FINE STRUCTURE AND MECHANICAL DESIGN OF CYLINDRICAL TENSION-TRANSMITTING CYTOSKELETONS

John Victor Wellings

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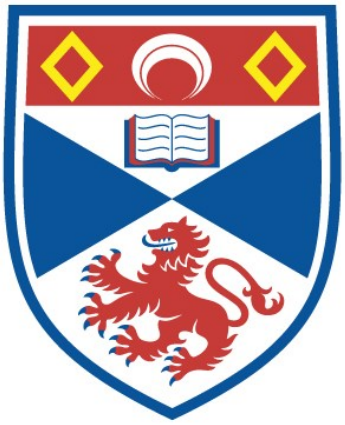

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M. DOWNIE
Printers and Bookbinders
(Baltic Chambers)
50 Wellington St. G2 6HJ
041-221 2469

FINE STRUCTURE AND MECHANICAL DESIGN OF

CYLINDRICAL TENSION-TRANSMITTING CYTOSKELETONS

J. V. WELLINGS

Ph. D. Thesis

CONTENTS

PART B : The cytoskeleton in oogenesis in **Drosophila melanogaster**

SUMMARY

All cells contain cytoskeletal components which perform static 'spatial organizing' and dynamic 'motile' functions. The studies described in this thesis concern two types of cytoskeletal component of universal biological importance: microtubules and microfilaments, and various other structures which are associated with them. The general properties of microtubules and microfilaments and of other cytoskeletal components are reviewed in the introduction. Examples of the functions they perform in various systems are described, with emphasis on Protozoa and metazoan morphogenetic systems.

The ciliate Nassula aurea was the first system studied. The cytopharyngeal basket is a tube shaped feeding organelle through which rapid cytoplasmic streaming occurs. The basket becomes deformed during transport of a food vacuole through its lumen. Ultrastructural examination of deformed baskets reveal that part of the basket, the tubular 'sheath', becomes stretched, probably in an elastic manner. Links between the microtubules in the sheath also appear to become stretched, so these could be responsible for the elastic properties. The nature of the links between microtubules within the 'rods', components of the basket responsible for its longitudinal rigidity, is also discussed, as are some other features relevant to the function of the basket as a feeding organelle.

The second system studied was oogenesis in Drosophila melanogaster. Several stages in the development of the oocyte and the surrounding follicle cells and nurse cells were examined ultrastructurally. The arrangement of microtubules and microfilaments is described, as are the changes that occur in these and other structures during oogenesis, and the related changes in morphology. A circumferentially oriented layer of microfilaments was discovered in vitellogenic oocyte follicles. The oocyte expands rapidly during vitellogenesis, as cytoplasm flows into it from the nurse cells, and as yolk proteins and nutrients flow in across the follicle. The microfilamentous layer could cause the oocyte to elongate as it expands by restricting its increase in diameter. The layer is destroyed by cytochalasin B, and can be decorated by heavy meromyosin. Therefore it is concluded that the microfilaments are actin-like. Various other structures in the developing egg chamber (the oocyte plus follicle and nurse cells) are described.

DECLARATION

I hereby declare that this thesis is my own composition, and that the experimental work was performed by me alone. None of the material in this thesis has been submitted for any other degree.

16 September 1983.

STATEMENT OF HIGHER STUDY AND RESEARCH UNDERTAKEN BY CANDIDATE

I graduated from Queen's College, Oxford, in July 1975 with an Honours degree (B.A. 2nd class) in Zoology.

The research described in this thesis was undertaken while I was employed as a research assistant (SRC grant) to Dr. J.B. Tucker in the Department of Zoology, St. Andrews Univerity, with the permission of the SRC and of the University to register for a Ph. D. I registered for the degree of Ph. D. in October 1976.

STATEMENT BY SUPERVISOR

I certify that the conditions of Resolution of the University Court, 1967, No. 1 and such regulations of the University Senate that are applicable to the conference of the degree of Ph. D. by the University of St. Andrews have been fulfilled by the candidate.

16 September 1983.

INTRODUCTION

This thesis is concerned with two types of cytoskeletal element and the functions they perform in two very different systems. The elements (microtubules and microfilaments) can be detected within the cytoplasm of most cells (at least during some part of the cell cycle) using conventional transmission electron microscopy. The aim of the investigations described below was to monitor the ultrastructural arrangement of cytoskeletal elements and to try to discover the basis of their functions in terms of mechanical behaviour in relation to the overall functions and behaviour of the systems studied. The functions of the cytoskeletal elements can in part be deduced from their arrangement, and in part by performing experiments which modify the elements and affect the activities performed by them.

One of the systems studied was the feeding organelle (cytopharyngeal basket) of the ciliate Nassula aurea. Some new discoveries relevant to the function of the basket are described in chapters 1 and 2. Analysis of cytoskeletal function was also pursued in an examination of cytoskeletal involvement during cell shape changes associated with oogenesis in Drosophila melanogaster (chapters 3 to 6). The chief aspect of oogenesis that was investigated was the elongation of growing oocytes.

These two particular systems were selected for study for the

following reasons. Both Nassula and Drosophila can be easily cultured in the laboratory. The large size and highly ordered architecture of Nassula's cytopharyngeal basket [Tucker 1968] provide excellent opportunities for cytoskelal analysis: how does the functional design of highly ordered microtubule arrays compare with that of the less highly ordered ones that are more common in cells generally? An examination of the control of oocyte shaping in Drosophila was undertaken because Tucker and Meats [1976] have argued that microtubules (which do not have an especially well ordered arrangement) help to control such shaping in a gall midge (Heteropeza pygmaea) which exhibits an unusual type of polytrophic oogenesis, and the cockroach (Periplaneta americana) with panoistic oogenesis. Do microtubules play a similar role during insect oogenesis generally? More especially, do they participate during polytrophic oogenesis in Drosophila which is unquestionably one of the most important 'laboratory animals' from a genetic and developmental standpoint? My studies show that actin-like microfilaments rather than microtubules play the major cytoskeletal role during Drosophila oocyte shaping.

The remainder of this introduction is taken up first with a description of the properties of various types of cytoskeletal elements and then with a selection of functions that they are known to perform, in Protozoa and in metazoan morphogenesis, which are relevant to the studies described in the rest of this thesis. The nature of cytoskeletons is a subject on which several volumes have already been written. It is not therefore appropriate, nor indeed is it practical,

to review the subject in detail here. Instead a general introduction is provided and review articles, in which more substantial accounts may be found, are cited.

What is a cytoskeleton? The term has been used to describe those parts of a cell (mainly for cells in tissue culture) which remain after detergent extraction [Brown et al. 1976, Osborn & Weber 1977] or (e.g. in smooth muscle cells) after extraction with high ionic strength buffers [Cooke & Chase 1971, Cooke 1976]. The former treatment leaves microfilaments and intermediate filament bundles intact (see below), as well as the nucleus. The latter leaves intermediate filaments intact but removes microfilaments (or the equivalent actin or thin filaments in muscle cells) and myosin (thick) filaments. However in this thesis the term cytoskeleton will be used in a more general sense to include all cytoplasmic structures which play a mechanical or skeletal role. The majority of these structures are filamentous. They include microfilaments (and thin filaments of muscle), intermediate filaments, myosin (or thick) filaments and microtubules.

Intermediate filaments are a diverse class of filament which exhibit diameters of 8 to 10nm [Lazarides 1980]. They include 'Keratin' type filaments of epithelial cells, the vimentin filaments of muscle, the desmin filaments of cells of mesenchymal origin, and certain filaments in neurons and glial cells. More than one type may be present in an individual cell. They have mainly been studied in the cells of certain vertebrates. They have not been observed in any of

the cells considered in this thesis.

Myosin filaments or thick filaments (about 15nm in diameter) are prominent features of muscle cells [Pollard & Weihing 1974]. Myosin-like proteins have been extracted from, or demonstrated *in situ* using anti-myosin immunofluorescence in several types of nonmuscle cells [Pollard & Wehing 1974]. Myosin is the only well known protein capable of producing movement by interaction with actin, so it is likely that myosin is usually present whenever actin filaments are present (i.e. in most or all cell types). However it is possible for motility to occur without the involvement of myosin. This is the case in the elongation of the acrosomal process of the sperm of some invertebrates. In *Limulus* elongation occurs by the conversion of a supercoiled arrangement of filaments in a bundle to a straight paracrystalline one [Tilney 1975, De Rosier *et al.* 1982]. In *Thyone* and *Piaster* elongation occurs by the rapid polymerization of actin filaments [Tilney & Kallenbach 1979]. It is possible that in other nonmuscle cells myosin is functional in forms other than the thick filaments of the type found in muscle cells. Thick filaments have not been observed in the cells considered in this thesis.

Microtubules are tubular structures with external diameters of about 24nm and side walls which are about 5nm thick. They are normally several micrometres in length. Specialized doublet and triplet microtubule complexes occur in cilia, flagella, basal bodies and centrioles. Microtubules are present in all eukaryotic cells and have been implicated in a wide range of functions [Reviews: Porter 1966,

Roberts 1974, Stephens & Edds 1976, Soifer (ed.) 1975, Mohri 1976, Dustin 1978, Roberts & Hyams (ed.) 1979, Stebbings & Hyams 1979]. These functions include the development and maintenance of cell shape, the intracellular transport of materials for secretion, and cell locomotion. In addition they are extensively employed in the construction of large motility associated organelles (especially in Protozoa).

Microtubules can be demonstrated to be made up of protofilaments if appropriate techniques are used, such as tannic acid / glutaraldehyde fixation [Mizuhira & Futaesaki 1971, Tilney et al. 1973] or negative staining of isolated microtubules [Behnke & Zelander 1967]. The majority have 13 protofilaments, but exceptions do exist [Chalfie & Thomson 1982]. The major protein component of microtubules, tubulin, is arranged in a bead like array along the length of the protofilaments [Amos 1979]. Tubulin is present in the cytoplasm in a state of dynamic equilibrium between soluble dimers (molecular weight about 110 000) and polymerized microtubules [Pipeleers et al. 1977a,b]. The dimer is composed of two slightly different polypeptide chains: alpha- and beta-tubulin [Luduena et al. 1974]. Each dimer has two guanosine nucleotides (GDP or GTP) bound to it [Shelanski & Taylor 1967, 1968, Weisenberg & Taylor 1968]. These are required for assembly to take place. Microtubules can be assembled in vitro if appropriate conditions are maintained. These include, for mammalian tubulin, very low Ca^{2+} concentration with the presence of Mg^{2+} and GTP at $37^{o}C$ [Weisenberg 1972, Olmstead & Borisy 1975]. Microtubule proteins can be purified by repeated

assembly/disassembly cycles. Certain proteins (microtuble-associated proteins, MAPs) copurify with tubulin and are believed to be required for assembly under physiological conditions [Weingarten et al. 1975, Borisy et al. 1975, Sloboda et al. 1976, Bloodgood & Rosenbaum 1976]. Some of these proteins can form 'fuzzy coats' on reassembled microtubules and may be related to the intertubule links which join microtubules together in certain situations [Sloboda et al. 1976]. Several other proteins can bind to microtubules, the most notable being dynein, which is involved in force production during the beating of cilia and flagella [Gibbons & Rowe 1965, Gibbons 1975, Warner 1979, Warner & Mitchell 1980]. Colchicine, vinblastine and several other drugs [Dustin 1978, chapter 5] bind to sites on the tubulin dimer and this inhibits the dimers from participating in microtubule assembly. Microtubules may also be disassembled by cold ($0^{o}C$) and high hydrostatic pressure. There is considerable variation in suscepibility to these treatments. Cytoplasmic microtubules are on the whole labile and readily disassembled by drugs or by cold. The microtubules of cilia, flagella and certain organelles such as the cytopharyngeal basket of Nassula are more stable and may (in Nassula) even assemble at high concentrations of colchicine [Tucker et al. 1975].

Actin-like microfilaments (5 to 7nm in diameter) are very similar to the thin filaments of muscle cells. They are present in most cell types [Reviews: Goldman & Knipe 1973, Pollard & Weihing 1974, Hitchcock 1977, Korn 1978, Stebbings & Hyams 1979]. The quality of preservation after conventional glutaraldehyde/osmium tetroxide

fixation is more variable for microfilaments than it is for microtubules [Pollard 1976]. The major protein component of microfilaments is actin (referred to as cytoplasmic actin or actin-like protein to distinguish it from muscle actin). Actin in the form of filaments is referred to as F-actin. The monomer is referred to as G-actin. Microfilaments composed of actin can be decorated with heavy meromyosin to distinguish them from other filaments of the same diameter (see chapter 6). Microfilaments have been implicated in motility both within individual cells (e.g. amoebae and certain cells in tissue culture) and in certain morphogenetic events that involve movements in which large numbers of cells participate. They may also play a static or skeletal role (preventing the movement of structures). G-actin is often present in considerable quantities in the cytoplasm where it contributes to colloid (gel) formation. The gel may itself have cytoskeletal functions [Pollard 1976].

Cytoplasmic actins have been isolated from a small number of nonmuscle cell types [Pollard & Weihing 1974, Korn 1978]. Actin may make up 10 to 15% of total protein in cells (e.g. amoebae, platelets, sea urchin eggs) compared to about 25% in rabbit muscle [Taylor & Condeelis 1979]. The molecular weight is always about 42 000. Most organisms contain a small number of actin variants [Korn 1978, Horovitch et al. 1979] some of which occur only in muscle, some in nonmuscle cells and some in both. Muscle contraction is known to be regulated by proteins which can be situated either on the actin filaments (typical in vertebrates) or on the myosin filaments (common in invertebrates) [Lehman 1976]. Nonmuscle contraction can be

regulated in either of these ways or by the control of the assembly of actin filaments [Pollard & Weihing 1974, Hitchcock 1977]. Tropomyosin which, together with troponin, is responsible for actin-filament-Ca^{2+}-dependent control of contraction, is present on some, but not all, cytoplasmic actin filaments. Both filaments with and without tropomyosin can occur within the same organism [Lazarides 1976]. The distribution of the two types of control of contraction in nonmuscle cells does not correspond to the largely phylogenetic one for muscle contraction [Lehman 1976, Hitchcock 1977]. Various other proteins are known which can interact with cytoplasmic actins. These include profilin and DNase I, which bind to G-actin and prevent it from polymerizing, and alpha-actinin, filamin and spectrin, which bind to actin filaments. The latter may possibly act as intermediates in the attachment of actin filaments to other structures. The drug cytochalasin B binds to G-actin, preventing the assembly of most microfilaments (see chapter 5). It is less specific than antimicrotubule drugs such as colchicine and is generally more toxic.

The study of Protozoa has contributed much to the understanding of the nature of cytoskeletons. Protozoa possess organelles that are usually built up of the same cytoskeletal components as are present in Metazoa. In Protozoa however the organelles sometimes perform functions which in Metazoa are performed more efficiently by multicellular systems. For this reason several of the most elaborate cytoskeletal specializations are to be found in Protozoa.

Several Protozoa have proved useful in the study of cell motility. The properties of cilia and flagella, and the mechanism of amoeboid movement, have been extensively studied in Protozoa (see microtubule and microfilament review references above). Several Protozoa have complex microtubular organelles other than cilia or flagella. These can be motile or skeletal in function. Motile systems include the axostyles of certain flagellates [Grimstone & Cleveland 1965, Bloodgood 1975, Woodrum & Linck 1980] and the kinetodesmal fibres of Stentor [Huang & Pitelka 1973]. Skeletal supports for cell extensions are found in the axopodia of Heliozoa and Radiolaria [Ockleford & Tucker 1973, Bardele 1975, 1977, Cachon et al. 1973], and the tentacles of Suctoria [Curry & Butler 1976, Tucker 1974]. In Suctoria the microtubules are arranged to form a tube in the tentacle. Side arms are possibly involved in the mechanism of cytoplasmic streaming through the tentacles [Tucker 1974]. Many ciliates have microtubular organelles associated with the cytostome [Lynn 1981]. These are often little more than enlargements of the microtubular bundles which are associated with the non-oral ciliature. However in certain Gymnostomatida, particularly those such as Nassula which feed on filamentous algae, a more complex organelle, the cytopharyngeal basket, is present [Tucker 1968, 1972b, Hausmann & Peck 1978]. During feeding a stream of cytoplasm through the basket propels an algal filament (within a food vacuole) into the organism (see chapter 1). Studies of the growth of cytopharyngeal baskets and the assembly of heliozoan axonemes have provided some evidence of how the assembly of microtubules into complex patterns is controlled [Tucker 1970a, 1977, 1979, Jones and Tucker 1981].

The early development of Metazoa involves considerable movement of cells and multicellular aggregates. In this 'morphogenetic phase' the primitive body plan of the organism is layed down [Trinkaus 1976]. The cells involved are relatively unspecialized. Later, during a 'cytodifferentiation phase' the cells acquire the specialized functions of the tissues they become a part of. Cytoskeletons are involved in both phases. The discussion which follows concentrates on developmental events in which cytoskeletons play an important role, especially those in which cytoskeletons are involved in cell locomotion or in cell shape changes. These occur more often during the morphogenetic phase. Situations in which cytoskeletons perform functions similar to those performed in mature tissues, as is common in the cytodifferentiation phase, will not be considered.

How are cytoskeletons involved in cell movements which take place during the morphogenetic phase? Cells may move individually or in sheets [Spooner 1975, Trinkaus 1976]. Examples of individual cell movement include neural crest formation (early stages) and primordial germ cell migration. Examples of sheet movement include gastrulation, neurulation and the formation of several organs by the invagination of cell layers.

The locomotion of individual cells has largely been studied in tissue culture because suitable systems for <u>in vivo</u> studies are rare. Cells generally spread out in the direction of motion, forming broad lamellipoda [Spooner 1975, Trinkaus 1976]. A cell may extend

thin filopodia before a lamellipodium is formed. Once a lamellipodium has spread parts of it adhere to the substratum. The spreading of a lamellipodium causes the cell to become elongate, but it periodically detaches at its trailing edge and shortens by contraction. Spreading and contraction are inhibited by cytochalasin B [Goldman *et al.* 1973] so microfilaments could be responsible for both processes. Microfilaments form a meshwork under the plasma membrane of the lamellipodium and are present as bundles (referred to as 'stress fibres' from their appearance in phase contrast light microscopy or 'actin cables') in the contracting 'tail'. Microtubule inhibitors reduce cell spreading and prevent the unidirectional spreading necessary for locomotion [Vasiliev and Gelfand 1976]. It is possible that microtubules play a role in controlling spreading, and are not directly involved in spreading itself, as this can occur in the presence of microtubule inhibitors.

There is some evidence that the *in vivo* movement of single cells occurs in a similar manner to *in vitro* movement [Trinkaus 1976]. A few organisms such as sea urchins and tunicates have early stages of development which are transparent enough to permit direct observation of moving cells. Cells often appear to move by extending a long protrusion which adheres at the tip and then shortens, to pull the cell along. Cells sometimes extend filopodia in several directions; some of these adhere to points on the substratum (normally the layer of cells which they are moving over). Larger cell extensions then follow in the direction of those that adhere. After these have attached the trailing edge detaches and movement is achieved by contraction as occurs in cells in tissue culture.

