Fine Structure and Function in *Stentor polymorphus*

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ABSTRACT

The fine structure of the ciliate *Stentor* has been studied by means of the electron microscope and the results have been correlated with observations made on the living organism by means of light microscopy; special reference has been made to structural features which may be responsible for contraction and extension in *Stentor*.

Descriptions have been given of the structure of the macronucleus, the vacuolated cytoplasm, mitochondria and the pellicle; a detailed study has also been made of the adoral membranelles. About 250 membranelles encircle the peristomal cap and each is composed of 3 rows of cilia, with 20 to 25 cilia in each row; a fibrillar root system connected with the membranelles depends into the endoplasm for about 20 μ and each is essentially in the shape of a fan, the terminal ends of each root bifurcating to connect to neighbouring roots. The membranelles thus form a cohesive unit and this morphological arrangement may have a bearing on the motion and coordination of the whole system.

Two structural features extending throughout the length of the animal have been identified per cortical stripe in the body wall of *Stentor*; first, km fibres lying just beneath the pellicle are composed of stacks of fibrillar sheets and are identical with the birefringent fibres observed in the living animal. The individual fibrils of the sheets are in turn connected to the kinetosomes of the body cilia; thus the km fibres are homologous to kinetodesmata. Secondly, M bands lie beneath the km fibres and form an interconnected system in contact with the surrounding vacuolated cytoplasm; the thickness of the M bands is greatest at the base of a contracted animal. The contractile and extensile properties of these organelles have been discussed in the light of experimental results and theoretical considerations.

INTRODUCTION

This paper describes and discusses the results of studies of the fine structure of the ciliate *Stentor polymorphus*, and to a smaller extent that of *S. coeruleus*, and relates wherever possible the fine structure to function. While the cytology of the ciliates is of considerable general interest, there are certain structural features of the genus *Stentor* which makes further work particularly attractive; many of these features have been known for a long time but are still little understood. Three of the best early accounts of the morphology and biology of *Stentor* have been given by Schüberg (56), Johnson (26), and Schröder (55). Members of the genus *Stentor*, all of which are found in fresh water, are highly contractile. While resting, the organism attaches itself by a posterior holdfast to debris or other suitable anchorage; in this state *Stentor* can become much extended and trumpet-shaped, with an over-all length considerably greater than that when swimming. In the extended state the greater part of the length of the animal is taken up by the narrow stalk-like posterior portion, but on supercontraction the organism is almost spherical. These changes of shape emphasize the structural attributes of contractility and extension associated with certain ciliates.

The history of the investigation of fibrillar and contractile structures in the *Protozoa* is a long story

of complexity and confusion which began as far as we know with the observations of Lieberkühn (32) on the contractile structures of *Stentor* which were named *Muskelfasern* by him, *Myonemes* by Bütschli (5), and *Myophanes* by Neresheimer (41). To add to the confusion, adjacent systems were also variously named: *e.g. neurophanen* (41) and *neuroids* (9). Previous investigators have attributed the functions of contractility, elasticity, supporting fibres, and neuromotor systems to the features observed, but it has been generally agreed amongst protozoologists since the work of Rouget (52) and Engelmann (11), both of whom made use of the polarizing microscope, that the contractile elements (or myonemes) are, in ciliates, long narrow structures lying parallel to rows of body cilia. The study of contractile structures in the *Protozoa* is to a large extent an example of inference made from insufficient experimental data. The difficulties were twofold: first, the early observations of longitudinal structures in the heterotrichous ciliates were often too near the limits of optical resolution to be exact; second, microphysiological techniques were non-existent, and there was an almost invariable acceptance without physiological proof of the functions of particular structural elements. It is one of the objects of this paper and of later investigations to clarify and identify the structure of these organelles in *Stentor* and to study their mode of contraction.

Fibrillar systems have been observed in electron microscope sections of protozoa by several authors. Thus in two recent papers (48, 50) evidence of fibrillar sheets in *Spirostomum* has been reported; it has been inferred that, in the absence of other subcortical structures, these are the structural units responsible for contractility in this organism. In the peritrich *Carchesium* (48, 49) the stalk is differentiated into an annulus and a central core, and the annulus has been shown to be filled with numerous long striated fibrils which are attached proximally to the scopula of the zooid. The contractility of the stalks of peritrichs has been discussed in the light of these findings. Fauré-Fremiet and Rouiller (12) observed similar fibrils in the stalks of other peritrichs and Fauré-Fremiet, Rouiller, and Gauchery (13) have also published a paper on the contractile structures of other ciliates, including *Stentor*, to which further reference will be made in the Discussion. So far, however, no proper link has been discovered which relates the contractile organelles of protozoa with the muscles of higher organisms. There seems to be little doubt that comparative studies of the morphology and physiology of contractile structures in lower animals will contribute notably to the general understanding of muscle structure and function.

In this paper attention is also paid to the ciliary structures to which the group of organisms as a whole gives its name. In their early successful application of thin-sectioning techniques to the study of ciliated epithelia, Fawcett and Porter (14), Sedar and Porter (57), and others (for further references see 45) have shown that the fine structure of the external cilium is substantially the same in material from widely different tissues and phyla, and appears also to be the same in the flagella of algal zoospores (*e.g.* 37 and 18) and in the tail-ends of animal and plant spermatozoa (*e.g.* 4 and 36). The history of the determination and the variations of ciliary structure has been traced in a recent Harvey Lecture (45) and will not be elaborated here.

This paper will describe the complex system of ciliary bodies and roots which form the adoral membranelles which almost encircle the peristomal cap of *Stentor* species. Earlier investigators, making use of light microscope techniques only, had concluded that the external membranelles were in the form of plates of fused cilia (55, and for example Bishop (1) on *Spirostomum*). Studies of the fine structure of *Stentor* during stages of conjugation and division, together with the use of grafting techniques (*e.g.* 60–63, 67) provide a means for investigating the morphogenesis of the cilium, the development of ciliary fields, and the differentiation of the membranelle system. The solution of these morphological problems should play its part in the ultimate understanding of the physics, biochemistry, and physiology of ciliary motion and metachronal rhythm. The rhythmic ciliary motion of ciliates is commonly associated with the existence of infraciliary kinetodesmata. Yet in higher animals where such rhythmic ciliary motion is also observed in some tissues, such underlying structures are not always found. Further comparative studies will be clearly worthwhile. The two structural systems of *Stentor*, common also to certain other ciliates, particularly to be discussed in this paper, the contractile structures, and the cilia and specialized membranelles, thus clearly form an important biologi-

cal link between some of the most primitive of organisms and the most highly developed.

EXPERIMENTAL

Materials

Stentor Cultures.—The specimens of *Stentor polymorphus* were derived from local ponds; the ciliates were isolated from other pond life and then maintained in culture at room temperature (17°C.) in covered dishes. The culture medium was composed of Chalkley's salt solution (pH 6.8–7) plus a small amount of thick gel obtained from previously boiled wheat grains which were soaked in the above salt solution. Subculturing was carried out about every 3 weeks, the *Stentor* being transferred to clean dishes. Clone cultures of *Stentor polymorphus* were also set up, and more recently *Stentor coeruleus*, obtained from the Carolina Biological Supply Company, North Carolina, were kept in mass culture and also in clone cultures.

Fixatives.—Various fixatives have been used, most of which were based on the use of 0.75 to 1 per cent osmium tetroxide as one component in the presence of appropriate amounts of physiological salts. The pH of the fluids used varied between 6.8 and 8.4. Six per cent neutral formalin in either Chalkley's salt solution or 0.44 M sucrose was also used as a fixative.

Plastic Embedding Media.—The medium most frequently employed consisted of a mixture of 85 per cent butyl and 15 per cent methyl methacrylate plastic with 1 per cent benzol peroxide as catalyst. Araldite and epicote resins were also used.

Heavy-Metal Staining Mixture.—1 per cent phosphotungstic acid was used during dehydration procedures in the first absolute alcohol stage.

Methods

Methods Employed for Observations on Living Organisms:

1. The general behavior of colonies of *Stentor* was studied in open dishes by means of the dissecting microscope.

2. The organisms were examined individually or in groups (either on a glass slide with coverslip or alternatively in a perfusion chamber made out of stainless steel) by means of transmitted light using phase contrast, Baker interferometer, and polarizing microscopes over a wide range of magnifications.

(*a*) The animals were kept either in their normal medium or the medium was modified by the introduction of boiled methyl cellulose, 30 per cent magnesium sulphate, copper sulphate, or various narcotics, to bring about the reduction or cessation of movement; supravital stains such as Janus green B and neutral red were also used and they were examined after extraction with glycerol.

(*b*) The organisms were micro-dissected with glass needles.

(*c*) Approximate measurements were made of the linear dimensions of the *Stentor* in all its normal shapes in rest and in motion, with the aid of an eyepiece graticule or from pictures taken of the living animal.

3. Illuminating systems of optical microscopes.

(*a*) Stroboscopic illumination was used to study in particular the motion of the adoral membranelles; suitably interrupted illumination was obtained by means of a revolving mirror (*e.g.* 59).

(*b*) For various types of photographic work, subsidiary illumination systems were introduced:

(*i*) A Kohler field lens used in conjunction with a monochromatic source obtained from the mercury green line (546 mμ) was used for black and white photography of *Stentor*. The Hg line was isolated from a stabilized 250 watt mercury compact-source arc by means of Wratten filters 77A and 58.

(*ii*) A 250 watt Xenon arc lamp with a Kohler field-lens was used for colour photography.

(*iii*) A flash system obtained from a point-o-lite source attached to a stroboscopic control unit was used with the necessary optical condensers to obtain individual exposures at high speed. 1/500 seconds exposure with Kodak panchromatic recording film (35 mm.) or Ilford "Pan F" was used for black and white photography, while for colour film ecktachrome B was used in conjunction with the interferometer microscope illuminated with white light.

Methods of Preparation of Fixed Material

Preparation of Sectioned Material:

Individuals or small groups of *Stentor* were isolated and left in a small drop of medium in a maximow slide until they were well extended. Ice cold fixative was then added quickly and the organisms were allowed to remain in the fixative for periods varying from 5 to 15 minutes at 0°C.; this method reduced the instantaneous contraction of *Stentor* at fixation, though contraction was still considerable. Some organisms were fixed in the extended state after treatment with relaxing fluids; some physiological changes, however, could be detected in the living animal on treatment with the fluids within a few minutes and upon later examination of such fixed specimens in the electron microscope, it was found that severe morphological disruption had occurred; few specimens have, therefore, been used after such treatment. Procedures subsequent to that of fixation were carried out at 0°C.; the animals were washed in two changes of Chalkley's solution, dehydrated by gradual stages in chilled graded alcohols, and thereafter impregnated in a mixture of *n*-butyl-methyl methacrylate or other resin. The organisms were usually embedded either individually or in small groups, and polymerisation was carried out either by dry heat at 60°C. or by irradiation with ultraviolet. Centrifugation was not used during any of the above

procedures, since the density of individuals is sufficient to cause sinking.

A hardened block of resin containing the specimen to be sectioned was mounted on a chuck so as to give the appropriate orientation of the organism for the section required; *e.g.*, a transverse section through the membranelle region. Initial control sections were then cut on an ordinary histological microtome at thicknesses of 1 to 7 μ; the angle of the knife was adjusted so as to be similar to that used subsequently on the thin-sectioning microtome. The histological section was usually mounted in oil (μ 1.54) and examined by means of phase contrast illumination. If necessary the orientation of the block was altered by remounting, and controlled sectioning was then continued until the desired area of the *Stentor* was being cut. The chuck and mounted block were then transferred to the thin-sectioning microtome and ribbons of sections were prepared. The sections were picked up, often serially, on carbon-coated electron microscope grids and examined in the electron microscope without chemical removal of the embedding material. This alternation technique of cutting first the control sections and then the thin-sections for electron microscopy was used until the whole individual *Stentor* had been sectioned.

By the use of the light microscope together with this controlled sectioning technique the general topography of any particular plane of section through the organism could be determined. Adjacent thin-sections were then surveyed at low magnification (2000 to 5000) in the electron microscope and sometimes a mosaic of micrographs was built up of the whole sectioned organism in any chosen plane. Subsequently, the sections were investigated at considerably higher magnifications. These techniques have enabled us to forge a direct and coherent link between the known orientation of the organism in the block, the direction and position of a particular section in relation to the *Stentor* as a whole, and the micro-structure of the section concerned. These topographical relationships have been of great assistance in the interpretations of the fine structures observed.

During the course of the investigation the organisms were studied in four different electron microscopes; a modified Metropolitan Vickers EM3, an RCA EMU 2u and a 3a, and the Siemens Elmskop Ia. Micrographs were taken at initial magnifications ranging from × 3000 to × 42,000.

Preparation of Fragmented Material:

Stentor were fixed with osmium tetroxide or with 6 per cent formalin; after preliminary teasing with glass needles, the material was subjected to ultrasonic vibration at a frequency of several megacycles per second. The fragments were collected, teased further, washed in glass distilled water, and concentrated by centrifugation. Specimens were mounted in carbon-coated electron microscope grids; some specimens were stained with 1 per cent phosphotungstic acid or shadowed with gold-palladium alloy at an inclination of 1 in 6. The grids were then viewed in the electron microscope. Digitonin, sucrose, and various other reagents were also used in attempts to get sufficiently thin fragmented specimens for use in the electron microscope.

Histochemical Staining:

Some of the 1 to 7 μ control sections were used; the plastic was removed either by toluene or amyl acetate and the tissue sections hydrated to the necessary state for subsequent staining by any of the following methods:

The periodic-acid Schiff technique.

The Feulgen technique.

The methyl-green pyronine technique.

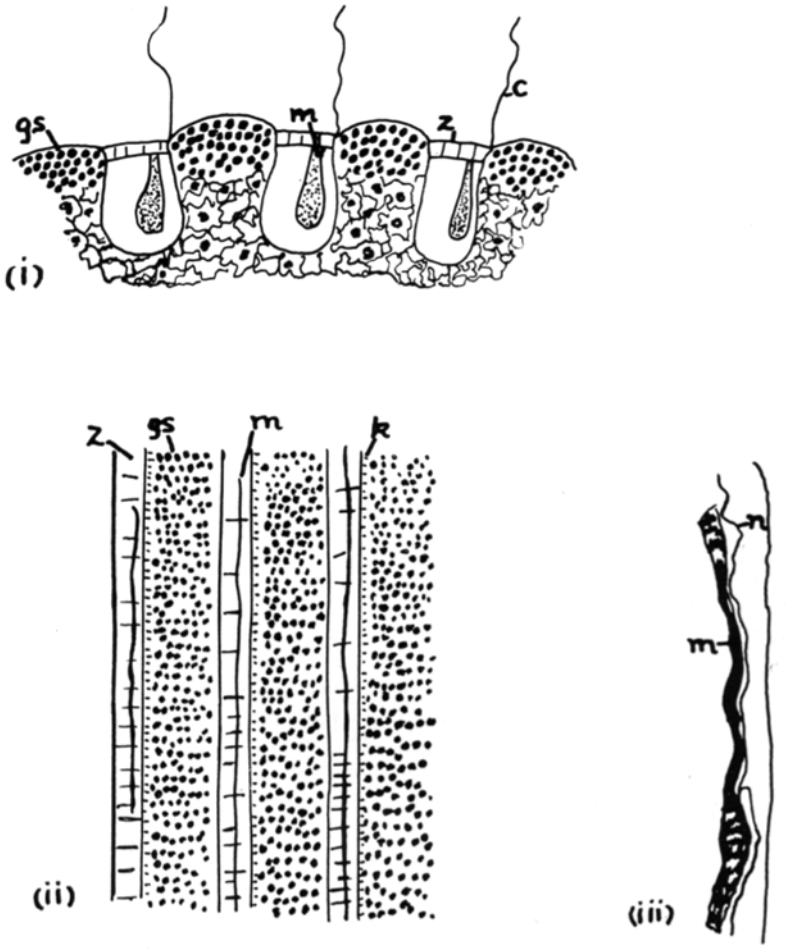
Pollac's trichrome stain.

RESULTS

In order to clarify the account of the fine structure of *Stentor* and the subsequent discussion, a brief description of the well documented major morphological features of the organism will first be given (*e. g.* 9, 10, 25, 55, 56, 64, and Figs. 1 to 10) including details of the terminology which has been used in this paper.

The exterior body surface of *Stentor polymorphus* is covered by a thin pellicle which is formed into a longitudinal series of granular ridges and clear narrow furrows, about 90 of each; the ridges vary considerably in width (Figs. 4 and 5). The body cilia originate in basal bodies or *kinetosomes* which lie beneath the surface of the furrows adjacent to one side of a ridge; they are disposed in single rows, *i.e.* the *kineties* (8), one row per furrow, and the kinetosomes are about 1 μ apart. In the living *Stentor* a well defined birefringent fibril lies within each clear stripe and is located laterally with respect to the kineties. Villeneuve-Brachon (64) has identified these structures as *kinetodesmal fibrils* though she was not able to demonstrate clearly whether they are linked to the kinetosomes. The rows of kinetosomes and their associated kinetodesmata is sometimes referred to as the *infraciliature* (7, 8). This term has also been used rather loosely in the past to refer to all structures lying immediately below the pellicle.

The ectoplasm also contains mitochondria, identifiable by the use of Janus green B, and other granules, about 0.5 μ in diameter, of high refractivity. In the subcortical zone, histological sections demonstrate the presence of long bodies, oval in cross section, which course beneath and slightly to one side of the kineties: these bodies are



TEXT-FIG. 1. Diagrams of "myonemes" in *Stentor* as found in the literature. (*i*) Transverse section of the ectoplasm (Schröder, 55). (*ii*) Surface view of three myonemes; section parallel to the long axis of the body of *Stentor* (Schröder, 55). (*iii*) Longitudinal section cut into and parallel to the long axis of the body of *Stentor* to show the arrangement of neuroids to myonemes (Dierks, 9).

m, myoneme; *c*, cilium; *z*, Zwischenstreifen; *gs*, granular stripe; *k*, kinetosome; *n*, neuroid.

usually referred to as *myonemes* (*e.g.* 55 and Text-fig. 1) (41). The rest of the ectoplasmic matrix is hyaline.

