further seem likely that the 2 planes of orientation lie at 90° to each other (Fig. 6), in which case a 3-dimensional solution to the structure can easily be constructed. A lattice of filaments forming interconnected hexagonal structures as shown in Fig. 6 might be expected to possess some mechanical stability, and the apparent rigidity of the fully extended trichocyst body agrees well with this proposal.

It is further seen that the thick filaments are possibly formed of intertwined thin filaments: the dense 60-nm period line could be formed by those regions in the thick filaments where the 2 thin filaments separate for a short distance, perhaps to change the handedness of the spiral which they form in adjacent rows of units. Many details of this substructure await further analysis.

The structure of the undischarged trichocyst matrix is less clear, and like the extended structure, awaits the application of modern methods of optical analysis for its detailed solution. However, it is possible to suggest a model for its organization if the following factors are taken into account. First, it is likely that the extended configuration is present in some contracted or distorted form in the undischarged
Fig. 7. Diagram showing a possible 3-dimensional model of the filamentous structure of the undischarged trichocyst matrix (see text). The thickness of the lines at a particular point represents their distance from the observer. Two complete 16-nm transverse bands are shown, rotated slightly to show the depth of structure, which is limited in this diagram to 2 vertical planes. The arrows indicate the positions of the 16-nm period lines, which are here depicted as distorted and tilted hexagons seen edge-on.

trichocyst; second, the ratio of extended length to unextended length is about 7:1 (Table 2), showing that one 16-nm transverse band in the unextended form corresponds dimensionally to 2 of the extended 60-nm bands; third, transverse sections of the undischarged matrix show a square lattice in the transverse plane (Fig. 27); fourth, several different fine patterns are seen in the tips of trichocysts, which appear to be different views of the same basic structure; fifth, each 16-nm transverse band in the undischarged trichocyst is a mirror image of the transverse bands on either side of it; sixth, superimposed on the 16-nm periodicity is a longer 32-nm repeat pattern.

Various models were constructed to embody these features, the most satisfactory of which is shown diagrammatically in Fig. 7. This is a distorted and contracted version of the extended pattern, composed of fine filaments. These are arranged to
form regular arrays of flattened interconnecting hexagons which form a complex lattice. All of the hexagons in a single transverse series are oriented at 90° to those in the adjacent transverse series with which they are interconnected by short straight filaments which run longitudinally. Each filamentous hexagon, in addition to being distorted laterally is also tilted in the plane lying perpendicular to its face and connected by filaments with the neighbouring hexagon towards which it is inclined, so that a zig-zag line is described when the row of hexagons is viewed edge-on. This zig-zag line corresponds to the dense 16-nm period line of the undischarged trichocyst body or the trichocyst tip, and the hexagons when seen face-on constitute the obliquely longitudinal structures visible between the 16-nm period lines (Figs. 29-34). The lateral distortion of the filamentous hexagons occurs in successive 16-nm bands to the left and to the right alternately to give the mirror symmetry of these bands. If a series of longitudinally connected hexagons is followed, therefore, the first, third, fifth and seventh hexagons will be successively mirror images of each other; the second, fourth and eighth hexagons, being seen edge-on, may or may not be laterally distorted. If they are, however, and form part of a regular spiral series oriented successively at 0°, 90°, 180° and 270° with respect to the observer, they would generate a 32-nm repeat pattern superimposed on the 16-nm periodicity. This corresponds to the 32-nm super-periodicity of the undischarged trichocyst and, when extended from 7-8 times, to the 240-nm super-periodicity of the discharged trichocyst (Fig. 39).

It will be seen from this model that the 16-nm period lines do not correspond exactly to the 60-nm period lines of the extended form. Each filamentous hexagon of the undischarged bands is thought to expand to form part of the intermediate zone between each pair of 60-nm period lines for which there is no special precursor in the undischarged trichocyst except for the straight unbranched filaments connecting longitudinally adjacent hexagons.

The model suggested here is of course one of many possible ones, and is only presented as the basis for further investigation by more direct methods. The precise structural transformations occurring at the time of trichocyst discharge cannot be assessed without a knowledge of the exact lengths and inclinations of the filaments of the 2 forms, although it seems likely from simple inspection that the filaments increase in length and in diameter during this process (see Figs. 34, 42, for example). Such changes might be the result of the entry of water into the spaces between the filaments causing alterations in the charges associated with the polypeptide chains (Steers et al. 1969), perhaps to alter the way in which they are intertwined or coiled. However, further biochemical study is required before these transformations can be understood in molecular terms.

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REFERENCES


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# ABBREVIATIONS ON FIGURES

- **f**: cytoplasmic filament  
- **it**: inner sheath of trichocyst tip  
- **isl**: lamina of inner sheath of trichocyst tip  
- **mt**: microtubule  
- **mtr**: outer ring of microtubules  
- **os**: outer sheath of trichocyst tip  
- **osl**: lacuna in outer sheath of tip  
- **pm**: pellicular membrane  
- **th**: trichocyst body  
- **tbs**: sheath of trichocyst body  
- **tm**: crystalline matrix of trichocyst  
- **tsm**: trichocyst sac membrane  
- **tt**: trichocyst tip
Fig. 8. Undischarged trichocysts (long arrows) in a compressed living *Paramecium* photographed with phase-contrast microscopy. Prolonged steady compression had caused the expansion of some trichocysts within the cell into spherical forms (short arrows). ×4000.

Fig. 9. Fully discharged trichocysts, showing conical tips and spindle-shaped bodies. Note the variation in body length. Phase-contrast, ×2000.

