

THE SECRETION AND DEVELOPMENT OF NEMATOCYSTS IN A SIPHONOPHORE

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SUMMARY

Nematocyst development was watched in living cells of a siphonophore, *Rosacea cymbiformis*. The secretion differentiates in the formative cell into an ovoid capsule, continuous with a long apical tube. The entry of this 'external' tube into the capsule creates the coiled internal 'thread' of the mature nematocyst. Electron microscopy shows that the tip of the external tube inverts and moves down the tube towards the capsule. Complex pleats form during inversion. Thus formation of the internal thread is the geometrical converse of discharge. Extensive repacking of the triply pleated, internal thread occurs, and subsequently the barbs form.

INTRODUCTION

The structure of the thread wall of mature nematocysts is complex (Skaer & Picken, 1965); nevertheless a similar structure can be generated from a cylinder of pliant material by buckling (Skaer & Picken, 1966). To demonstrate this, a length of discharged thread was modelled from a cylinder of velvet or cellophane, with barbs, cast in Araldite, stuck on the cylinder. If the model is turned outside-in and the barbs are packed hexagonally, as in the undischarged thread (Picken, 1953), the cylinder also becomes pleated as in the undischarged thread. The success of this demonstration raised the question whether the nematocyst thread is formed initially as a smooth-walled cylinder that subsequently buckles (if so, what part is played by the barbs in this process), or whether the undischarged thread is secreted directly as a triply pleated structure complete with rifling and pockets for barbs (Picken & Skaer, 1966).

These points could only be investigated by a study of the development of nematocysts using electron microscopy. So much controversy, however, has arisen from attempts to use fixed material to study the development of nematocysts that it was also decided to look out for suitable material in which the process could be observed in a living animal. Siphonophores are particularly suitable for this purpose as they are transparent, and some of their nematocysts are very large. Moreover, all the nematocysts of a gastrozooid and its associated tentacle develop in a region 1 mm or so wide and 6–8 cells thick that forms a cuff around the aboral region of the gastrozooid. This cuff consists almost entirely of developing nematocysts. In the polygastric phase of many siphonophores the stem that develops from the nectophores (or swimming bells) bears a string of cormidia of progressively increasing age. Thus gastrozooids of appropriate age, containing large numbers of developing nematocysts, can be selected. The siphonophore *Rosacea cymbiformis* (Chiaje) was chosen from the dozen or so genera

commonly encountered at Villefranche-sur-Mer, for its developing nematocysts are large and more refractile than those of other genera.

With phase-contrast microscopy, individual cnidoblasts could be watched continuously and photographed as they developed in the tissues of the living gastrozooid. The process of formation of all 4 types of nematocyst present in *Rosacea cymbiformis* was followed in this way. This account will be based on the development of the large, microbasic mastigophores that lie on either side of the cnidoband in the tentillum, and that discharge to produce a thread differentiated into a wide-bore butt region with large barbs and a narrow tapering thread with small barbs. The development of the 3 other types is essentially similar.

The approach and the main conclusions given in this paper were outlined in a talk to the Station Zoologique, Villefranche-sur-Mer in April 1970. A paper based on this approach and in which some of my observations have been confirmed in the siphonophore *Muggiaeae* has already appeared (Carré, 1972).

MATERIAL AND METHODS

Young gastrozooids of the siphonophore *Rosacea cymbiformis* were picked from the stems of living animals and placed in dialysis tubing that had been soaked for 24 h in seawater. This was mounted beneath a coverslip in a Rose chamber (Rose, 1967), so the gastrozooid was slightly compressed, and perfused with aerated seawater at 18 °C. Under these conditions the gastrozooids survived for many hours.

Specimens for electron microscopy were fixed for 2 h at 4 °C in a freshly prepared, 2·5% solution of glutaraldehyde in 0·05 M cacodylate buffer at pH 7·8. The fixative also contained 0·8 M sucrose and 4 mM calcium. The fixed specimens were given a prolonged wash in cold 1·1 M sucrose solution buffered to pH 7·8, were then postosmicated for 1·5 h at 4 °C in 1% osmium tetroxide solution in 1 M sucrose, buffered to pH 7·4 with 0·1 M phosphate buffer, and dehydrated in a graded series of ethanol. They were then passed through 2 changes of chloro-epoxypropane and embedded in Araldite.

Nematocysts at particular stages of development were selected for electron microscopy by cutting thick sections freehand from the blocks of polymerized Araldite. These sections were mounted in liquid paraffin and were examined by Nomarski interference optics. The section was trimmed around the appropriate cell, the liquid paraffin washed off with soapy water and the trimmed section was remounted with Araldite glue on an Araldite stub.

Thin sections were doubly stained in uranyl acetate and lead citrate and examined in an AEI EM 6B electron microscope operated at 60 kV.

RESULTS

Observations on living cnidoblasts

In all types of cnidoblast, the first sign of differentiation visible in the living animal was the ovoid vesicle that ultimately forms the capsule of the nematocyst. An external tube, external to the capsule but within the cell, grows out from this vesicle by accretion of secretory droplets. The droplets are produced in a region of granular cytoplasm – apparently the Golgi apparatus – around the tip of the external tube, and can be seen to fuse on to the pointed tip of the tube. When it is fully developed in those cnidoblasts that will form large, microbasic-mastigophore nematocysts, the tube may be 7–10 times the length of the developing capsule. The external tube of these nematocysts tapers gently from base to tip (Fig. 1A).