The *endoplasm* is composed chiefly of a sponge-like mass of vacuoles and cytoplasmic matrix, and contains a beaded macronucleus, usually in the form of about 8 serial lobes; numerous small micronuclei; food vacuoles; the contractile vacuole; and many mitochondria and other similar sized refractile granules. Our specimens of *Stentor polymorphus* also contain a large number of symbiotic *Zoochlorella* which lie at random in the cytoplasm. A note on these *Zoochlorella* will shortly be published.

The anterior of *Stentor* consists of the peristomal cap (*cf.* Fig. 1) encircled by a complex system of adoral membranelles (Figs. 1 and 5) which spirals clockwise into the body and thus also forms the mouth, or *cytopharynx*, and the internal *buccal cavity* (Figs. 5 and 6). Large and long adoral cilia are attached to the outer membranelles (Fig. 8), and a root system can just be distinguished descending into the endoplasm of the *Stentor* when the organism is fully extended into its typical trumpet-shaped form (Fig. 9). The posterior end of *Stentor* is composed of a blunted point of cytoplasm, the *holdfast* (Fig. 4); this spreads out unevenly, either over the glass surface of the culture dish or over debris when the *Stentor* becomes anchored.

The living organism can assume a variety of

shapes and sizes which have been defined in this paper as:

(*a*) *The normal or swimming length* in which *Stentor* is roughly conical with the membranelles nearly coincident with the basal perimeter of the cone (Fig. 1). That part of the pellicle which covers the granular strips is often corrugated during swimming (Figs. 1 and 4) or when contracted. The average length of the *Stentor* when swimming is 0.5 to 0.7 mm., and the diameter of the peristomal cap is about 0.5 mm.

(*b*) *The partially contracted length* in which the membranelles lie closer to each other and sometimes force the peristomal cap upwards and outwards while the posterior region becomes more rounded. In this state the organism is roughly oval in shape (Fig. 5) and the peristome is about 0.25 mm. in diameter.

(*c*) *The supercontracted animal* is roughly spherical and the peristomal cap is enclosed within the more tightly compressed membranelles (Fig. 3). The length of the animal is then about 0.25 mm.; the widest region of the body 0.3 mm., and the membranelle region 0.2 mm.

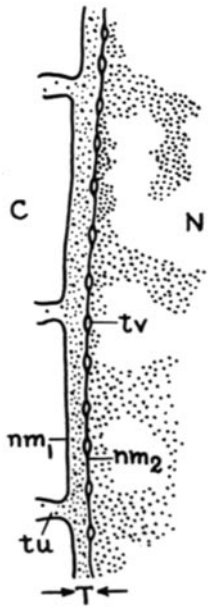
(*d*) *The extended length* of *Stentor* may be observed after anchorage of the organism; the swimming (axial) length is increased when the posterior half of the quasi-conical body extends to form a stalk; the diameter of the peristomal region becomes enlarged to about 0.7 mm. and the adoral cilia curve outwards (Fig. 2).

(*e*) *On superextension* the stalk region becomes considerably longer, and often measures 1.5 to 2 mm.; the body does not increase in length beyond about 0.8 mm.

The main dimensions of many animals have been measured in three of these configurations, the supercontracted, the normal or swimming length, and the length at superextension. The results of such measurements on the living organism can only be approximate but the same criteria have been used throughout. The average volume-ratios of *Stentor polymorphus* in the three positions (*c*), (*a*), and (*e*) as described above are about 4 : 9 : 16.

Observations by Electron and Light Microscopy

Nuclear Structure.—The lobar-shaped bodies of the macronucleus have proved a great convenience for identification purposes in low magnification micrographs, but we have not yet observed any bodies which may be clearly identified with micronuclei. The body of the macronucleus is characterized by the presence of large numbers of irregular shaped dense masses (Fig. 11). In some micrographs these masses are much more dispersed than in others and give the appearance of fairly coarse structures of unknown length with a diameter of at least 600 A. The larger masses sometimes appear to be aggregates of entangled beaded filaments with a diameter about 300 A; the beading may, of course, be an artifact due to fixation, but even so, it probably represents some local differences in the nature of this component. The matrix of the nucleus in which these filamentous masses are embedded appears less electron-dense; it also contains some filaments of medium density which are linked irregularly to the main masses. Other more compact dense bodies are present and range in size from 0.75 to 5 μ; these may be equivalent to nucleoli.



TEXT-FIG. 2. Diagrammatic representation of the structure of the macronuclear membrane of *S. polymorphus*. *N*, nucleus; *C*, cytoplasm; *T*, over-all thickness of membrane system; nm_1, outer membrane; *tu*, tubular openings; nm_2 inner membrane; *tv*, tubular vesicles.

The macronucleus viewed in section is bounded by a complex system of membranes and tubular vesicles about 2000 A in over-all thickness ((*T*) denoted in Text-fig. 2, and Fig. 12). The outer nuclear "membrane" (nm_1) appears to be broken

rather irregularly and occasionally presents the appearance of a tubular orifice. It seems possible that the outer "membrane" (nm_1) is provided with a number of tubular openings, which occupy a considerably smaller cross-sectional area comparable with the intervening sheet-like material. The thickness of the outer membrane, which is in intimate contact with the neighbouring cytoplasm, is about 300 A. The material between the outer and inner membranes has an electron density roughly the same as that of the irregular shaped masses contained inside the nucleus (Fig. 12). In many of the micrographs parts of the inner nuclear membrane (nm_2) appear to be composed of a system of numerous tubular vesicles joined to one another by interconnecting sheets of material about 300 A wide. The vesicles also have a wall thickness of about 300 A, and the lumen is about 1000 A in diameter; quite often the lumen is somewhat flattened in section with its long axis approximately parallel to the nuclear surface, but this orientation may be due to pressure of the knife edge during sectioning.

Cytoplasmic Matrix and Vacuoles.—The cytoplasmic matrix, which lies between numerous vacuoles, is somewhat hyaline, though small particles and vesicles are scattered throughout the region at random. The vacuoles in *Stentor polymorphus* may be divided into three groups:

(*i*) *The endoplasmic vacuoles*, the greater number of which are responsible for the spongy appearance of the *Stentor*. These vacuoles may generally be distinguished as in entity, and they are present in great numbers near the mitochondria and ciliary root systems (*e.g.* Figs. 24 and 35). The vacuoles may be as small as a few hundred A in linear dimensions or as large as 1 or 2 μ. In general, the vacuoles are bounded by a well defined membrane system. Sometimes the membrane is covered either internally or externally with a less dense layer about 500 A thick; this appearance may be caused by the plane of the section being almost tangential to the surface of the membrane (Fig. 26). Neighbouring vacuoles are sometimes deficient in sharp bounding membranes at what otherwise might have been described as areas of contact.

(*ii*) *The contractile vacuole* has so far been identified tentatively from its size, shape, and position. No structural features consistent with the specialized functions of this organelle have yet been observed.

(*iii*) *An organized system of vacuoles* lying in close conjunction with one another just below the buccal cavity (Fig. 15). These vacuoles increase in size when at some distance from the edge of the cavity; this observation suggests that the vacuoles may originate at the surface and gradually migrate inwards. Each vacuole is bounded by a system of triple membranes; some vacuoles have been observed with one triple membrane, others with two. In the latter instance, the triple membranes are concentric with one another and separated by about 125 A. The outer layers of the triple membrane are dense and are about 25 A thick and 75 A apart. Each membrane thus extends over a total width of 125 A (Fig. 14 and 14 *a*). Immediately adjacent to the membranes is a system of fibrils, each about 250 A in diameter and about 100 A apart from each other. These fibrils appear to depend into the body of the *Stentor* (Fig. 16).

If living *Stentor* are dissected in sucrose solution, it has been found that there is a protoplasmic connection between the mouth region and the holdfast, and when the rest of the animal has disintegrated this fibrillar-like connection can still be retained between needles. The observation does not in itself demonstrate a true fibrillar connection.

Mitochondria.—The mitochondria of *Stentor polymorphus* occur in large numbers near the cortical region, but not exclusively so. The mitochondria are about 1 μ long, and their shape is roughly cylindrical with rounded ends (Fig. 13). Each mitochondrion is bounded by three membranes or layers of equal thickness (180 A). The outer two layers show a greater apparent density; as a result it has been more usual to describe such a system as a double membrane. The material between the outer and inner membrane cannot however be ignored. The mitochondria are filled with many tubular vesicles which often terminate on the innermost membrane. Tubules are occasionally formed from invaginations of the innermost membrane. The lumen of the vesicles is often about 300 A in diameter. It is characteristic of both the bounding membranes of the organelle and of the tubules that they are not uniformly dense, but appear dotted with small particles fairly evenly along their lengths. It is not clear whether these particles are confined to one side only of the membranes of the vesicles or whether they actually form the membrane. Such granularity may be a characteristic nucleation due to osmium tetroxide rather than a reflection of the presence of particular sub-

stances at definite sites. The intramitochondrial matrix is of intermediate density and finely particulate. Each mitochondrion has a density on the micrographs well above that of the surrounding matrix. Mitochondria without obvious bounding membranes have also been observed.

In addition to these characteristic mitochondria there are often present, and adjacent to them, numbers of an extremely "empty" type in which the tubular vesicles are much less numerous and largely confined to the peripheral regions. The density within these bodies is extremely low and much less than that of the surrounding cytoplasm.

Cortical and Subcortical Structures:

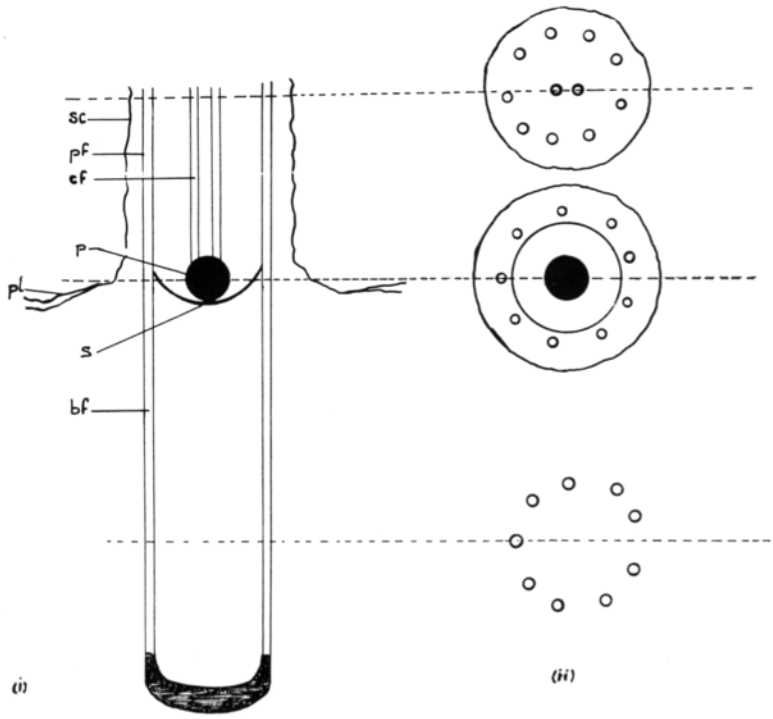
(*a*) *The Pellicle.*—In longitudinal sections of the cytosome (Figs. 20 and 21) the pellicle is well defined and usually appears wrinkled. It is composed of at least two dense membranes, the over-all thickness is about 400 A; each of the dense membranes is about 130 A thick and they are separated from each other by roughly the same distance. The measurements given above have been taken from micrographs of sections cut approximately perpendicular or parallel to the long axis of the animal and may therefore be regarded as minimal. These membranes are extremely electron-opaque and this effect may in part be due to a staining reaction with the osmium tetroxide used in the fixative. In places the pellicle appears to be composed of three dense membranes closely apposed to each other. This appearance may be due to a single cell membrane lined by rows of flattened vesicles (Fig. 20). In the adoral zone the pellicle is sometimes found to consist of four dense membranes (Fig. 17); in this region the two outer membranes are in comparatively close contact, but are separated in places by a distance of several thousand angstroms from the inner pair. Both sets of membranes, however, are joined together at certain points, *e.g.* at the kinetosomes (Fig. 17). Fig. 5 of Johnson's paper (26) of the adoral zone of *S. coeruleus* is very similar, while Sedar and Porter (57) recorded the same type of structure in *Paramecium multimicronucleatum.*

Since the *Stentor* are known to be contracted in the fixed state some of the undulations of the pellicle may have been caused by killing the organism, though undulations of pellicular ridges have been noted in micrographs of the living *Stentor* (page 812 and Fig. 1). Measurements demonstrate that the average ratio of the length of the pellicle between two points, *a*, *b*, to the shortest distance between these points is 1.6:1.

(*b*) *Cilia and Kinetosomes.*—The cilia of *Stentor* conform to the normal structure now so well known. The cylindrical sheath in which the peripheral and two central filaments are confined is continuous with the outermost membrane of the pellicle. Electron micrographs of whole fragments of the pellicle with its attached cilia show the body cilia to be about 20 μ long and 0.2 μ in diameter over the greater part of their length. The body cilia are, however, longitudinally differentiated and have a narrower tip or distal portion which extends for about a third of the total length and is some 0.1 μ in diameter; the internal structure of this region of the cilium is not known. From measurements made on photographs of living *Stentor*, the average length of the cilia that can be resolved is 10 μ; the cilia have not therefore shortened on fixation.

A whole complex of structures form the kinetosome which is about 2000 A in diameter and 0.75 μ long. The peripheral filaments of the cilia fuse to from the outer boundary of the kinetosome which is terminated by a dense disk of material perpendicular to the longitudinal axis. Figs. 17, 18, and Text-fig. 3 show more clearly the structure of the kinetosome and its relationship with the external cilium. The two quasi-axial filaments of the external cilium terminate at approximately pellicular level in a large particle (Fig. 18) below which there is a curved membrane extending the full width of the basal body; the particle, however, is too small to be observed in the light microscope. The kinetosome occasionally contains considerable numbers of dense granules each about 250 A in diameter: in longitudinal sections the granules are aligned in rows parallel to the long axis of the kinetosome (Fig. 18 *a*). These granules are observed predominantly in conjugating and mitotic *Stentor*. Histological sections of *Stentor* have been stained by the Feulgen technique. In longitudinal sections of the membranelle region, the kinetosomes are stained a comparatively deep magenta by this method; owing to the small size of the kinetosomes it has been necessary to employ stringent optical conditions for the detection of the Feulgen stain.

(*c*) *km Fibres.*—Part of the infraciliature of all *Stentor* consists of a complex system of long narrow microfibres running approximately parallel to the length of the animal, one per cortical body



TEXT-FIG. 3. The structure of a kinetosome in *Stentor* (ref. Figs. 18 and 29). (*i*) Longitudinal section of a kinetosome. *pl*, pellicle; *sc*, ciliary sheath; *pf*, peripheral filaments; *cf*, central filaments; *bf*, outer boundary of the kinetosome formed in direct continuity with the peripheral filaments of the cilium (24); *p*, particle on which the central filaments terminate; *s*, septum. (*ii*) Diagrams of the appropriate transverse sections at various levels of the cilium and kinetosome.

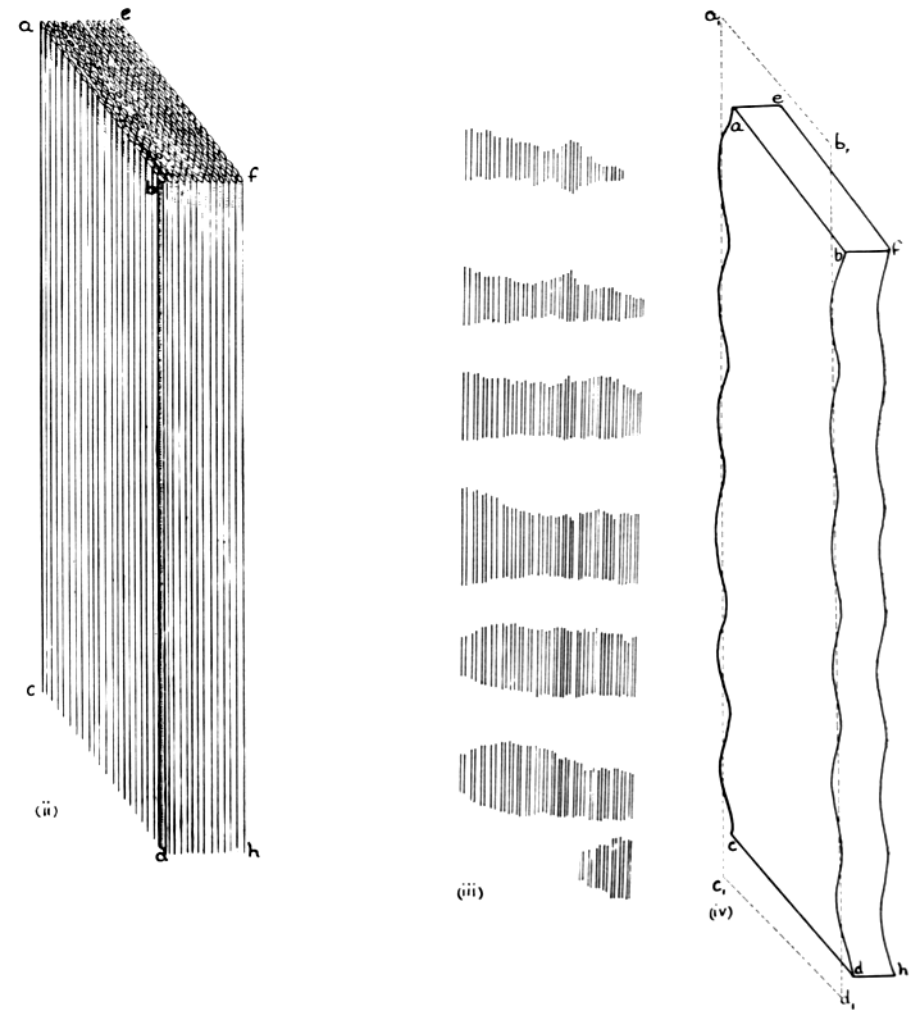
stripe, and by definition lying about 0.3 μ beneath the pellicle. For convenience we refer to this system of microfibres as km fibres.