The types of movement of cell sheets that occur during morphogenesis include spreading, contraction (with resultant thickening), invagination and other types of folding [Spooner 1975, Trinkaus 1976]. The spreading of sheets with a leading edge often involves the formation of lammelipodia at the leading edge. These possibly adhere to the substratum and generate a tension to pull the rest of the sheet along. It is not certain what other factors contribute to the spreading of such sheets, and to the spreading of sheets which do not have a leading edge. Cell sheets which contract and thicken appear to do so by the elongation of their component cells in a direction normal (perpendicular) to the planes of the sheets. Often microtubules are present in considerable numbers parallel to the long axes of the cells. Colchicine can inhibit elongation. However it is not clear how the microtubules produce elongation, or if they are only indirectly involved. Invagination and folding are thought to occur as the result of the constriction of the ends of the cells in a sheet on one side only (i.e. that which will become the concave side). A band of microfilaments is often found around the inside of the ends of the cells that constrict. This band may produce the constriction by a 'purse string' mechanism [Burnside 1971, 1973, Spooner 1975, Trinkaus 1976]. Cytochalasin B inhibits invagination.

Microtubules and microfilaments have been implicated in the formation of specialized structures by cells. For example the extension of axons from nerve cells involves a growth cone with small rod-like protrusions (microspikes) which contain microfilaments and

which probably pull the axon along in a manner similar to that of the lamellipodia in cells in tissue culture [Trinkaus 1976]. Cytochalasin B stops axon growth. Axons contain numerous microtubules which are essential for elongation [Yamada et al. 1971]. Colchicine causes retraction. Microtubules (and intermediate filaments) are also involved in the transport of material along mature axons. In insects microtubules and microfilaments are thought to play a role in the development of cuticular structures such as the fine 'interference filter' ridges of butterfly scales [Overton 1966, Ghiradella 1974]. Microtubules, often as components of ciliary structures, play an important part in the formation and function of some insect sense organs and glands. These include duct formation in glands such as the tergal gland of cockroaches [Sreng & Quennedey 1976], the formation of hair sensilla [Altner & Prillinger 1980] and the development of ommatidia of the eye [Perry 1968a,b]. Microtubules and microfilaments are also involved in the development of retinal cells in the vertebrate eye [Burnside 1976, Matsusaka 1976].

A morphogenetic system in insects which has been much studied is the eversion of dipteran imaginal discs [Fristrom & Fristrom 1975, Poodry & Schneiderman 1970]. This is inhibited by cytochalasin B [Mandaron & Sengel 1973]. However the relative extent to which microfilaments and other factors such as cell rearrangement are involved has not yet been resolved [Fristrom 1976]. Furthermore, changes in cell shape in the disc epithelium (from elongate to cuboidal) are accompanied by loss of microtubules [Poodry & Schneiderman 1970]. Microtubules have also been reported to be

involved in the movements of cells in the imaginal histoblasts in the abdomen of Diptera [Bautz 1976].

In insect oogenesis (considered further in chapters 3 and 4) microtubules have been reported to be present in very large numbers in the nutritive tubes of the teleotrophic ovaries of Hemiptera [Macgregor & Stebbings 1970, Hyams and Stebbings 1977]. Ribosomes, and, in some hemipterans, other cytoplasmic constituents, are transported along the nutritive tubes from the trophocytes to the oocyte. It is not known what role the microtubules play in this transport. Microtubules have also been previously reported in Drosophila oocytes [Mahowald 1972b] and in follicle cells [Quatropani & Anderson 1969] (see chapter 4). Microfilaments have been observed in the oocytes of Amphibia [Franke et al. 1976], but not (as far as I have been able to ascertain) in insects.

It is often assumed that microtubules and/or microfilaments are involved in events which occur within cells on the basis of ultrastructural observation and the effects of drugs such as colchicine or cytochalasin B. This type of evidence does not show how directly the microtubules or microfilaments are involved. Cytoskeletal elements which are not readily affected by drugs or are not well preserved after conventional (glutaraldehyde/osmium tetroxide) fixation for electron microscopy are likely to be overlooked. There is a considerable body of evidence to show that the ground substance of cytoplasm possesses considerable structure [Pollard 1976, Wolosewick & Porter 1979], which is not revealed in electron micrographs of

conventionally fixed material. It is likely that the ground substance has a more important role in the events that occur within cells than has hitherto been realised. It is probable that microtubules and microfilaments (and indeed other cytoskeletal elements) often interact synergistically, as may be the case in secreting cells [Feldman & Maurice 1975]. In some systems there is evidence that microtubules direct the arrangement of microfilaments: for example in phagocytosis and concanavalin A induced cap formation in leukocytes [Berlin et al. 1979]. It is probable that one of the major roles of microtubules is to provide spatial information for the concentration and orientation of other cytoplasmic elements [Tucker 1979].

The mechanical forces per unit area of cell surface needed to perform motile events in certain nonmuscle cells (e.g. those in the cleavage furrow in sea urchin eggs or cytoplasmic streaming in Nitella) has been estimated to be of the order of 10^{-4} to 10^{-5} Nm^{-2} [Wolpert 1965]. It has been calculated that these forces could be provided by from 1 to 7 myosin filaments per $1\mu m^2$ of cell surface, if the force produced per myosin filament is the same as has been measured for skeletal muscle [Wolpert 1965]. Actin filaments and myosin filaments in skeletal muscle occur in a ratio which varies from 2:1 to 6:1 depending on the type of muscle [Aidley 1971]. Therefore a relatively small number of actin filaments, say 40 per square micrometre of cell surface, would be capable of providing the mechanical forces needed for motile events. The actual figure may well be greater than 40 as the filaments are not as well organized as they are in skeletal muscle, and actin and myosin filaments can only

generate force if they are close together with the correct orientation. However the possibility that the very numerous microfilaments that are often found close to the surface of cells may perform functions in addition to force production has to be born in mind. It is possible that large numbers are necessary to form a stable network of filaments which is capable of preserving spatial information (such as information about the orientation of structures). An oriented array of filaments (or any cytoskeletal element in fact) should be capable of transmitting spatial information over considerable distances, and even, with the aid of various intercellular junctions, between cells [Tucker 1981].

Microtubules and microfilaments are arranged in a great variety of configurations depending on the type of cell under consideration. They interact and complement each other cytoskeletally in a variety of ways. The chapters that follow explore two extremes in this range of variation. They deal with a large protozoan organelle on the one hand, and a multicellular complex concerned with insect oocyte growth and shaping on the other. The former system effects rapid and powerful unidirectional cytoplasmic streaming for periods of a few minutes at a time. It is a tubular structure constructed in a manner which provides longitudinal rigidity while permitting considerable distension of the lumen. The latter brings about carefully and continuously controlled spatial changes in cell shaping and cell association over a period of several days. Spatiotemporal fluctuations in the deployment of microtubules and microfilaments have been assessed using several microscopical procedures under naturally occuring and experimentally

induced conditions.

MATERIALS AND METHODS

CULTURE

NASSULA AUREA

Cultures were maintained by inoculating Nassula into flasks containing Phormidium inundatum which had been grown as described previously [Tucker 1977].

DROSOPHILA MELANOGASTER

Fruit flies were maintained on cornmeal-molasses-agar [Sang 1957] or David's medium [David 1962] at 25°C in small milk bottles. Adult females were normally used from 5 to 9 days after emergence to obtain a wide range of stages of egg chamber development (see chapter 3).

MANIPULATION AND DISSECTION

All manipulations of ciliates and dissections of Drosophila were

performed with the aid of a binocular microscope (Zeiss Oberkochen Ltd., Stereozoom III) using transmitted or reflected illumination. Ciliates were withdrawn from culture medium for examination using fine drawn out Pasteur pipettes. Drosophila were anaesthetized with diethyl ether and dissected in drops of liquid using watchmakers forceps and sturdy tungsten needles. The liquid was either an appropriate fixative or, in studies of unfixed material, DPBS (Drosophila phosphate buffered saline [Robb 1969]) or certain other solutions selected for experimental purposes (see chapters 5 and 6). The ovaries were teased apart with tungsten needles. Egg chambers were released from the epithelial sheath by rupturing it with tungsten needles.

LIGHT MICROSCOPY

Preparations were examined and photographed with a Zeiss Oberkochen Ltd. WL or Universal microscope using bright field, phase contrast or Nomarski interference contrast optics. Measurements were made with an eyepiece graticule or were taken from photographs. The eyepiece graticule was calibrated and the camera magnification was assessed using a micrometer slide.

STAINING PROCEDUES FOR LIGHT MICROSCOPY

Silver staining [Corliss 1953]

Egg chambers were fixed for 2 - 3 minutes in Champy [1911] followed by 24h in Da Fano (1% $Co(NO_3)_2$, 1% NaCl, 10% v/v commercial Formalin). They were pipetted onto microscope slides and coated with saline gelatine (8% gelatine, 0.05% NaCl) melted at 60°C. The slides were cooled on ice to set the gelatine and placed in ice cold 3% $AgNO_3$ in the dark for 20min. They were rinsed with cold distilled water and irradiated (while covered with iced distilled water) with an ultraviolet lamp until an orange colour developed. They were placed in 50% ethanol, dehydrated and mounted in Canada balsam.

Feulgen staining

Egg chambers were fixed (1h) in 3:1 ethanol:acetic acid [Clarke 1851] or in formaldehyde-saline [Baker 1966], pipetted onto microscope slides and coated with 8% gelatine, melted at 37°C. The slides were cooled to set the gelatine and fixed overnight in formaldehyde-saline (initially at 0°C, but allowed to warm to room temperature). They were hydrolysed in 1M HCl (10min at 60°C), rinsed in distilled water, placed in Schiff's reagent (1.5h in the dark) followed by sulphurous acid (15min) and a wash in running water (5min). They were dehydrated and mounted in Canada balsam.

Methylene Blue staining

Resin embedded material (see below) was sectioned at 1μm and stained (60°C) with 1% methylene blue (in 1% borax, 70% v/v ethanol) for light microscopical examination.

ELECTRON MICROSCOPY

Material was fixed for transmission electron microscopy using one of the procedures set out in the table. Procedure A was used in an experiment decribed in Chapter 2. Procedure B was employed for all other fixations of Nassula and in early investigations of Drosophila. The concentration of the phosphate buffer used in procedures C and D was increased to reduce the slight cell swelling that occurs in Drosophila ovarioles when procedure B is used. Procedure C was adopted for later studies of Drosophila. Procedure D caused some shrinkage (material fixed using procedure D is illustrated in plates 71 and 81).

The fixed material was embedded in 2% agar (to faciliate handling and reorientation), dehydrated and embedded in thin layers of Araldite

resin as described previously [Tucker 1967]. Ciliates or egg chambers were selected for thin sectioning using phase contrast microscopy and their orientation in the section observed. They were cut out of the resin sheet and were glued to a resin stub at the desired orientation (i.e. they were oriented for transverse or longitudinal sections, of the cytopharyngeal basket in the case of Nassula, or of the egg chamber for studies of Drosophila oogenesis).

Thin sections (grey to silver interference colours) were cut with glass knives using an LKB Ultratome III. Sections were collected on formvar/carbon coated (chapters 1 to 4) or uncoated (chapters 5 and 6) copper grids. They were stained for 90min in uranyl acetate (a saturated solution in 50% ethanol) and 2min in lead citrate [Reynolds 1963]. They were examined with a Philips 301 electron microscope at 60kV (except plate 56: 80kV). Photographs were taken on Ilford EM4 glass plates or sheet film or on Kodak 4489 sheet film.

OTHER PROCEDURES

Fixation of Nassula cytopharyngeal baskets at the start of feeding, detergent isolation of baskets, drug treatments (colchicine, cytochalasin B), and heavy meromyosin decoration of actin filaments in Drosophila ovarian follicles were also undertaken. Details of these procedures are provided in the individual chapters concerned.

COMPOSITION OF FIXATIVES USED FOR ELECRON MICROSCOPY

A	Heliozoan Fixative [Bardele 1977, Roth *et al.* 1970]	1st Fixative (30s)	2.5% Glutaraldehyde 14mM PB, pH 7.6 3.7mM Sucrose 4.5μM $MgSO_4$ 6.5% Culture medium.
		2nd Fixative (30min)	After 30s an equal volume of 1% OsO_4 in 25% v/v culture medium, 75% v/v buffer (PB, sucrose, $MgSO_4$ as above) was added to to produce: 0.5% OsO_4 1.25% Glutaraldehyde 14mM PB, pH 7.6 3.7mM Sucrose 4.5μM $MgSO_4$ 45% v/v Culture medium.
		Rinse (3 X 5min)	28mM PB, pH 7.6 7.3 mM Sucrose 9μM $MgSO_4$.

B	Fresh water Ciliate Fixative [Tucker 1967]	1st Fixative (30min)	2.5% Glutaraldehyde 16mM PB, pH 7.6 33mM Sucrose.
		Rinse (3 X 5min)	18mM PB, pH 7.6 110mM Sucrose.
		2nd Fixative (30min)	1% OsO_4 8mM PB, pH 7.6 110mM Sucrose.
C	Drosophila Fixative	1st Fixative (30min)	2.5% Glutaraldehyde 45mM PB, pH 7.6 33mM Sucrose.
		Rinse (3 X 5min)	50mM PB, pH 7.6 110mM Sucrose.
		2nd Fixative (30min)	1% OsO_4 25mM PB, pH 7.6 110mM Sucrose.

D	Double strength *Drosophila* Fixative	1st Fixative	2.5% Glutaraldehyde
			90mM PB, pH 7.6
		(30min)	33mM Sucrose.
		Rinse	100mM PB, pH 7.6
		(3 X 5min)	110mM Sucrose.
		2nd Fixative	1% OsO_4
			50mM PB, pH 7.6
		(30min)	110mM Sucrose.

PB : KH_2PO_4 / Na_2HPO_4 Buffer.

PART A

FINE STRUCTURE AND MECHANICAL DESIGN OF THE CYTOPHARYNGEAL BASKET IN THE CILIATE *NASULA*

CHAPTER 1

CHANGES IN THE MICROTUBULE PACKING DURING STRETCHING OF THE ELASTIC SHEATH

INTRODUCTION

Microtubules are known to be involved in a very diverse range of functions in cells (see introduction to thesis). The microtubules often or always have a mechanical role to play in the systems they are part of. This applies to cytoplasmic microtubules, such as those involved in cell division or in the transport of materials, as well as to microtubules in systems such as cilia or flagella, where the mechanical role is more obvious. In systems where many of the components structurally and functionally associated with microtubules have been identified it appears that these components, rather than the microtubules themselves, are responsible for many of the active functions performed by the systems. This chapter contains evidence that components other than microtubules are responsible for the elastic properties of the cytopharyngeal basket of the ciliate Nassula. It is possible to prepare this organelle for electron microscopy in two mechanically different states. This provides an opportunity to observe the behaviour of various components of the organelle and gives a rare insight into the types of mechanical

properties possessed by microtubules and associated structures.

The cytopharyngeal basket of Nassula becomes stretched during one stage of the ciliate's feeding process. This stage was examined using electron microscopy to discover if any of the components show changes from the unstretched resting (i.e. non-feeding) state. This gave information about which components are deformable. One of the deformable structures (the sheath) showed notable changes in appearance on stretching and therefore information was gained about its constituents. Because the sheath appears to behave elastically it is concluded that some of its components must have elastic properties. The most likely candidates for the elastic elements in the sheath are links between the microtubules. The possible nature of such links will be discussed.

The organization of the cytopharyngeal basket in **Nassula aurea**

A brief description of the cytopharyngeal basket will be given here to help orient the reader. The structure has been described in detail previously [Tucker 1968] for a larger unidentified species of Nassula, the cultures of which have unfortunately been lost. The species used in this study, N. aurea is very similar, the main differences being in the number and sizes of the basket components.

Algal filaments enter the organism at the cytostome which is situated at the bottom of a pit in the pellicle, the oral atrium, in non-feeding organisms, but which is everted from the oral atrium

during feeding. The cytostome is surrounded by a thickened portion of the pellicle, the collar, to which the top of the basket is attached. A circular palisade (55μm long, 12μm in diameter) of microtubular rods runs from top to bottom of the basket (see plate 4 and figure 1.1 which illustrates the arrangement of the components of the basket in the resting (a) and feeding (b) states). The rods are not exactly parallel to the longitudinal axis of the basket but slope at a slight angle. About 12μm from its top the palisade is encircled by the dense annulus where the microtubules are embedded in dense staining material. Below this level it is encircled by the microtubular sheath. Beneath the collar, the rods are encircled by the fibrous annulus which is composed mainly of fibrous or microfilamentous material.

The crests are ridges of microtubules attached to the rods above the dense annulus and to the sheath below. Over the sheath they spiral at an increased angle to the longitudinal axis and reduce in size until they disappear at about the mid-level of the basket.

FIGURE 1.1

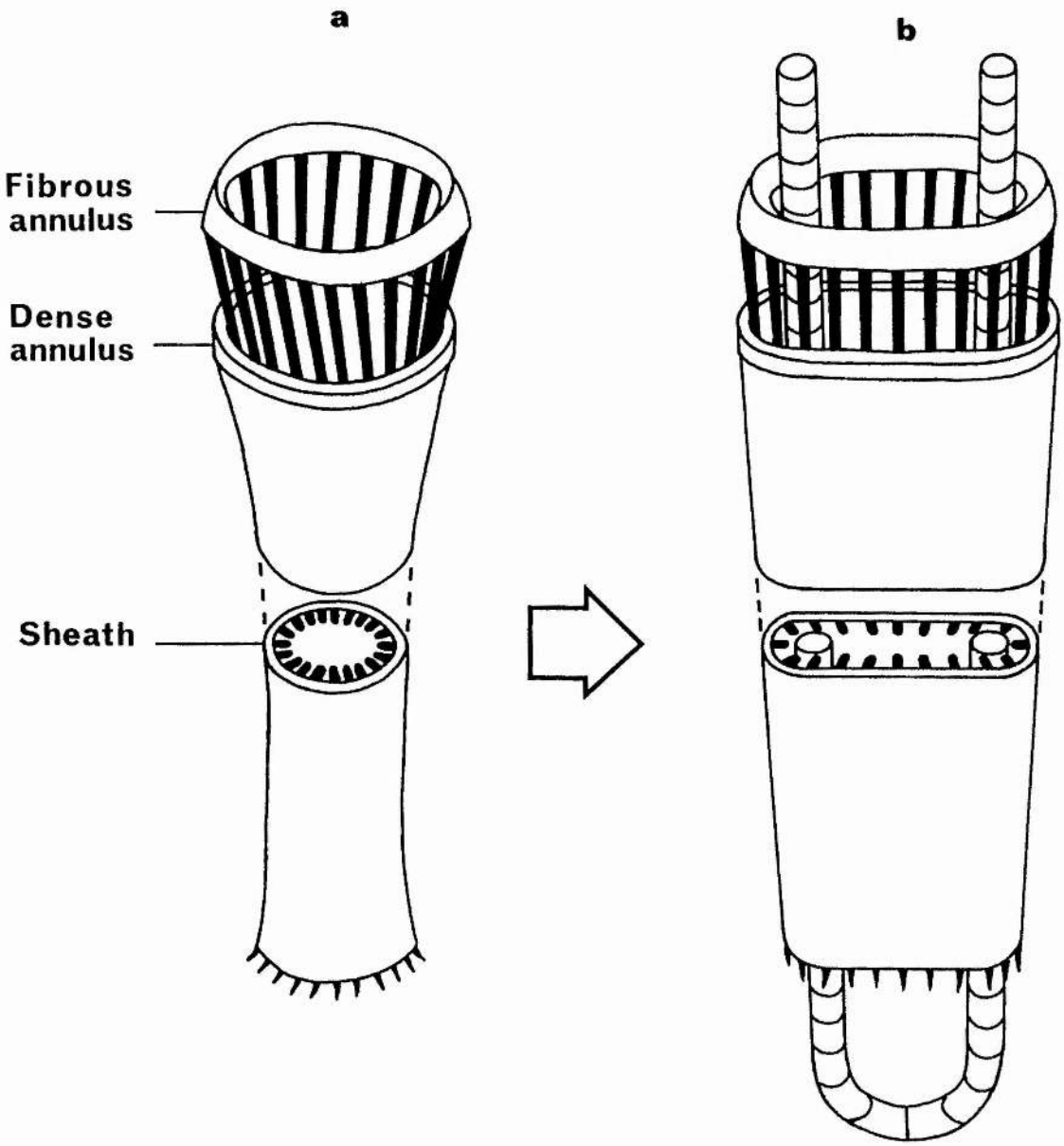
Schematic diagrams showing lateral views of cytopharyngeal baskets drawn with a portion of each basket removed to show the cross-sectional shape of its sheath and the arrangement of rods (blocked in black) inside it. Crests have been omitted for clarity.

a Resting basket.

b Stretched basket during the ingestion of an acutely bent portion of an algal filament

This figure is taken from Wellings and Tucker (1979) and was drawn by Dr. J.B. Tucker, to whom I am very grateful for permission to include it.