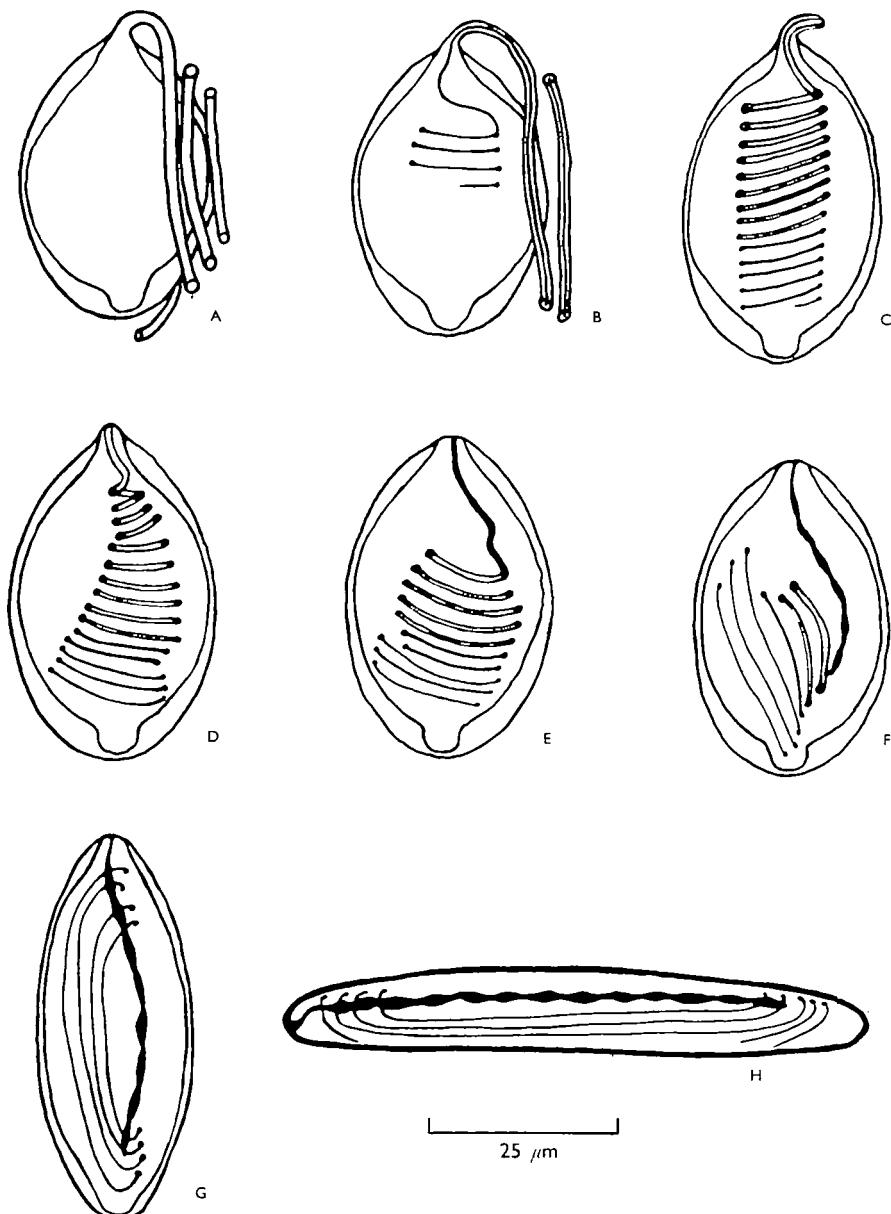


Fig. 1. Diagram of the development of a microbasic-mastigophore nematocyst, based on phase-contrast microscopy of living specimens. None of the formative cell is shown. For clarity much of the external thread has been omitted and the internal thread is shown in optical section. A, Formation of cylindrical thread in the cytoplasm. B, Entry of thread into capsule. C, Coiling of thread in capsule. D-F, Remodelling of thread in capsule. G, Stage at which migration occurs. H, Mature nematocyst.

In rhopaloneme nematocysts, on the other hand, the external tube is much shorter; it may be $30 \mu\text{m}$ long – only twice the length of the capsule. Though less than $1 \mu\text{m}$ in diameter near the capsule, the tube widens distally to approximately $2 \mu\text{m}$, but the tip itself is sharply pointed.

The tip of the external tube then apparently turns outside-in and passes back down the external tube into the capsule. In microbasic mastigophores it seems it is the tip of the tube that enters first, for the thread that first arrives inside the capsule is the thinnest (Figs. 1B, 5A). The part of the thread that enters the capsule later is greater in diameter (Figs. 1C, 5D). The fact that inversion begins at the tip of the external tube was first clearly observed in rhopalonemes, for in these nematocysts the pointed tip of the external tube disappears first, then the widest distal part of the tube, and finally the narrow part next to the capsule disappears as the internal tube forms in the capsule.

The further development of microbasic mastigophores is as follows. The internal tube coils to form a helix about the long axis of the capsule. Approximately 3 coils of internal tube are added every 4.5 min at first (Figs. 1B, 5B), but later, coils are added more slowly. Coiling is evidently an intrinsic property of the tube and is not initiated nor directed by the capsule wall. The coils are suspended inside the capsule and do not touch the wall. They are wound round a straight axis (Fig. 1C). As the internal tube forms, the volume of the capsule increases, for it lengthens from approximately 37 to $42 \mu\text{m}$ while the width remains at approximately $25 \mu\text{m}$.

The external tube shortens as more coils of internal thread form, and when about 15 coils have formed the external tube has almost disappeared (Figs. 1C, 5D). Although at first all the coils are of the same diameter, those near the free tip of the tube soon become more lax, and those near the point of attachment to the capsule tighten (Figs. 1D, 5E). This tightening of the coils continues until the latter part of what may now be called the thread is almost straight (Figs. 1E, F, 5F). This part also becomes more highly refractile and helical pleats can be seen in its wall. It forms the butt of the mature nematocyst. The rest of the internal thread becomes much less visible and comes to lie longitudinally in the capsule as a result of the straight axis about which the coils are wound itself becoming curved and finally helical (Figs. 1D–F, 5E). As the thread becomes differentiated into 2 regions, the containing capsule elongates to $50 \mu\text{m}$ and the width decreases to approximately $19 \mu\text{m}$. Elongation is correlated with a noticeable decrease in the thickness of the walls of the capsule, especially near the poles. (Günzl (1968) claims that this is due to expansion of the capsular contents.) A small operculum forms at the point of attachment of the thread to the capsule.

At this stage (Figs. 1G, 5E, 12) the cnidoblasts migrate to their final site in the batteries on the tentacles, where further elongation and slimming of the capsules occurs (Figs. 1H, 14). They move through the tissues at speeds up to $15 \mu\text{m}$ per min. As the cell moves, the nematocyst comes to lie with the operculum region trailing; if the cell reverses its direction of movement, the nematocyst flips over so that it again comes to lie with the operculum region trailing.

Even after the microbasic-mastigophore nematocysts have arrived at their final site beside the cnidoband of the tentillum and are oriented with the operculum outwards, they are still not mature. The butt region is still very distinct and the rest of the thread

very indistinct (Fig. 14). All that can be seen of the rest of the thread at this stage is rows of 5 or 6 dots arranged approximately longitudinally in the capsule. These dots represent the sites of whorls of developing barbs. In the fully mature, large, microbasic mastigophore (Fig. 16) the capsule wall is thin, the butt region less distinct than before, and the thread is oriented largely longitudinally in the capsule. The thread appears alternately dark and light from the arrangement of the refractile barbs.

The whole process of development in cnidoblasts of *Rosacea* is very rapid – from the completion of the external tube to the beginning of migration the process takes just over 4 h.

Electron microscopy

Cnidoblasts in the early stages of secretion of nematocysts resemble many types of cell that produce large amounts of an exocrine secretory product. In stained sections many spherical mitochondria 1–2 μm in diameter with numerous vesicular cristae are visible, and rough endoplasmic reticulum is abundant throughout the cell (Fig. 6). The contents of the cisternae of the endoplasmic reticulum are generally pale, though in some places the pale cisternae end in a sphere up to 1 μm in diameter with dark contents. These spheres, coated with ribosomes, usually occur near the Golgi apparatus (Figs. 6, 7), which is very large and forms a cap on top of the developing capsule and, later, over the growing point of the external tube (Fig. 7). A very large number of small vesicles (0.1 μm diameter) with darkly stained contents occur around and between the stacks of flattened Golgi cisternae (Fig. 7). When stained these cisternae exhibit electron-dense contents. Those nearest the external thread of the nematocyst enlarge, apparently by fusion with the small spheres (Figs. 7, 8), for in several places on the enlarged Golgi cisternae, protrusions occur whose diameter and staining properties are the same as those of the small spheres. The Golgi cisternae enlarge to form spheres up to 2 μm in diameter (Fig. 7). These are clearly the spheres observed in the living cell that fused with the growing tip of the external tube.