When the living organism is studied in phase-contrast illumination the surface of a slightly compressed *Stentor* shows numerous parallel linear structures, *i.e.* the km fibres (Fig. 7). The sinuous movements of the km fibres in different states of contraction and extension of the *Stentor* can be clearly seen, and in polarized light they are strongly birefringent, as first recorded by Engelmann in 1875 (11). Some regions of the km fibres are extremely convoluted (Fig. 7), while in areas of the ectoplasm which are clearly extended the structures are quite linear and not more than 0.5 μ wide (Fig. 7). In contracted areas, and in particular in the posterior half of a contracted *Stentor* (Fig. 10) the km fibres again appear linear but are about 2 μ wide, this dimension varying with the degree of contraction. Though it is difficult to establish conclusively by the light microscope alone, the visual evidence supports the view that these structures are homologous with true kinetodesmata, since the kinetosomes, which are at the limit of resolution, appear to be attached to them. This observation can be made more clearly at the foot than in other parts of the body.

The chief characteristics of the km fibres as deduced from electron micrographs are:

(*i*) The km fibres cannot accurately be described as lying beneath either the furrows or the ridges of the pellicle; they are found along the right side when viewed from the anterior, but extend somewhat into the neighbouring ridge and furrow. They are thus displaced from the position in which birefringent structures have been reported previously in the light microscope (Figs. 20 to 24).

(*ii*) Longitudinal sections of both *S. polymorphus* and *S. coeruleus* confirm that the km fibres are to be found from the posterior extremity to the adoral zone.



TEXT-FIG. 4. Diagrams of km fibres as seen in longitudinal section. (*i*) Sketch of an organism to show the position of the km fibres in relation to the diagrams, (*ii*), (*iii*), and (*iv*). The plane of the section parallel to the periphery of the *Stentor* is *a*, *b*, *c*, *d* and that within the cortex is represented by *e*, *f*, *h*. (*ii*) Sheets of fibrils arranged in longitudinal units or stacks to form the km fibres. Groups of fibrils are sometimes arranged as in (*iii*); this appearance is due to the sectioning of an undulating structure in the plane a_1, b_1, c_1, d_1, as indicated in (*iv*).

(*iii*) It has also been clearly established that structures are present beneath the peristomal cap. Rather ill defined rope-like structures have also been seen in this region.

(*iv*) The km fibres are composed of fibrils disposed in layered sheets; each sheet contains from 24 to 30 fibrils of about 200 A in diameter. The fibrils are joined to each other by narrow strips of material about 100 A in width (Fig. 21 and Text-fig. 4); it is this feature which gives to each fibrillar unit its sheet-like character.

(*v*) The sheets are arranged in longitudinal units or stacks. The number of sheets per unit is greatest at the foot of the animal where it is about 24, whereas about halfway along it is about 13 (Fig. 21).

(*vi*) The length of the individual sheets is unknown, but the variable number of sheets suggests that few, if any, manage to extend from one end of the animal to the other.

(*vii*) It is unusual to obtain micrographs of longitudinal sections of km fibrils which show continuity over long distances. It is much more usual to obtain views such as those of Fig. 20, where the apparent discontinuities in the fibrils could be caused by failure to cut in their plane, as illustrated diagrammatically in Text-fig. 4.

(*viii*) The km fibres may be identified as kinetodesmata (8, 34, and 64) since there is indisputable evidence of lateral links between the km fibres and the kinetosomes of the kineties (Figs. 19, 23, and Text-fig. 5). Such connections are particularly evident near the foot of the animal, where, contrary to the earlier observations of Johnson (26), there are many cilia arranged in double rows, as opposed to the single rows of cilia and kinetosomes higher up the body.

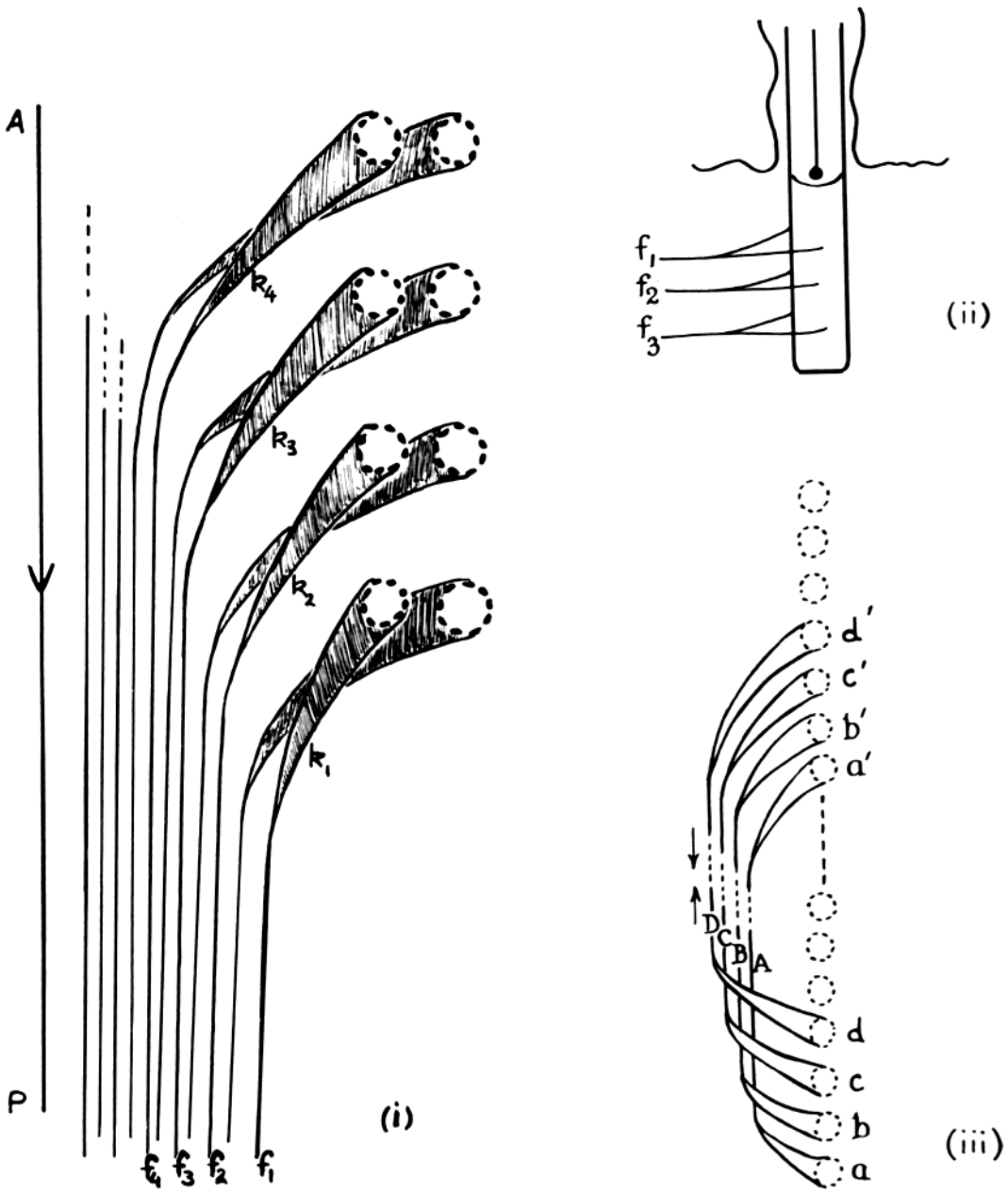
(*ix*) Each fibril of each sheet appears to terminate on a kinetosome. These connections, between the fibrillar components of the km fibres and the kinetosomes, are formed by individual fibrils f_1, f_2, . . . diverging from the sheets of km fibrils at appropriate points (Fig. 23 and Text-fig. 5). After diverging from its sheet each fibril splits into at least two subunits; these components then curve towards the respective kinetosome and the distance getween them gradually increases (Fig. 23). At higher magnifications, it appears that after bifurcation, these subunits are joined together by interconnecting filaments (Fig. 19); subsequently each of the subfibrils joins the kinetosome at opposed points (Fig. 19). From micrographs it is possible that more than one km fibril may be joined to each kinetosome, the individual fibrils diverging from different sheets of the km fibre system. (Text-fig. 5 (*ii*)). Where there are two rows of cilia, as near the posterior end of the organism, kinetodesmal connections are made with both rows.

(*x*) It was stated in (*iv*) above that each sheet of km fibrils contains an average value of 27 fibrils per sheet, and in (*v*) that the number of sheets varies along the length of the organism; 18 sheets however is about the mean figure. Hence this provides 486 fibrillar connections to the kinetosomes. There are an estimated 1000 kinetosomes per kinety; hence there appear to be about twice as many kinetosomes as fibrils.

(*d*) *M bands*. No other type of fibrous structure can be resolved in the upper portion of the organism other than the km fibres by means of the light microscope, but in the lower half of a somewhat contracted *Stentor* a second system has been identified. To distinguish them from the km fibrils, these additional structures are referred to as M bands. The M bands lie beneath and slightly to one side of the km fibre system described above. This second system also seems to show a one-to-one correspondence with the cortical stripes of *Stentor*; they are very weakly birefringent and of only low density in phase-contrast illumination. The structures appear narrowly elliptical in cross-section with the longer axis lying in an approximately radial direction (Fig. 27 and Text-fig. 6). These M bands appear to be laterally joined to each other by considerable numbers of side branches. When further contraction of the *Stentor* occurs they become thicker and seem to be firmly attached at the foot though at the resolution obtained with the light microscope no clear structural junction can be distinguished. In histological sections (Fig. 27) stained by trichrome methods, a typical colour reaction is obtained for a protein-like substance, but no result was obtained from the use of the periodic-acid Schiff technique.

Examination of many thin sections of *S. polymorphus* in the electron microscope has confirmed the existence of these dense elongated subcortical bodies with comparatively ill defined internal structure. Their chief morphological characteristics are as follows:

(*i*) M bands occupy a subcortical position about 2.5 to 8 μ below the surface of the animal and are to be found in positions roughly parallel to the



TEXT-FIG. 5. (*i*) Diagrammatic illustration of the attachment of the fibrils $f_1, f_2, f_3, f_4, \ldots$ of each sheet of the km fibres to the corresponding kinetosome k_1, k_2, k_3, k_4; AP represents the direction of the anterior-posterior axis of the ciliate. By the law of desmodoxy, the kinetodesmal fibrils are always at the right of the kinetosomes when viewed from the anterior end.

(*ii*) A longitudinal section of a kinetosome; evidence indicates that more than one sheet of km fibrils is concerned in the attachment of the individual km fibrils to each kinetosome as shown by f_1, f_2, f_3.

(*iii*) Diagram showing a possible stepped arrangement of km fibrils. A, B, C, and D attached at both ends to different kinetosomes, $a\ a'$, $b\ b'$, $c\ c' \ldots$ respectively; this figure is essentially diagrammatic and represents only one possible method of attachment. Such an arrangement could have rhythmical consequences.

pellicle from the adoral membranelles to the holdfast (Fig. 24).

(*ii*) The width varies at the base depending on the degree of contraction of the fixed animal and is not more than 0.1 μ at the anterior end of the *Stentor* (Fig. 26).

(*iii*) There is one M band per cortical stripe (Fig. 24).

(*iv*) In spite of careful search we have failed to observe any clearly defined structural attachment of these bodies to any part of the cortical system, although the separation from the pellicle and/or km fibres is sometimes not more than a fraction of a micron.

(*v*) There is an intimate connection of the M bands with the surrounding cytoplasm, and with the membranes of the cytoplasmic vacuoles described earlier. Finger-like processes extending from the M bands are interwoven into the cytoplasmic matrix (Fig. 25).

(*vi*) Occasional branching of M bands has been observed. This probably occurs at a point of bifurcation of the body strips.

(*vii*) Adjacent M bands are joined to each other by side branches (Fig. 25 and Text-Fig. 6).

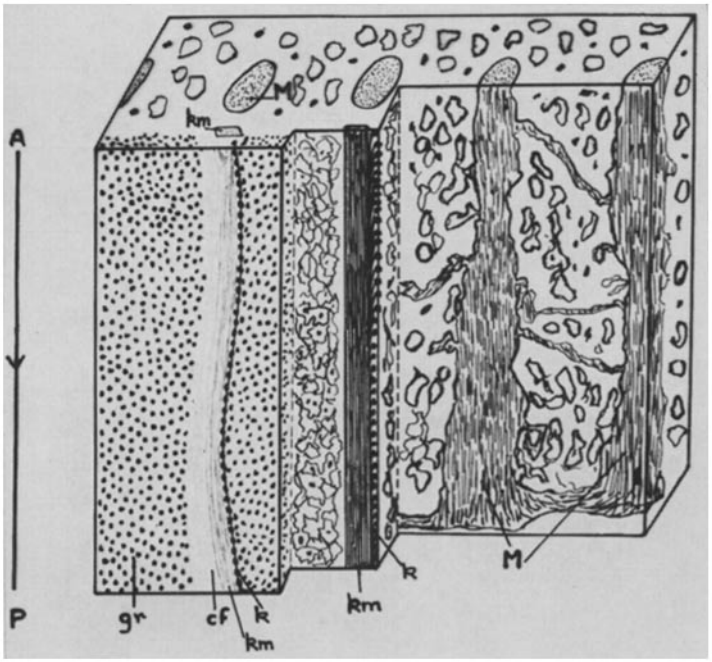
(*viii*) It appears from some micrographs that the distal or posterior portions of the M bands are joined to each other by broad strands of identical material.

(*ix*) The substance of the M bands appears to be largely structureless, although some very fine, short filaments randomly arranged, but with their long axes in general alignment with the long axes of the M bodies, can also be distinguished (Fig. 25).

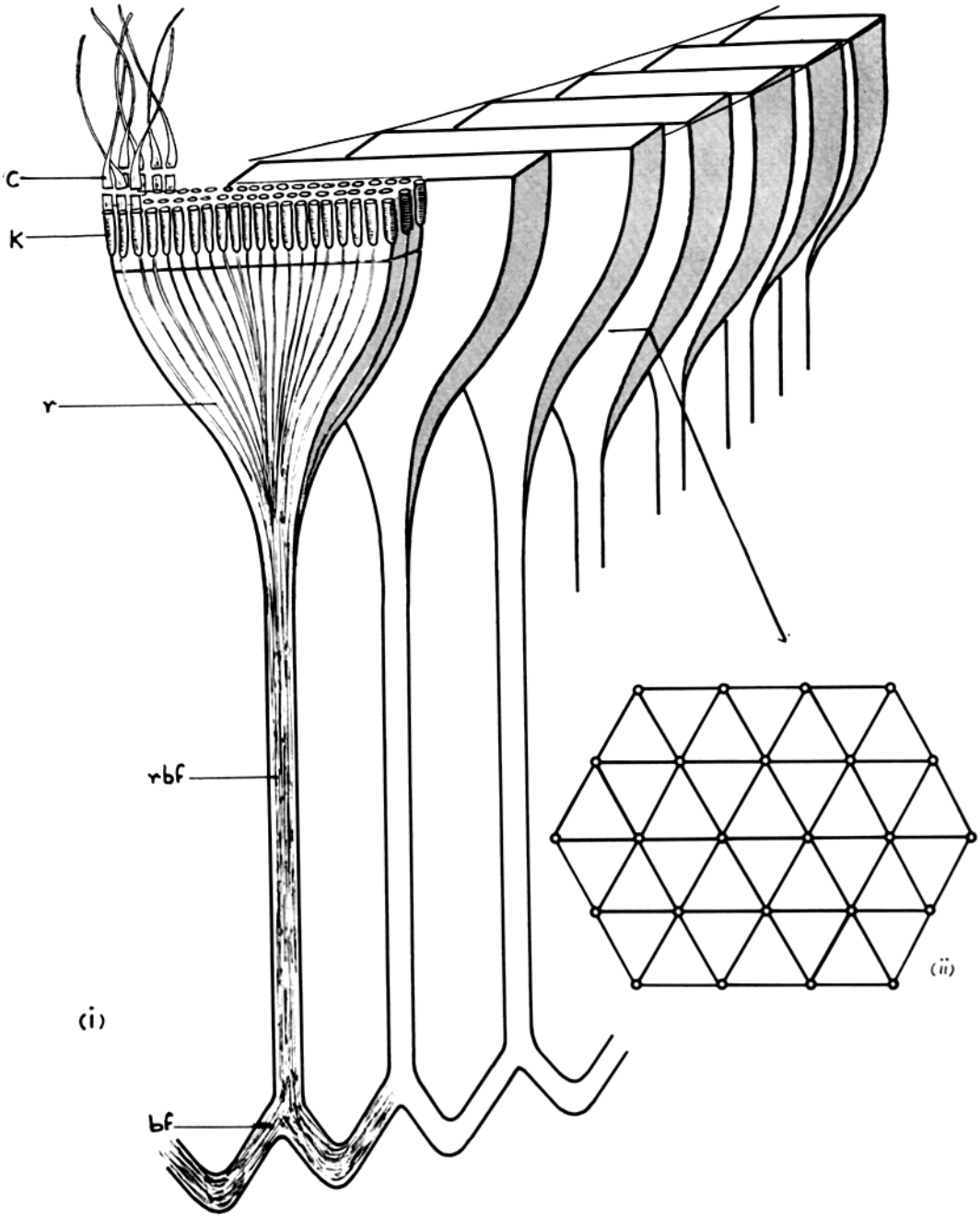
The Adoral Membranelles:

The adoral membranelles almost completely encircle the peristome and with modifications spiral into the cytopharynx almost to the base of the buccal cavity. The membranelles are shown by our electron microscope studies to have an extremely complex organization. The chief structural features are:

(*i*) Each membranelle is made up of three rows of cilia with 20 to 25 cilia in each row (Fig. 29).



