ELECTRON MICROSCOPE OBSERVATIONS ON TRICHOCYSTS

by

BARBARA P. POTTS

Physics Department, University of Adelaide (South Australia)

The trichocysts of *Paramecium* have been extensively studied with the light microscope[1], and they were first examined in the electron microscope by JAKUS and co-workers[2,3,4]. The shaft of the discharged organelles proved to have an interesting submicroscopic structure, being composed of a fibrous protein with prominent cross striations. The period of the cross striations was less than that for collagen fibres, and some of the chemical properties of the shaft were shown to resemble those of collagen. Other investigations on the structure of the trichocysts have been made with the electron microscope by a number of workers[5,6,7,8,9,10,11].

Concurrently with an investigation on the cilia of *Paramecium*[12], some observations were made on the structure of the trichocysts. This paper is an account of these findings.

EXPERIMENTAL

Paramecia, of unidentified species, were grown in Osterhaut-vegemite culture media[13], two concentrations of vegemite (0.05–0.1% and 0.2–0.3%) being used. The organisms were transferred to distilled water and concentrated by gentle centrifugation. After the supernatant liquid had been kept overnight in the refrigerator it contained discharged trichocysts. Particularly after harder centrifugation, cell fragments, including pieces of the pellicle and resting trichocysts, were also found in the suspensions.

Trichocysts were examined, both with and without fixation in 2% osmium tetroxide, after drying drops of the suspensions onto collodion films.

The method used for preparing sections has already been indicated[12], the embedding being carried out essentially as in PALADE's procedure[14]. Sections of resting trichocysts were obtained from organisms promptly fixed after they had been transferred to distilled water, while sections of the discharged organelles were mostly obtained from a preparation in which fixation was delayed for several hours.

The electron microscope used for these investigations was a Philips "Metalix" instrument operated at 60 or 80 kilovolts. The magnification meter was calibrated against polystyrene latex particles.

RESULTS

Resting trichocysts

Whole undischarged trichocysts were occasionally found on specimens, and they each consisted of a dense body and a tip similar to the structure seen on the discharged trichocysts (Fig. 1). As the whole organelles were electron optically opaque it was necessary to examine sections in order to study their internal composition. Fig. 2 is a section of *Paramecium* showing three trichocysts which appear to be located in cavities in the cytoplasm. Cone-shaped structures surround the tips and lead to the surface of the

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cell. The body is surrounded by a membrane adhering closely to its contents, which consist of a dense and apparently homogeneous material.

In sections, the resting trichocysts were often traversed by striae produced in sectioning; this suggested that they were harder in composition than the surrounding tissue. The denser regions on one side of the trichocysts in Fig. 2 were also probably produced during sectioning.

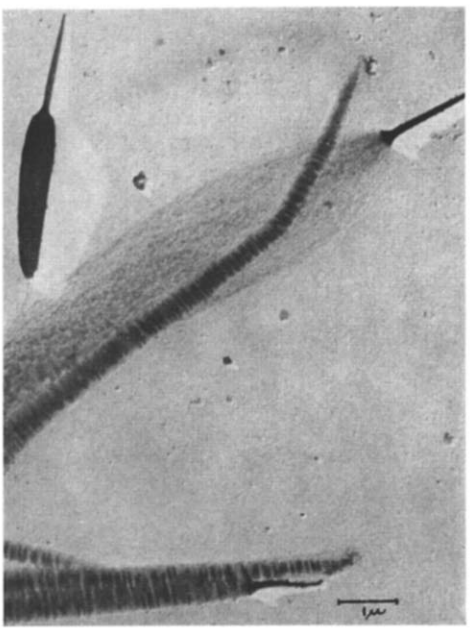

Fig. 1. Resting trichocyst and parts of several discharged trichocysts (× 10,000).

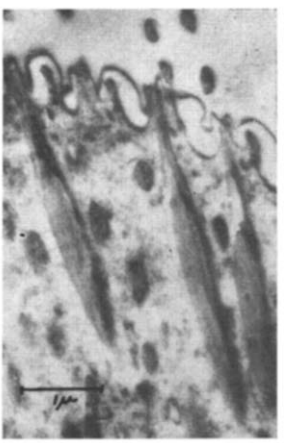

Fig. 2. Section of *Paramecium* showing resting trichocysts (× 14,000).