The external tube is 1–2.5 μm in diameter, and contains a fine, granular, electron-dense material in a clear matrix (Fig. 6). The contents of the external tube are identical in appearance with the contents of the capsule (Fig. 6), although the overall electron density of both is much less than that of the Golgi vesicles from which they are formed. The wall of the external tube has 3 layers (Fig. 7). One layer is very electron-dense after staining and is approximately 12 nm thick. The other 2 layers lie on either side of it; each is approximately 0.04 μm thick and of medium electron density. The last 1 μm of the external tube where it joins the capsule is twice as thick and stains darkly and uniformly.

At the developing tip of the external tube, and extending approximately 4 μm back from the tip, are 70–80 microtubules aligned with their long axis parallel to the long axis of the external tube. Their presence may perhaps be correlated with the localized deposition of Golgi vesicles (Roberts & Northcote, 1970).

Although in the living cell the external tube tapers uniformly from base to tip, as seen in the electron microscope, the developing tip is often larger in diameter than regions further from the tip (Fig. 8). Moreover in sections for the electron microscope

but not in the living cell, the external tube occasionally appears kinked, locally constricted or dilated (Fig. 7). These appearances may be artifacts.

As soon as the external tube begins to invert to form the internal tube, the inverted portion is complexly pleated (Fig. 9). The pleats arise at right angles to the long axis of the thread; approximately half-way along some pleats there is a further right-angled bend, so the other portion comes to lie parallel with the long axis of the thread. All pleats bend to some extent across their width; the angle through which they bend varies from 30 to 90°. It is interesting that at this stage, when some of the external tube still contains no internal thread (Fig. 9), no barbs are present. All that can be seen are tiny electron-dense spheres approximately 0.03 µm in diameter at the junction between the pleat and the axial cylinder of the internal thread. The pleated wall is largely transparent to electrons, but it is bounded by a layer that stains strongly and shows up as a fine dense line (Fig. 9).

At this stage the endoplasmic reticulum has become reduced in amount; in sections it often appears as concentric circular cisternae 0.5 µm in diameter with numerous ribosomes on the outer face of the outer, and inner face of the inner, profiles. The cisterna itself, between the inner and outer profiles, contains electron-dense material; within the space bounded by the inner concentric profile are numerous granules approximately the size of ribosomes but less electron-dense, and vesicles approximately 0.1 µm in diameter with electron-dense contents. These formations may be early stages of the 'ER isolation bodies' described by Lentz (1966) in developing cnidoblasts of *Hydra*.

By the time 7–10 coils of internal tube have formed within the capsule (Fig. 10), the barb material has increased in amount and forms electron-dense spheres approximately 0.1 µm in diameter regularly distributed along the entire length of the internal thread (Fig. 11). The wall of the internal thread is almost completely electron-transparent, and shows up in the capsule only because the capsular fluid stains darker. The pleats in the thread wall appear to be more widely spaced at this stage than when the thread was first inverting.

When these cnidoblasts start to migrate (Fig. 12) the internal thread is clearly triply pleated (Fig. 13). The barb material has undergone redistribution so there is now less barb material in the greater part of the thread than at the preceding stage (Fig. 11), but a large amount is present in the triply pleated region of the thread that is conspicuous in the light microscope and that will form the butt of the nematocyst. Here the barb material forms groups of 3 large spheres (Fig. 13).

Microbasic-mastigophore nematocysts that have just stopped migrating and are in their final position (Fig. 14) have a butt that is triangular in cross-section but has no distinct pleats. The butt contains 3 helices of barbs that are themselves sigmoidal in cross-section (Fig. 15). The rest of the thread has 3 distinct pleats and in many cross-sections 3 barbs can be seen. These are electron-dense and have the form of pyramids the sides and axes of which are curved and the height of which is approximately equal to the length of the base. The tip and edges of the pyramid are coated with material that stains strongly with uranium and lead. There are 3 pleats in the threads not only of the large microbasic mastigophores but also of the desmonemes of *Rosacea*.

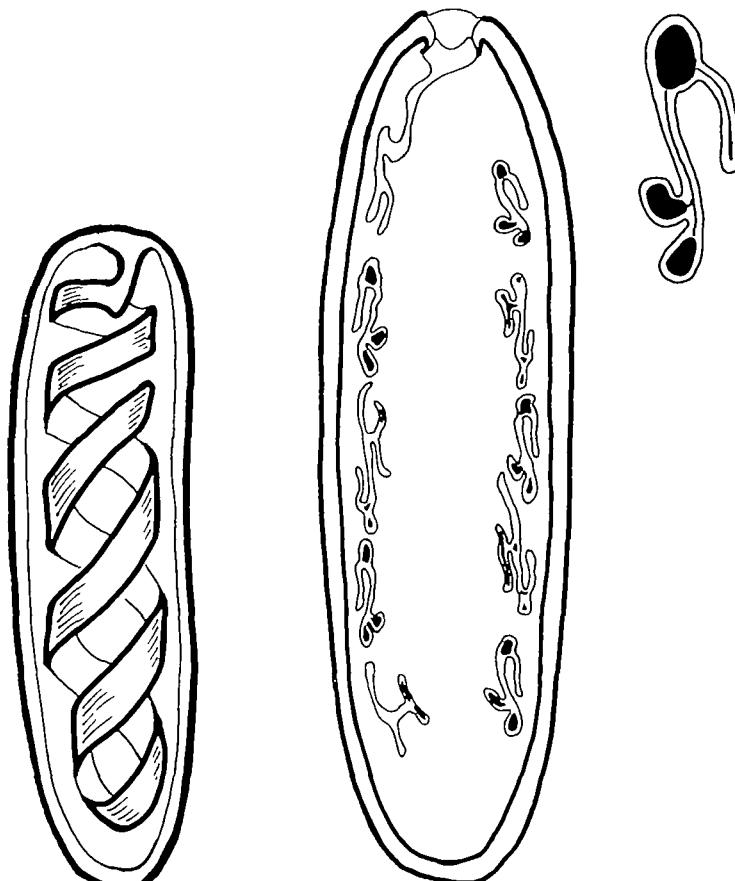


Fig. 2

Fig. 3

Fig. 2. Diagram of the coiling of the internal thread of a developing rhopaloneme. No pleating of the thread is shown. The arrangement of the thread in the capsule can be thought of as a U-shape which has become twisted several times around the long axis of the capsule. This results in the gyres arranged around the long axis of the capsule being alternately descending (formed from the descending branch of the U that is anchored to the operculum) and ascending (formed from the ascending branch of the U that ends with the free tip of the thread).

Fig. 3. Diagram of the pleating of the thread in a longitudinal section of a rhopaloneme. Sections of the thread down each side of the capsule pass through alternately descending and ascending gyres. The shape of the thread varies regularly along its length and cannot be produced by extrusion or extrapolation from the shapes shown here. Notice the 3 darkly staining regions in some sections of the thread (as in the inset which is of a transverse section of the thread at higher magnification).