TEXT-FIG. 6. Three-dimensional diagrammatic representation of the cortical region of *Stentor polymorphus*, showing the spatial organization of the granular ridges (*gr*) and clear furrows (*cf*) seen at left of block. The km fibres (*km*), kinetosomes (*k*), and M bands (*M*) are also shown. This arrangement of organelles is found at the foot of the *Stentor*, and the broad-base connections between the M bands have been indicated. In the contracted state the km fibrils and M bands would be more closely apposed to each other; this point has been ignored in the diagram. *A-P* represents the direction of the anterior-posterior axis of the ciliate.



TEXT-FIG. 7. (*i*) Schematic diagram of the organization of the adoral membranelles. *c*, cilia; *k*, kinetosome; *r*, root fibrils of individual kinetosomes; *rbf*, bundle formed of root fibrils; *bf*, basal fibre.

(*ii*) Transverse section made in the fan-shaped region. The arrangement of root fibrils and small lateral bridges form an hexagonal net structure (ref. Fig. 34).

The rows are fairly close together so that the cortical portions of the kinetosomes are almost touching (at least in some fixed and embedded preparations). The cilia in each row are spaced about 1000 A apart from edge to edge, and the over-all width of the triple row is about 1.5 μ at the surface of the animal; the over-all dimensions of a single membranelle at this point are 7.5 $\times$ 1.5 μ in the fixed condition. Light microscope observations show that the dimensions of the surface region of a membranelle in a living *Stentor* in a partially contracted state are about 10 x 2.3 μ (Fig. 6).

(*ii*) The external cilia of the membranelles seem to be covered with small irregular hair-like protuberances, the membranes of which are continuous with those of the cilia themselves (Fig. 18).

(*iii*) The terminal distal part of each membranelle does not appear to be bounded by any membrane as implied by the older light microscope observations, yet the cilia evidently maintain some kind of spatial arrangement with respect to one another.

(*iv*) The membranelles in the neighbourhood of the buccal cavity each contain two rows of cilia, not three.

(*v*) The kinetosome of each component cilium of a membrane is about 2000 A in diameter and 0.75 mμ long with similar internal structure to that found in the body kinetosomes (Fig. 18 and Text-fig. 3).

(*vi*) The membranelles have a very complex "root" system extending into the endoplasm for about 20 μ (Fig. 30). Numbers of fibrils, usually about 10, are attached to each kinetosome. Each membranelle thus possesses about 600 to 750 such fibrils. It is the specialized organisation of these fibrils which constitutes the root system. From micrographs it could be inferred that the fibrils attached to each kinetosome are arranged in two rows rather than in a circular or cylindrical array.

(*vii*) Longitudinal sections show that the roots gradually converge into a bundle for the distal two-thirds of their length, the proximal third being in the shape of a fan (Fig. 33). The distal extremities of the bundles divide to link with similar neighbouring roots on either side (Fig. 35 and Text-fig. 7); the roots of the membranelles are thus linked together to form a basal ring.

(*viii*) In transverse sections the root fibrils are joined together transversely by a regular system of small bridges, and there is no bounding membrane. These bridges appear in all transverse or near-transverse sections, but have only occasionally been observed in appropriate longitudinal sections. The bridges are presumably thin filaments of about 60 A thick (Fig. 34).

(*ix*) The pattern made by the root fibrils in transverse section is almost hexagonal; the fibrils are about 220 A in diameter and 1100 A apart.

(*x*) The membranelles are joined together in the cortical region by a thick strand of fibrous material (Fig. 32).

(*xi*) The over-all picture of the adoral membranelle system may be seen schematically in Text-fig. 7.

DISCUSSION

The Morphology of Stentor

The Macronucleus.—The dense regions described as present within the nuclei are clearly homologous with similar areas which give positive reaction with the Feulgen stain for desoxyribonucleic acid. It is disappointing, however, that the macronucleus has not so far revealed any of the fine fibrillar structures that have been observed in the nuclei of *Amoeba proteus* (*e.g.* 42) or in developing spermatids of a number of invertebrates (*e.g.* 40), but features of this kind may possibly show up in later studies of the animal in fission. Though micronuclei have been distinguished in stained sections, they have not been identified in electron micrographs.

The nuclear membrane system would seem to ensure a close relationship between nucleus and cytoplasm, but it is apparently much less precisely organized than some others that have been examined. Pappas (43), for example, has described the structure of the nuclear envelope of *Amoeba proteus* and shown that it consists of two membranes containing pores which are formed where continuity between the inner and outer membrane occurs. The main features in *Stentor* are somewhat similar but the degree of perfection and structural organization are less than in *Amoeba*.

Cytoplasmic Matrix and Vacuoles.—The body of *Stentor* contains numerous vacuoles of various sizes which are enclosed by a cytoplasmic matrix composed of many small particles and vesicles. The enveloping membranes of many vacuoles have been found to consist of two dense components separated by a narrow less dense region. On extension of the *Stentor*, there is a considerable intake of water and an increase in size of the

vacuoles; this indicates that the membranes of the vacuoles must be capable of stretching to allow for the increase in size, but it is not known if this is equivalent to an elastic stretch.

The very numerous vacuoles observed in the region of the buccal cavity may well be formed by invagination of the surface of the cavity; such a system would be equivalent to the pinocytotic activity of metazoan cells demonstrated in tissue culture (31). Many fibrils have been found near the surface of these vacuoles (*e.g.* Fig. 16), but their extent and the location of the extremities has not been determined. They may represent the "myonembundel" of Dierks (9) who proposed the existence of an axially oriented bundle of unattached fibrils situated in the center of the body of *Stentor* and which were connected in the holdfast region to the "myonemes" observed under the body stripes. The element we isolated by micro-dissection may be homologous with Dierks' structure.

Mitochondria.—The tubules found in the mitochondria of *Stentor* are similar to those reported as present in other protozoa by, *e.g.*, Sedar and Porter (57) and Sedar and Rudzinska (58); the internal finger-like projections are characteristic for most protozoa so far examined. Lever (30) has observed similar structures in the mitochondria of the *zona reticularis* of the adrenal cortex of the rat, but the typical internal mitochondrial structure of metazoan cells is essentially different from that described in protozoa. The external triple membrane of mitochondria in *Stentor* is well defined and appears to be a feature common to most mitochondria. The "empty" type of mitochondria found in *Stentor* may well reflect a particular metabolic condition; since they lie in close association with the above described mitochondria, the internal lack of structure seems unlikely to be due to fixation artefact. On the other hand, whether adjacent mitochondria are necessarily in the same metabolic state is open to question; thus it may well be that the described features represent either a degenerative state or alternatively newly formed mitochondria.

Pellicule.—The body pellicle is composed of at least two dense membranes and in section shows considerable convolutions; convolutions are also visible in the contracted living *Stentor* (*e.g.* Fig. 4). In the fixed *Stentor* measurements on sections show that the ratio of the pellicle to the underlying surface is 1.6:1. This, however, is not a true shortening since it is due entirely to the convolutions. It seems unlikely that the pellicle is responsible for the mechanism of contraction.

The Adoral Membranelles.—The structure of the adoral membranelles of *S. polymorphus* has been summarized in Text-fig. 7. If we compare this figure with that of Schröder's figure (55) and with that of von Gelei (15), we see that there are several gross similarities and differences which could only have been revealed by the higher resolution studies with the electron microscope. We have demonstrated that the exterior parts of the membranelles consist of groups of cilia arranged mostly in triple rows, though the extension of the adoral membranelles into the buccal cavity reveals double rows; the cilia within the rows are not confined at their base by membranes as frequently suggested in the past. There is one small difference, however, between the cilia of the membranelles and those of the kineties apart from the greater length of the former; the membranelle cilia are covered with small hair-like protuberances, the membranes of which are continuous with the ciliary membranes. Porter (46) has noticed similar structures in *Paramecium*, and Roth (51) has observed interciliary extensions to the ciliary membranes in the groups of cilia which form the cirri in *Euplotes patella*.

It has often been suggested that kinetosomes are to be regarded as self-reproducing cytoplasmic particles (7, 24) and it is of great interest to determine their biochemical constitution. The intimate structural relationship which exists between the kinetosomes, the external cilia, and the ciliary roots in many organisms and tissues makes it clear that the kinetosomes are, also, in all probability intimately concerned in the synthesis of fibrous proteins. In *Stentor* we have shown in histological sections that the kinetosomes of the membranelles stain magenta after using the Feulgen technique. On the basis of this we are of the opinion that the kinetosomes contain DNA. Experiments are still in progress and we hope to confirm this finding by the use of other methods, and we are also investigating the nature of other components of the kinetosomes by several methods.

The portion of the membranelles lying below the kinetosomes bears a superficial resemblance to the basal plate of the old light microscopists; a notable feature of this root-like arrangement is the existence of one large root shaped like a fan for each membranelle. The roots are a highly organised and complex system of fibrils, which form an hexagonal net when seen in transverse section; the roots bear no bounding membranes

and the small lateral bridges presumably serve as confining structures. Each root is connected to its neighbour by a "basal fibre," thus forming a ring-like termination as predicted by Schüberg (56) from early light microscope observations; our electron microscope studies show that the "fibre" has the same detailed structure as the axial roots and that it is sinuous in form. Each membranelle is connected with the next one by a thick strand of fibrils in the cortical region. Thus the membranelles are formed into a cohesive unit at both the anterior and posterior extremities. The membranelles, however, do not form a closed ring around the peristomal cap since one end spirals into the cytopharynx (*e.g.*, Figs. 5 and 6); it is, therefore, reasonable to assume that the corresponding basal fibre of the root system follows a similar outline.

While this paper was in preparation, one by Rouiller and Fauré-Fremiet (63) on the skeletal fibre of the peritrich *Campanella umbellaria* has appeared in which is illustrated a network closely similar in appearance and dimensions to the micrographs of transverse sections of the roots of the adoral membranelles of *Stentor*. They also show that the network is linked to the kinetosomes. We note that, whereas Rouiller and Fauré-Fremiet interpret the objects forming a three-dimensional net as particles linked together by fine filaments, the apparent corresponding objects in our micrographs have been shown by the study of longitudinal as well as transverse sections to be fibrils linked together by side filaments. We strongly suspect that the network observed by our French colleagues is also of a similar character. There would seem to be no sound evidence at this stage to suggest that the described network in *Campanella* bears more than a superficial resemblance to the Descemet membrane of the vertebrate eye (25), and hence that it may be composed of collagenous protein.

It seems likely that the complex root system we have described is associated through its biochemical and physical properties with the transmission of substances which may control the motion and coordination of the membranelles. The three-dimensional networks of the roots which are completely immersed in the endoplasm of *Stentor* are suitable placed for intimate biochemical interchange. This leaves entirely unsolved, however, the question of the mechanism of energy transmission through the network. Some form of biochemical stimulus could perhaps, if propagated along the network with uniform velocity from one end of the basal fibre, lead to a suitably phased metachronal activity; the timing of transmission of these substances along the membranelle system would in itself constitute control of coordination. What form such a stimulus should take and at what point or at which end of the system it might start is completely unknown at present, but Sleigh (59) has found that the mechanical process involved in ciliary activity functions independently of the coordination process. This finding is of interest since the morphological arrangement of the membranelles is such that they are linked to each other both at the kinetosome region of the root system as well as at the basal fibre, and these facts may have a bearing on the functional significance. In this respect, it is also of interest that when *Stentor* retracts, the cortical portions of the membranelles move closer together, but the distal basal fibre joining the roots appear sinuous in the fixed tissue. These facts suggest that contractions of the adoral zone may be due to a contractile mechanism at the anterior of the membranelle system, but it is not known whether the basal fibre is more sinuous in the contracted state or whether it may have a contractile function.

The km Fibres and M Bands.—Examination of the body wall of *Stentor* by the various techniques used in this work has demonstrated two major features: a fibrous system lying just beneath the pellicle and termed km fibres; and other bodies, lying a little deeper, one per cortical stripe and termed M bands (Text-fig. 6). The analysis demonstrates that the whole of the body of *Stentor* from the posterior holdfast to just below the adoral zone is covered by rows of the km fibres asymmetrically placed with respect to the center of each ridge, and electron microscopy has shown conclusively that the km fibrils are connected to the kinetosomes; thus they can be identified with the kinetodesmal fibrils proposed by Chatton *et al.* (8). Villeneuve-Brachon (64) has concluded that each kinetosome of *Stentor* conforms to the rule of desmodexy formulated by Chatton and Lwoff (7); by this rule the kinetodesmata are said to lie to the "right" of the kinetosomes; such a feature is consistent with the curved termination of the kinetodesmal fibrils (*cf.* Figs. 19, 23).

Fauré-Fremiet *et al.* (13), however, have identified only one system by means of light and electron

microscopy (see their Figs. 1 to 3 and 15); they have named this "myoneme." This structure lies just beneath the pellicle; while a denser portion of the same organelle, which lies adjacent to the cilia, has been identified by them as the kinetodesmata.[1] We agree with the French workers that the km fibres (*i.e.* their myonemes) are long and narrow, but in our detailed interpretation we differ from Fauré-Fremiet *et al.* We hold that the km fibres are stacks of fibrillar sheets (Text-fig. 4) somewhat similar to those observed in our earlier analysis of *Spirostomum Ambiguum* (50), and that they are not a series of long narrow ribbons arranged in piles. It is clear that the individual components of the km fibres (or kinetodesmata) do not extend the full length of the body because of their attachment to the kinetosomes. It is highly probable that when all the fibrils of one sheet have been attached to the appropriate kinetosomes another sheet in the pile is utilized. It is possible that each kinetodesmal fibril may be attached at both ends to different kinetosomes, as shown for example in Text-fig. 5, but we have not obtained structural evidence for this arrangement though the probable numbers of fibrils and kinetosomes (page 817) supports this argument. It is clear, however, that the structure of the kinetodesmal fibres described in this paper is such that the arrangement of the fibrillar sheets could fulfill a role in the coordination of ciliary activity. If the kinetodesmal fibrils were connected to the kinetosomes in the stepped arrangement shown in the diagram, such an arrangement could have rhythmical consequences.

The M bands are a constant feature of the cortical region of *Stentor*; they appear as thick structures at the posterior end of the organism in the contracted and fixed state; by means of the electron microscope they have been traced throughout the length of the organism; the diameter of the M bands diminishes towards the anterior, and the dimensions in this region are such that they would not be resolved by light microscopy. The organelles are embedded within a reticular cytoplasmic matrix; the surrounding sheath of the M bands often appears to be continuous with the bounding membranes of adjacent vacuoles. There can be little doubt that the contacts between the M bands and the surrounding vacuolar system are considerable. Further, each M band is itself connected to the adjacent M band by quite well formed branches. At the posterior end of the *Stentor* the M bands are fused together in broad strands. No well defined morphological connection has been found, however, between the M bands and the km fibres, nor with the pellicle, though there is a one-to-one correspondence between the number of M bands, km fibres, and body stripes of *Stentor*.

In the light of our own observations on living and sectioned *Stentor*, a rigorous analysis of the descriptions given of fibrous systems in *Stentor* in the earlier original papers (*e.g.* 5, 9, 11, 15, 19, 26, 35, 41, 54–56) and textbooks (*e.g.* 6, 10, 20, and 29) on the *Protozoa* has led us to the following conclusion: in living *Stentor* the structures usually referred to by these authors as "myonemes" and assigned a contractile function, are in reality those elements now designated km fibres, *i.e.* the kinetodesmata. On subsequent fixation and histological examination most of these same authors, however, identified the "myonemes" as the underlying structures named M bands in this paper (*e.g.* 55, 56). Hence in some of the papers the properties of two separate elements have been thought to represent one fibrous system (*e.g.*, 55); on the other hand, in reports in which two fibrous structures were distinguished, the identity of each structure has usually been reversed when comparisons of the properties were made between living and fixed tissue (41).

For clarity, the summation of our analyses of earlier work has been set out in Table I. From this it is possible to understand how the observations, though individually extraordinarily precise, have been somewhat misleading. In histological preparations the M bands are well displayed, especially in transverse sections (Text-fig. 1, Fig. 27); on the other hand, the km fibres (or kinetodesmata) can only just be resolved by the light microscope, though in tangential sections of the surface they may be more clearly seen, since the widest dimension of the km fibres lies parallel to the surface. In the living *Stentor*, however, it is the km fibres which are so clearly seen, and the M bands which can only just be distinguished in the contracted animal. As will be observed from the table, the M bands have seldom been identified in living material; in fact in the literature it is often not clear whether the authors are recording observations made on living or fixed material. There is no doubt that the evidence before us upholds our conclusion that in the literature the descriptions

[1] Please see footnote with regard to a further publication at the end of this paper.