Figure 1.1



RESULTS

Algal filaments are bent into a hairpin-shape as they are initially drawn into the basket [Tucker 1968]. The basket and its sheath are maximally stretched as the bent portion of the filament passes through the basket's interior (figure 1.1). This initial stage of ingestion is only 1s or 2s in duration, but can be distinguished when supensions of starved ciliates and algae in drops of culture medium in watchglasses are examined with a dissecting microscope using transmitted light.

Ciliates were fixed with the basket in the stretched position as follows. Small drops (about 0.05ml) of culture medium containing starved Nassula in suspension were pipetted into watchglasses that each contained a drop (0.05ml) of culture medium with a small clump of algal filaments included. The ciliates started to feed shortly afterwards. The glutaraldehyde fixative was added to each watchglass as an algal filament started to bend into the basket of a ciliate in the watchglass in question. Ciliates which were fixed while their baskets were maximally stretched were isolated at this stage by pipetting them individually into a fresh watchglass containing fixative. Thereafter fixation and embedding were conducted as described above (see Materials and Methods, procedure B).

A ciliate may attach at any point along an algal filament. At such a point the filament becomes bent to form a hairpin-shape as it enters the top of the basket. The basket becomes more or less elliptical in

cross section as the 'hairpin' is drawn into it. At this stage the top end of the basket is the most distended. When the bent portion of the filament has moved out of the bottom of the basket the two strands of the filament become clenched together side by side, apparently because of elastic recoil of the basket, which occurs once the distortion caused by the bent portion of the filament is removed from the basket. The basket returns nearly to its nonfeeding size and shape. At an intermediate stage when the bent portion is just passing out of the basket the top end of the basket begins to close on the filament, which now forms a loop in the basket. The lower portion of the basket is the most distended at this stage. The changes in shape of the basket are produced by the resistance to bending of the filament and the resistance to stretching of the basket, as the filament is propelled through the basket.

The sectioned baskets considered below were in the intermediate stage. In this stage the dense and fibrous annuli are stretched to only a relatively small extent. Their structure does not differ to any marked extent from that found in unstretched baskets (plates 1,2). The portions of the annuli between the rods become extended. Therefore the fibrous material in the fibrous annulus and the region with dense staining material between the microtubules in the dense annulus are deformable. The microtubules in the dense annulus become further apart (plate 2). In this respect the dense annulus is similar to the sheath (see below).

During this stage the midportion of the sheath becomes stretched

so that it has a more or less elliptical cross-sectional profile (plate 3) with a circumference of about 50μm (an increase of about 90% relative to the nonfeeding condition: compare with plate 4), but there is no marked change in its breadth (i.e. radial thickness) along the sides of the elliptical cross-sectional profile. The two strands of the algal filament are situated near opposite ends of the sheath's elliptical cross-sectional profile as would be expected if the stretching and elliptical deformation is due to the strands pressing the basket and its sheath outwards at right angles to the longitudinal axis of the basket (plate 3). At the ends of elliptical cross-sectional profiles the sheath breadth is sometimes greatly reduced and occasionally the sheath appears to be discontinuous as if it has split longitudinarily during stretching (plate 6). This is especially apparent in sections close to the bottom of the sheath. It is not clear if splitting and thinning of the sheath actually takes place in Vivo or whether these distortions occur during fixation. Microtubules flanking the outer surfaces of rods are attached by intertubule links to some of those lining the inside of the sheath. The centre-to-centre spacing of adjacent rods is about 1.4μm in resting baskets but about 2.5μm in stretched baskets. However there is no change in the spacing or arrangement of microtubules within individual rods.

Microtubules are arranged in slightly curved but widely spaced rows in stretched sheaths (plate 5). Each row is oriented more or less radially with respect to the basket's median longitudinal axis. The distances between pairs of microtubules (in the maximally stretched

regions of the sheath away from the close vicinity of the rods) in radial (i.e. within a row) and circumferential (between rows) directions were measured. For unstretched baskets the mean centre-to-centre radial separation was 42.6nm ± 1.3nm (mean ± standard error), but the mean centre-to-centre interrow spacing of microtubules measured in directions parallel or nearly parallel to the sheath circumference was 57.3nm ± 1.5nm. The difference is statistically significant at a 1% level. Errors due to variation in magnification were avoided by taking radial and circumferential measurements from the same micrographs. Radially oriented rows can occasionally be detected in micrographs of unstretched baskets (plate 7). The rows appear to be an important structural feature of the sheath. They move apart during stretching. For stretched sheaths the mean centre-to-centre radial separation of microtubules is 42.4nm ± 1.2nm: almost the same as in unstretched sheaths. Detailed statistical comparison of measurements from different baskets, such as unstretched and stretched specimens, is not practical. The baskets vary in size and in number of rods and it is not possible to identify the level in a basket which any particular section comes from sufficiently accurately to make comparison valid. The variances of measurements from unstretched and stretched baskets are not homogeneous (e.g. as tested by Bartlett's test) so simple analysis of variance procedures could not be used to separate the effects of stretching from the other causes of variation, even if a large number of baskets were sectioned. The mean centre-to-centre circumferential spacing of microtubules in stretched sheaths is 136.9nm ± 9.0nm, except in the vicinity of some rods (see below). This is 2.4 times

greater than the interrow spacing of an unstretched sheath. Thus the radial rows do not stretch in the radial direction, but become further apart in the circumferential direction.

Fine circumferentially oriented strands of dense material which perhaps represent extensible links can sometimes be detected running between microtubules in adjacent radial rows (plate 8). Sometimes these strands do not run directly from microtubule to microtubule but rather appear to be part of a meshwork in which microtubules are embedded. Structures which possibly represent thicker links can sometimes be seen running radially between microtubules (plate 5).

Crest microtubules are packed hexagonally and are joined together by well defined intertubule links (plate 2); like rod microtubules they do not appear to become more widely spaced when the basket is stretched. Rod and crest links thus appear to represent links of the relatively inextensible variety. Stretching is very much less marked than elsewhere for each portion of a sheath that lies between a rod and a crest (plate 5). This is to be expected if rods and crests are not deformed during stretching and if sheath microtubules are linked to those of rods and crests, as appears to be the case where the surfaces of rods and crests are positioned against the sheath.

Extensible models

The ways in which microtubules that are joined by combinations of

flexible elastic links and rigid inelastic links might be rearranged when tension is applied to a microtubule/link array at right angles to the longitudinal axes of the microtubules has been explored with extensible models (plate 9). In the models cork discs represent microtubules, portions of elastic rubberbands represent flexible elastic links, and wooden matchsticks marked with a crossbanding pattern represent inextensible links. In model A discs are arranged hexagonally and each disc is linked to its neighbours by flexible elastic links (plate 9A). None of these 'rows' of discs in the array is oriented at right angles to the direction of tension applied during stretching. The array decreases markedly in breadth (i.e. the decrease occurs in a direction at right angles to the direction at which tension is applied) when stretching occurs (plate 9A'). Discs are similarily arranged in model B, but discs in one of the sets of disc rows, those running diagonally across the longitudinal axis of the array, are joined by stiff inextensible links. All the other links are elastic and flexible (plate 9B). Before stretching the inextensible links run at an angle of 60^{o} to the direction in which tension is subsequently applied. During stretching the discs twist so that the inextensible links lie more nearly parallel to the direction of tension applied, and the breadth of the array decreases (plate 9B'), but not as markedly as in model A. In model C discs are arranged hexagonally and all the links are elastic (plate 9C), but one set of disc rows runs at right angles to the direction of tension application. These rows shorten and the array decreases markedly in breadth during stretching (pate 9C'). Links in the rows are subjected to compression. Discs are identically arranged in model D (plate 9D),

but the rows do not shorten and the breadth of the array remains constant during stretching because the links joining discs together in the rows at right angles to the direction of tension application are stiff and are not deformed by the compressive forces to which they are subjected (plate 9D'). Discs have a square packed arrangement in model E and all the links are elastic and flexible (plate 9E). The breadth of the array remains constant during stretching (plate 9E'). In this model, as in all others, rows of discs that run across the array (either diagonally or at right angles to the direction of tension application) become more widely spaced during stretching.

DISCUSSION

Microtubule packing and linkage in the sheath

Sheath microtubules are apparently not packed and linked together in a very well ordered arrangement. They are arranged in rows that are oriented more or less radially with respect to the cross-sectional profiles of both stretched and unstretched sheaths. In unstretched sheaths the rows sometimes appear to be packed together to form a hexagonal arrangement of microtubules; sometimes the arrangement is closer to a rectangular array (plate 7). The case for the existence of links will be discussed in terms of radial links which join sheath microtubules in the same radial rows and circumferential links oriented parallel or nearly parallel to the sheath's circumference.

Why are so few clearly defined links detected in the sheath? Presumably some material must connect the microtubules or the basket would split apart during feeding. There are several possible reasons why any links present should not stand out clearly in electron micrographs. The links might have a rather loosely coiled or convoluted configuration, especially when unstretched, so would not have a typical 'link-like' appearance. Links of this type would explain why sheath microtubules are not packed in a very regular pattern as they would not place as vigorous constraints on packing as rigid links. The links could be thin or might not be consistently oriented in the plane of section (i.e. in a plane normal to the longitudinal axis of the basket). Then only links which happen to fall

in the plane of section would stand out clearly in electron micrographs. Extensible links between the doublets of cilia and flagella [Dentler & Cunningham 1977, Warner 1976a] are also often difficult to detect when cross-sections of the microtubules to which they are attached are examined (c.f. the problems of detecting rod links in longitudinal sections, chapter 2).

Are links really present? There is not suficient evidence to state unequivocally that links are present in the sheath. There are alternative possibilities that can not be ruled out. For example it is possible that the sheath microtubules are connected by a meshwork of material rather than by links in the conventional sense (i.e. intertubule connections more or less permanently attached to microtubules at both ends). However the occasional discovery of link-like structures and their prevalence elsewhere in the cytopharyngeal basket perhaps makes the existence of links more plausible.

The case for elastic links

It is of course not possible to measure directly changes in physical parameters (such as deformation, tension) for any links in the cytopharyngeal basket, or even for the whole basket. However the sheath and annuli return to close to their normal dimensions and circular cross-sectional shapes in less than 1s immediately after the acutely bent portion of an algal filament has passed through them. Therefore they appear to have elastic properties (i.e. they deform

when tension is applied to them, in this case as a result of cytoplasmic streaming propelling an object larger than the lumen of the nonfeeding basket through the basket [Tucker 1978], and return to their former dimensions when tension is removed). Components that elastically resist stretching in the sheath and annuli could provide restoring forces for the return of the basket to its unstretched shape. Do circumferential links break when the the circumferential spacing of sheath microtubules increases by a factor of 2.4 during stretching? Examination of baskets fixed no longer than 5min after stretching reveals that sheath microtubules are packed in the normal resting arrangement [Tucker 1978]. The apparent elastic behaviour of the sheath after stretching also suggests that the circumferential links do not break, that they can be stretched in vivo, and probably resist stretching elastically. The appearance of fine strands of material running circumferentially between the radial rows of stretched sheaths (plate 8) is more compatible with link stretching than it is with link breakage.

No change is observed in any of the microtubules that make up the basket. It is probable that some of them bend slightly when the basket is stretched, and so act as minor elastic components. However the major elastic properties must reside in the material between the microtubules. The properties of the rigid elements of the basket such as the rods must also be related to the material (links) between their microtubules. Certain microtubular bundles which perform active bending movements or are otherwise associated with active intracellular movements exhibit quite marked shape changes.

Examinations of such bundles, for example ciliary and flagella axonemes [Warner 1976b, Olson & Linck 1977] certain axostyles [Bloodgood 1975], suctorian tentacle axonemes [Tucker 1974], and cytopharyngeal baskets [Tucker 1968, 1972a], are beginning to indicate that from a mechanical point of view there are two main types of intertubule links: inextensible links which prevent microtubules sliding past each other or from becoming more widely spaced, and links of a less rigid nature which can be stretched in vivo and tolerate a certain amount of microtubule sliding and/or change in microtubule spacing without detaching from the microtubules they interconnect at either of their ends. For example, there is evidence that the interdoublet nexin links of cilia and flagella are extensible and stretch elastically during the sliding of the microtubule doublets which they interconnect [Warner 1976a, Olson & Linck 1977]. Links of similar appearance interconnect microtubule doublets in the axonemes of Sciara sperm flagella which do not have the usual 9 + 2 organization. These links also seem to be extensible [Dallai, Bernine & Giusti 1973]. The links that interconnect sleeve microtubules in the tentacular axonemes of the suctorian Tokophrya stretch when the tube-shaped sleeve increases in circumference during feeding; links lengthen by about 700% and decrease in thickness by about 50% [Tucker 1974].

Sheath stretching and the possible mechanical organization of its links

Many elastically extensible biological structures are fairly

flexible especially when in a markedly expanded configuration. This is also likely to be the case for the circumferential links so that flexible rubber band links in the extensible models may mimic the action of circumferential links fairly closely.

Model A (plate 9A,A') does not behave like the sheath during stretching. It decreases considerably in breadth. Correlated with this none of its disc rows are oriented as nearly at right angles to the direction of stretching as the radial rows in the sheath. The models clearly indicate that the near perfect radial orientation of microtubule rows could be a major factor in determining that sheath breadth does not decrease markedly during stretching. For example, models D and E (plate 9D,D',E,E') mimic the deformation of the sheath fairly closely in so far as the disc rows that correspond spatially to radial sheath microtubule rows become more widely spaced, spacings of discs within these rows do not change, and the breadths of the arrays also remain constant. This constancy is ensured in model D, and to a lesser extent in model B (plate 9D,D',B,B') by stiff inextensible 'radial' links but all links in model E are extensibleand flexible. Are radial and circumferential sheath links identical? Are they all elastic and extensible? In regions of the sheath where microtubules are more or less hexagonally packed the radial rows are likely to shorten during stretching as do the 'radial' rows of discs in model C (plate 9C,C') unless microtubules in the same row are prevented from moving more closely together. It is not unreasonable to suppose that extensible links might have considerable resistance to compression and shortening to less than their resting lengths. Such links would

however be subject to tension during stretching even when they run along radial microtubule rows in cases where these are not oriented perfectly radially. Some parts of some of the radial microtubule rows run at angles of up to 40^{o} to the true radial orientation with respect to the cross-sectional profile of stretched sheaths. Whether this would result in sufficient extension and flexion of radial links in the event that they have the same mechanical properties as the circumferential links) for production of clearly detectable changes in sheath breadth and intra-row microtubule spacing (as in model A) cannot be predicted with any certainty in the absence of quantitative data about the mechanical properties of the links. Hence the behaviour of the models during stretching does not unequivocably demonstrate whether radial links are inextensible or not.

However, there are other indications that radial links are inextensible and that they differ in dimensions and composition from circumferential links. Firstly, the radial spacing of microtubules in the unstretched sheaths of resting baskets is less than their circumferential spacing. Since the spacing of microtubules in microtubule bundles is apparently defined by link length and arrangement [Bardele 1977, Tilney 1971, Tucker 1977], this is a strong indication that the lengths of radial links are different from those of circumferential links. Secondly, resistance to stretching of the portions of the sheath that are situated between the inextensible rods and crests will be very marked and as described above if radial links are stiff, inextensible, and join sheath microtubules to crest and rod microtubules. Extensible radial links would be much less effective at

transmitting resistance to circumferential stretching across the sheath between a rod and a crest. Hence the sheath microtubules could be joined by inextensible radial links holding them together in radial rows that can be stretched apart because they are interconnected by elastic extensible circumferential links. The non-rigid nature of the latter could allow some freedom in the pattern the rows pack together in.

CHAPTER 2

VARIOUS FEATURES OF THE ORGANIZATION OF THE CYTOPHARYNGEAL BASKET RELATED TO ITS FUNCTION AS A FEEDING ORGANELLE

INTRODUCTION

This chapter brings together certain minor experiments and discoveries made during the course of my studies on Nassula aurea which all relate to the function of the cytopharyngeal basket. The general structural arrangement of the basket has been described above (see introduction to chapter 1). Three sets of evidence for the function of certain parts of the basket will be described in this chapter. First a type of microtubular bundle which has been discovered extending out from the sheath, and which could act as a 'guy rope' holding the basket in position during feeding. Second the nature of the links in the rods, the most rigid microtubular components of the basket, will be examined, and the problem of why they are difficult to detect in sections parallel to the longitudinal axes of the rods will be discussed. Third, changes in the electron microscopical appearance of the cytoplasm in regions known to be highly gelated from light microscopical examination, and the position of the cytostomal lamellae during the formation of the cytoplasmic extrusion will be described.

RESULTS

Sheath extensions

A new component of the cytopharyngeal basket was discovered during attempts to isolate cytopharyngeal baskets for biochemical analysis. In preparations of cells gently lysed in buffer containing Triton X-100 long thin structures were found extending out of the basket. Electron microscopical examination of the region surounding the sheath revealed both small and large bundles of microtubules apparently splaying off the sheath. These extensions could explain the steady decrease in the number of microtubules in the sheath as the distance from the dense annulus (towards the bottom of the basket) increases. The possibility that the extensions help distribute forces applied to the basket will be discussed.