The coiling (Fig. 2) and pleating of the threads of nearly mature rhopalonemes is very complex for there is no obvious central core on which pleats are inserted as in other nematocysts (Fig. 3). Instead the region on which the pleats insert is itself flattened and no thicker than a pleat. Cross-sections of the thread, visible in longitudinal sections of the capsule, show a minimum of 3 slightly inflated regions containing

darkly staining, amorphous material. More than 3 inflated regions, visible in some sections, could be caused by obliquity of sectioning. Thus it is possible that the 3-dimensional structure of the threads of rhopalonemes is not fundamentally different from the threads of other nematocysts.

Electron microscopy confirms the light-microscope finding that not all nematocysts in their final position are fully mature, for the capsular fluid still stains darkly and the thread wall is pale (Fig. 15). At maturity, when the nematocyst is functional, the capsular fluid is clear in the electron microscope and the thread wall stains strongly (Skaer & Picken, 1965).

DISCUSSION

Nematocyst development can be successfully followed in living material by phase-contrast microscopy. Correlation of information obtained in this way with electron microscopy enables a reconstruction of the developmental sequence. These observations on living cells show clearly that the external tube is an essential stage in the development of the internal tube of nematocysts, rather than an artifact produced by precocious discharge on fixation, as held by Weill (1934). Fixation in glutaraldehyde does not provoke the discharge of even the mature nematocysts of siphonophores. Moreover the constant association of a cap of Golgi apparatus with the developing external tube, as first pointed out by Slautterback (1961), and the fact that the external tube never penetrates the cell boundary in living cells, are both incompatible with Weill's view. The external tube transforms into the internal tube of the nematocyst; and is *not* incorporated into the elongating wall of the capsule as claimed by Westfall (1966). Furthermore, the internal tube is *not* formed within the matrix of the capsule from unaggregated precursor material as suggested by Lentz (1965).

The external tube is a smooth-walled cylinder (Fig. 4). It becomes pleated as it inverts. The region of inversion moves along the external tube towards the capsule. Thus the formation of the internal thread is the geometrical converse of its discharge (Fig. 4). These results focus our attention on the inversion processes as a key step in the formation of pleats. Although the complex geometry of the internal thread is produced, at least in part, by buckling, the localized forces that cause the tip of the external tube to invert and pleat are unknown. The barbs play no part for they do not form until later in development.

Some remodelling of the internal thread in the capsule as well as redistribution of barb material must occur, for, whereas the external thread tapers smoothly from base to tip, the discharged thread has an enlarged region at its base – the butt (Fig. 4D, E). I have observed that a rather similar remodelling of the thread in the capsule must occur in the microbasic mastigophores of the siphonophore *Hippopodius hippocampus*. The external thread tapers smoothly; the discharged thread has not only a butt region, but also has a regular series of localized enlargements along the entire length of the thread. Localized enlargements of the discharged thread are sometimes, however, preformed in the external tube and are not the result of remodelling in the capsule. I find, for example, that the external thread of developing stenoteles in the Siphono-

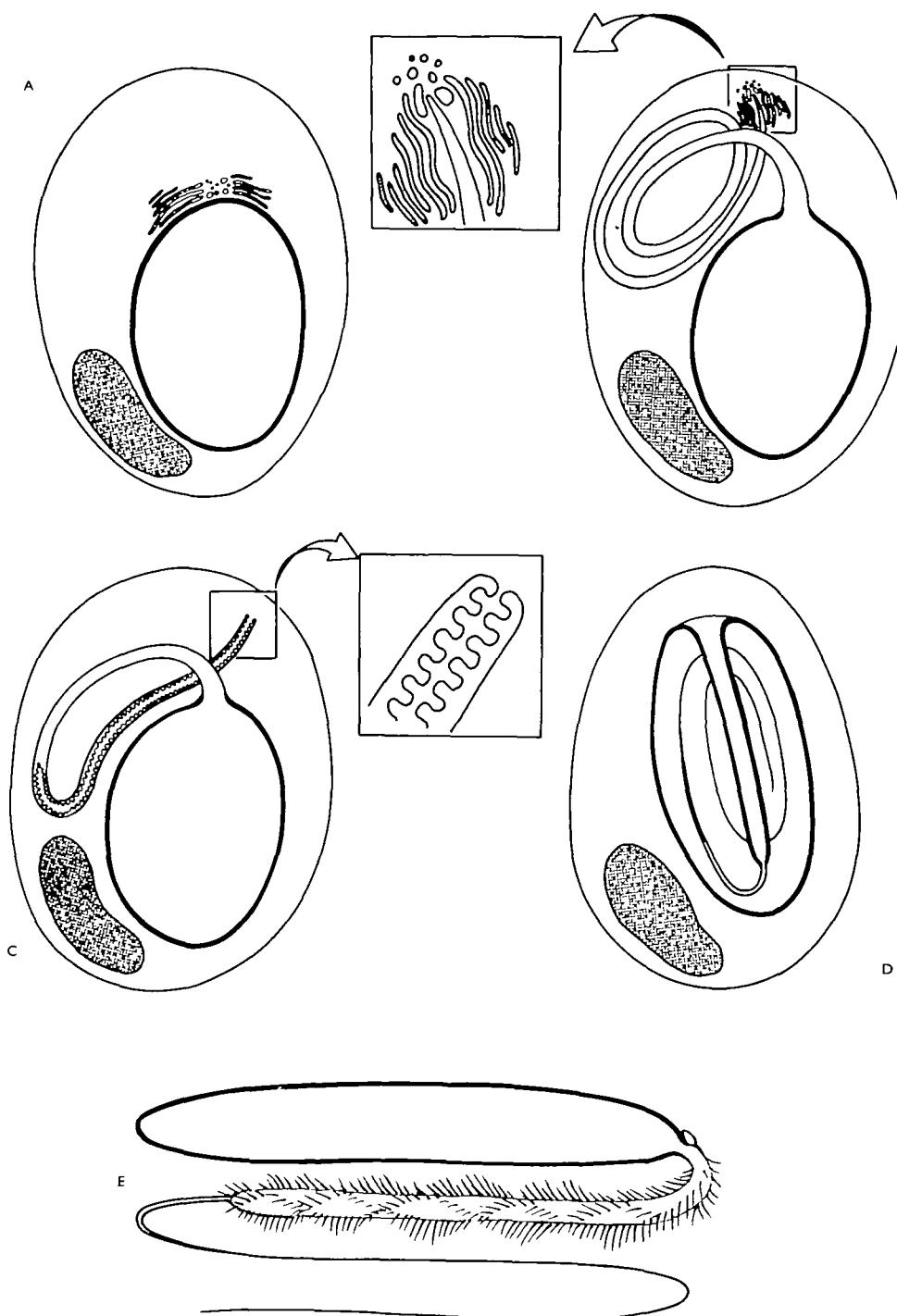


Fig. 4. Generalized diagram of the formation and discharge of the large microbasic-mastigophore nematocysts of *Rosacea*. For clarity, details of the cytoplasm of the formative cell, and the dark staining of the capsular contents of the developing nematocysts are omitted. A, Secretion of the capsule. B, Secretion of the external tube showing the Golgi cap. C, Inversion of the tip of the external tube to form the internal thread. Inset, the tip at higher magnification; the pleating has been stylized. D, Remodelling of the internal thread in the capsule to form a butt. E, Discharged nematocyst showing butt with barbs. Only part of the length of the discharged thread is shown. The barbs on most of the thread are too small to be shown.

phore *Physophora* has a region next to the capsule that corresponds to the butt of the discharged thread, but without the barbs.