TABLE I

Summary of Early Work on Identification of Fibrous Systems in Living and Fixed Stentor

Author	Clear stripe	km Fibres		M bands	
		Living	Fixed	Living	Fixed
Lieberkühn (32)		Muskelfasern			
Engelmann (11)		Myonemes			
Bütschli (5)	Zwischen-streifen	Myonemes			Myonemes
Schüberg (56)		Myonemes			Myonemes
Johnson (26)		Myonemes			Myonemes
Neresheimer (41)		Myophanes	Neurophanes	?Neurophanes	Myophanes
Schröder (55)	Zwischen-streifen Neurophanes	Myonemes			Myonemes
Dierks (9)		Myonemes	Neuroid	?Neuroid	Myonemes
von Gelei (15)		Myonemes	Neuroid	?Neuroid	Myonemes
Villeneuve-Brachon (64)		Kinetodesmata	Kinetodesmata	Myonemes	Myonemes (non-contractile)

of "myonemes" in living *Stentor* refer to km fibres (*i.e.* kinetodesmata), whereas in fixed tissue they refer to M bands.

Theoretical Considerations of Structural Mechanisms in Contraction and Extension

At the present time our knowledge of the morphology of the *Protozoa* is increasing rapidly, but we are still lamentably ignorant of the functions of the structures we observe. Our earlier investigations on *Stentor* (48, 49) and the work of Fauré-Fremiet, Rouiller, and Gauchery (13) have shown the existence of complex cortical elements which we have here called km fibres. In the absence of any other suitable longitudinal elements, it would at least have been reasonable to ascribe a contractile function to km fibres. In these earlier reports, this inference was made, but it is now clear that further critical examination of the data is required.

First, we must examine briefly some of the more probable structural desiderata of contractile systems. These considerations are by no means exhaustive, but are regarded as a necessary preliminary to the proper assessment of the data. To judge from the intensive research which has lately been carried out on the muscles of higher animals, no simple answers may be expected to the problems of contractility as a whole. In our considerations of contractility in protozoa we may define a contractile mechanism as one capable of inducing a contracted state in the organism; that it is necessary to make such an apparently obvious broad definition rather than one precisely limited to fibrous structures will be seen later. Two possible types of contractile system readily come to mind: (1) contractions, presumably syneretic, of protoplasmic gels, and (2) fibrillar systems such as those already known to exist in various forms in higher animals.

The Contraction of Gels.—The potential contractility of gels may be of importance in all contractile protozoa, and there is certainly considerable evidence in the *Amoebidae* that the motile characteristics of this family and related forms (39, 47) are dependent solely on the syneretic properties of gels in which the initial gelation involves a positive increase of volume and an absorption of heat. Studies of thin sections of *Amoebae* in the electron microscope have not so far revealed the presence of any cortical or subcortical fibrils. It has been suggested by Marsland (38) in his review of this subject that the proposals of Goldacre and Lorch (16) and Goldacre (17) relating to the folding and unfolding of protein chains, particularly in *Amoebae*, are consistent with the hypothesis of gel contraction and also with the results of the large amount of research

which has been carried out on the effects of hydrostatic pressure on amoeboid shape and movement. On this basis, gelation is a consequence of the formation of a three-dimensional network of protein fibrils, and the contraction of the gel is determined by the rapid folding of these fibrils and the resultant syneresis and decrease of volume. Marsland considers that the volume of some gels after contraction may be no more than 10 per cent of the original value. If the shrinkage is uniform, this corresponds roughly to a halving of linear dimensions. If effective in the living state, such contractions might thus account qualitatively for the movements of *Amoebidae*.

The well known significance of adenosinetriphosphate (ATP) as an energy source in vertebrate muscular tissues indicated that this substance might also be the source of energy for a sol-gel cycle in cells. Indeed Kriszat (27, 28) has shown that the movements of *Amoeba proteus* are modified in the presence of ATP; and Loewy (33) has extracted an actomyosin type of protein from another amoeba, *Pelomyxa*, and shown that the properties of the gels are dependent on ATP. The work of Weber and his collaborators on glycerol-extracted fibroblasts (66) is also well known.

The difficulties of obtaining more direct physiological proof of the gel-contraction hypothesis as the main mechanism of contractility in protozoa as a whole are formidable; but as we have seen, there is now substantial evidence in favour of the hypothesis in *Amoebidae*. Such a mechanism cannot yet be excluded from any attempt to account for the motion of other groups such as *Gregarinidae* in the sporadin phase. Since the work of Watson (65), it has generally been assumed that this motion arises from the presence of observable "myoneme" structures. Our own observations (to be published) lead us to rather different conclusions which do not exclude the possibility of a contractile gel mechanism. So far as we are aware, no experiments at high hydrostatic pressures have yet been carried out on the ciliates, and comparative studies of contractile and non-contractile forms could be useful.

Fibrillar Systems.—The recent studies of Hanson and Huxley (*e.g.* 21, 23) bring into sharp relief two important structural aspects of contractility. The older conception of molecular contraction was based on the work of Astbury who showed that protein chains could exist in extended and contracted forms. As yet, however, there is no certain evidence that any phenomenon of animal contractility can be explained satisfactorily on the basis of the molecular contraction of protein chains, in spite of the fact that a reversible extension of 30 to 50 per cent can be achieved in fibrils containing keratin. Existing evidence obtained from the high angle x-ray diffraction of skeletal muscle which should be strictly relevant is rather confused and seems to bear little relation to tissues in known physiological states. Both the configuration of the Pauling-Corey α-helix and that of the extended chain (β-configuration) are known to be stable under suitable conditions of hydrogen-bonding or of tension (44). The Hanson-Huxley hypothesis (*e.g.* 21, 23) of muscular contraction depends chiefly, not on molecular contraction, but on the relative motion of two interdigitating morphologically and chemically distinct sets of fibrils, actin and myosin. The implications of these results on the fine structure of striped muscle for theories of contraction have been fully considered by A. F. Huxley (22). With regard to molecular contraction the stability of a protein chain in the configuration of, say, the α- or β-helix will be dependent on the existence of appropriate hydrogen bonds between specific points of the helix. In any conceivable mechanism of molecular contractility the reversible breaking of hydrogen bonds is thus a necessary preliminary to extension. Both in the gels already considered, and in such fibril systems the processes of contraction will be sharply dependent for their action on the physical and chemical conditions of the protozoan cortex, and on the presence of suitable enzymes and substrates for the provision of the necessary energy.

It has already been shown by Boedtker and Doty (2, 3) that changes of pH alone are sufficient to change the configuration of chains of synthetic polypeptides in solution from that of a helix to that of a random coil. This change occurs as a result of the breakdown of hydrogen bonds as the pH is reduced, and is reversible. It is interesting to note that any protein fibre system in which the chains are normally in helical configuration could be extended in length by the provision of appropriate conditions for the breaking of hydrogen bonds. Change of pH is one possible agency for bringing this about. In addition, pH change will also affect the value of the net electric charge on protein chains carrying acidic and basic side groups. This charge will, in turn, probably affect the linkage between one structural unit (perhaps a twin or triple helix) and another.

Since true molecular contraction may yet be found to play a part in contractile mechanisms, perhaps especially in lower animals, we have tried to set out some of the more obvious implications. In a simple system involving only one molecular species of fibril, the relevant high-angle x-ray diffraction diagrams should differ in the relaxed and contracted states and may thus provide a distinguishing feature.

Since apparently all animals outside the phylum *Protozoa* make use of fibrils for contraction, it has been natural to seek for similar origins of contractility in the *Protozoa* themselves. It is important to realize that the existence of subcortical fibrils is not in itself proof of potential contractility; nor does it necessarily distinguish between the hypothesis of sliding fibrils and molecular contraction—both of which could conceivably be involved.

Contraction and Extension in Stentor

The problems of contraction and extension in *Stentor* will now be examined in the light of the experimental results and theoretical considerations of the preceding pages.

First, the km fibres, *i.e.* the kinetodesmata, may form the contractile elements. Our present evidence on function is restricted to our observations on the living animal made by means of light microscopy, but the observed behaviour of the km fibres is consistent with contractility and extension of these fibres. Villeneuve-Brachon (64) recorded that on contraction the kinetosomes of living *Stentor* came closer together and that the kinetodesmata remained straight and thickened considerably; on the basis of these observations she has attributed to the kinetodesmata contractile properties. Further, our analysis of the literature on *Stentor* shows that fibrillar elements which were held to have a contractile function in the living organism are in fact the km fibres. Taken together, these observations support the contention that the km fibres or kinetodesmata form at least part of the contractile system.

Electron microscopy has demonstrated that the km fibres are composed of well orientated parallel fibrils as indicated by their birefringent properties. From our theoretical considerations we must infer that the mechanism of contraction involves either a true molecular folding without visible microscopical folding; or, a relative sliding motion of parallel fibrils with respect to each other. Such a movement of fibrils, however, would be restricted, to a degree, if the individual km fibrils terminate at both ends on kinetosomes as has been suggested, or if they were attached at one end to a kinetosome as we have shown is the case, and at the other end to the pellicle. The possibility that *Stentor* makes major use of elastic fibrils with properties similar to those of elastin in higher animals seems unlikely; if this were so we would expect the contracted state of a ciliate to bring about not only a change in shape and size of the km fibrils, but also marked signs of crumpling of the fibrils. In fact, the reverse occurs; crumpling of the km fibrils is apparent only when they are not under any form of tension, either that of contraction or extension. Our present evidence is thus insufficient to indicate what type of mechanism is involved, since we also have to account for the known extensile property of the km fibres.

Secondly, the M bands may also have a contractile function. Our own evidence clearly indicates that these structures are thicker at the base of the living organism, especially when extreme contraction has taken place; the M bands extend throughout the length of the *Stentor* and form an interconnected system, both axially and at right angles and these organelles are also in intimate contact with the surrounding vacuolar cytoplasm. It is thus possible that the M bands may form a contractile system in that region of the body where the greatest contraction and extension occurs; but support for such a mechanism would be stronger if we had firm evidence that the M bands were attached either to the pellicle or to the km fibres. It must be noted that the M bands are not fibrillar in the sense of containing long parallel arrays of fibrils; rather their internal structure is somewhat akin to that of smooth muscle fibres, and this analogy may be relevant. If this system of M bands were responsible for some of the changes in form of the animal the discussion on gel systems above would appear to be pertinent, since any theory of their function may have to depend on transmission of force through a gel-like cytoplasm.

It is obvious that existing data do not permit us to decide conclusively between the relative contractile functions of km fibrils and M bands; it seems probable that both are involved. The fact that for every body stripe there is one km fibre unit and one M band is very suggestive in spite of the lack of demonstrable morphological connection between them. In *Spirostomum* no M bands have been found, only fibrillar sheets extremely similar

to those described here for *Stentor*; it appears that such contraction as *Spirostonum* exhibits must depend on its km fibre system alone. At the present time our conclusions cannot be more than tentative in this particular respect. We propose that the km fibres in *Stentor* play an important role in minor contractions and extensions, especially in the anterior portions of the organism and that the M bands form a complementary system which reinforces the contractile and extensile properties of the km fibres in the middle and posterior region of *Stentor* where the greatest alterations in form occur.[2]

This work has been supported by a grant from the Rockefeller Foundation. One of us (J. T. R.) is indebted to the President and Staff of the Rockefeller Institute for hospitality and laboratory facilities during the Winter 1956–57, when part of this work was carried out in both the Laboratories of Dr. Keith R. Porter and Dr. George E. Palade, and that of Dr. Paul A. Weiss.

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[2] Since this paper was submitted for publication to the Editors of this *Journal*, Professor Fauré-Fremiet has kindly shown us the manuscript of a paper by himself and Dr. Charles Rouiller entitled "Myonèmes and cinétodesmes chez les cilies du *Stentor*." The authors describe two types of fibres and come to a somewhat similar conclusion concerning the contractile function of km fibres (*"myonèmes ectoplasmiques"*) and M bands (*"myonèmes endoplasmiques"*). In discussion they query whether all or only part of the "myonèmes ectoplasmiques" are homologous to kinetodesmata and stress the need for further investigations before the precise functions of these organelles can be decided. We are very much indebted to the authors for allowing us to see their manuscript.

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Explanation of Plates

Plate 408

Light microscope pictures of living *Stentor polymorphus* taken in transmitted light by means of a flash exposure of duration 1/500 seconds. The photomicrographs show the general appearance and the variety of shapes and sizes of the organism in different states of contraction and extension. The magnification of all five figures is the same, thus demonstrating clearly the alterations in size. × 160.

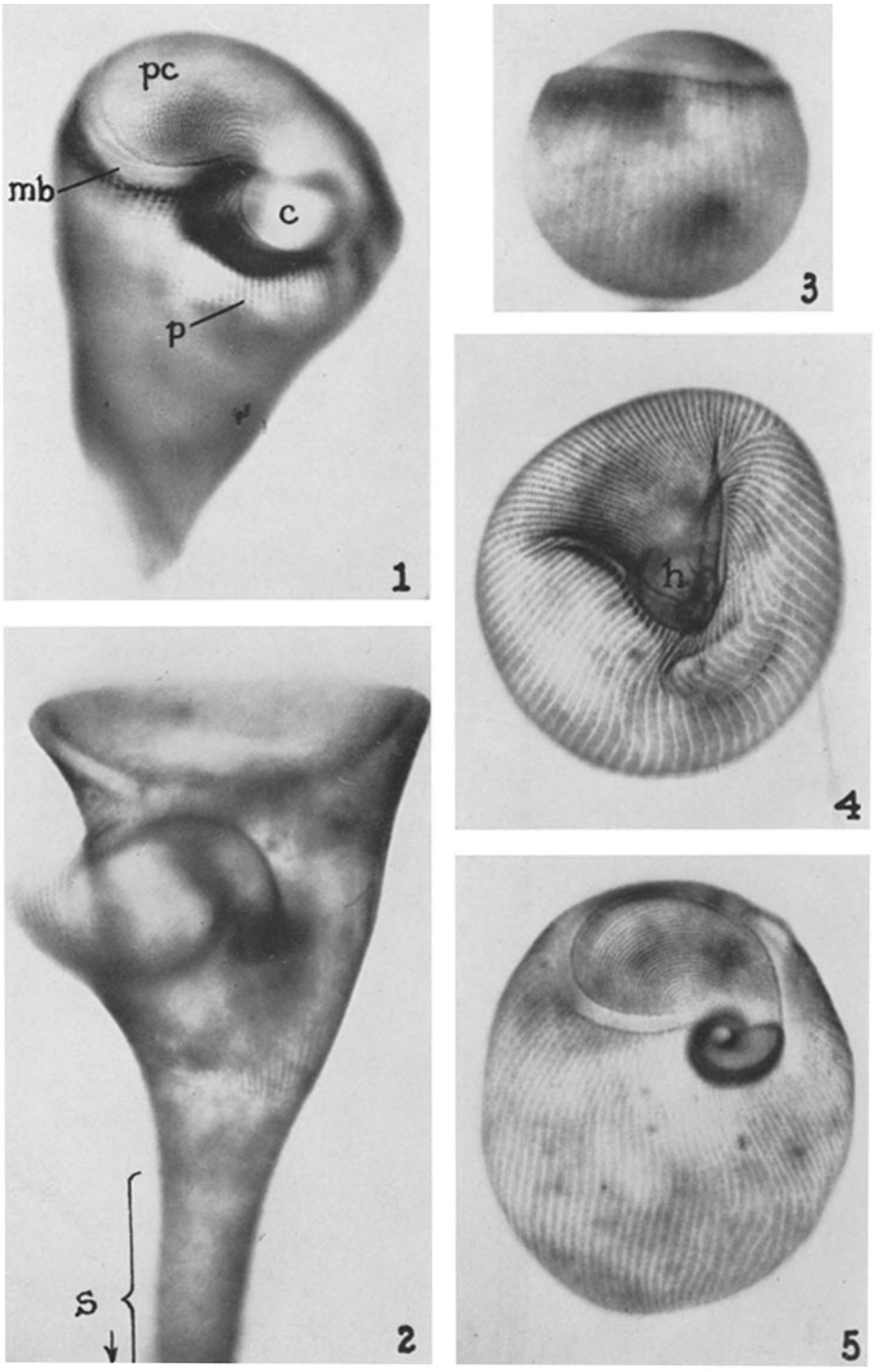
Fig. 1. *Stentor;* at normal or swimming length the conical shape is apparent and corrugations of the pellicle (*p*) can be distinguished. *pc*, peristomal cap; *mb*, adoral membranelles; *c*, cytopharynx.

Fig. 2. An extended *Stentor*; only a small portion of the stalk region (*s*) is shown. In this photograph the granular stripes are denser than the clear ones.

Fig. 3. A supercontracted *Stentor*; the size of the compressed adoral membranelle region should be compared with the enlarged region shown in the extended organism (Fig. 2).

Fig. 4. A posterior view of *Stentor* showing the apex of the holdfast (*h*); the variations in the width of the ranular stripes and the corrugation of the pellicle are clearly demonstrated.

Fig. 5. A partially contracted *Stentor*; the stalk has retracted so that the posterior region has become rounded nd the membranelles are also compressed.



(Randall and Fitton Jackson: Structure and function in *Stentor*)

PLATE 409

Light microscope pictures of living *Stentor* taken under phase-contrast illumination.