The pellicle

It is of interest to compare the appearance of detached pellicular fragments[15] with that seen in sections obtained in the present investigations. Sections (Figs. 2 and 3) showed that the pellicle itself was a double membrane, with outer layer continuous with the ciliary membranes and inner layer continuous with a membrane closely applied to the cytoplasm. The pellicle was separated from the cytoplasm by large gaps, except at the depressed ciliary entry points, and at trichocyst exit points and other ridges in the cytoplasm (Figs. 2 and 3). These ridges undoubtedly correspond to the edges of polygons seen on the surface of *Paramecium*[15,16]. Indeed, the general picture revealed in these sections is in accord with previous observations, although a fibrous network defining the polygons[15] was not seen.

Fig. 3. Section of *Paramecium* showing the entry of a cilium and the structure of the pellicle (× 36,000).

Discharged trichocysts

Whole discharged trichocysts, similar in appearance to those described by other authors[3,11], were observed. Such shafts were low in density and very flat

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(Figs. 1 and 4), but occasionally less collapsed shafts were seen. Some shafts with stratified deposits of denser material on them were found in most preparations (Fig. 1). However, when the trichocysts were discharged from organisms grown in the more concentrated culture medium, besides trichocysts with such low density shafts, others were observed which were either homogeneously dense structures or which had dense cores (Fig. 5). The high density of the former appeared to be the result of the deposition of denser material on the outside of the shaft, and, in such cases, the shafts did not flatten greatly on drying.

The low density shafts usually appeared slightly denser at the centre than at the peripheries, an effect which, it is suggested, is caused by the solid nature of the collapsed shaft. Those found in preparations from *Paramecia* grown in more concentrated culture media often had sharp and extremely dense cores, which were higher than the less dense

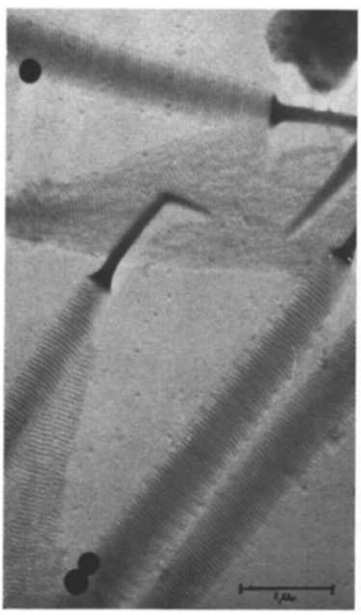

Fig. 4. Discharged trichocysts showing the superperiodicity (× 16,000).

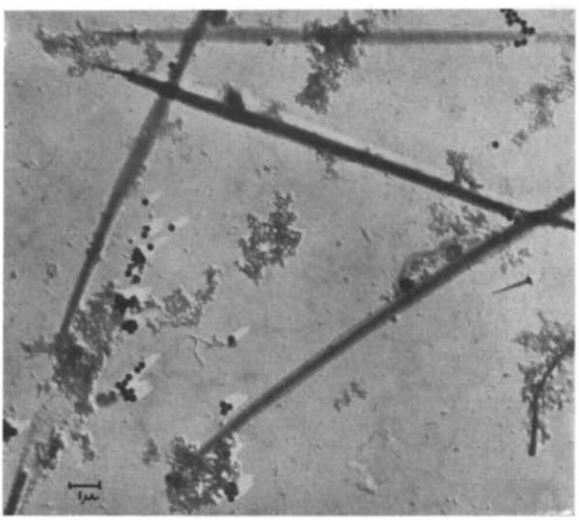

Fig. 5. Discharged trichocysts from organisms grown in the more concentrated culture medium (× 5,000).

peripheries of the shaft. The cross striations passed across the whole shaft, but the impression was given that the cross striated material surrounded a central denser core (Fig. 5).

Sections of trichocysts from the more concentrated culture medium showed no clear evidence of a central core different in composition from the outer layer, although sometimes, there were regions of slightly different density in the centre of the shaft. The cross striations were prominent in sections, and each dark band appeared to consist of three fine lines (Fig. 6).

The period of the cross striations in whole trichocysts was of the same order as found by JAKUS[3]. In the few sections obtained, the period varied over a much wider range; the apparent period might be expected to increase with obliquity of the section, but the minimum observed was about 400 Å, which is considerably less than the 520 -

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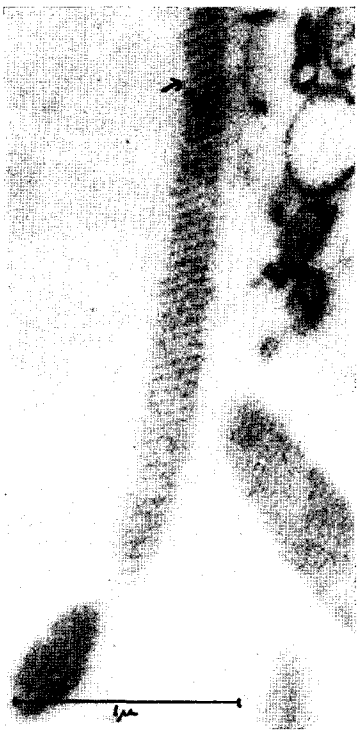

Fig. 6. Sections of discharged trichocysts showing fine structure (arrow) in the cross striations (× 40,000).