Nassula were lysed under very gentle conditions to produce a broken open pellicle with the basket still attached. The lysis medium consisted of 0.25% Triton X-100 in 3mM $MgCl_2$, 30mM Tris-HCl buffer, pH 7.5 (based on axostyle isolation buffer [Mooseker & Tilney 1973] with reduced Triton X-100 concentration). Nassula were concentrated by centrifugation and the lysis medium added in the centrifuge tube. The tube was given a quick shake by hand to break open the pellicle. The Nassula were then allowed to settle without further centrifugation and were pipetted onto microscope slides. The preparations were examined using Nomarski interference contrast and phase contrast microscopy.

This method results in better resolution of the basket than is possible for living animals, largely because food vacuoles and other materials which normally obscure the basket are removed. The crests can be observed along the length of the basket (plate 10). Long thin structures can be observed extending out from the bottom of the basket (plate 11). These are easier to observe using phase contrast microscopy. This is probably due to the greater depth of focus compared to Nomarski interference contrast microscopy. The extensions tend to curve around and do not lie in one plane. They end free in the medium. They range from about 35μm to about 90μm in length. Therefore they are long enough to reach across the organism from the basket to the pellicle on the opposite side of the organism (approximate dimensions: length of basket 60μm, length Nassula 175μm, width 100μm).

Transverse sections of the basket were cut at various levels from the dense annulus to the bottom of the basket. Small (20 or fewer microtubules) bundles of microtubules were often observed near the sheath from the mid-level down to the bottom (plate 12). Some also occur at greater distances from the basket (up to at least 15μm away) in the region with large numbers of cytopharyngeal vesicles which surrounds the basket. Towards the bottom of the basket the number of microtubules in, and the thickness of, the sheath becomes much reduced (plate 13). The splaying off of small bundles of microtubules could explain these reductions.

The small bundles of microtubules described above are probably too small to represent the extensions discovered using light microscopy. Occasionally larger bundles (more than 100 microtubules) can be observed (plate 13a). These probably are the structures that were observed by light microscopy. Links between the microtubules in these bundles can occasionally be observed (plate 13b).

No attempt was made to follow the bundles of microtubules through the cytoplasm away from the basket. However bundles of microtubules can be observed close to the pellicle and occasionally inserting on it (plate 14). Therefore it is conceivable that the extensions from the sheath are anchored on the pellicle. However subpellicular microtubules, which are often associated with the basal bodies of the kineties, are common in ciliates [Franke 1971, Tucker 1972a] so there may not necessarily be any relationship between the bundles near the basket and those near the pellicle.

Links between rod microtubules

When cytopharyngeal baskets are sectioned transversely links can be readily observed between the rod microtubules (plates 2,23). The microtubules are hexagonally packed and correspondingly have six links per microtubule, approximately but not exactly regularly spaced [Tucker 1968]. The links appear practically as dense as the microtubule walls [Tucker 1968]. However in longitudinal sections the links can not be clearly observed using conventional fixation and staining. Material can sometimes be observed joining the microtubules.

This can have the appearance of irregular sheet-like, or, more rarely, peg-like links [Tucker 1968 (for the larger unidentified species of Nassula: the links in N. aurea are very similar)]. Two methods which improve the appearance of links in heliozoans were tried, in order to improve the appearance of the rod links. First a special type of fixative [Roth et al. 1970, Bardele 1977], in which the solution containing osmium tetroxide is added to the glutaraldehyde fixative after 30s of fixation have elapsed, was used (Procedure A in Materials and Methods chapter). Organisms fixed in this way are very similar in appearance to those fixed with conventional separate glutaraldehyde and osmium tetroxide fixatives (such as procedure B in Materials and Methods), and no difference in the appearance of the rod links were observed. However fixation procedure A was used for all the studies described in this chapter except one observation from a feeding organism obtained as described in chapter 1. The other method employed was an additional staining step in 2% potassium permanganate, after or replacing staining in uranyl acetate, followed by a rinse in 2% citric acid [Tilney and Byers 1969]. The combination of potassium permanganate and lead staining produces a very dense and contrasty result. This leads at best to a slight improvement in the appearance of the links in longitudinal sections. However there is some indication that there are more small links present than have previously been reported. The nature of the links and the reasons why they stand out clearly in transverse sections and poorly in longitudinal sections will be discussed.

Various times for staining in potassium permanganate were tried

(10min - 30min). Ten minutes proved adequate to produce a large increase in density and contrast of staining. Omitting the uranyl acetate stain did not noticeably affect the results so this was done in later experiments. In regions of longitudinal sections of the rod which appear well oriented the microtubules are very densely stained and much material can be observed between them (plates 15, 16). However the arrangement of the material cannot be clearly made out. The density is not constant along the space between the adjacent microtubules, rather there are dense and less dense patches (plate 15:1). The variation in density is not regular (interval 60nm - 140nm) and in places it is absent. Smaller links can occasionally be distinguished. They are approximately 12nm long (intertubule distance), 8nm wide and they occur at intervals of about 12nm (plate 15:2). They can occasionally be detected where a rod passes out of the thickness of a section (plate 16). Careful examination of the micrographs reveal that small links are also present in the less dense patches between the microtubules as well as in the dense ones (plate 15:3). The small links are probably present along the length of the rod microtubules.

The cytoplasmic extrusion and changes in the appearance of the cytoplasm

The first stage in feeding is the evertion of the oral atrium and the formation of an extrusion of cytoplasm to which the algal filament becomes attached [Tucker 1978]. This stage was, by chance, discovered in longitudinal sections of an organism from the experiment described

above (the investigation of rod links with potassium permanganate staining). This organism was presumably in the process of making an extrusion against an algal filament when fixative was added. The corrugations of the collar and the cytostomal lamellae attached to them can be observed to point upwards into the extrusion. The appearance of the cytoplasm in the extrusion is unusual, correlating with its gelated nature as observed by light microscopy. Some changes in the appearance elsewhere in feeding organisms will also be described.

The positions of the collar and the cytostomal lamellae (plate 17) are raised in the extrusion compared to the resting position (plate 18). The subcytostomal lamellae hang downwards from the raised collar away from the rods. In sections grazing across the edge of the extrusion (plate 19) the close association between the cytostomal lamellae and the thick corrugations of the collar can easily be made out. The cytoplasm in the extrusion is full of the small cytopharyngeal vesicles which are normally associated with the basket. The cytoplasm has a fine granular appearance (plate 19). The granules are more tightly clumped than in normal cytoplasm, and they often appear aggregated around the lamellae. This could possibly represent changes in the cytoplasm responsible for gelation.

Some sections stained with potassium permanganate and lead citrate show a slight change in appearance in the cytoplasm at the boundary between the highly vesiculate region which surrounds the basket and the normal cytoplasm. The cytoplasm is again more finely granular in

the vesicular region, with some clumping of the granules between the vesicles (plate 20). This could represent the remnants of the cytoskeletal system which retains the vesicles close to the basket.

Plates 21 and 22 show a region of cytoplasm in a feeding organism (conventional fixation) where the algal filament has passed out of the bottom of the basket. The cytoplasm here is slightly fibrous in appearance. This could again represent the remnants after fixation of some system responsible for gelation.

DISCUSSION

Sheath extensions

The splaying off of small bundles of microtubules from the sheath could explain the reduction in number of microtubules in the cytopharyngeal basket as distance below the dense annulus increases. As it is not possible to detect the ends of microtubules without following them in serial sections this study does not reveal if any microtubules end in the basket. For the same reason it is not known whether the small bundles just terminate or whether they run for long distances through the cytoplasm.

The larger bundles observed near the bottom of the basket can probably be identified with the structures discovered by phase contrast microscopy. If so they are long enough to reach across the cell to the pellicle. It seems likely that they do insert on the pellicle, as they would then be able to act as 'guy ropes' and help distribute the forces on the basket during feeding. These forces must tend to pull the basket in the opposite direction to the movement of the algal filament (i.e. out of the cell). The processes are correctly positioned to resist such forces.

Links between rod microtubules

The use of the mixed glutaraldehyde/osmium tetroxide fixative did not lead to any improvement in the definition of the rod links in

longitudinal sections. It is unlikely that the problem is one of poor preservation.

The potassium permanganate/lead citrate staining combination leads to much more dense and contrasty staining than the conventional uranyl acetate/lead citrate. However this has not lead to any substantial improvement in the definition of the links. It appears that both types of stains work in the same nonspecific manner. The denser staining may show up the small link structures slightly better than the conventional stain, probably as a result of the higher contrast.

The problems involved in detecting the links in longitudinal sections can be better understood by considering what would be included in a section, say 50nm thick, through the hexagonal array of microtubules and links observed in transverse sections, but parallel to the longitudinal axes of the microtubules. Because the electron microscope has a large depth of focus all objects lying above or below one another are superimposed in the image. The microtubules in transverse section are linked in lines each of which defines a plane of microtubules in the rod. There are three sets of planes which intersect at angles of 60^{o} and 120^{o}. The baskets in this study were oriented before sectioning so that longitudinal profiles of the rod microtubules would be obtained. The angles between the planes described above and the plane of the section are also critical in determining the appearance of links in the section. This cannot be adjusted before sectioning, but, as the basket is circular, it varies from level to level and favourable regions can be selected. In less

favourable regions the links are totally obscured by microtubules lying above or below them. Figure 2.1 shows the orientations of sections relative to the planes of links that give the most information. Sections x and y are parallel to one set of planes. They contain the microtubules and links at different depths, but because of the large depth of focus, and the resulting superimposition, they will produce identical images in the elctron microscope. Figure 2.2 shows some of the dimensions of a regular lattice of microtubules and links, based on a microtubule diameter of 24nm and a link length of 12nm. It is clear that any section of normal thickness (50nm or more) of type x or y will have parts of microtubule walls obscuring the links. A section thinner than 38nm would be necessary to avoid this problem.

Section z in figure 2.1 shows another orientation of section that could produce useful information. This is at 30^{o} to the sets of planes. The links are oriented normal to the plane of the section and therefore will be observed end on in the image. The links are again obscured by the walls of microtubules above and below them. The dimensions of figure 2.2 indicate slightly more than half of each of two microtubules will be included above and below one of the links. Nevertheless micrographs of this type have been published for another nassulid ciliate, Pseudomicrothorax dubius, which convincingly demonstrate the sheet-like nature of the links [Hausmann & Peck 1978]. Unfortunately no sections with end on links were obtained in this study. Probably an exceptional combination of thin sections and favourable positioning is required. One situation in which obscuration of links might be reduced is where a rod passes out of the section.

FIGURE 2.1

Diagram showing arrangement of microtubules and links in a transverse section of a rod. The material which would fall within three possible longitudinal sections, each 50nm thick, is delimitted by pairs of dotted lines. See page 54 et seq. for discussion of the effects the different orientations of the sections relative to the planes of microtubules have on the appearance of the rod links.

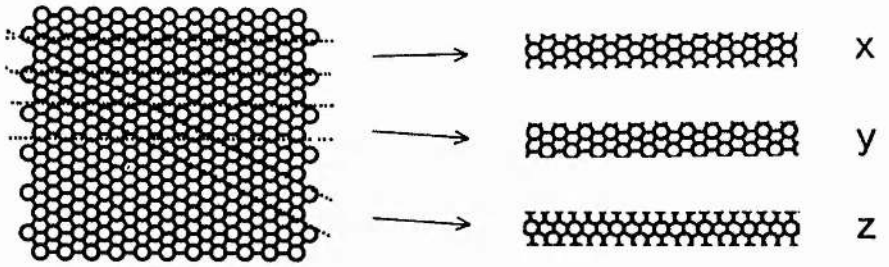
FIGURE 2.2

An enlargement of part of figure 2.1 with the distances (in nanometres) between various planes marked.

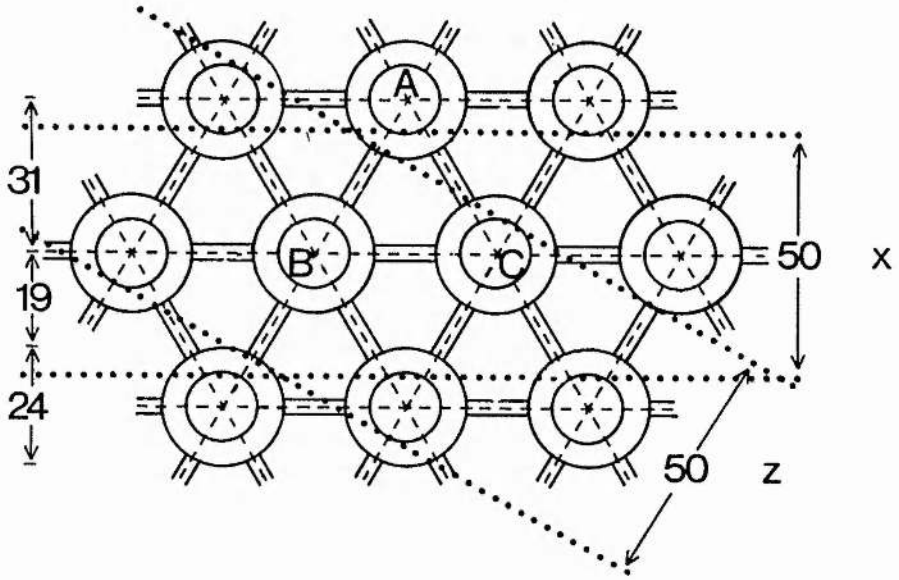
FIGURE 2.3

Diagram showing four possible arrangements of links between rod microtubules. See page 56.

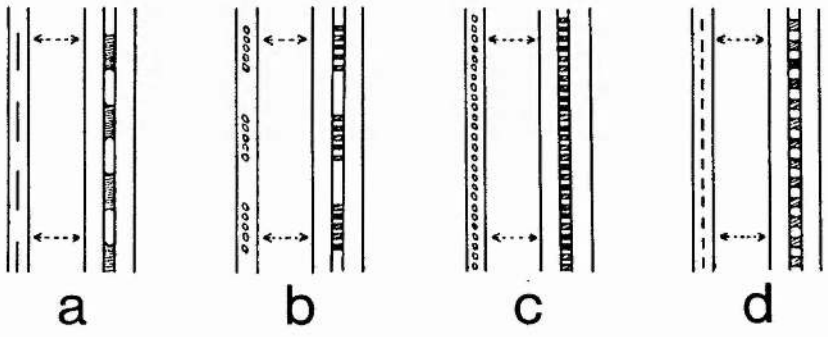
2.1



2.2



2.3



There is some indication that this is the case (plate 16). Small links can be observed in such regions.

The alternation of dense and less dense areas between the microtubules was described previously [Tucker 1968] with the suggestion that it represents sheet-like links (figure 2.3a). Occasional smaller links were observed. This was for the larger unidentified species of Nassula used in previous studies, but the nature of the links in N. aurea is probably very similar, as they appear identical in transverse sections. However the dense patches are not of regular size. Therefore it is possible that they are composed of a variable number of subunits (figure 2.3b). Some of the denser patches do appear to be made up of small links which would support this model. The occasional detection of small links in the less dense patches however suggests that the links are equally spaced along the microtubules, as in figure 2.3c or 2.3d.

Any model for the arrangement of the links must explain their good definition in transverse sections. The explanation in the case of sheet-like links would be that they are viewed edge on in transverse sections so that much more material intercepts the electron beam than it does when the sheet lies flat in longitudinal section. With small peg-like links the explanation would be that several lie above one another in transverse section, while they are examined singly in longitudinal sections. In transverse section the links usually appear to be approximately 6nm thick but occasionally thinner strands (about 3nm: plate 23). It is possible that the thicker appearance is due to

several thinner links only approximately in register. If this is the case the links could actually be about 3nm thick.

It is suggested that the denser and less dense patches do not represent any definite structure, such as sheet-like links. It is possible that they are due to variations in the amount of material in the section. Figures 2.1 and 2.2 illustrate that any links which are in a favourable orientation for observation will be partially obscured by other links (at angles of 60^{o}) and parts of microtubule walls. Microtubules and the links which connect them are never arranged in an absolutely precise pattern (as observed in transverse section [Tucker et al. 1975]). Slight variations in the positions of microtubules and links could produce the variation in the amount of material in the section and hence the variation in density. The appearance of small links in the less dense patches as well as in the denser suggests that a continuous arrangement of links (figure 2.3c or 2.3d) is the most likely. If the links are in fact 3nm thick the arrangement is that illustrated in figure 2.3d: small sheet-like links.

From consideration of the evidence discussed above it is concluded that the rod links are probably sheet-like (approximate dimensions: length, microtubule-to-microtubule, 12nm, width 8nm, thickness 3nm) and are continuous along the microtubules with a periodicity of about 12nm. Therefore the links could be similar to those in Pseudomicrothorax dubius [Hausmann & Peck 1978] but narrower and closer together. The links are probably smaller and more numerous than has previously been suggested [Tucker 1968]. This arrangement should

be as strong as one with larger, less numerous, links, and would explain the rigid nature of the rods equally well.

The cytoplasmic extrusion and changes in the appearance of the cytoplasm

The formation of the cytoplasmic extrusion has previously been described on the basis of light microscopy [Tucker 1968, 1978]. The discovery of an organism fixed at the cytoplasmic extrusion stage of the feeding process provides electron microscopical confirmation of these observations. The positions of the cytostomal and subcytostomal lamellae are as had previously been deduced.

The appearance of the cytoplasm in the extrusion and the basket could represent some remnants of systems responsible for gelation of the cytoplasm, or for holding cytopharyngeal vesicles in position. The vesicles remain in position around the basket despite the general flow of cytoplasm through this area. The appearance of the cytoplasm around the portion of the algal filament that has passed through the basket corresponds with a region that appears gelated in living organisms observed with Nomarski interference microscopy [Tucker 1978]. No thin or thick filaments such as have been observed for example in amoebae [Pollard & Weihing 1974] can be detected. If an actin/myosin system is responsible for cytoplasmic streaming it does not survive the fixation for electron microscopy.

PART B

THE CYTOSKELETON IN OOGENESIS

IN *DROSOPHILA MELANOGASTER*

CHAPTER 3

CHANGES IN SIZE, SHAPE AND ORGANIZATION OF THE FOLLICLE AND OOCYTE DURING OOGENESIS

INTRODUCTION

This chapter contains a description of changes at a cellular level in the Drosophila egg chamber as it develops, and which are relevant to the results to be described in subsequent chapters. This description is based on observations and measurements made with the light microscope. It is an extension of earlier work by R. C. King and others, and is intended to provide information in a more useful form for the interpretation of the cytoskeletal structures discovered in ultrastructural studies. The principle improvements in understanding the events which take place have been made by using measurements from material not distorted by fixation and by paying more attention to oocyte shape changes.

The introductory description of Drosophila oogenesis which follows will concentrate on the stages of egg chamber development most studied: the vitellogenic stages. This description is based on earlier studies [King et al. 1956, King & Vanoucek 1960, King & Koch 1963, Cummings & King 1969, King 1970, Mahowald 1972a,b]. The various stages in development are illustrated with light micrographs taken using

Nomarski interference contrast microscopy (plates 24 - 28) and also in figures 3.1, 3.5 and 3.6.