The evidence is unequivocal that nematocysts with a pleated internal thread inside the external tube are not mature but are at a stage of development. The claim by Sutton & Burnett (1969), that the scyphozoan *Chrysaora quinquecirrha* has 10 categories of nematocyst is invalid, for several of their categories are developmental stages of the relatively few mature types. The staining properties of nematocysts, on which they base some of their categories, must also be interpreted with caution.

The reversal of staining of the capsular fluid and thread wall at maturity, such that the capsular fluid that was strongly stainable ceases to be so, and the thread wall that was unstained becomes strongly stainable, re-emphasizes the point made in early papers on the electron microscopy of nematocysts (e.g. Chapman, 1961) but often overlooked – that most pictures are of *nearly* mature nematocysts, rather than of mature ones (except in Skaer & Picken, 1965). Fully mature nematocysts are difficult to infiltrate with resin. Thus the distinctions between nematocysts and spirocysts made by Doumenc (1971) on the basis of their staining properties are vain, for she compares immature nematocysts with mature spirocysts.

The orientation of nematocysts with the operculum trailing, observed in migrating cnidoblasts of *Rosacea*, is also visible in Günzl's (1967) film of the hydroid *Dipurena reesi*, and is described in his published text of the film (Günzl, 1971). If this behaviour pattern of the cnidoblasts were retained when the nematocyst reached its final site, the operculum would lie towards the interior of the animal. More observations are needed to find out when and how the orientation changes so the operculum comes to lie towards the outside of the animal.

In all nematocysts where pleating has been examined, 3 helical pleats have been found. This has been shown here to be true both of the microbasic mastigophores and the desmonemes of *Rosacea*. Even the rhopalonemes have a pleated thread and at least some components are arranged in threes. A pleated thread with parts in threes may be a general feature of nematocysts.

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Fig. 5. Group of 3 microbasic-mastigophore nematocysts developing in a gastrozooid. Phase-contrast microscopy of living specimen. $\times 700$.

A, 4 coils of thread in the capsule. Dark profiles of external thread can be seen outside the capsule.

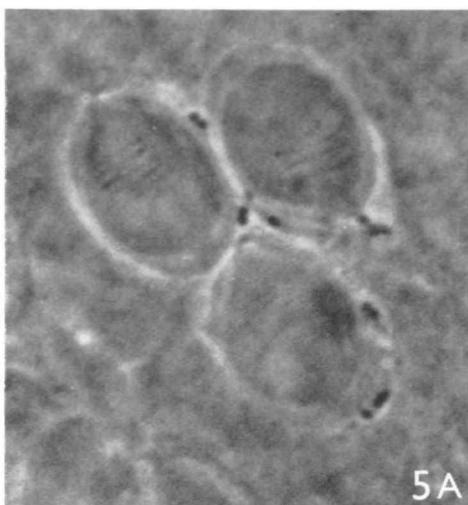
B, The same 2 min later.

C, 8 coils of thread in the capsule. A dark length of external thread can be seen alongside the uppermost capsule. 37 min after B.

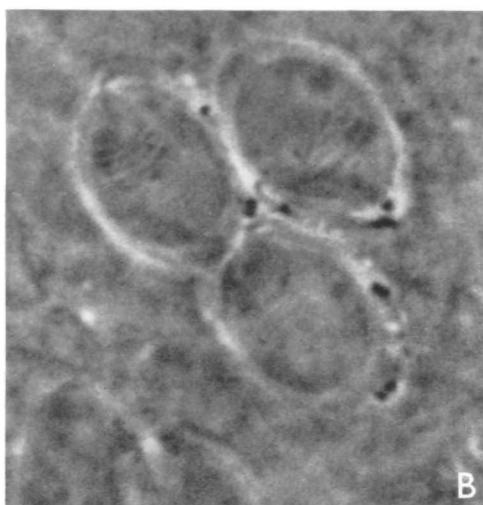
D, 15 coils of thread in the capsule. All the external thread has entered the capsule. 30 min after C.

E, Remodelling of the thread in the capsule. A migrating cnidoblast containing a developing, large, microbasic mastigophore has collided with the group and displaced them. 21 min after D.

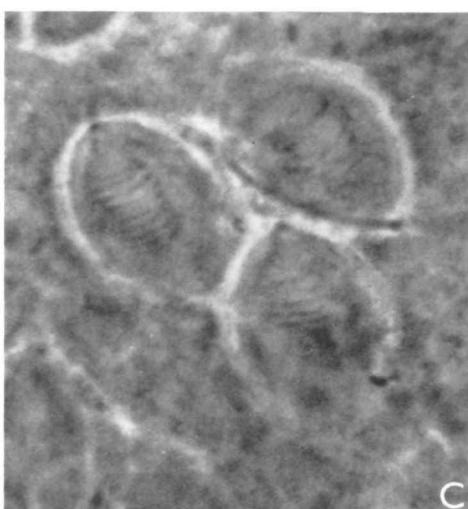
F, Extensive remodelling of the thread and formation of butt. The migrating cnidoblast has moved on. 74 min after E.