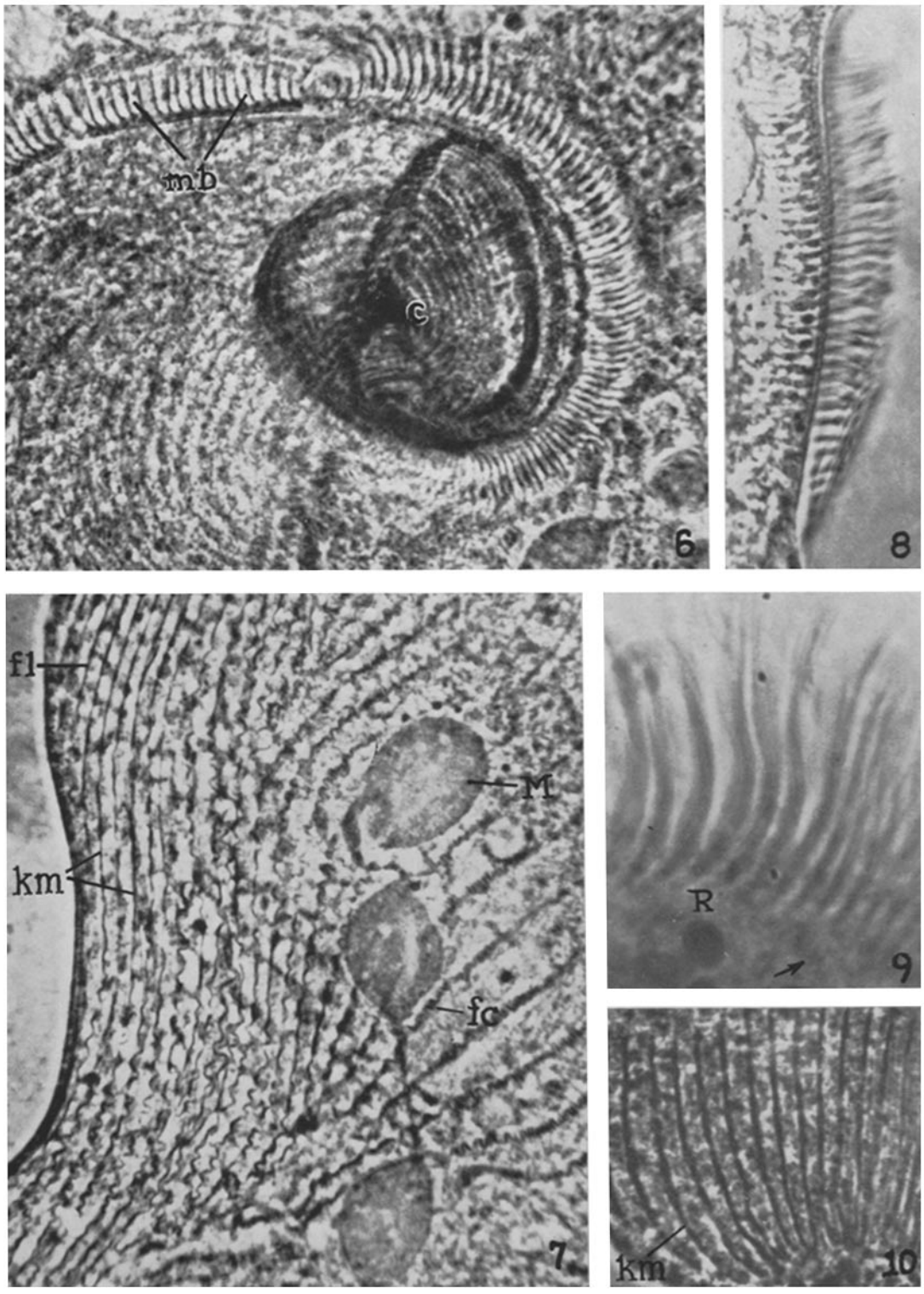
FIG. 6. Part of the adoral membranelle system of *Stentor* showing the clockwise spiral as viewed from above which forms the cytopharynx (*c*). Individual membranelles (*mb*) are clearly resolved; pictures of the whole system taken at different focal levels show that there are about 250 membranelles encircling the peristomal cap, but the number lining the cytopharynx has not been determined. × 840.

FIG. 7. Part of the body of *Stentor* showing the km fibres (*km*) which lie approximately parallel to each other. Some km fibres are extremely convoluted (*fc*), but in a region where the organism is extended the km fibres are linear (*fl*). Three lobes of the macronucleus (*M*) are also seen. × 840.

FIG. 8. Part of the adoral membranelles which show the cilia; the metachromal wave is evident. × 840.

FIG. 9. At higher magnification the root system of the membranelles (*R*) can be more clearly resolved, and a sinuous fibril (arrow) can just be distinguished at the base of the root. × 3,000.

FIG. 10. The km fibres (*km*) at the posterior of a contracted *Stentor*. Note the increase in width as compared with those seen in Fig. 7. × 840.



(Randall and Fitton Jackson: Structure and function in *Stentor*)

PLATE 410

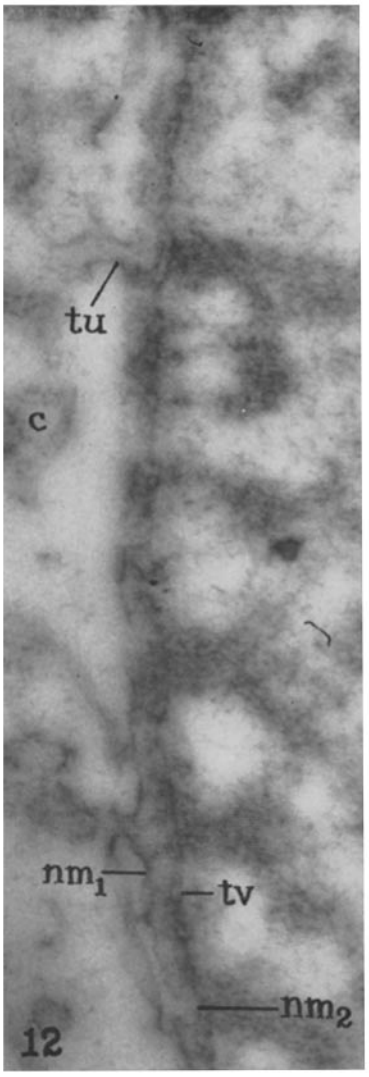
FIG. 11. Electron micrograph of a section through part of the macronucleus showing (i) a content of irregular shaped dense masses in which small particles and beaded filaments (*f*) can be distinguished; these masses are Feulgen-positive; (ii) a less dense matrix containing randomly arranged filaments often merging into the denser masses, and (iii) the nuclear membrane (*nm*) and adjacent cytoplasm. × 30,000.

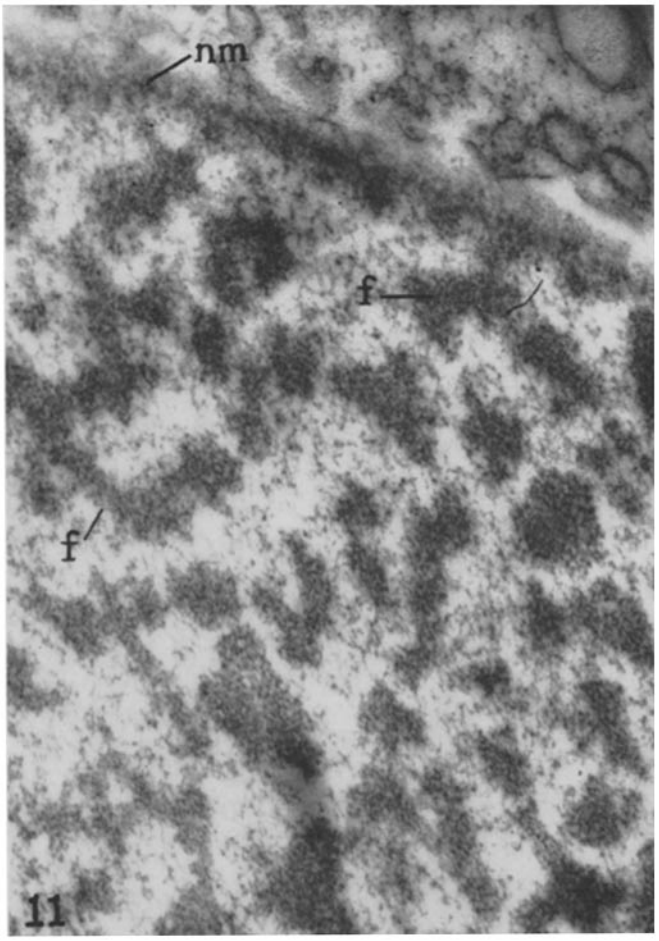
FIG. 12. A transverse section of the nuclear membrane of the macronucleus showing the outer membrane (nm_1); irregularly disposed openings (*lu*), the bounding membrane of which merges into the cytoplasm (*c*); small vesicles (*lv*) compose part of the inner membrane (nm_2). Dense masses extend from the membrane into the nucleus and fine filamentous structures are evident. A diagram of the nuclear membrane is shown in Text-fig. 2. × 30,000.

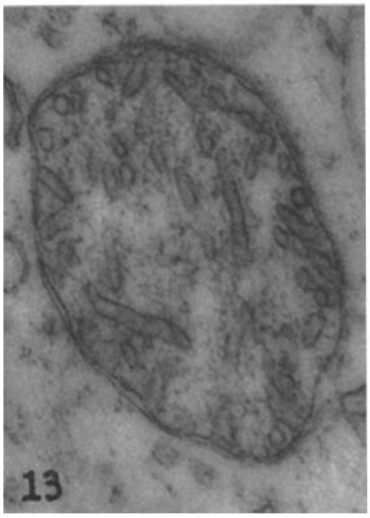
FIG. 13. Section of a typical mitochondrion which is bounded by a well defined system membrane; the internal tubular vesicles are seen in transverse and longitudinal section. × 45,000.

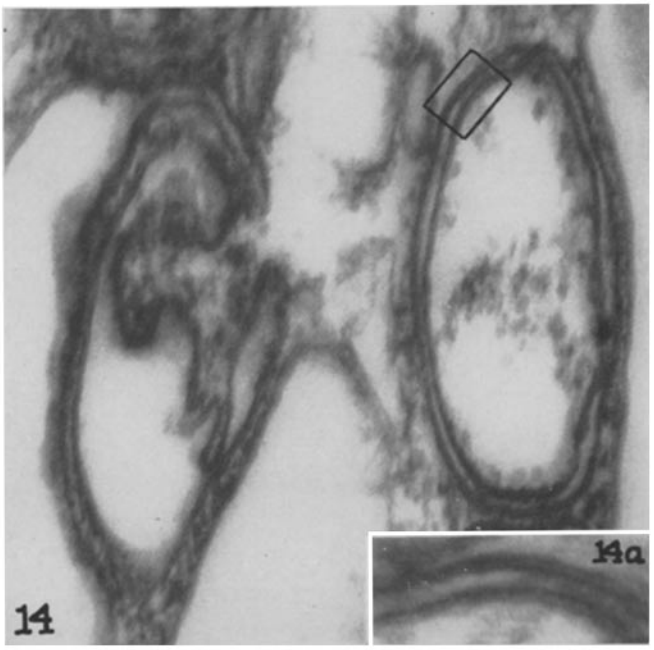
FIG. 14. The membrane system of the vacuoles found near the buccal cavity (see also Fig. 15). Two triple membranes separated by about 125 A bound the vacuole seen at the right of the micrograph. Each dense layer is separated from the other by about 75 A, and each individual dense layer is about 25 A thick. × 120,000.

FIG. 14 *a*. Enlargement of area outlined in Fig. 14. × 230,000.







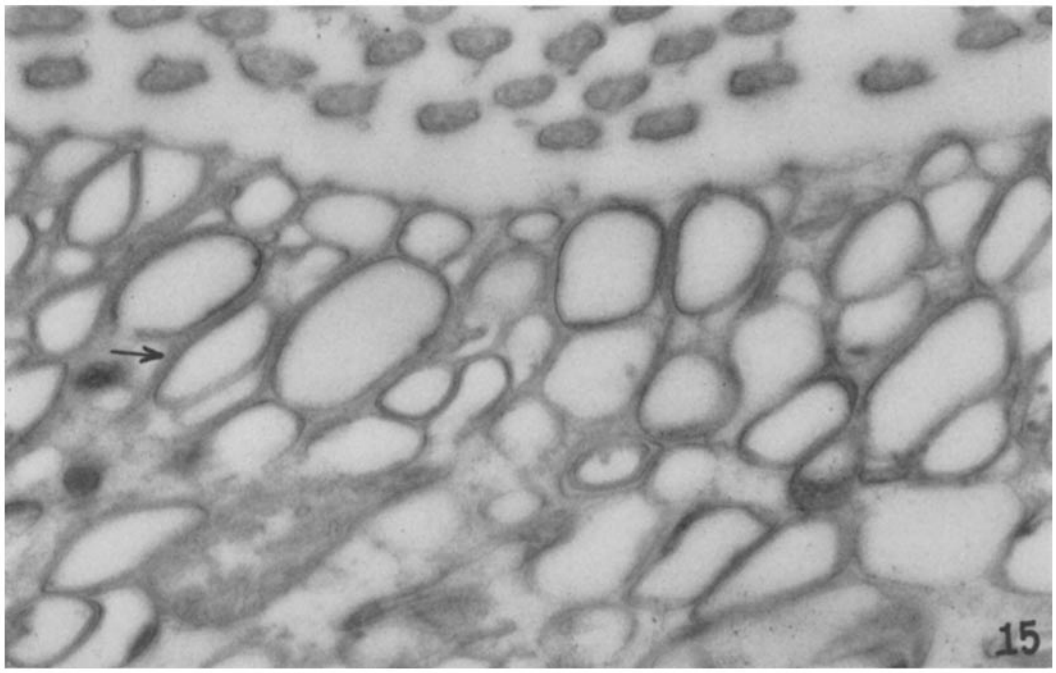


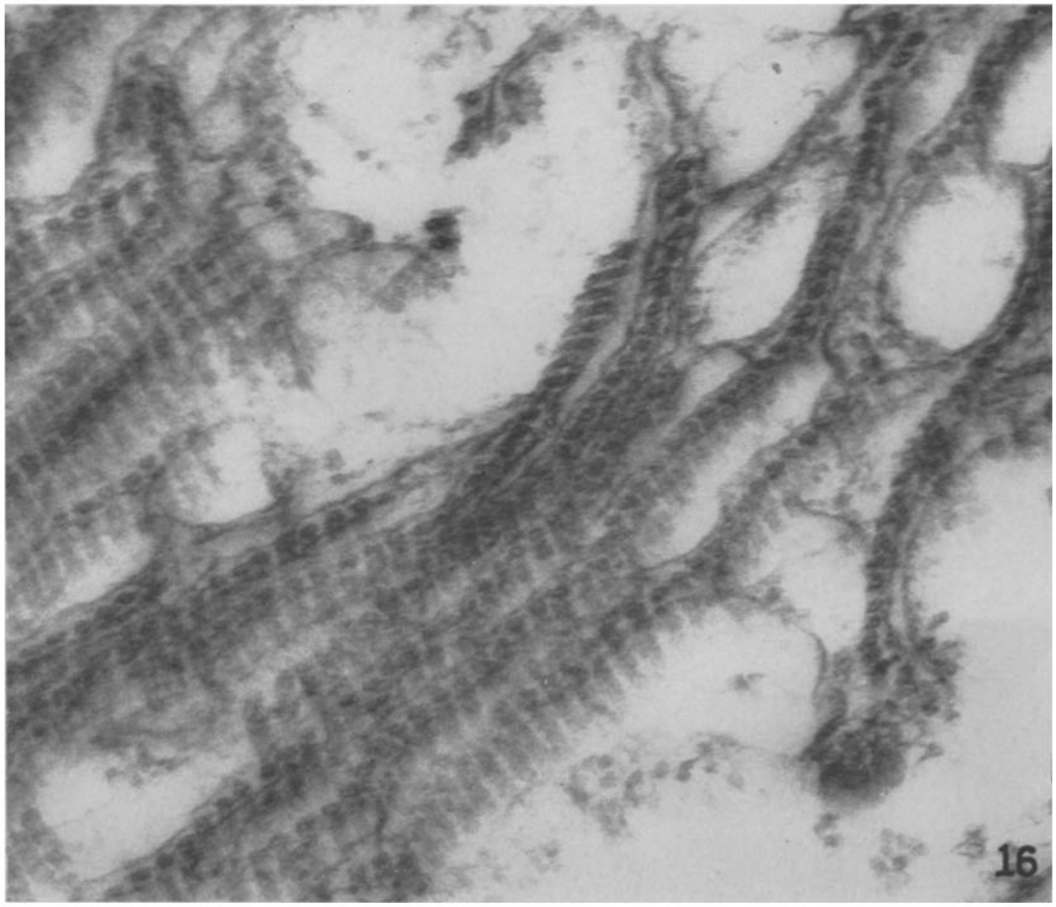
(Randall and Fitton Jackson: Structure and function in *Stentor*)

PLATE 411

FIG. 15. The vacuole system of the subcortical region below the buccal cavity; the membrane system of the vacuoles is shown more clearly in Fig. 14. Transverse sections of cilia are seen in the cavity, and there is evidence of transverse sections of fibrils lying adjacent to and in between some of the vacuoles (arrow). × 30,000.

FIG. 16. Electron micrograph of the fibrils noted in Fig. 15 seen in oblique and transverse section. Many micrographs of this area representing sections in various planes indicate that these fibrils depend into the central region of the *Stentor* body. Each fibril is about 125 A in diameter. × 100,000.