The Drosophila ovary is divided into 12 ovarioles, which are enclosed in a peritoneal sheath. Each ovariole consists of a thin muscular tube, the epithelial sheath, and the developing egg chambers which lie within. The epithelial sheath is attached at its anterior and dorsal end to the terminal filament of the ovariole and at its other end to the oviduct through which mature eggs leave the ovary. The germarium is situated within the epithelial sheath at its anterior end and is also attached to the terminal filament. New egg chambers are formed in the germarium. Each chamber consists of a cluster of 16 cells (cystocytes) which become surrounded by follicle cells. The posteriormost cystocyte becomes the oocyte, the remaining 15 the nurse cells. The cystocytes are interconnected by ring canals: large intercellular bridges formed by incomplete cell division (plates 29 and 48). The exterior surface of the follicle cell layer is covered by a basement lamina, the tunica propria. The newly formed egg chambers separate from the germarium, but remain attached to it, and to each other, by interfollicular stalks (plate 24). Each ovariole contains 5 or 6 egg chambers, each one at a later stage of development to that anterior to it. Normally only one vitellogenic or later stage egg chamber is found in each ovariole (exceptions are found in old flies which often retain mature eggs in the ovarioles, or in flies prevented from mating or denied medium to lay on).

The development of the egg chamber has been divided into 14 stages

[King, Rubinson & Smith 1956, Cummings & King 1969, King 1970] on the basis of a number of morphological criteria. The dimensions and some of the characteristics of the various stages are summarized in table 3.1. Stage 1 is the terminal 16-cell cyst in the germarium. Stages 2 to 6 are slightly elongate chambers of increasing size characterized by various features of the oocyte and nurse cell nuclear morphology (with Feulgen staining). Stage 6 is more or less ellipsoidal but in stage 7 the anterior end becomes slightly more elongate than the posterior producing a 'pear-shaped' chamber. Vitellogenesis begins at stage 8 and the rate of chamber enlargement increases substantially. The oocyte and the follicle cells that surround it are referred to as the oocyte chamber, the nurse cells and the follicle cells which cover them as the nurse chamber. From stage 9 the oocyte begins to enlarge more rapidly than the nurse chamber. By stage 10 it half fills the egg chamber. From this stage on cytoplasm is transported out of the nurse cells through the ring canals into the oocyte. During stages 11 and 12 the nurse cells decrease in size until only a small anterior cap remains. At the end of stage 12 the oocyte is nearly as large as the whole egg chamber. The vitelline membrane (the innermost layer of the egg shell) is layed down in stages 9, 10 and 11 (it does not become continuous until vitellogenesis is completed), the endochorion in stages 11 and 12 and the exochorion (the outermost layer) in stage 13. Development of the egg shell is more advanced at the posterior end of the oocyte than at the anterior end where the cellular movements described below are occuring. Stage 14 ends with the final maturation of the egg. Measurements of oocytes and whole egg chambers, primarily for stages 6 to 14, which illustrate these events will be found in the

results section.

This study is largely concerned with stages 9 and 10 where the most dramatic changes in size and shape of the oocyte occur. These changes, and the concomitant changes in the follicle cells will be described in more detail here. Some new observations and diagrams may be found in the results section. In stage 7, before vitellogenesis starts, the cuboidal follicle cells are evenly distributed round the 15 nurse cells and the oocyte. During stage 8 a posterior 'migration' (the cells move as a unit retaining a constant topological arrangement, they do not migrate individually) of the follicle cells begins and the anterior cells become thinner, and eventually squamous. At first the transition from tall columnar cells at the posterior end to thin squamous cells at the anterior is gradual (plate 25, see also figure 3.3). By stage 9 the transition is marked. By the end of stage 9 there is a sharp change between tall columnar follicle cells (about 20μm high) which cover the oocyte and very thin (0.5μm - 2μm) squamous cells which cover the nurse chamber (plate 26). As the oocyte expands into the egg chamber, elongating as it does so, the oocyte follicle moves with it. The cells flatten as this occurs, increasing their surface area in contact with the oocyte and with the tunica propria. During stage 10 the most anterior columnar cells of the oocyte follicle begin to extend between the nurse chamber and the oocyte (figure 3.1). These anterior centipetally migrating cells will form the anterior vitelline membrane and chorion, except for that in the vicinity of the micropyle. During stages 9 and 10 a group of follicle cells migrate from the anterior of the egg chamber, between

the nurse cells, to a final position on the oocyte/nurse chamber boundary close to the oocyte nucleus. These 'border cells' will form the micropyle and the surrounding vitelline membrane and chorion. Two groups of cells within the anterior centripetally migrating cells of the oocyte follicle, near what will become the dorsal surface of the egg, begin to differentiate in stage 11. These will form the two dorsal appendages which lie beside the remains of the nurse chamber in stage 12 and which elongate out of the anterior end of the egg chamber in stage 13, reaching their maximum length in stage 14. These become the respiratory horns of the mature egg which permit diffusion of air into eggs buried in food, through air passages in the horns and endochorion [Hinton 1960].

TABLE 3.1

The stages of development of the Drosophila egg chamber, their duration and representative dimensions of whole egg chambers and oocytes.

SYMBOLS

l_c	:	length of whole egg chamber	(/μm)
d_c	:	diameter of whole egg chamber	(/μm)
l_o	:	length of oocyte	(/μm)
d_o	:	diameter of oocyte	(/μm)

NOTES

a	excluding 0 to 200 μm of dorsal appendage
b	excluding about 250μm of dorsal appendage
[1]	David and Merle 1968
[2]	Cummings and King 1969

Dimensions in parentheses () for stage 12 are based on measurements of a very small sample of egg chambers and therefore could be unreliable.

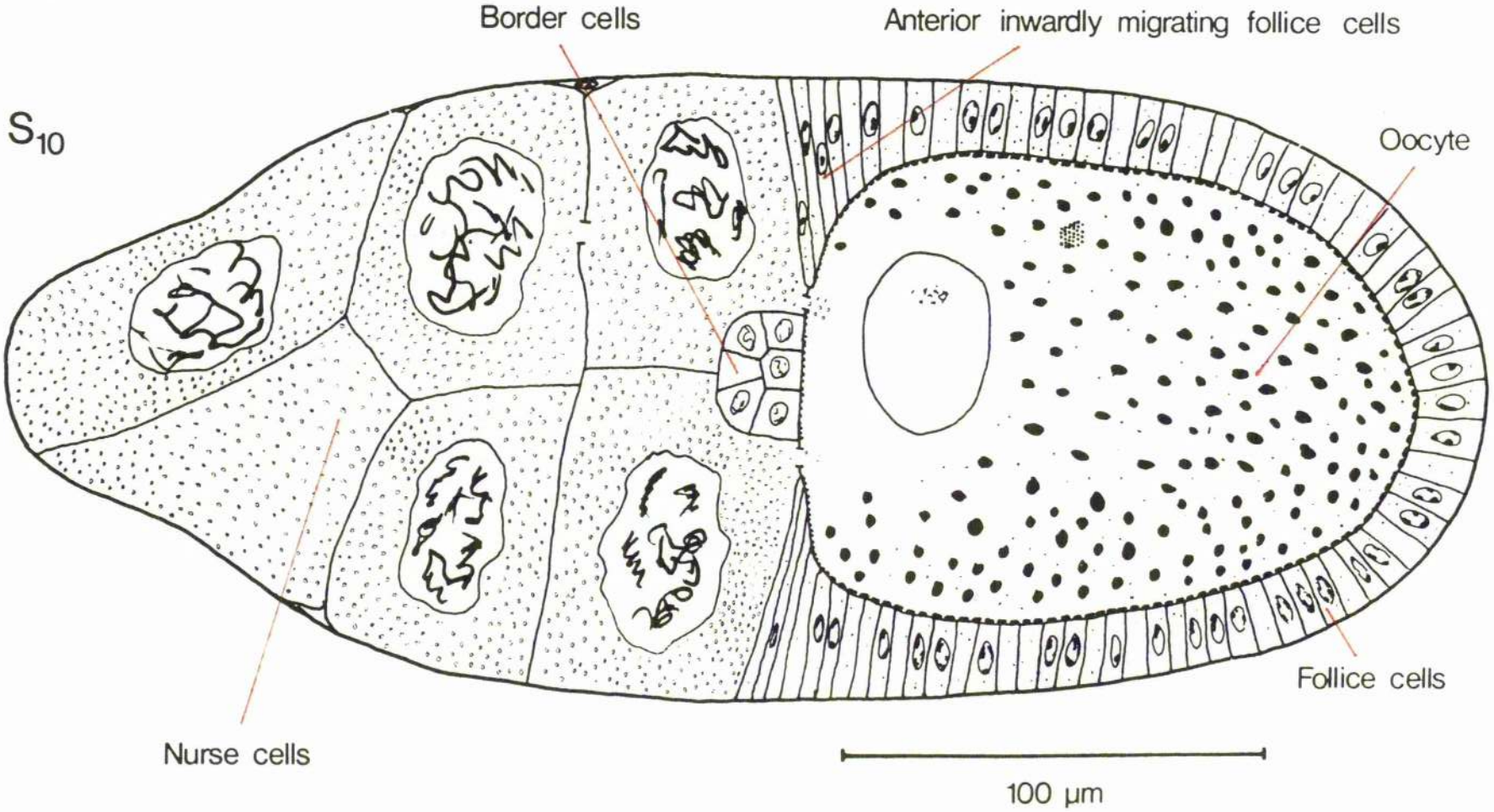
TABLE 3.1 Approximate dimensions of egg chambers and oocytes (/μm)

STAGE	DURATION /h[1]	WHOLE EGG CHAMBERS						OOCYTES				IDENTIFYING FEATURES
		Fixed[2]		Unfixed				Unfixed				
				min.		max.		min.		max.		
		l_c	d_c	l_c	d_c	l_c	d_c	l_o	d_o	l_o	d_o	
1	9.56	15	15	17	17	23	23					Terminal germarial 16 cell cyst
2	9.56	20	20	23	23	34	40					{
3	9.56	30	30	34	40	46	40					{ Spherical or slightly elongate
4	9.16	40	35	46	40	55	44					{ chambers with changing nurse cell and
5	2.61	50	40	55	44	90	55					{ oocyte nuclear morphology
6	8.45	65	50	90	55	120	65	10	20	20	40	{
7	8.69	100	60	120	65	160	80	15	30	25	55	Change in shape of anterior end of chamber
8	5.21	130	70	160	80	235	100	25	55	50	75	First yolk appears in oocyte
9	5.61	230	80	235	100	430	155	50	75	170	120	Oocyte 1/3 to 1/2 chamber length
10	5.13	260	110	430	155	500	170	160	100	240	170	Oocyte about 1/2 chamber length
11	0.40	400	150	470	155	520	170	240	100	400	170	Oocyte about 2/3 chamber length
12	1.90	400	160	(470	155	520	170	400	155	450	170)	Nurse chamber reduced to anterior cap
13	0.79	400	140	450[a]	155	520[a]	170	450	155	520	170	Appendages elongate up to 3/4 final length
14	1.98	450	140	470[b]	155	520[b]	170	470	155	520	170	Appendages reach final length, follicle cells die, egg matures.

FIGURE 3.1

Diagram of a stage 10 egg chamber as observed in median longitudinal section.

Figure 3.1


Border cells
Anterior inwardly migrating follice cells
S_{10}
Oocyte
Follice cells
Nurse cells
100 µm

RESULTS

Changes in egg chamber and oocyte dimensions

The staging system which has been described above is very convenient for outlining the main features of the morphological changes which occur as oogenesis progresses. However, it is not so suitable for comparing the dimensions and shapes of developing egg chambers. This is because the development of the egg chamber is in reality continuous, it is not divided into discrete steps. There is often a considerable increase in size from the beginning to the end of a stage. No average value for the dimensions of a stage can be taken as representative. Moreover there is never an abrupt transition between the characteristics of one stage and the following stage. Therefore stage identifications are necessarily approximate. For these reasons the length of the egg chamber, which is readily obtained for any chamber, will be used below as a reference point to which diameters and shapes can be compared. The approximate staging will however be retained to permit ready comparison with morphological descriptions.

The dimensions of egg chambers in table 3.1, quoted from Cummings & King 1969, are 'representative' ones for each stage, taken from fixed and embedded material, or from freeze-dried whole mounts. For this study a continuous set of measurements has been made from unfixed freshly isolated material in DPBS (Drosophila phosphate buffered saline: see Materials and Methods), using Nomarski interference

contrast microscopy. This avoids the problems of distortion and compression (occuring particularly in chambers not released from the epithelial sheath which contracts during fixation, compressing its contents) which tend to obscure the slight elongation of early egg chambers and the anterior elongation of stage 7 and subsequent egg chambers.

The dimensions (lengths and diameters) of a selection of egg chambers have been plotted in figure 3.2. The egg chambers were selected to give a range of sizes and not in proportion to their occurence. The measurements concentrate on stages 6 to 14 (as do all measurements in this chapter). However a few points have been added to figure 3.2 to illustrate the changes in shape that occur before stage 6. The lengths of stages 13 and 14 do not include the appendages, which elongate for up to 250μm from the anterior end of the egg chamber, and therefore are not entirely comparable to the lengths measured for the earlier stages. However the length measurements used are representative of the part of the egg formed by the majority of the structures in the egg chamber. The lengths of stage 10 to 14 egg chambers overlap to some extent and so have been represented by different symbols. These stages can be identified by characteristics such as the proportion of the egg chamber occupied by the oocyte and the stage of development of the appendages. The earlier stages cannot always be so readily identified for reasons given above so boundaries in terms of lengths which are more or less representative have been arbitarily chosen (these are also given in table 3.1). The precise position of the boundary between any two stages is not important to

any of the arguments that follow. The cross-sectional profiles of the later stages (10 to 14) are not perfectly circular. Therefore variation in the orientation of the dorso-ventral axis of egg chambers in the preparations from which measurements were taken probably introduces additional variation into the measured diameters.

A moderate change in shape of the whole egg chamber occurs in the early stages. Stage 1 is approximately spherical (d_c=l_c: symbols as in table 3.1). The chamber becomes gradually more elongate during subsequent stages until by stage 5 or 6 the ratio of diameter to length is approximately 1:1.4 ($d_c = 0.7\ l_c$).

A regression line has been calculated for stages 6 to 14:

$$d_c\ /\mu m = 36 + 0.26\ l_c\ /\mu m$$

This line is a close fit to the plotted points (/μm indicates that measurements are in micrometres). The intecept (a = 36μm) and the slope or regression coefficient (b = 0.26μm) are both significantly different from zero (SE_a = 0.9μm, t_a = a/SE_a = 40, df = 87, P<<0.001; SE_b = 0.006μm, t_b=43, df = 87, P<<0.001: SE_a and SE_b are the standard errors of a and b, t_a and t_b the Student's t ratios, and df the degrees of freedom). The existence of a straight line implies that the rate of increase of diameter with respect to length (dd_c/dl_c in the notation of calculus) is approximately constant. However as the intercept (a) is not equal to zero the shape of the chamber is not constant (d_c/l_c is not constant): therefore growth is not isometric.

Figure 3.3 illustrates the change in oocyte length with respect to egg chamber length for stages 6 to 14. The oocyte lengthens only very slightly during stages 6 and 7. From stage 8 the oocyte begins to grow more rapidly than the rest of the chamber. When the oocyte diameter is plotted against oocyte length (figure 3.4) there is again an obvious transition between stages 6 and 7 and stage 9. During stages 6 and 7 the oocyte is wider than it is long. It forms a 'cap' at the posterior end of the chamber, within the follicle, and is smaller than the individual nurse cells. It rapidly elongates during the following stages, and increases in diameter until it reaches its final diameter during stage 10, when it is approximately half its length. From then on elongation occurs at the expense of the nurse chamber as cytoplasm flows into the oocyte from the nurse cells. No simple mathematical relationship to relate the diameter to the length of the oocyte has been discovered. The relationship is obviously not linear. No regression analysis has been attempted. The growth of the oocyte is not isometric.

Figure 3.5 illustrates the changes in size and shape, that occur from stage 6 on, in the form of tracings of the outlines of whole egg chambers and of oocytes. The change of shape of the anterior portion of the chamber from the rounded form of stage 6 and earlier stages, which is similar to the shape of the posterior portion, to the more elongate 'pear-shaped' form of the subsequent stages is apparent. Another feature not discernable from the measurements is the flat boundary between the oocyte and the nurse chamber, which is maintained until the final reduction of the nurse chamber is complete.

FIGURE 3.2

Graph illustrating the variation of egg chamber diameter with length. A regression line is included for stages 6 to 14:

$$d_c\ /\mu m = 36 + 0.26\ l_c\ /\mu m$$

The approximate range of lengths for each stage is shown above the bottom axis.

Figure 3.2

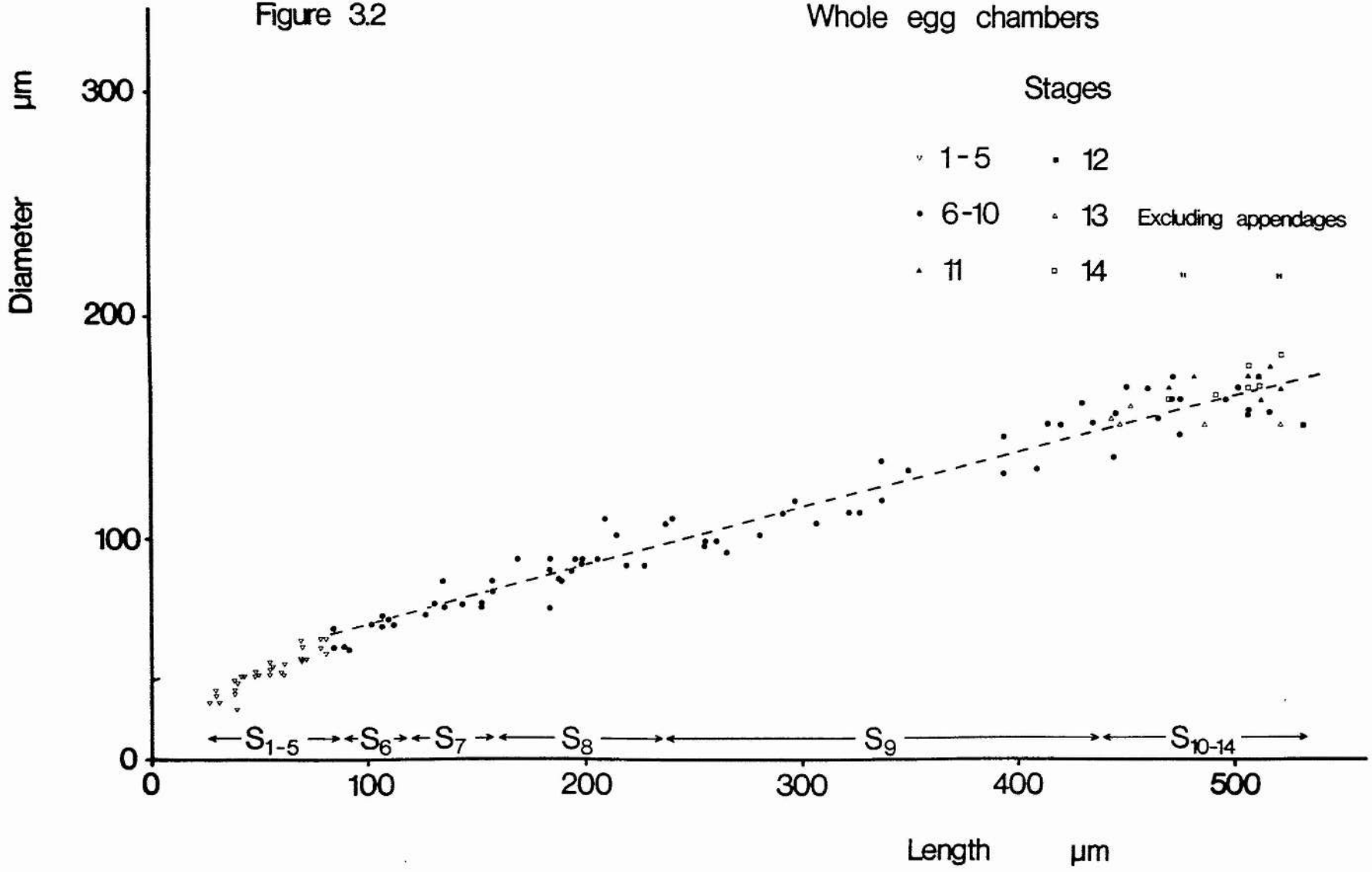

Whole egg chambers
Stages
1-5
6-10
11
12
13
14
Excluding appendages
Diameter µm
300
200
100
0
0
100
200
300
400
500
Length µm
S1-5
S6
S7
S8
S9
S10-14

FIGURE 3.3

Graph of length of oocyte against length of whole egg chamber for stages 6 to 14. The approximate range of lengths for each stage is shown. Symbols as in figure 3.2. The line $l_o = l_c$ is plotted.