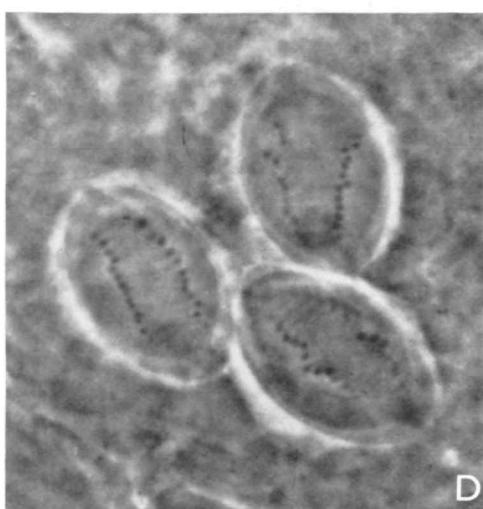
5A



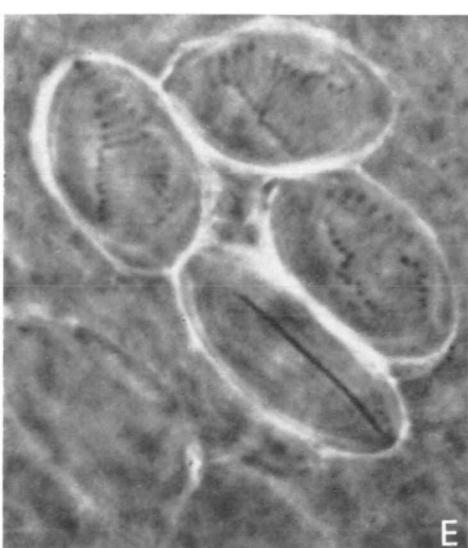
B



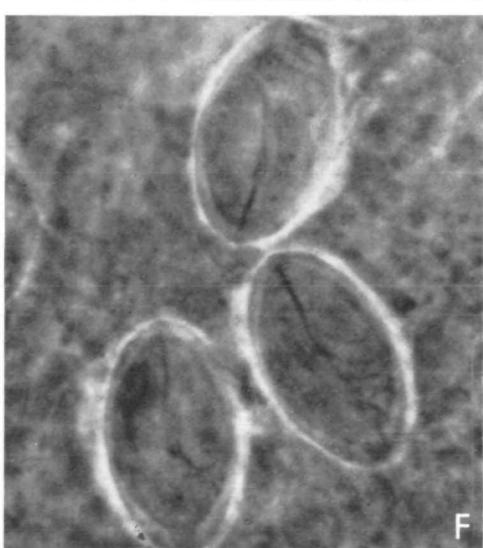
C



D



E



F

Fig. 6. Section through a cnidoblast with a developing large microbasic mastigophore and much rough endoplasmic reticulum. Seven profiles of external tube (*e*) can be seen in the cytoplasm. The wall of the capsule (*cw*) is very thick and the capsular fluid (*cf*) stains in the same way as the contents of the external thread. *m*, mitochondrion.
× 24000.

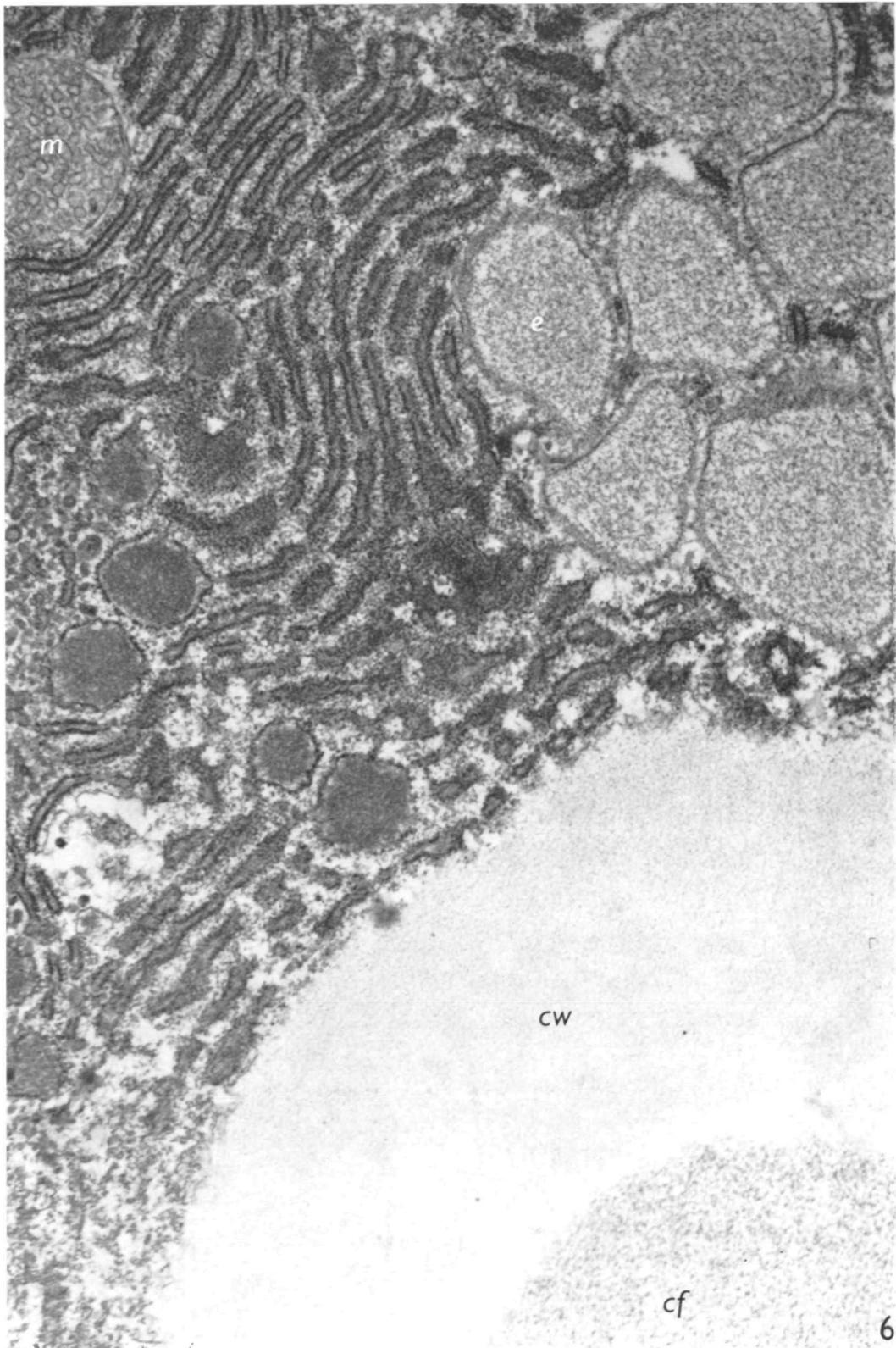


Fig. 7. Longitudinal section through the developing tip of the external tube (*e*) of a large microbasic mastigophore showing the extensive cap of Golgi apparatus. Microtubules lie along the wall of the thread at the tip. $\times 26000$.