(Randall and Fitton Jackson: Structure and function in *Stentor*)

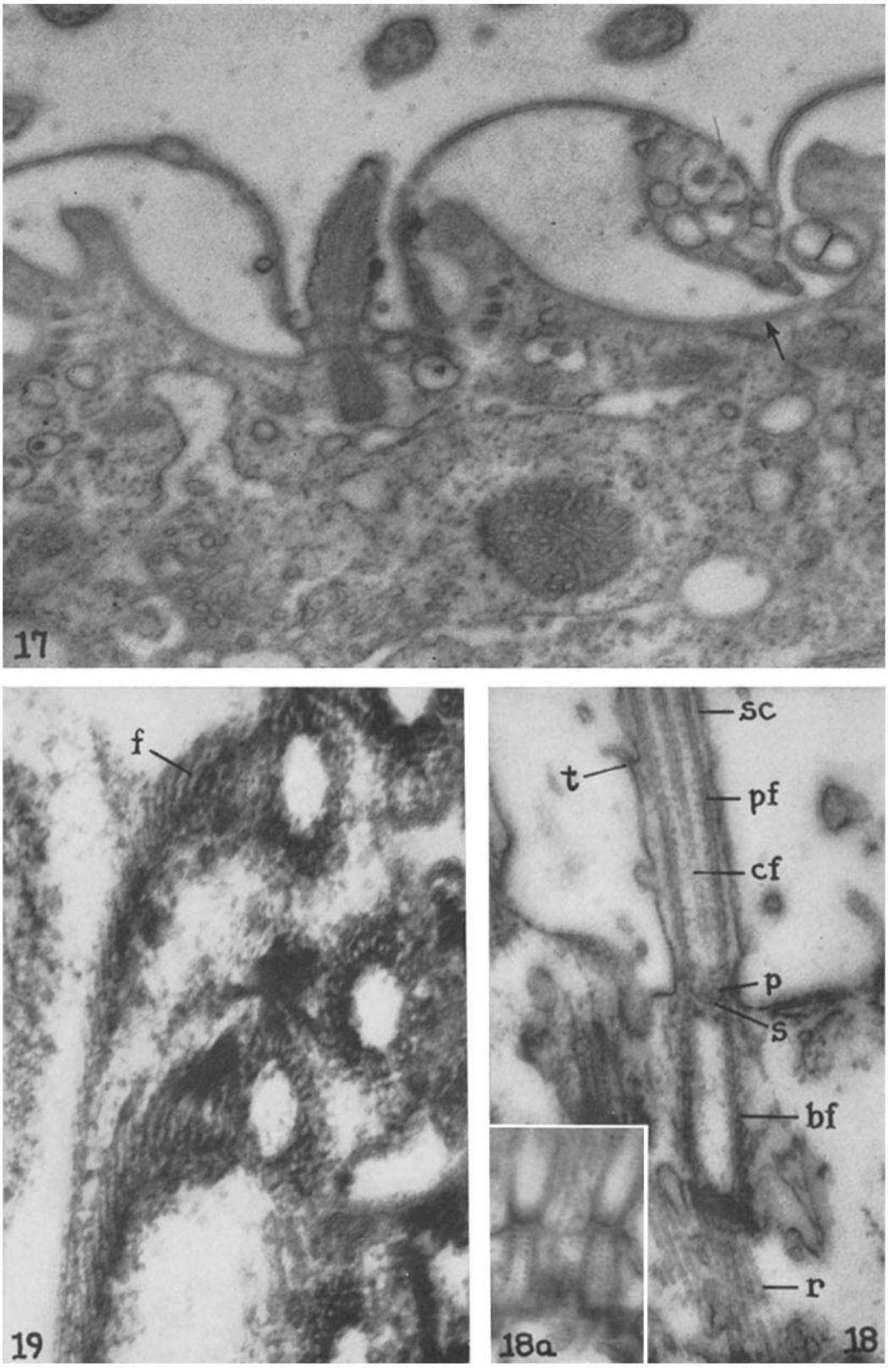
PLATE 412

FIG. 17. Transverse section of part of the cortical zone of *Stentor* near the adoral region. The pellicle is composed of two paired membranes, the outer pair being well separated from the *Stentor* body, but it is firmly connected at regular points usually close to a cilium. The general complexity of the cytoplasm is evident, and a cilium and its kinetosome can be seen in longitudinal section. Note the row of four bodies lying beneath the pellicle at the left of the micrograph and a similar row to the right of the kinetosome. × 30,000.

FIG. 18. Longitudinal section of a cilium and kinetosome from the membranelle system seen at higher magnification and showing the outer sheath of the cilium (*sc*) with small tubular protuberances (*t*); the peripheral filaments (*pf*) and central filaments (*cf*) of the cilium, the latter terminating at a particle (*p*); a curved septum (*s*) extends across the anterior of the kinetosome; the peripheral filaments of the cilium extend into the body to form the outer boundary (*bf*) of the kinetosome (24); the roots of the kinetosome (*r*). × 47,000.

FIG. 18 *a*. Longitudinal section of 2 kinetosomes showing small internal granules. × 19,000.

FIG. 19. Transverse sections of kinetosomes and the junction of the twin subfibrils formed from bifurcation of individual km fibrils with the respective kinetosome. Each twin subfibril is joined together by interconnecting filaments (*f*). See also Fig. 23 and Text-fig. 7. The structure of the periphery of the kinetosomes suggests that the continuations of the filaments from the cilium which form the wall are at least double. × 100,000.



(Randall and Fitton Jackson: Structure and function in *Stentor*)

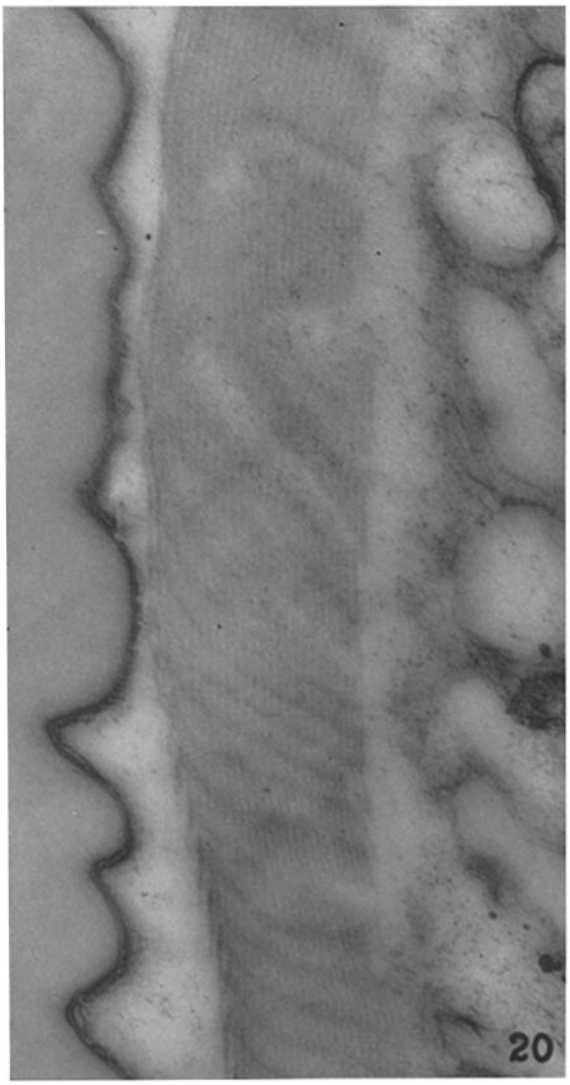
PLATE 413

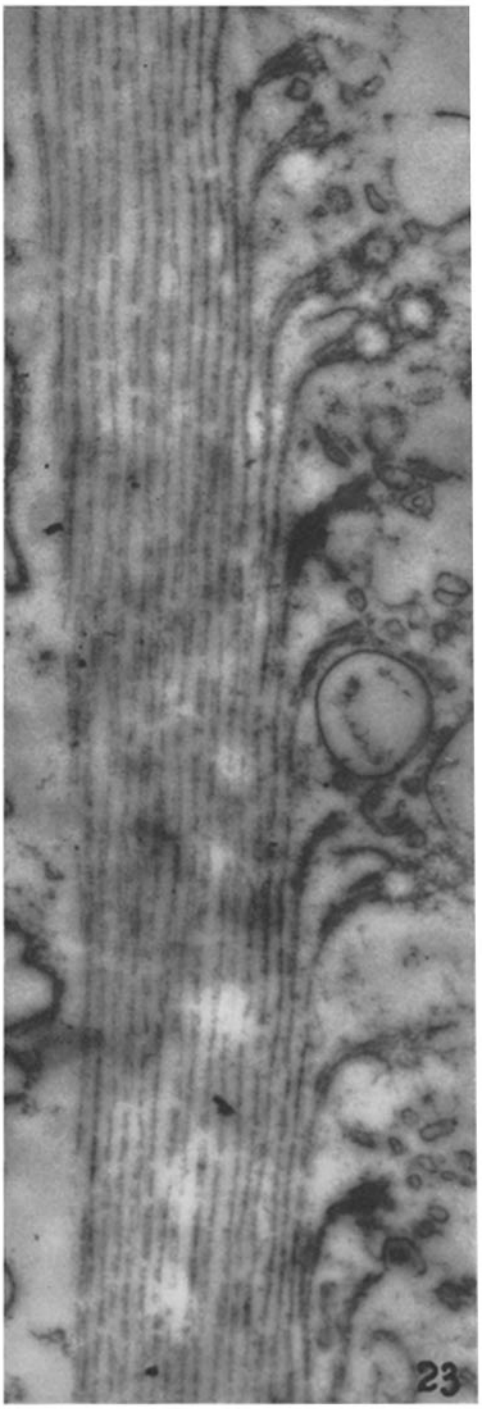
FIG. 20. Electron micrograph of the pellicle and the underlying km fibrils seen in longitudinal section. The pellicle is composed of 3 closely apposed dense membranes; the parallel arrangement of the individual sheets of the km fibrils is well shown. More than 20 sheets may be clearly distinguished in parts of this micrograph which should be compared with the different view in Fig. 23. × 35,000.

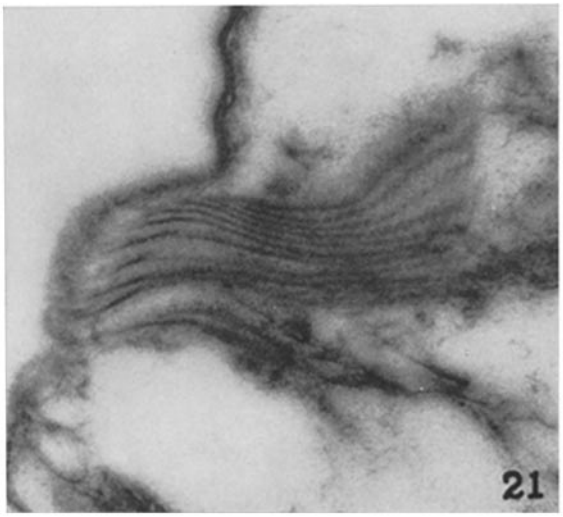
FIG. 21. A near transverse section of the km fibrils; this micrograph comes from about halfway up the side of the organism and shows 13 layers. This micrograph demonstrates close association between one of the long edges of each sheet of km fibrils and the pellicle. × 39,000.

FIG. 22. At high magnification an almost exact transverse section of one layer shows the fibrous nature of the structure. × 120,000.

FIG. 23. This micrograph shows the connections between individual km fibrils and the kinetosomes which are seen in transverse section. (For higher magnification view of junction see Fig. 19.) Thus the km fibrils may be identified with the kinetodesmata of Chatton and Lwoff. × 30,000.







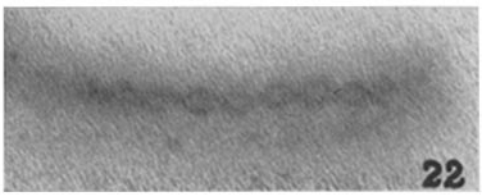


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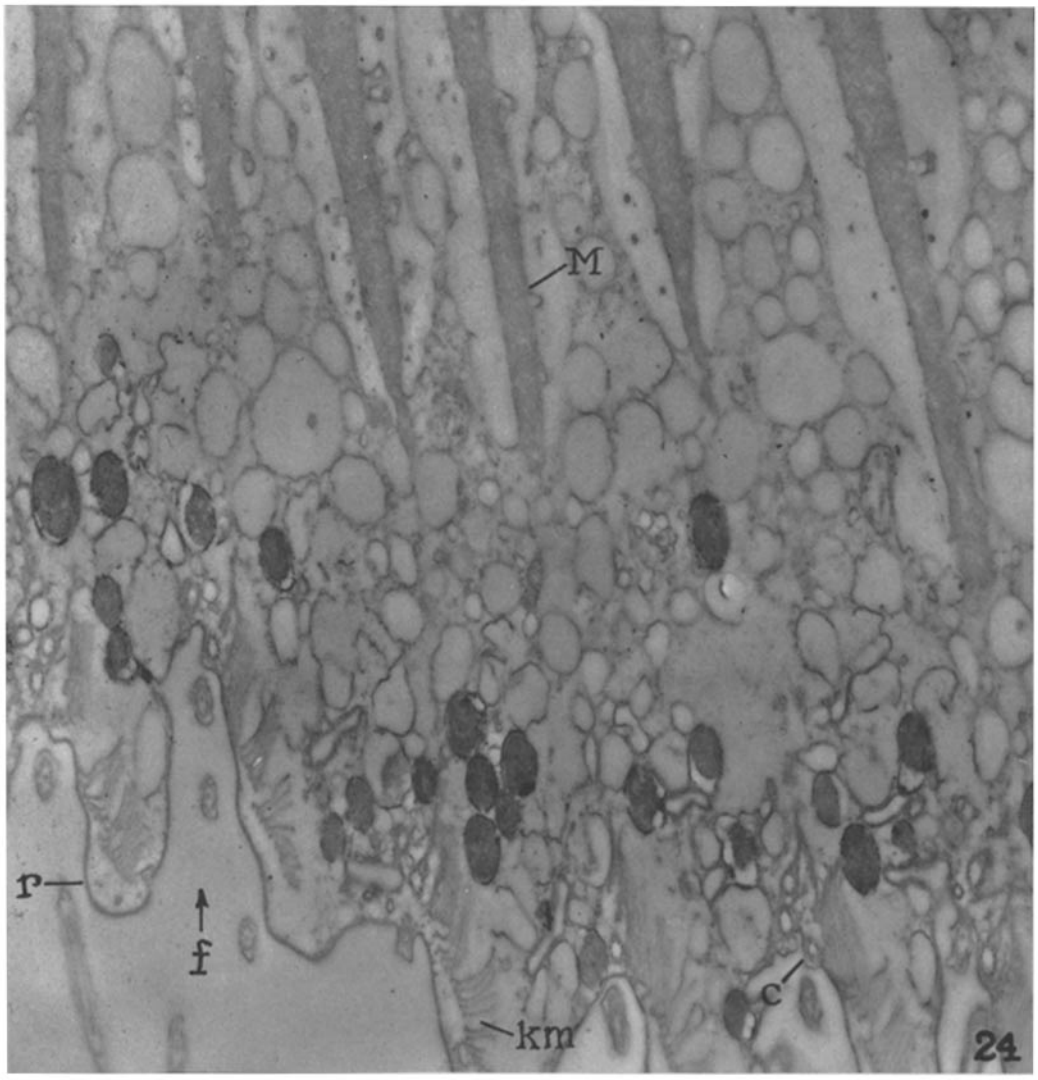
FIG. 24. Oblique section of the cortical region of *Stentor* near the foot of the organism. The section shows six ridges (*r*); six furrows (*f*); the cilia (*c*) lying adjacent to one side of the ridges; six units of km fibrils (*km*) spreading into the appropriate adjacent ridge; six M bands (*M*); the general vacuolated cytoplasm; most of the dense oval structures are zoochlorella. × 8,000.

FIG. 25. Two adjacent M bands at higher magnification; an interconnecting branch is shown, and also the close association of the bounding membrane with the adjacent vacuoles. The rather indistinct filamentous material of the M bands is also shown. × 32,000.



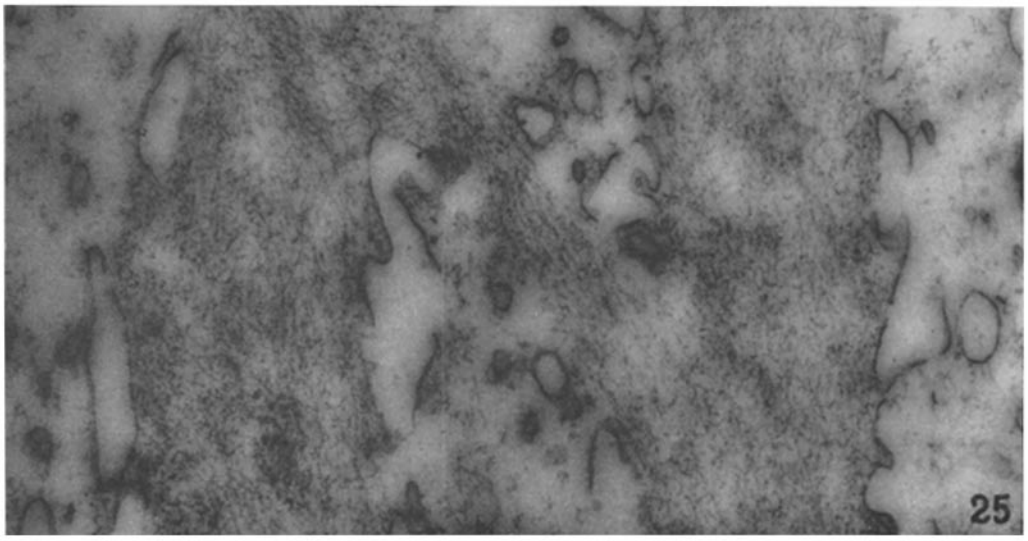


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FIG. 26. Section of a more anterior region of the inner cortical zone which shows part of one M band (*M*) which in serial sections was traced throughout the length of the organism; lateral connections between M bands are not seen in this region of *Stentor*. The general vacuolar nature of the cytoplasm is well displayed; part of two zoochlorella (*z*) and a mitochondrion (*m*). × 39,000.

FIG. 27. An histological section taken in phase contrast illumination showing the organization of a few M bands (*M*); they become highly refractive on fixation with osmium tetroxide. × 1,500.

FIG. 28. Body cilia seen *in toto* after fragmentation of the pellicular region of *Stentor* by ultrasonics; the narrower tip is shown (arrows). × 9,800.