Figure 3.3

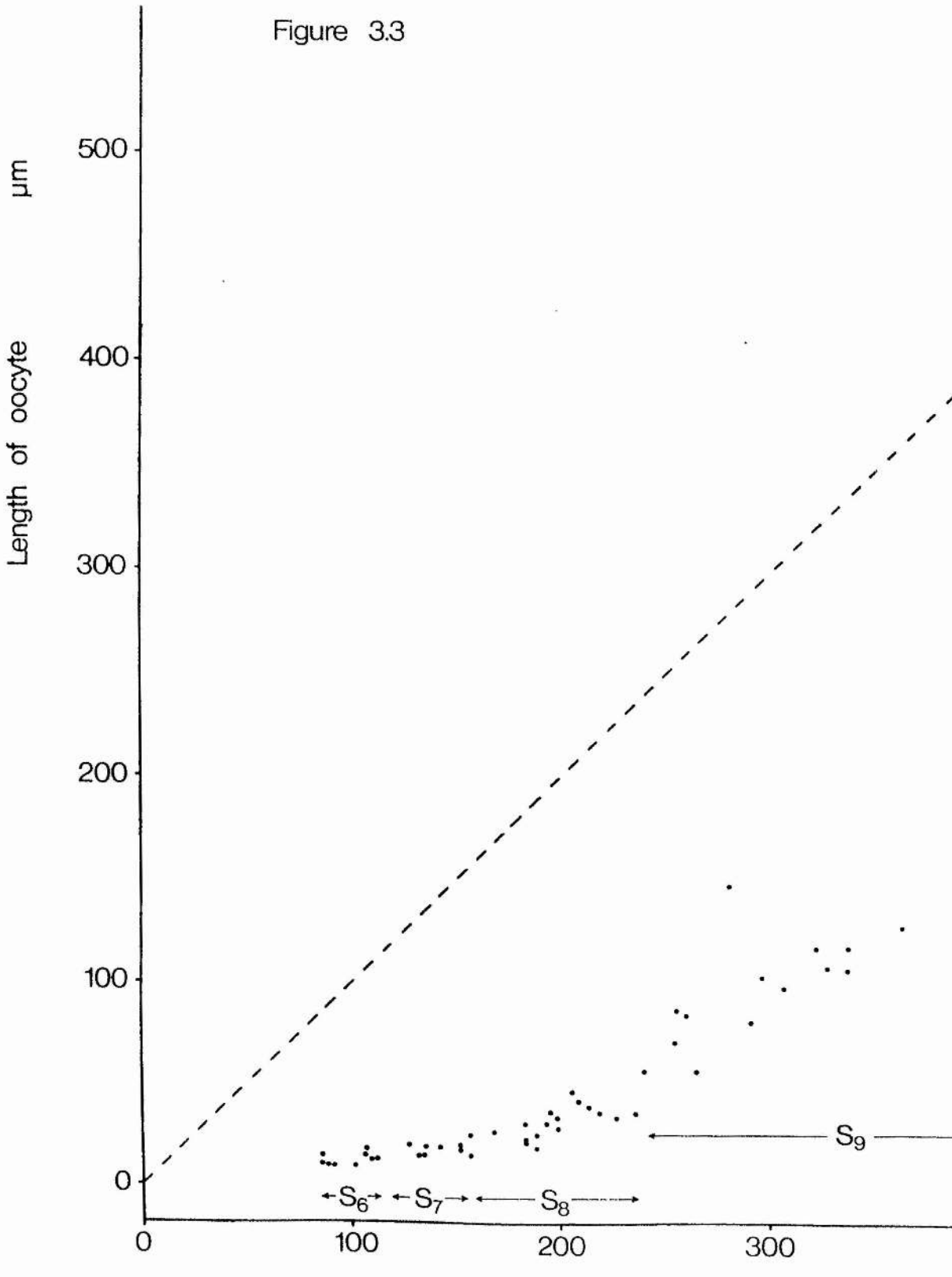

Length of oocyte µm
500
400
300
200
100
0
0
100
200
300
Length of whole egg
S6
S7
S8
S9

FIGURE 3.4

Graph of oocyte diameter against oocyte length for stages 6 to 14. The approximate range of lengths for each stage is shown above the bottom axis. Symbols as figure 3.2.

Figure 3.4

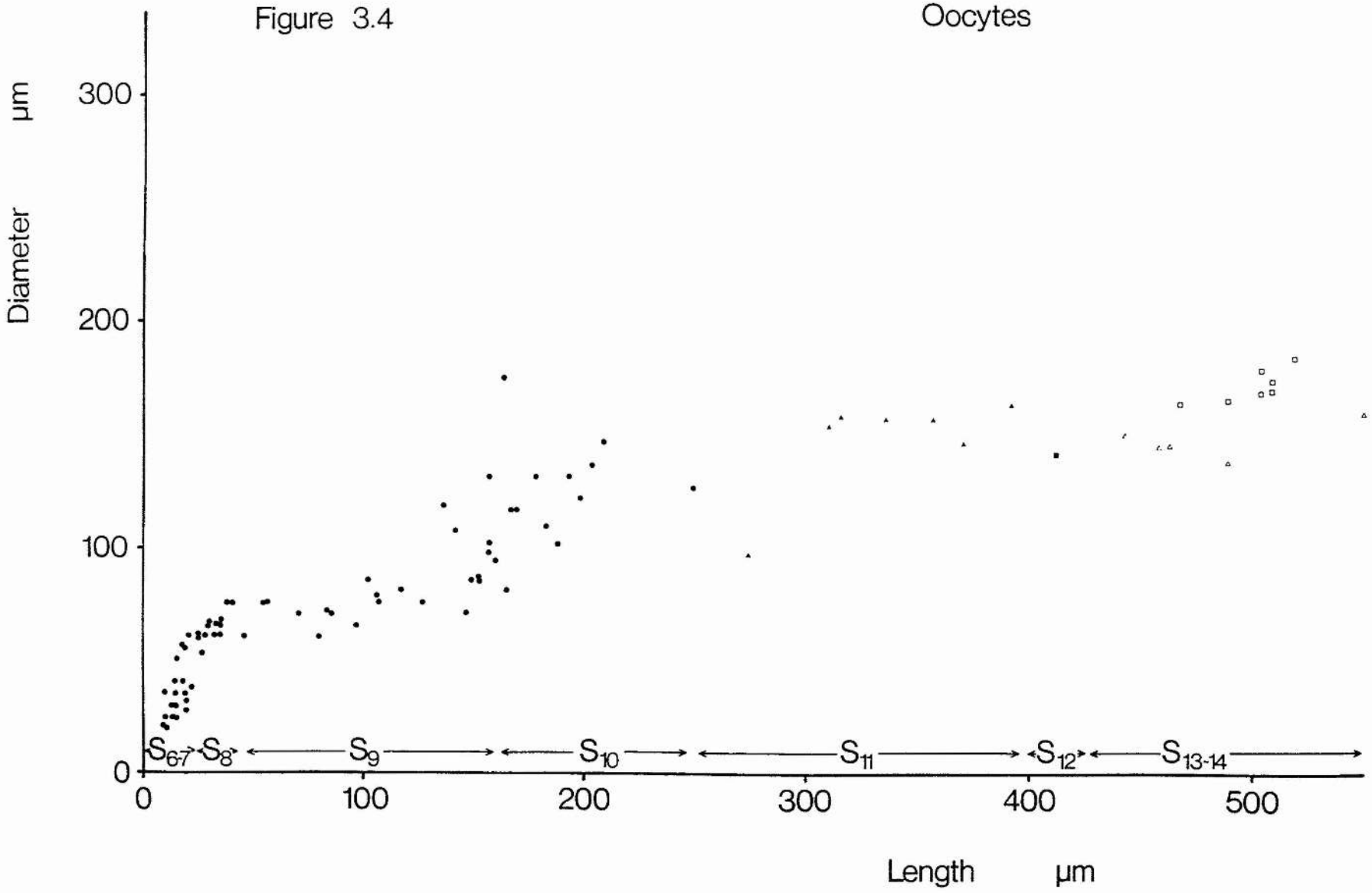
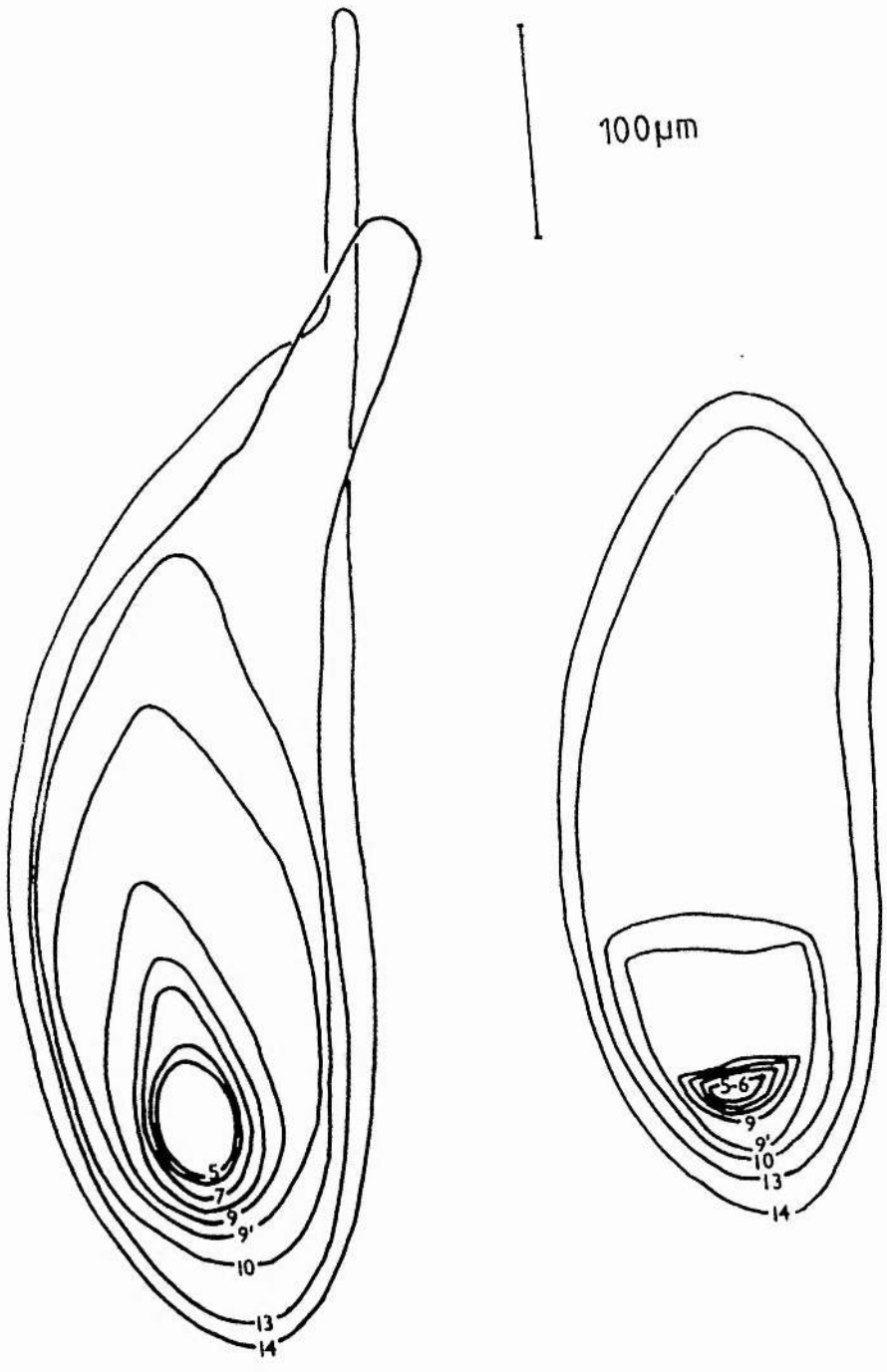

Oocytes
Diameter µm
300
200
100
0
0
100
200
300
400
500
Length µm
S6-7
S8
S9
S10
S11
S12
S13-14

FIGURE 3.5

Tracings of the outlines of egg chambers and oocytes (taken from light micrographs) for stages 5 to 14 (9' is a late stage 9 chamber).

Figure 3.5



Whole egg chambers

Oocytes

The behaviour of the follicle cells

The position and shape of the follicle cells at various stages was determined by the following methods: 1. Direct observation of egg chambers in DPBS using Nomarski interference contrast microscopy. 2. Silver staining of fixed material. 3. Observations of the positions of the follicle cell nuclei using Feulgen staining. Methods 1 and 2 allow direct observation of cell boundaries in favourable conditions (e.g. with tall cells with their sides approximately parallel to the axis of the microscope, and with little obscuration by refractile yolk or lipid droplets). For situations where this is impossible method 3 was used and cell boundaries were assumed to be situated midway between adjacent nuclei. Examples of the appearance of cell boundaries and nuclei revealed by these methods are shown in plates 30 to 40.

The probable arrangement of the follicle cell boundaries, as obtained by these methods, is illustrated in figure 3.6. The arrangement of cells in the elongating dorsal appendages has not been studied in detail because of the difficulty of obtaining sufficient material, and because Nomarski interference contrast microscopy and silver staining do not reveal the cell boundaries. The arrangement shown in figure 3.6 for stage 13 in the appendage region is therefore provisional, and may be inaccurate. However no markedly elongate cells have been observed in this region (see, for example, plate 38 where the shape of a few cells can be made out). This being so it is

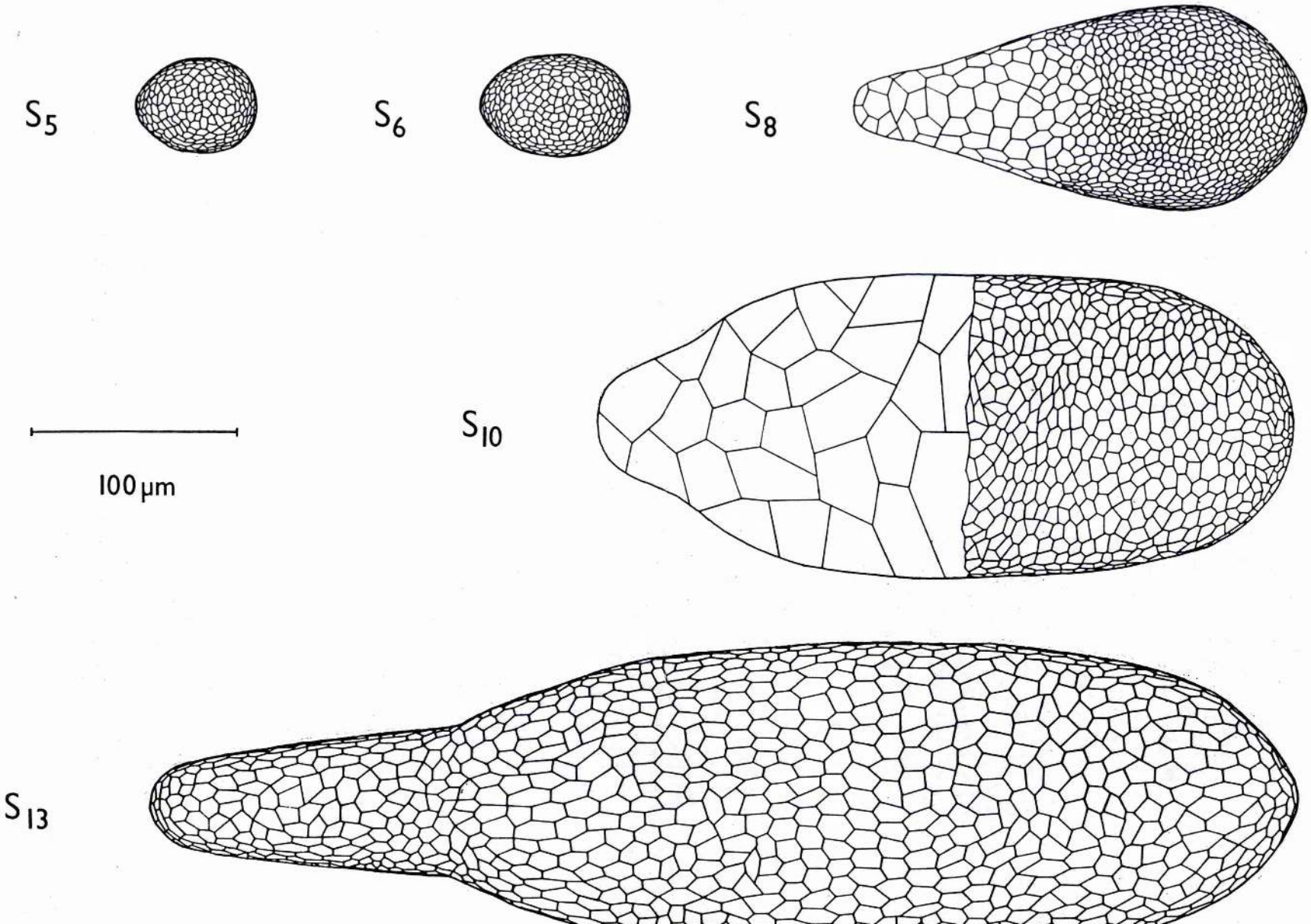
probable that the cells in this region rearrange (by moving past one another) as the appendage elongates.