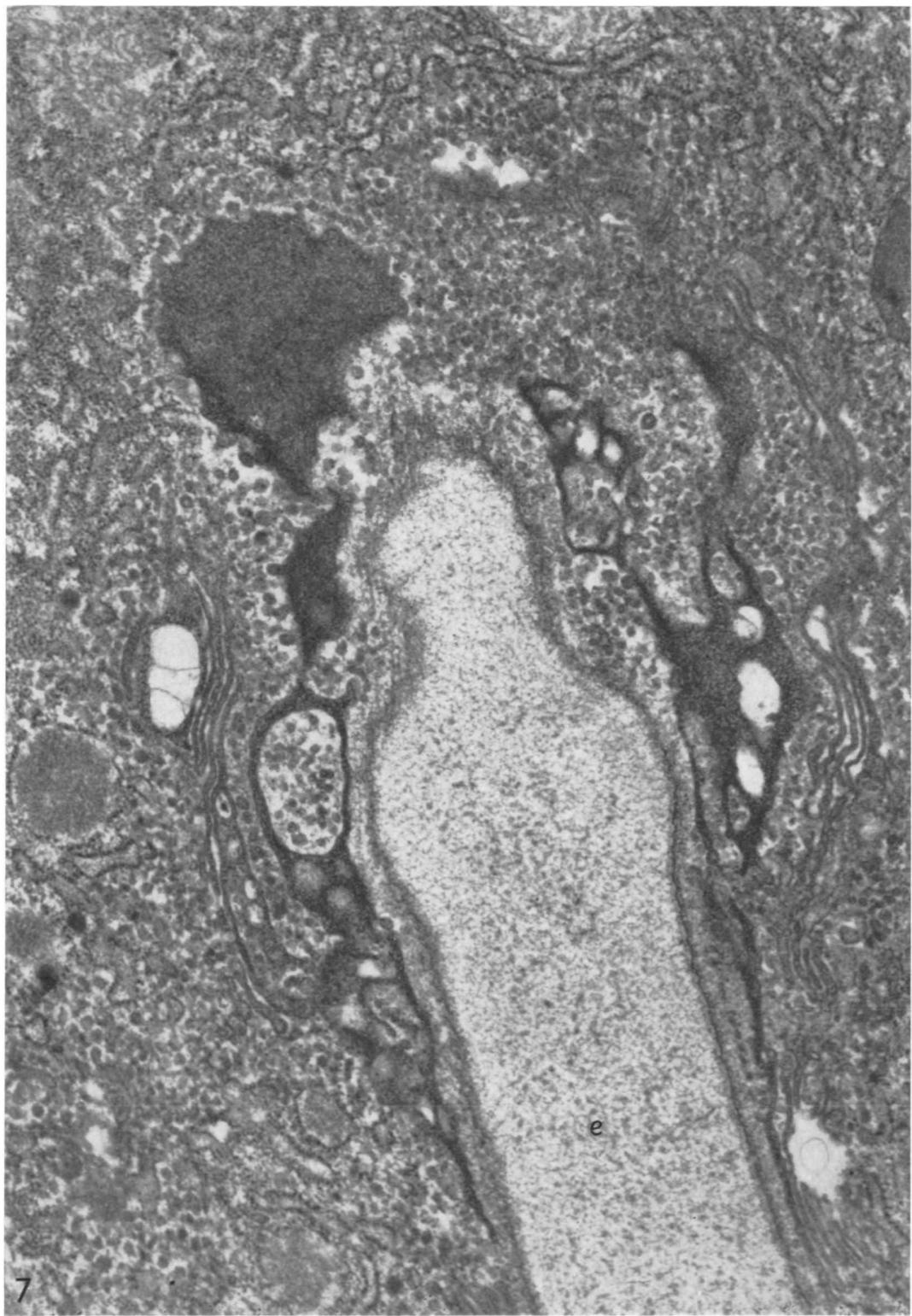
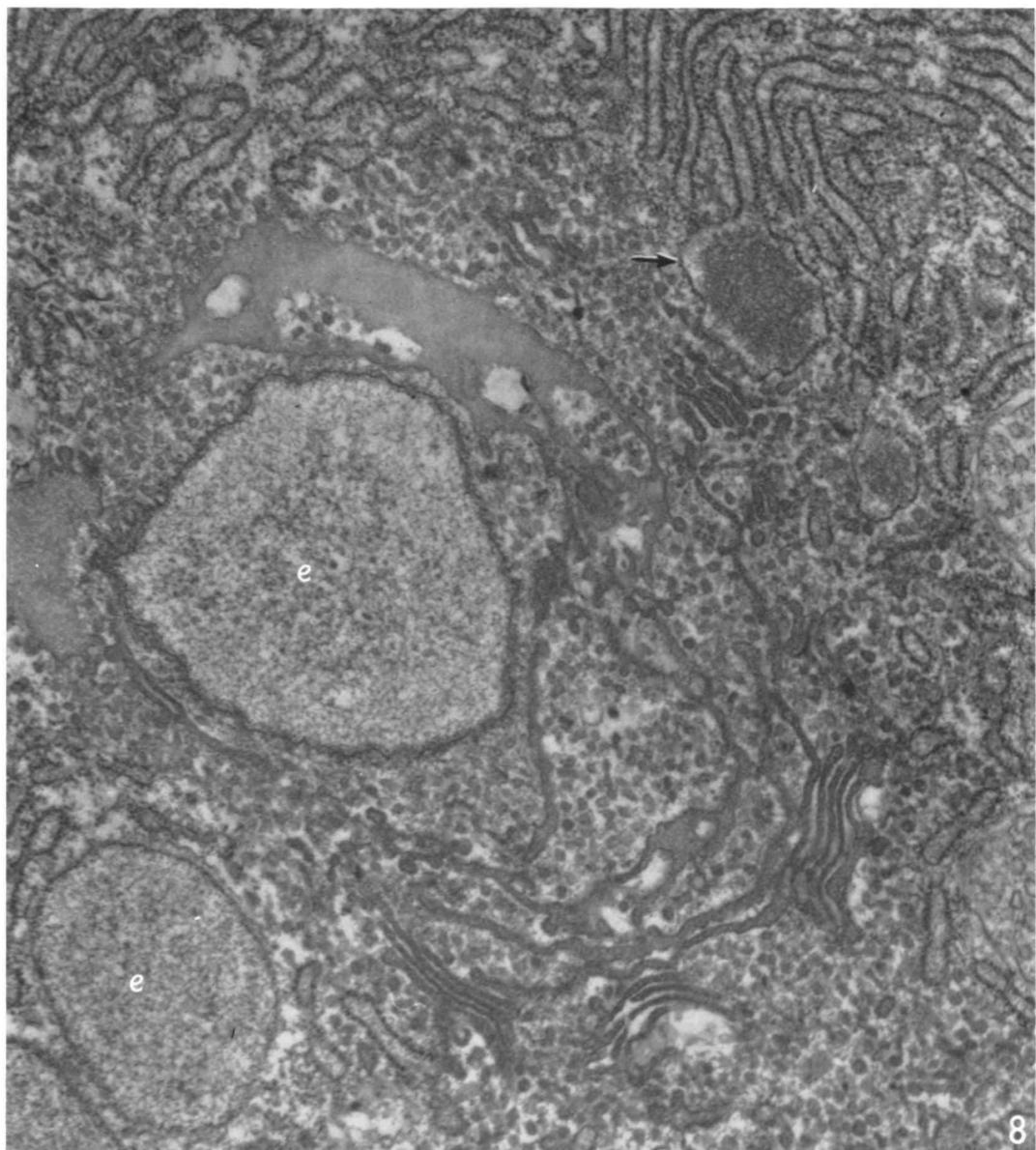
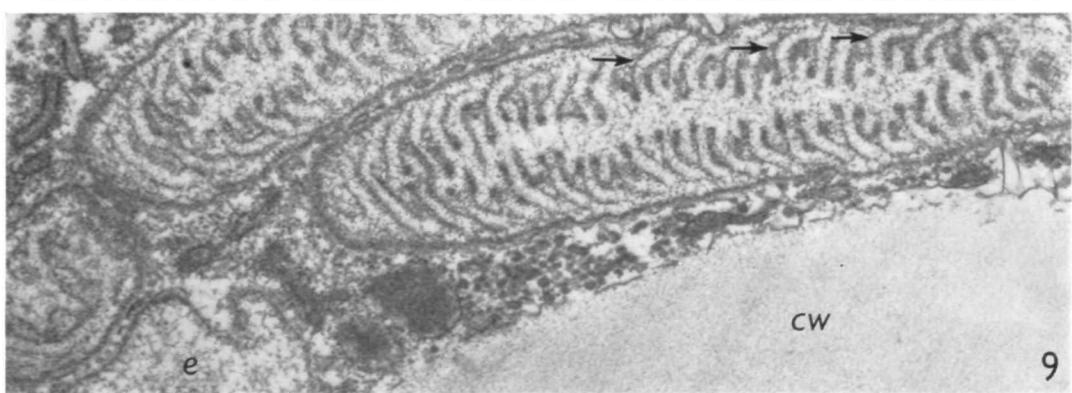