580 A usually found in the whole shafts. This difference may be the results of differences in treatment.

In both whole and sectioned trichocysts, no signs of a membrane surrounding the cross-striated material were seen.

The superperiodicity in the shafts of discharged trichocysts

As other authors[3,7,11] observed, a superperiodicity is superimposed on the cross striations of the trichocyst shaft; the period is four times that of the cross striations. It usually consists of lines of greater density traversing the shaft at an angle to the cross striations, so that on the peripheries of the shaft, the ends of the lines coincide with dark bands of the cross striations. Each line takes twice the period of the cross striations or half of the superperiod to cross the shaft (Fig. 4). When shadowed so that the direction of shadowing is perpendicular to the cross striations, these lines appear as ridges which are most prominent at the edges of the shaft (Fig. 4). Projections of these ridges beyond the edges of the shaft appear to correspond to the alternate tufts or spots previously described[3,11]. In less collapsed shafts the contours of the edges of the shaft are related to the superperiodicity. In a few of the trichocysts which had been treated with phosphotungstic acid, the shaft appeared to be surrounded by a fine helical cord with pitch equal to the superperiod.

The superperiodicity was sometimes retained by the component fibrils in shafts which had frayed. In Fig. 7, for example, it is manifested as a wave-like configuration.

Many trichocysts did not exhibit the superperiodicity at all, while in others it was apparent in only part of the shaft. Sections usually showed no traces of the superperiodicity, but in some, denser lines traversing the shaft at an angle to the cross striations and projecting beyond the edges had a period four times that of the cross striations.

The effect of heating trichocysts

Discharged trichocysts, on being heated to temperatures of the same order as the shrinkage temperature of collagen, underwent a pronounced change in appearance. After being heated to about 80° C the shaft was a relatively dense narrow structure, surrounded by what appeared to be a helical cord (Fig. 8). In the less dense shafts, this appearance was seen to result from the shape of the surface of the shaft, rather than from a separate helical component

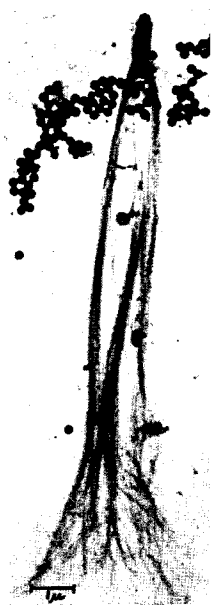

Fig. 7. Discharged trichocyst in which the shaft has frayed into fine fibrils (× 6,500).

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(Fig. 8). The cross striations traversed the whole structure, and the pitch of the helix was again four times that of the cross striations. In frayed shafts the helical character was evident in the components as a wave-like configuration.

Because of the wide range of lengths found for unheated trichocysts, it was difficult to determine whether these contracted longitudinally on heating. However, frequently the shafts appeared to be unusually short, and in such instances, the pitch of the associated helix was as much as half of the normal superperiod.

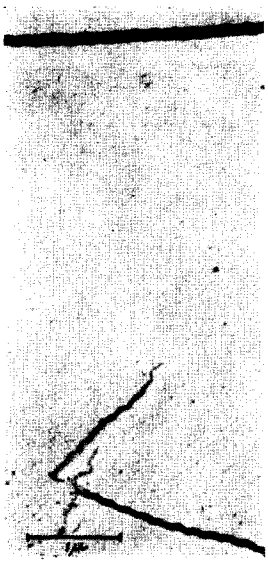

Fig. 8. Parts of the shafts of discharged trichocysts after heating to about 80° C (× 16,000).

After a suspension containing trichocysts discharged from organisms grown in the more concentrated culture medium had been heated to about 65° C, similar trichocysts were observed, but in addition, in some of the shafts, separation into a dense core and a less dense surrounding layer occurred. This gave the impression that in the more compact parts of the shaft (Fig. 9) the cross striated material surrounded the denser core. In such cases the helical structure was usually confined to the central region. The cores, themselves, in contrast to those in unheated trichocysts, were wave-like in appearance, suggesting contraction of the surrounding material. Heating to 40° C did not affect the trichocysts.