A sharp boundary between thin squamous cells of the nurse follicle and taller cuboidal or columnar cells of the oocyte follicle develops during stage 9 (see figures 3.1 and 3.6, plates 34 and 35). This boundary corresponds with the position of the anterior centripetally migrating cells (see introduction). At the beginning of stage 9 the change in cell shape is gradual (plates 32 and 33). Once this boundary is established the oocyte follicle maintains a constant relationship with the oocyte. The cells appear to maintain a constant topological relationship, and probably do not migrate past each other, as the oocyte enlarges. The cells become elongate as revealed by the separation of their nuclei (the ratio of the separation of follicle cell nuclei parallel to the anterior-posterior axis of the egg chamber to the separation perpendicular to the axis is approximately 1:1 in stage 9 or 10, and 1:1.4 in stage 13). Thus there is an intimate relationship between the oocyte and its follicle. They elongate together from stage 9 on.

FIGURE 3.6

Diagram showing the probable arrangement of follicle cells on the surface of egg chambers at various stages. See page 69 for description of techniques used to determine the position of the cell boundaries. The follicle cells are evenly distributed during the early stages but during stage 8 the cells over the nurse chamber (lefthand side) start to flatten. By stage 10 a sharp boundary has been set up between the nurse chamber follicle (lefthand side) and the oocyte follicle. The nurse chamber follicle is not present during stage 13. The narrower portion at the lefthand side is the dorsal appendage follicle. The arrangement of cells in this portion is uncertain as the cell boundaries in this region are difficult to detect. A possible arrangement is shown. It is unlikely that the cells in this region are markedly elongate.

Figure 3.6



DISCUSSION

The major changes in size and shape of the egg chamber and its constituent parts have been described. The most significant changes in whole egg chamber shape are the gradual elongation which occurs from stage 1 to 6 (figure 3.2) and the change in shape of the anterior end from a rounded to an elongated, 'pear-shaped', form at stage 7 (figures 3.5 and 3.6). The elongation of the dorsal appendages during stage 13 of course also changes the shape of the whole chamber. This special case will be discussed after the oocyte shape changes.

The major changes in shape of the oocyte do not correspond with those of the whole chamber. From stage 1 to stage 7 it occupies a small proportion of the chamber, and its diameter is greater than its width. From stage 8, when vitellogenesis starts, it begins to grow and elongate. During stage 9 the elongation is at its most rapid, and it continues until the oocyte fills up nearly all the volume of the egg chamber in stages 12 to 14.

The growth of both the whole egg chamber and the oocyte is never isometric (i.e. the shape and hence the diameter:length ratio never remain constant). There are many reasons why isometric growth is not generally to be expected. Many of the physical properties of biological structures depend on the surface area to volume ratio [Alexander 1968]. Such properties include the rate of oxygen diffusion into, or the mechanical strength of a structure. The surface area to volume ratio does not remain constant for objects of the same shape

but of different sizes.

The change in whole egg chamber shape is gradual and occurs thoughout the period the chamber is growing. The changes in oocyte shape however are most dramatic during the vitellogenic stages 8 to 10, with the greatest rate of change occuring in stage 9. This difference between the whole chamber and the oocyte probably reflects the interaction of factors, such as oxygen diffusion, for which the size and shape of the whole chamber is probably more critical than those for the oocyte or any other constituent parts of the chamber, and factors, mostly associated with the oocyte, related to the final form of the egg. The elongate form of the eggs of Drosophila and the majority of other insects is thought to be related to the mechanical properties such as resistance to buckling on the application of mechanical stress [MacMahon 1973, Legay 1977].

The follicle cells undergo several changes during the development of the egg chamber, which have been described by King and others (see introduction). This study highlights the relationship between the oocyte follicle and the oocyte. During the previtellogenic stages the whole follicle is of similar thickness, and the cells are cuboidal. During stage 8 the thickness begins to vary over the surface of egg chamber. In stage 9 a sharp boundary is formed between the very thin squamous nurse follicle cells and taller, columnar, oocyte follicle cells. Once this boundary is established the oocyte follicle remains intimately associated with the oocyte and elongates along with it. The follicle cells do not appear to rearrange while this occurs, and

presumably each oocyte follicle cell also elongates with the oocyte. These cells form the posterior portion of the egg shell (vitelline membrane and chorion). In addition to the oocyte follicle cells which remain in contact with the oocyte, some follicle cells take on a more specialized role in forming particular egg structures (specialization of the egg shell). The anterior centripetally migrating follicle cells move inwards during stage 10 and separate the oocyte from the nurse cells. These follicle cells form the anterior egg shell and the dorsal appendages. Once migrated into position they also retain their relationship with the oocyte. A group of cells from the anterior of the egg chamber migrate through the nurse chamber to a position anterior to the oocyte, adjacent to its nucleus. These 'border cells' form the micropyle and the egg shell next to it.

Two groups of cells within the anterior centripetally migrating follicle cells start to make the dorsal appendages. Follicle cells surround the appendages as they elongate out of the anterior end of the egg chamber. These cells have not been observed to elongate, although their cell boundaries are very difficult to detect using the light microscopical techniques employed. It is likely that these cells rearrange by moving past one another as the appendages elongate. No cell division has been observed in the follicle subsequent to stage 6 [King & Vanoucek 1960], and none have been observed in this study. However it is possible that some division does occur in the specialized regions of the follicle that form the appendages and the micropyle (the number of cells in contact with the oocyte decreases due to the migrations [King & Vanoucek 1960]: so these cells do not

divide). The follicle cell nuclei are very small (about 3μm long) and division might be rapid and infrequent, so it is possible that some undetected division does occur.

The nurse chamber follicle cells remain thin and squamous. However they reduce in size as the nurse chamber contracts. They could contribute to the breakdown of the nurse chamber.

CHAPTER 4

ULTRASTUCTURAL OBSERVATIONS ON DEVELOPING EGG CHAMBERS. THE ROLE OF MICROFILAMENTS IN THE FOLLICLE CELLS

INTRODUCTION

This chapter contains description of the ultrastructure of Drosophila egg chambers with emphasis on the follicle cells. It is principally concerned with the vitellogenic stages, but other stages will also be described.

The ultrastructure of Drosophila egg chambers has been extensively studied [King & Devine 1960, King 1970, Cummings & King 1970a, Mahowald 1972b]. More recent research has mainly centred on polar body formation [Illmense et al. 1976], secretion of the egg shell [Cummings et al. 1971, Giorgi 1977], and the deposition of yolk [Giorgi & Jacob 1977a,b]. This study concentrates on cytoskeletal aspects and their possible morphogenetic role. Description of structures that have been described previously will be omitted unless directly relevant to growth and shape control. The most striking cytoskeletal structure discovered in the follicle cells was a circumferentially oriented layer of microfilaments in the oocyte follicle. Microfilaments were also present in the follicle surrounding

the developing appendages and in the nurse cells.

RESULTS

Previtellogenic stages and stage 8

The following terms will be employed in describing follicle cells. The surface in contact with the tunica propria will be described as the outer surface. The surface in contact with the oocyte or nurse cells (or in the later stages in contact with the egg shell that covers the oocyte) will be described as the inner surface. The surfaces in contact with other follicle cells will be referred to as the sides. The distance between the inner and outer surfaces will be referred to as the height.

The general organization of a previtellogenic follicle cell is represented in figure 4.1. Microtubules are present in the cytoplasm close to the outer plasma membrane. These are commonly circumferentially oriented, and so appear as longitudinal profiles in transverse sections of whole follicles (plate 41). Cross-sections of microtubules are also found. Microtubules are never present in large numbers. Small patches of microfilaments are occasionally present close to the plasma membrane (Plate 42).

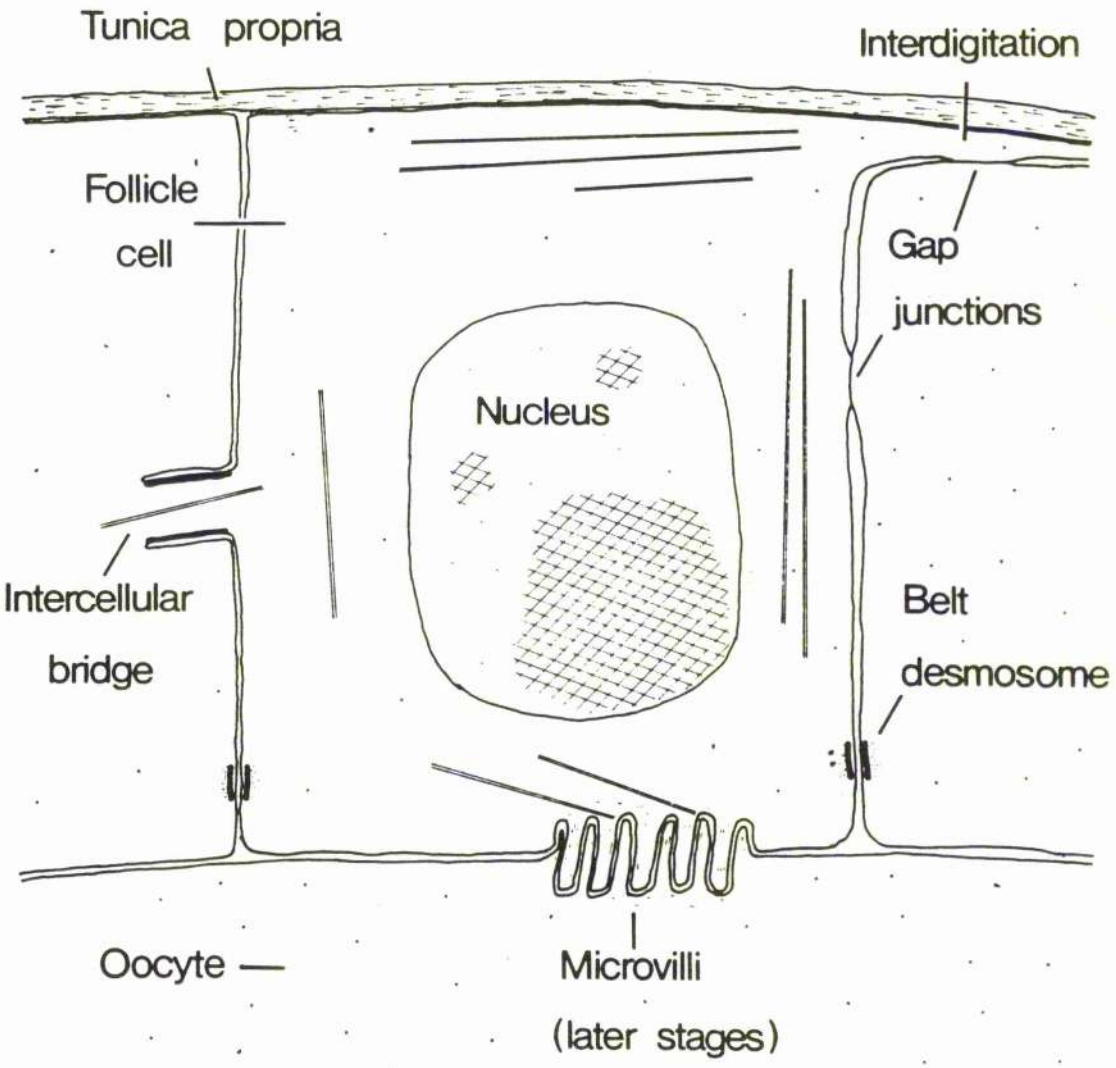
Follicle cells are occasionally joined by intracellular bridges (formed by incomplete separation after cell division [Giorgi 1978]). These occasionally have microtubules passing through them (plate 49). Gap junctions [Mahowald 1972b] also occur between the cells. A belt desmosome [Staehelin 1974] encircles the sides of each cell, close to

FIGURE 4.1

Schematic diagram of the arrangement of prominent structures in a previtellogenic follicle cell. The pairs of thin parallel lines () represent microtubules. The positions they are shown in are in the regions where they occur most frequently.

Figure 4.1

Diagram of a previtellogenic follicle cell



the oocyte/follicle boundary. This has filamentous material associated with it (plate 43), plus the occasional microtubule (plate 44) [Mahowald 1972b]. Microvilli occur on the inner surface of follicle cells in stages 7 and 8. These contain microfilaments (plate 45), as do all microvilli in the egg chamber. Microtubules are frequently found associated with microvilli (plate 45).

There are a few microtubules in the oocyte [Mahowald 1972b] (plate 46). One stage 8 oocyte was discovered with a large number of microtubules (plate 47). It is possible that microtubules become numerous for a short period. Microtubules also insert on the oocyte lip of the ring canals (plate 48). They are absent from the nurse cells except for a small number associated with the microvilli which occur at intervals along the nurse cell membranes.

The cytoplasm of the nurse cells of stages 5 to 8 contain occasional bodies of a fibrous appearance (plates 50 - 52). These are about 0.5μm in diameter and several micrometres long (e.g. 8.5μm in plate 52). They are made up of filaments with a diameter of about 5nm, which sometimes appear to be crosslinked. They are often associated with endoplasmic reticulum in stage 8. The filaments in the bodies have a rather different appearance to the microfilaments found in later stages (see below), however it is possible they are related. Otherwise they could perform some other function, for example they could be storage organelles.

The tunica propria is generally of uniform thickness (about

200nm in stages 7 to 8). It is thicker in the slow growing early stages than in the rapidly growing stage 9 or later stages where it is 50nm or less in thickness. However in the anterior end of stages 7 and 8 egg chambers internal ridge-like thickenings of the tunica propria have been observed (plates 53 - 55). These are approximately circumferentially oriented. Some of the best examples were discovered in lightly glycerol extracted egg chambers (plate 53: 30min in 50% glycerol [Goldman et al. 1976]) prepared in early unsuccessful attempts to decorate microfilaments with heavy meromyosin (see chapter 6).

The ultrastructure of the follicle cells in stage 8 is similar to the previous stages although the shape changes described in chapter 3 start during this stage.

Stages 9 and 10

The general organization of a stage 9 or 10 follicle cell is illustrated in figure 4.2. There are no more microtubules in the cytoplasm than in the earlier stages. The distribution of the microtubules is similar. A small number run close to the outer plasma membrane while others run into the microvillous region at the inner surface of the follicle. A few run up and down the sides of the cell, either side of the nucleus. These microtubules are radially oriented with respect to the whole egg chamber. Microtubules are observed less frequently in the oocyte.

FIGURE 4.2

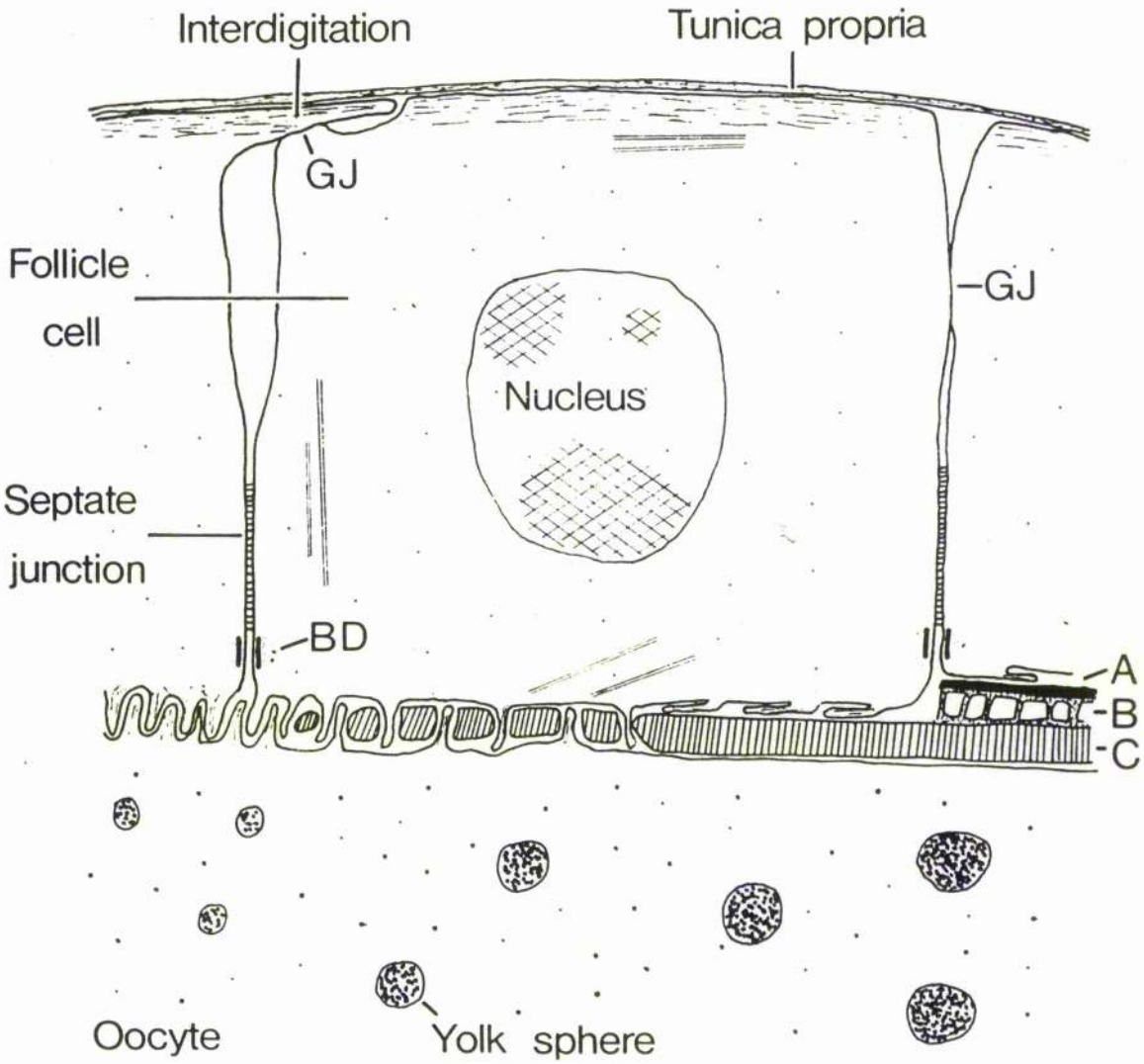
Schematic diagram of the arrangement of prominent structures in a vitellogenic oocyte follicle cell. The pairs of thin parallel lines (=) represent microtubules. The positions they are shown in are in the regions where they occur most frequently. **BD**: belt desmosome, **GJ**: gap junction.

The egg shell is shown in its mature form on the righthand side (**A** exochorion, **B** endochorion with air spaces, **C** vitelline membrane). The vitelline membrane is shown in increasing degrees of maturity from left to right.

The intercellular bridges present in previtellogenic follicle cells (figure 4.1) are probably not present during vitellogenic stages, except in the anterior inwardly migrating follicle cells [Giorgi 1978].