Fig. 8. Transverse section through the tip and 2 other profiles of the external tube (*e*) of a large microbasic mastigophore. The tip is larger than the other coils and is surrounded by microtubules and Golgi apparatus. A dark-cored vesicle of the endoplasmic reticulum is shown (arrow). $\times 23000$.

Fig. 9. Longitudinal section of complexly pleated, internal thread in the external tube of a large microbasic mastigophore. The internal thread has not yet reached some parts of the external tube (*e*) or the capsule. Darkly stained barb material is present as tiny spheres where the pleats arise from the central core of the internal thread. *cw*, capsule wall; arrows indicate where pleats bend at right angles. $\times 22000$.



8



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Fig. 10. Large microbasic mastigophore with 8 coils of internal thread and some external tube. Phase contrast of living specimen. $\times 1080$.

Fig. 11. Electron micrograph of the stage shown in Fig. 10. The pleated thread can be seen because of the darkness of the capsular fluid. Barb material (arrow) can be seen as dark spheres regularly arranged along the thread. *cw*, capsule wall. $\times 42000$.

Fig. 12. Two large microbasic mastigophores at the migrating stage, showing the long refractile butt in the capsule. Phase contrast of living specimen. $\times 945$.

Fig. 13. Electron micrograph of the stage shown in Fig. 12. The thread has 3 pleats and the barb material is largely present as whorls of 3 large dark spheres in the butt region shown in the bottom right hand corner. $\times 26000$.

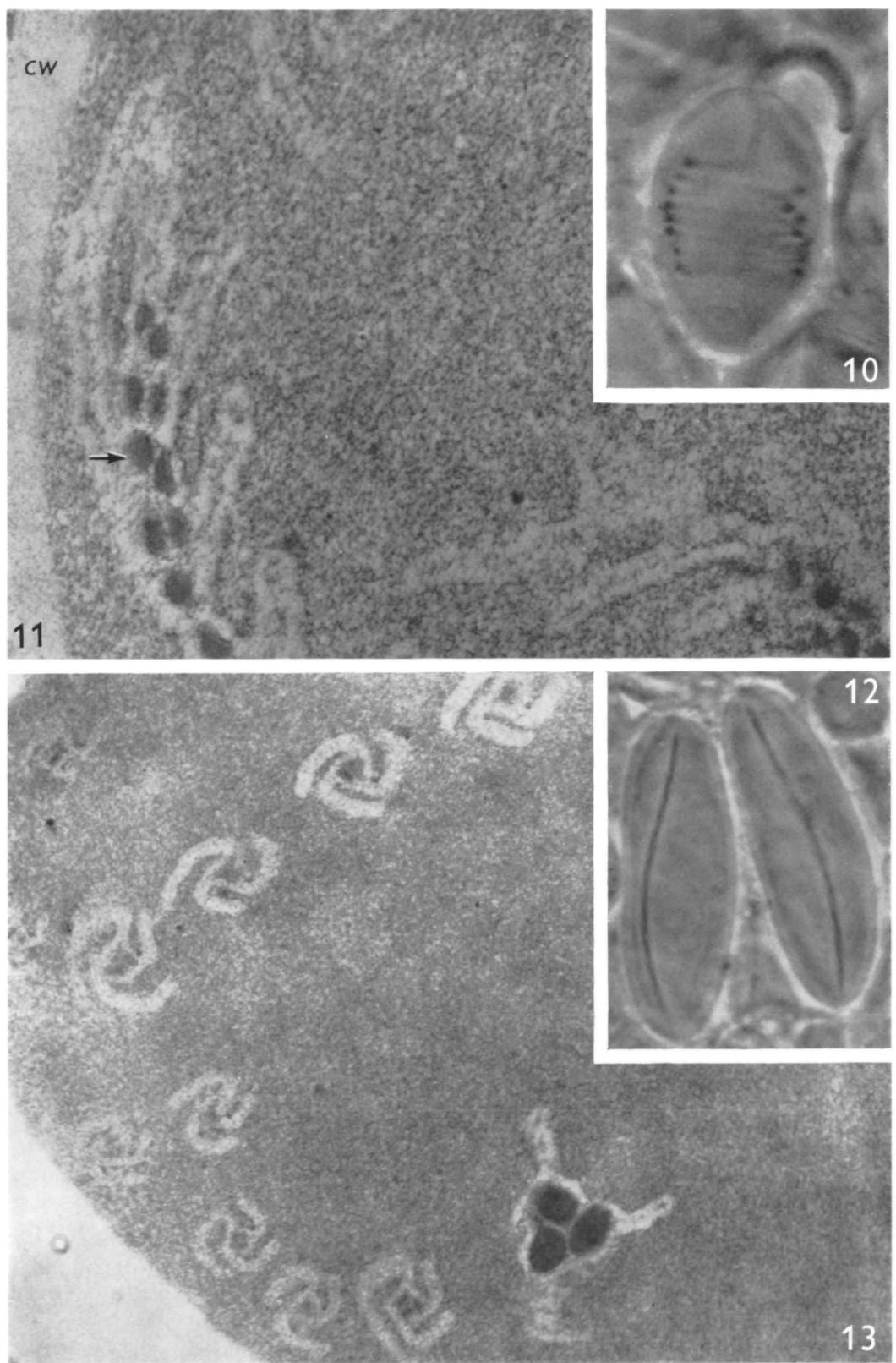


Fig. 14. Two large microbasic mastigophores that have completed their migration but are still not fully mature. The butt region is still very distinct and the capsules have become very long. Lines of 5 or 6 tiny dark dots show where whorls of barbs are developing in the thread. Phase contrast of nematocysts from a living specimen. $\times 870$.

Fig. 15. Electron micrograph of a transverse section of the stage shown in Fig. 14. The butt region in the centre contains sigmoidal barbs, and is not pleated. The rest of the internal thread has 3 pleats and 3 barbs. The latter are best observed in precisely transverse sections such as those in the lower left. The capsule wall can be seen in the bottom right-hand corner of the picture. The capsular fluid still stains darkly, and the thread wall is pale. $\times 43500$.

Fig. 16. Fully mature large microbasic mastigophore isolated from living material. The butt has become less distinct, and the rest of the thread more distinct when compared with Fig. 14. $\times 1580$.