Fig. 10. Negatively stained undischarged trichocysts clustered amongst the remains of a disrupted specimen of *Paramecium*. Bodies and tips are clearly distinguishable. Note the dense compact appearance, in contrast to the trichocyst in Fig. 35. ×13000.
Fig. 11. Vertical section through the tip and part of the body of an undischarged trichocyst showing the outer sheath (ot) in which microtubules (mt) are present, surrounding the trichocyst tip (tt) which points towards the pellicular membranes (pm). The trichocyst body sheath (tbs) invests the trichocyst body (tb). × 40,000.

Fig. 12. Vertical section through part of the tip of an undischarged trichocyst showing the subunit structure of the inner sheath laminae (iil) and the granular outer sheath. When the page is tilted to foreshorten the tip, discontinuities in the inner sheath become apparent (arrows). × 133,000.

Figs. 13–15. Transverse sections through the undischarged trichocyst tip and surrounding structures at various levels as shown diagramatically in Fig. 1, including the external microtubule ring (mtr), microtubules of the outer sheath (mt) and cytoplasmic filaments (f); the inner sheath of the tip is sectioned obliquely in Fig. 15 showing one filamentous component of the net-like substructure (arrows). × 100,000.

Fig. 16. Transverse section through the outer cylinder of microtubules surrounding the apex of the trichocyst sac. The trichocyst has been discharged. × 100,000.
Fig. 17. The tip of a negatively stained undischarged trichocyst with the outer sheath intact and showing longitudinal striations indicating the presence of microtubules (arrows). × 50000.

Fig. 18. Part of the inner sheath of a negatively stained discharged trichocyst tip seen at its lateral edge where the sheath is folded over. The subunit structure of 4-nm globular units arranged in slanting rows (arrows), is visible. The longitudinal axis of the trichocyst runs vertically and the crystalline matrix of the tip (tm) is to the left. × 450000.

Fig. 19. A negatively stained portion of a discharged trichocyst tip in which the crystalline matrix has disintegrated to allow flattening of the net-like inner sheath. The long axis of the trichocyst runs vertically (arrow). × 270000.

Fig. 20. A negatively stained trichocyst tip in which the crystalline matrix has fractured, the 2 pieces apparently being held together by the unbroken inner sheath. × 166000.

Fig. 21. A vertical section through the base of the tip and apex of the body in an undischarged trichocyst, showing the 16-nm transverse banding of tip and body matrix, and the beaded sheath of the body (tbs) in profile. The outer sheath of the tip (os) and the membrane of the trichocyst sac (tsm) are also shown. × 63000.

Fig. 22. A vertical tangential section of the sheath of the body in an undischarged trichocyst. Note the reticulum of fine fibrils connecting the dense bead-like bodies. × 250000.
Trichocysts in Paramecium
Fig. 23. Part of the body of a negatively stained undischarged trichocyst (see Fig. 10) showing the 16-nm transverse banding. × 56000.

Fig. 24. Detail of Fig. 23 showing the banding pattern at higher magnification. Compare with Figs. 28-34. × 131000.

Figs. 25, 26. Longitudinal sections through a trichocyst tip which has fractured, showing the altered appearance on either side of the break. Note the difference in pattern, indicating that a change of orientation has occurred in the two pieces. Note also the 16-nm period lines (large arrows) and the less-dense intermittent intermediate lines (small arrows). × 275000.

Fig. 27. The crystalline matrix of the tip in transverse section showing an indistinct net pattern of 8-nm squares. × 275000.

Fig. 28. A detached negatively stained trichocyst tip in which the outer sheath is lost, demonstrating the alternating 16-nm banding pattern giving a 'herring bone' appearance. × 87000.
Trichocysts in Paramecium
Fig. 29. Detail of a negatively stained fractured trichocyst tip, showing an apparent change in orientation between the parts on the 2 sides, evidenced by a difference in the 16-nm banding pattern. \( \times 350,000 \).

Figs. 30–32. Different types of appearances found in negatively stained trichocyst tips. In Fig. 32, a 32-nm 'super-periodicity' is visible in the profile of the edge of the tip matrix (arrows). \( \times 350,000 \).

Figs. 33, 34. Two stereoscopic views of portion of a negatively stained trichocyst tip showing tip matrix substructure and the profile of the inner sheath at the sides. Approximately 15° of tilt. \( \times 400,000 \).
Trichocysts in Paramecium
Fig. 35. A negatively stained trichocyst of the partially expanded type, corresponding to 'mature' undischarged trichocyst of some authors (see text). Note the amorphous, moderately dense trichocyst body. × 9000.

Fig. 36. Another type of partially expanded trichocyst, negatively stained to show the 60-nm banding on the exterior of the body. Note the apparently deformable nature of the body. × 9000.

Fig. 37. The basal region of a fully extended trichocyst body which has fractured in 2 places during specimen preparation, indicating a non-pliable nature. Negatively stained, × 17000.

Fig. 38. A highly magnified section through part of an amorphous trichocyst body showing fine filaments (arrows). × 250000.

Fig. 39. Detail from Fig. 37 to show the alternating 240-nm 'super-periodicity' of the discharged trichocyst body negatively stained. × 500000.

Figs. 40–42. Detailed structure of the extended trichocyst body in 3 different specimens. × 400000.

Fig. 40. Longitudinal section through a trichocyst, positively stained to show the dense 60-nm period lines and the pattern of intermediate bands between them.

Fig. 41. From a thick portion of a negatively stained trichocyst body showing a similar pattern of banding in reverse contrast (see the microdensitometric trace in Fig. 5).

Fig. 42. From a thin portion of a negatively stained trichocyst body, showing the detailed patterns visible between the 60-nm period lines, consisting of 2 alternating patterns A and B (a and b) (compare with Fig. 6).