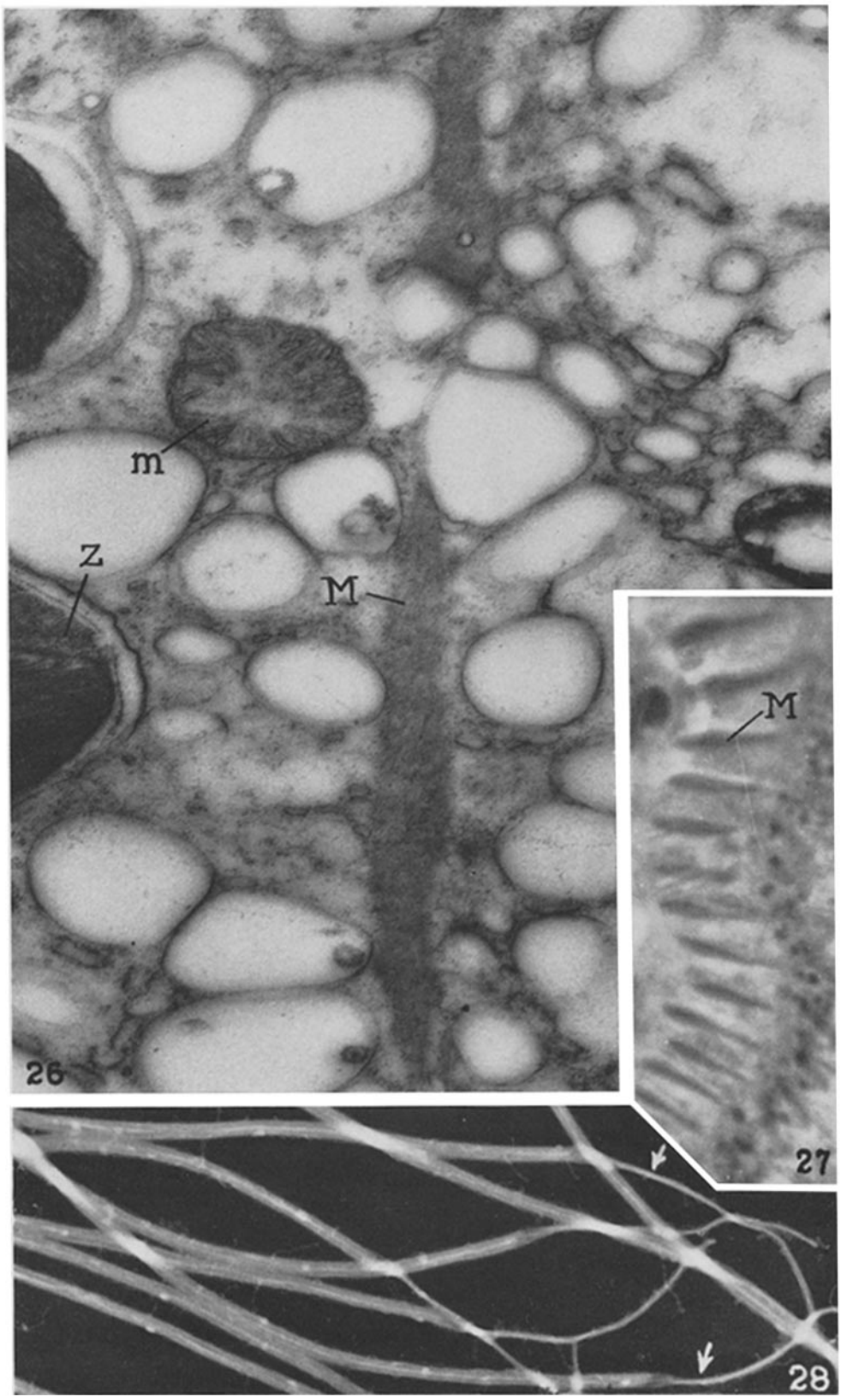

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PLATE 416

The Membranelle System I

FIG. 29. Transverse section though various levels of the kinetosomes of two adoral membranelles; this micrograph demonstrates the arrangement of the cilia into 3 rows per membranelle. The particle (*p*) at the proximal end, surrounded by a septum (*s*), and the arrangement of the peripheral filaments (*bf*) to form the outer boundary of the kinetosomes are also shown. × 32,000. See also Fig. 18 and Text-fig. 3.

FIG. 30. A micrograph which shows the end-on view of two adjacent membranelles. Longitudinal sections of the membranelle cilia and kinetosomes are seen at the top of the micrograph, and their long roots extend into the body of the *Stentor*. × 32,000.

FIG. 31. Longitudinal section of the roots which shows the individual component fibrils; each fibril is about 200 A in diameter. No clearly defined cross-striations have been observed. × 100,000.

FIG. 32. Electron micrograph which shows the fibrillar connection between membranelles in the cortical region. × 30,000.

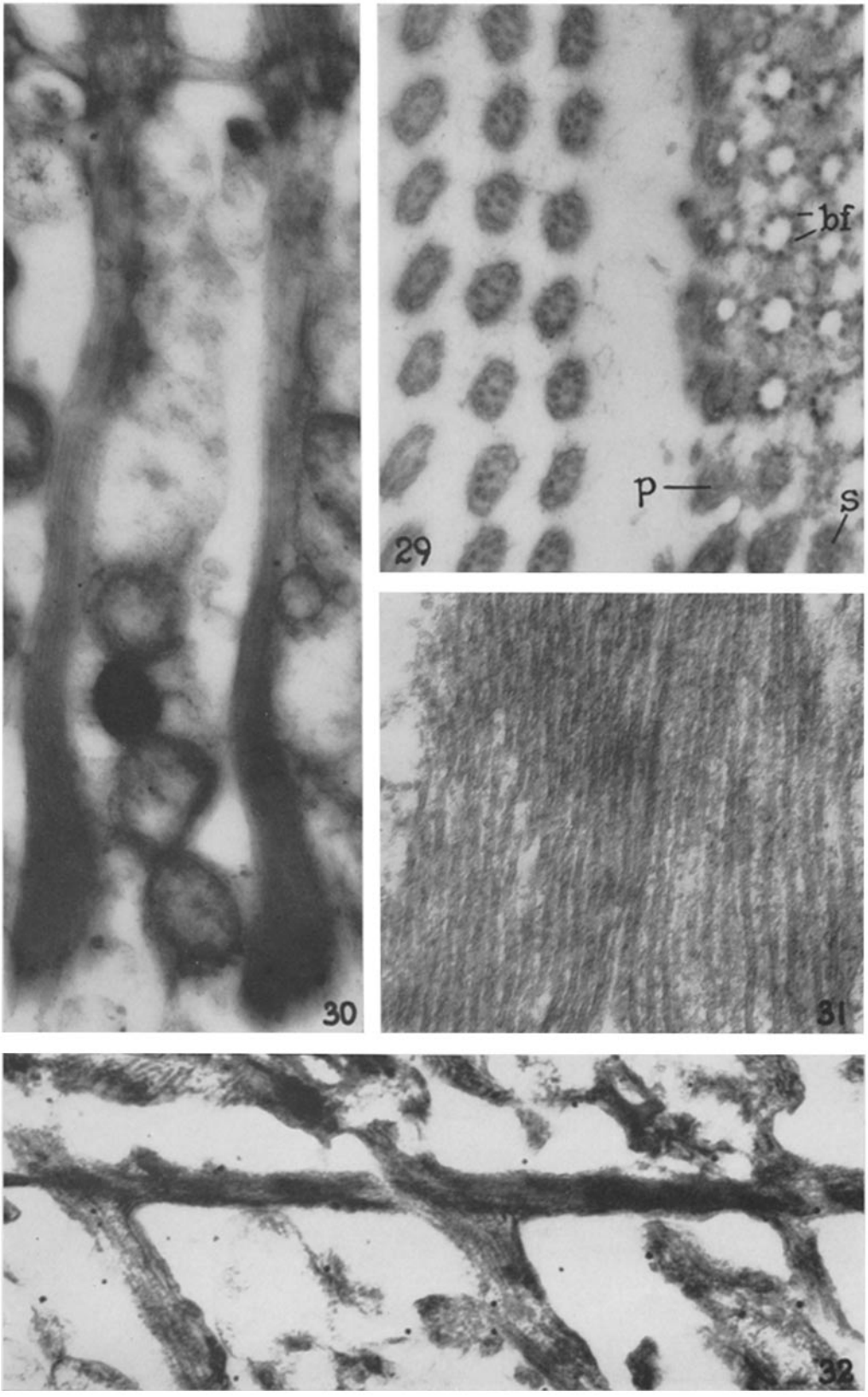

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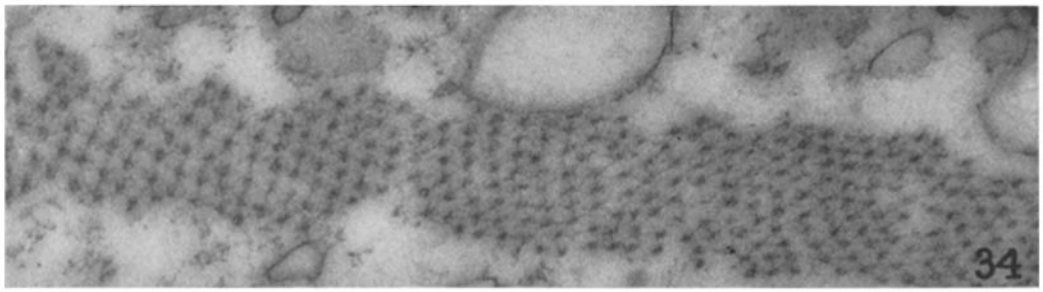
PLATE 417

The Membranelle System II

FIG. 33. A longitudinal section through the proximal third of a root of one membranelle, showing the fan-shaped arrangement of fibrils which gradually converge into a bundle. × 30,000.

FIG. 34. A transverse section through the fan-shaped region of the root. The individual fibrils of the root are clearly shown and are organised into an approximately hexagonal array. Individual fibrils are connected to each other by fine filaments. × 50,000.



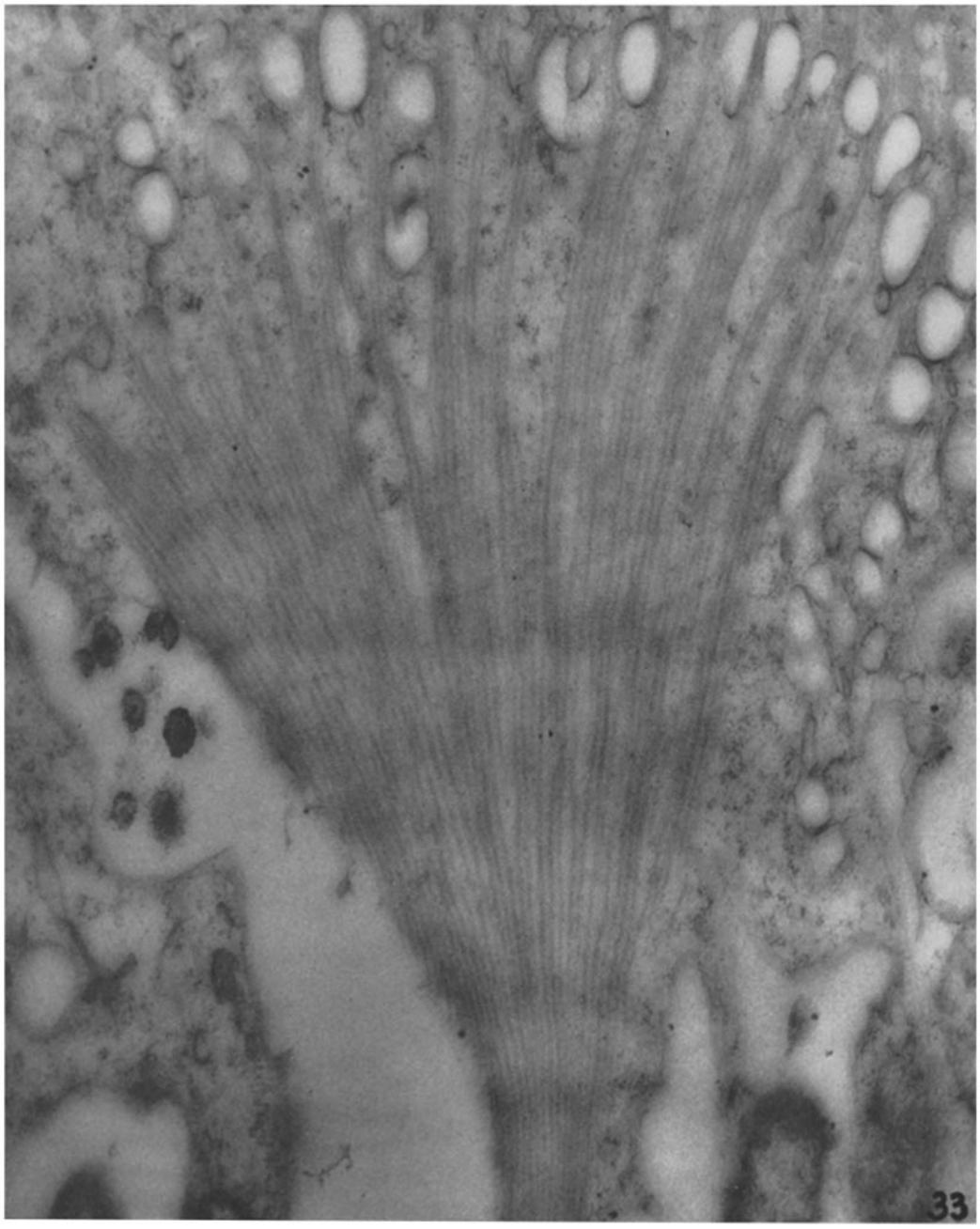


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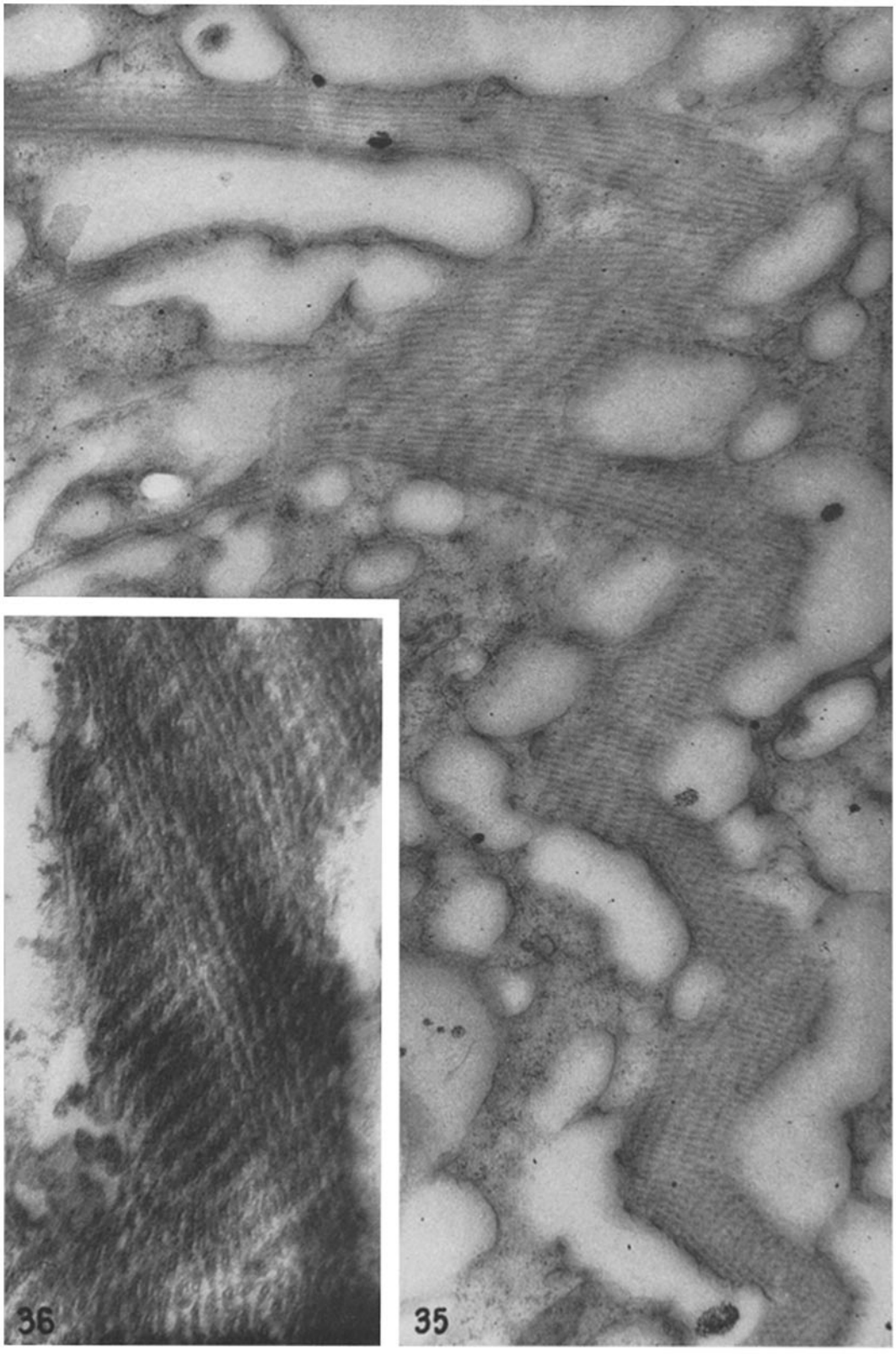
PLATE 418

The Membranelle System III

FIG. 35. Micrograph of the sinuous basal fibre which joins the roots of each individual membranelle. At the top of the micrograph, one bundle of root fibrils may be seen, and would connect with the fan-shaped structure as seen in Fig. 33. Each apex of the basal fibre at the left is joined to a membranelle root bundle, but the plane of the section is such that these are not observed. × 30,000. See also Text-fig. 7.

FIG. 36. Micrograph of the basal fibre to show the complex organization of the component fibrils. × 100,000.



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