Fig. 9. Part of the shaft of a discharged trichocyst, from the more concentrated culture medium, after heating to about 65° C (× 20,000).

DISCUSSION

Resting trichocysts

JAKUS[3] observed only whole resting trichocysts with caps surrounding their tips. In the present investigations, preparations yielded only trichocysts without these caps. However, in sections, cone-shaped structures were observed surrounding the tips of the trichocysts.

As KRUGER[1] observed, the resting trichocyst is surrounded by a membrane. The body, after fixation, appears to be occupied by a dense, hard and apparently homogeneous material.

Discharged trichocysts

As random sections of trichocyst shafts are prominently cross-striated, it is clear that the bulk of the shaft must be occupied by the cross-striated substance. The usual very flat character of whole trichocyst shafts must, therefore, be the result of the loss of large quantities of water in drying.

It is not surprising that the shaft is not surrounded by a separate membrane in view of the elongation involved in discharge. JAKUS[3] regarded the cross-striated layer as

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a membrane, which KRUGER[1] had described from observations with the dark field microscope. He also found within the membrane, a "Quellkorper", the swelling of which was responsible for the elongation of the shaft on extrusion. JAKUS[3] did not find this structure, but it appears from the present investigations, that the cross-striated material itself is the "Quellkorper".

It might appear from the observations on whole trichocysts that the core of the shaft is occupied by material different in composition from the outer layer, but this is not confirmed from the sections.

The dense deposits occurring on the outside and inside of the shafts of trichocysts from organisms grown in the more concentrated culture medium, are probably slats[10]. Their occurrence could be associated with the uptake of water involved in discharge, or it may reflect an osmo-regulating function as WOHLFARTH-BOTTERMANN[10] suggested.

The cross-striated fibrils

As JAKUS[3] has pointed out, the cross striations of the trichocyst shaft superficially resemble those of collagen. The superperiodicity, which is clearly of a helical character, is a more unusual feature. Some authors[11] considered it to be the result of a helical fibre surrounding the shaft. While there is evidence to support this view, it cannot be reconciled with the appearance of frayed shafts, of heated trichocysts, or of some sections. Though sometimes apparently restricted to the denser core of the shaft, it is more probably an inherent structural feature of the organised cross-striated fibrils.

JAKUS[3] has shown that in some ways the trichocyst shaft resembles collagen, and although like collagen, it is profoundly changed on heat denaturation, it does not appear to contract to the same extent, and unlike collagen, it retains the cross striations.

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SUMMARY

Some features of the structure of trichocysts have been determined from observations on sectioned trichocysts and from further observations on whole trichocysts. The resting trichocyst consists of tip and body which is surrounded by a membrane and occupied by apparently homogeneous material. The shaft of the discharged trichocyst is largely composed of cross-striated fibrils, and it has no surrounding membrane. A superperiodicity of helical character is manifested in several different forms; it is probably a structural feature of the cross-striated fibrils rather than a separate helical fibre. The appearance of the trichocysts depends on the original culture medium, and it is profoundly changed on heating.

RÉSUMÉ

Les observations sur des trichocystes sectionnés et sur des trichocystes entiers ont permis de déterminer quelques traits de la structure de ces organites. Le trichocyste au repos est constitué par une extrémité et un corps qui est entouré d'une membrane et rempli d'une substance apparamment homogène. La flèche du trichocyste développé est composé essentiellement de fibrilles striées

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transversalement et n'est pas entourée d'une membrane. Une superpériodicité de caractère hélicoïdal est observable dans plusieurs formes différentes; elle tient à un trait structural des fibrilles striées transversalement plutôt qu'à une fibre hélicoïdale distincte. L'aspect des trichocystes dépend du milieu de culture initial et est profondément modifié par chauffage.

ZUSAMMENFASSUNG

Einige Tatsachen über die Struktur von Trichocysten wurden durch Beobachtung von durchschnittenen und zusätzliche Beobachtung von ganzen Trichocysten festgelegt. Die ruhenden Trichocysten bestehen aus Spitze und Rumpfteil, welche von einer Membran eingeschlossen und mit scheinbar homogenem Material gefüllt sind. Der Schaft der entfalteten Trichocysten besteht grösstenteils aus quergestreiften Fibrillen und besitzt keine Membran. Eine übergeordnete Periode von schraubenartigem Charakter zeigte sich verschiedentlich an ihnen, doch handelt es sich hier eher um eine strukturelle Eigenschaft der quergestreiften Fibrillen, als um eine getrennte schraubenartige Faser. Das Aussehen der Trichocysten hängt von der ursprünglichen Nahrflüssigkeit ab und wird durch Hitze völlig verändert.

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