Figure 4.2

Diagram of a vitellogenic stage follicle cell



A layer of microfilaments is found below the outer surfaces of follicle cells immediately beneath the plasma membrane. In cross-sections of egg chambers these microfilaments are observed as longitudinal profiles (parallel to the circumference of the chamber: plate 56). In median longitudinal sections they are cut in cross-section (plate 57). Longitudinal sections which graze the surface of the egg chamber reveal longitudinal profiles perpendicular to the longitudinal axis of the chamber (plate 58). All these results are consistent with the microfilaments being circumferentially oriented with respect to the egg chamber.

Measurements of the diameter of microfilaments ranged from 5.7 to 7.2nm (mean 6.5). This is within the range of 5nm to 8nm reported for actin-like microfilaments [Pollard & Weihing 1974]. The wide range in measurements of diameter reported here and elsewhere is probably largely caused by inaccuracies in calibration of the magnification of electron micrographs. A comparison of the diameter of the microfilaments and of the diameter of thin filaments in the muscle fibres of the epithelial sheath was made (table 4.1). Measurements of both types of filaments were taken from the same electron micrograph (20 of each per micrograph) to avoid errors arising from inaccurate calibration of magnification. In two electron micrographs selected for having sufficient numbers of each type of filament the diameters were not significantly different when compared using a Student's t test ($P>>0.05$).

Table 4.1

Comparison of the diameters (± standard error) of microfilaments and muscle thin filaments from the same electron micrograph

Plate	Diameter (/nm)		t	df	P
	Microfilament	Thin filament			
58	6.2 ±0.21	6.5 ±0.22	0.76	38	0.45
-	6.4 ±0.20	6.3 ±0.16	0.45	38	0.65

Intercellular spaces are present between the sides of follicle cells during the vitellogenic stages (plates 59 - 61). The spaces sometimes reach half or three quarters of the way down the sides of the cells. They are widest immediately below the tunica propria where they are 5μm or more across. For most of their length they are 0.2μm to 0.5μm wide. Cell processes often cross these spaces, especially in the vicinity of the tunica propria, and interdigitate with the neighbouring follicle cell (plate 59). Microfilaments continuous with the microfilamentous layer are present in interdigitations at this level (plate 59).

Belt desmosomes encircle the base of each vitellogenic follicle cell as they do in earlier stages (figure 4.2). Septate junctions form above the belt desmosomes [Mahowald 1972b] on the sides of the follicle cells, beneath the intercellular spaces. Gap junctions are also present between the follicle cells [Mahowald 1972b], often in local occlusions of the intercellular spaces. They are also common on the interdigitations beneath the tunica propria (plate 60), which they may completely encircle. No microtubule-associated spot desmosomes of the type discovered in Heteropeza [Tucker & Meats 1976] have been observed. Occasionally structures which could be junctions are observed. One type is similar to the spot desmosomes of Heteropeza, but with less dense staining material on the inner surface of the plasma membrane (plates 61a,b). There is only a slight increase in extracellular material between the junctional membranes. Microtubules and microfilaments are not found associated with these junctions. Another type frequently observed at all stages of oogenesis consists of closely opposed regions of plasma membrane. The plasma membrane itself is densely stained and the 'unit membrane' trilaminar structure is very clearly defined (plate 62). The arrangement of these structures varies considerably. Some are simple unconvoluted structures, but others are arranged in tightly curled whorls (plate 62b). Microtubules and microfilaments have not been found associated with this type of structure.

The centripetally migrating cells of the anterior oocyte follicle have microtubules parallel to their anterior longitudinal axes (plate

63). Both the cells and the microtubules are oriented radially with respect to the egg chamber. During stage 10 however they are displaced so that their inner (nearest the anterior-posterior axis of the egg chamber) ends are more posterior than their outer ends. In median longitudinal sections of egg chambers cross-sections of microtubules are frequently observed in the region of the anterior centripetally migrating cells close to the inner, vitelline membrane forming, surface. These microtubules are oriented in a ring around the anterior-posterior axis of the egg chamber, within the vitelline membrane forming surfaces of the cells.

The border cells which migrate through the nurse chamber during stage 9 have areas which possibly represent microfilaments near their posterior (oocyte) surface (plate 64). Microtubules are also common in this region. Both the centripetally migrating anterior oocyte follicle cells and the border cells have invaginations on their nurse chamber surfaces (plates 65, 66). An extension, resembling a solitary microvillus, of the neighbouring nurse cell is located in each invagination. These nurse cell extensions are attachment sites for bundles of microfilaments, which can sometimes be observed to run several micrometres through the nurse cell cytoplasm (plate 66). Some of the bundles of microfilaments are made up of straight, parallel, microfilaments (plate 65), which differ considerably in appearance from the usual wavy microfilaments. This type of microfilament will be further considered in the next section.

The follicle cells covering the nurse chamber are extremely thin

(1 to 2μm) during stages 9 and 10 (plate 67). Microfilaments are present in the numerous microvilli which occur at the follicle cell/nurse cell boundary, but not elsewhere in the follicle cells (i.e. there is no microfilamentous layer). An occasional microtubule can be observed (plate 67) but there are no obvious cytoskeletal specializations. Invaginatons closely resembling those on the anterior centripetally migrating follicle cells and border cells (plate 68) also occur on the nurse chamber surfaces of the nurse chamber follicle cells.

Stages 11 and 12

The oriented layer of microfilaments in the oocyte follicle is still present in stages 11 and 12, although thinner than in the previous stages (plates 69 and 70). The layer is thinnest at the posterior end of the oocyte follicle, and thickest in the region of the anterior centripetally migrating follicle cells. The vitelline membrane forms earlier in the posterior region: it presumably prevents further deformation of the oocyte, allowing the microfilamentous layer to disassemble earlier in this region. The microfilamentous layer has not been extensively studied in these stages because the presence of the vitelline membrane and chorion causes sectioning difficulties, and because stages 11 and 12 are of short duration and therefore of low frequency of occurence (see chapter 3).

During stage 11 parts of the nurse chamber follicle cells form extensions which penetrate between the nurse cells (plates 71 and 72).

These are often associated with regions of nurse cell cytoplasm containing numerous microfilaments (plate 73). Both the straight, parallel, microfilaments and the more usual wavy microfilaments are present. The diameters of both types of microfilament were compared using measurements taken from the same electron micrograph (20 of each for the first micrograph, 30 of each for the second: table 4.2). The diameters are not significantly different when compared using a Student's t test ($P>>0.05$). Bundles of microfilaments are also present in the nurse chamber cytoplasm away from the cell boundaries (plate 74). The extensions of the follicle cells have occasional gap junction contacts with the nurse cells that they pass between (plate 71).

Table 4.2

Comparison of the diameters (± standard error) of normal and straight microfilaments from the same electron micrograph

Plate	Diameter (/nm)		t	df	P
	Normal	Straight			
73	5.9 ±0.14	6.2 ±0.17	1.22	38	0.23
-	7.2 ±0.21	6.9 ±0.21	1.00	58	0.33

The dorsal appendages start to form during stages 11 and 12. Many vesicles are present in the cytoplasm close to the forming appendages (plate 75). The vesicles contain a dense staining material which is probably a precursor for the assembly of the appendages. Microtubules are common in the cytoplasm between the vesicles. No structures, cytoskeletal or otherwise, have been observed in stage 11 or 12 egg chambers which could be controlling or shaping the assembly of the appendages.

Stages 13 and 14

The follicle cells alongside the developing appendage have several specializations [Quattropani & Anderson 1969]. These include vesicles containing dense staining material (plate 76) as described for stages 11 and 12 above. There are also elaborate septate junctions between the cells (plate 77). These are of the pleated sheet type [Staehelin 1974] as is revealed when the junctional membrane is parallel to the plane of section. There are microtubules which run parallel to the developing appendages [Quattropani & Anderson 1969] (plate 76). There is sometimes an appearance of filamentous material in the follicle cell close to the developing appendage (plate 78). This could have some role to play in the secretion and arrangement of appendage material.

A substantial layer of circumferentially oriented microfilaments is found underneath the outer plasma membrane (i.e. that beneath the

tunica propria) of the appendage follicle (plates 79 and 80). This is similar in appearance to the microfilamentous layer found in the oocyte follicle of stages 9 and 10. This layer is present during the period when elongation of the appendages and the surrounding follicle occurs.

DISCUSSION

Microtubules and various other structures

The circumferentially oriented microtubules observed close to the outer surfaces of the follicle cells are not nearly as numerous as those previously observed in Heteropeza pygmaea and Periplaneta americana [Tucker & Meats 1976]. The egg chambers of these insects undergo an elongation during their development similar to that which occurs in Drosophila (chapter 3). However their development is simpler. There are no nurse cells involved in the panoistic oogenesis of Periplaneta (Hemimetabola, Dictoptera) [Mahowald 1972a]. The paedogenetic viviporous development of Heteropeza (Holometabola, Diptera, Cecidomyiidae) is a highly anomalous variant of polytrophic meroistic oogenesis [Went 1977]. The oocyte spontaneously develops into an embryo inside the follicle which is released into the haemolymph of the female larva. There are no follicle cell 'migrations' such as occur in Drosophila and other Diptera. Tucker and Meats discovered a system of highly organized circumferentially oriented microtubules within the follicle cells of Heteropeza. These cells are mechanically connected by spot desmosomes and are highly interdigitated. The circumferentially oriented microtubules are associated with the desmosomes so that together they form "a mechanical continuum capable of transmitting tensile forces throughout the epithelium." Circumferentially oriented microtubules are also present in the follicle cells of Periplaneta, at least during the early stages of oogenesis, so a similar mechanical continuum could

also be present in Periplaneta. Tucker and Meats propose that this continuum "may provide greater resistance to circumferential expansion than to elongation of the follicle parallel to its polar axis." This resistance, in combination with tension in the follicular epithelium produced by the expanson of the oocyte, may 'mould' the growth of the oocyte, causing it to elongate. Although the number of microtubules necessary to operate this type of system is not known the small number present in Drosophila, which have not been observed to be associated with desmosomes, suggests that they are not the main element in producing oocyte and follicular elongation in Drosophila. However it is possible that they are involved indirectly, perhaps helping to control the orientation of other systems (see below).

Microtubules with orientations other than circumferential are occasionally found close to the outer plasma membrane of the follicle cells. These and the microtubules distributed throughout the cytoplasm away from the outer surface probably represent the general type of microtubules found in the cytoplasm of most cell types. The distribution of these microtubules and their association with microvilli is a typical one for insect epithelia (c.f. imaginal disc epithelia [Poodry & Schneiderman 1970]). Similarly the occurence of belt desmosomes and intercellular bridges is typical. Many of the microtubules, particularly those associated with the microvilli could be involved with the transport of materials within the cytoplasm.

The function of microtubules in the oocyte is unknown. It has been suggested that they are involved in the movement of yolk granules

[Mahowald 1972b]. A high concentration of tubulin is present in eggs after oviposition [Green et al. 1975]. It is possible that there is some accumulation in preparation for spindle formation during the extremely rapid nuclear divisions that occur during the formation of the syncitial blastoderm of the embryo. It is also possible, but there is no supporting evidence, that they help organize some mosaic system of cytoplasmic determinants which will control development of the embryo. However no association with the only possible determinants which are ultrastructurally recognizable, the polar granules, has been detected. The highest number of microtubules (per unit area of section) was observed in the stage 8 oocyte. This is earlier than would be expected if they are being stored for blastoderm formation or are involved in setting up a mosaic. If they are a storage product the highest number would be expected towards the end of oogenesis. Polar granules are first observed in stage 10 [Illmensee et al. 1976]. It may be that they perform yet another unforseen function.

The microtubules parallel to the longitudinal axes of the anterior centripetally migrating follicle cells (and oriented radially with respect to the egg chamber) are typical of elongate cells. They could be directly or indirectly involved in elongation of, or in transport of material along, the cells. However if the microtubules radiate from an organizing centre within the cell more longitudinal profiles of microtubules would be found in regions away from the organizing centre, as a result of simple geometric restrictions on the way the microtubules can fit into the volume available.

Significant numbers of microtubules are found within the migrating border cells, during stage 9, close to the posterior (oocyte-facing) surface. These microtubules could have a role to play in border cell locomotion. It is not clear how they could directly bring about movement of the cells. It is possible that they represent part of a system for transporting material into a highly metabolically active region of cytoplasm. This could contain part of the system responsible for locomotion (part might also be in the nurse cells).

The role of the fibrous bodies (plates 50 - 52) discovered in the nurse cells is unknown. The presence of endoplasmic reticulum around the bodies suggests that it could secrete them, in which case they could be storage organelles. However no endoplasmic reticulum has been observed to be associated with them in the earlier stages. This might be a matter of chance, or there could be more than one type, or a change in function could occur. It is also possible that they represent a modified form of microfilament bundle similar to those which occur in later stages, as the filaments that make up the bodies are of similar diameter to actin filaments. However their appearance is significantly different from the microfilament bundles observed in later stages, and it is not usual for microfilaments to be associated with endoplasmic reticulum.

The circumferential thickenings in the tunica propria of stages 7 and 8 could represent a mechanism for producing shape change. The anterior end of the egg chamber changes from a rounded to a slightly more elongate 'pear-shape' (see chapter 3) during stage 7.

The thickenings, because of their orientation, could produce elongation by restricting the increase in circumference of the chamber. The mechanism would be similar in principle to the microtubule one proposed by Tucker and Meats for Heteropeza (see above), or the microfilamentous one considered below. There is evidence for a shape determining role for the tunica propria in Periplaneta, where oriented fibrils are present [S.A. Mathews, personal communication]. In Drosophila the tunica propria decreases in thickness during vitellogenesis. This could be a result of increased tension as it becomes stretched while the egg chamber expands. Thus the tension necessary to produce elongation of the anterior end could be generated. The tunica propria is believed to be secreted by haemocytes which migrate over the surface of the egg chamber [King 1970]. These presumably only secrete tunica propria on early stage chambers, or cannot secrete it fast enough to keep up with the expansion of the later stages. The appearance of thickenings on the inner (follicle) surface during stages 7 and 8 suggests that the follicle cells could also play a role in the secretion of tunica propria.

Microfilamentous layer

The circumferentially oriented microfilamentous layer beneath the outer plasma membrane in the oocyte follicle cells of the vitellogenic stages is the most striking cytoskeletal element observed in the Drosophila egg chamber. It is proposed that these microfilaments could bring about the elongation of the oocyte that occurs during

these stages (see chapter 3). The mechanism proposed is analagous to that proposed by Tucker and Meats for the microtubule system in Heteropeza. The oocyte is expanding rapidly during these stages: it is reasonable to assume that tension is produced in the follicle as a result of this expansion. If the circumferentially oriented microfilaments generate force by contraction parallel to their longitudinal axes, as microfilaments of the actin-like type do in other systems [Pollard & Weihing 1974, Spooner 1975], they would restrict increase of circumference, and hence radial expansion. The oocyte follicle then would be forced to elongate, as would the oocyte within it. This might be achieved by a passive elastic system. However evidence for active actomyosin mediated contraction will be presented in chapters 5 and 6.

The expansion of the oocyte is the result of two mechanisms. The oocyte grows by absorbing material and metabolizing it as any other cell would. However the growth is accelerated by rapid transport of materials through the follicle cells, and by the preformation of one major component, the yolk protein, elsewhere in the female abdomen, in the fat body [Gelti-Douka et al. 1974]. The intercellular spaces between the follicle cells probably facilitate the diffusion of materials from the haemolymph across the follicle. The second mechanism is the direct transfer of nurse cell cytoplasm into the oocyte through the ring canals.

The follicle cells probably remain in the same relative positions topographically while the oocyte elongates (see chapter 3). The

individual cells appear to elongate, in a direction parallel to the anterior-posterior axis of the egg chamber, rather than rearranging as the cells of the developing imaginal disc are believed to do [Fristrom 1976]. Therefore it is possible that the reduction in the follicle cell height and the increase in length parallel to the axis is a passive result of the restrictions placed on follicle growth by the microfilamentous layer.

The interdigitations of the follicle cells which occur just below the tunica propria probably increase the strength of adhesion between the cells. It is evident that the cells must adhere tightly if tension is to be transmitted around the circumference of the egg chamber. Interdigitations could be necessary to increase the area of cell to cell contact, especially as this decreased by the presence of intercellular spaces between cells. No structures that can be certainly identified as desmosomes were detected at the level of the microfilamentous layer. This contrasts with the spot desmosomes which are associated with the circumferential microtubules in Heteropeza. Extensive gap junctions are present in this area, sometimes completely encircling the interdigitations. If these junctions have the same structure as those in systems that have been studied more fully (e.g. by freeze fracture) they represent regions of intimate contact between the membranes of opposing cells; particles which contain channels, through which material can diffuse, connect the membranes [Staehelin 1974]. It is possible that large areas of these junctions are necessary for maintaining intercellular communication (necessary for setting up and preserving the orientation of microfilaments over the

whole follicle?). It is likely that the junctions can resist a degree of tension, so that the large areas present for other regions could also provide the required mechanical connection between the cells. Evidence for a capability to resist tension comes from cells fixed in hyperosmotic (90mM phosphate buffer) fixative. The cells pull apart, but remain united at the gap junctions (plate 81). However it is possible that the normal adhesiveness of the cells is the major mechanical factor connecting the cells and that desmosomes are not needed in large numbers or at all, or that desmosomes are present but poorly fixed (the outer regions of the egg chamber may be particularly vulnerable to osmotic damage on fixation). Several of these factors could combine to provide sufficient resistance to tension.

The growth and elongation of the Drosophila egg chamber differs in principle to some extent from that of Heteropeza. In Heteropeza during the 'second growth phase' [Tucker & Meats 1976] the diameter of the follicle actually decreases slightly while the length increases rapidly. Overall growth occurs in the 'third growth phase' but the shape remains approximately constant (see figure 2 in Tucker & Meats 1976). In Drosophila both length and diameter are increasing rapidly while elongation is occurring. The microtubule system of Heteropeza (and of some stages of Periplaneta) thus could represent a rigid system for producing elongation in an egg chamber which is not undergoing the extremely rapid expansion that occurs during the vitellogenic stages of Drosophila. Perhaps in Drosophila such a rigid system would not be appropriate and a more flexible mechanism

for resisting tension which can keep pace with the overall increase in size (the length and diameter of the egg chamber nearly doubles in stage 9 which only lasts 5.61 hours: see chapter 3) and also the increase in oocyte length at the expense of the nurse chamber (see Legay 1977 for reasons why an elongate shape is advantageous to insect eggs). However it is possible that Heteropeza and Drosophila represent extremes of the same system. Microtubules and microfilaments could be involved in both, working together to produce elongation. Patches of filamentous material can occasionally be observed in Heteropeza [J.B. Tucker: personal communication] while microtubules are present in small numbers in Drosophila. In Periplaneta microtubules are numerous in the early stages [Tucker & Meats 1976] but microfilaments form a substantial layer in the later stages [S.A. Mathews: personal communication]. It is possible that the proportion of microtubules and microfilaments varies with different relative rates of increase of length and diameter.

Microfilamentous layer in the dorsal appendage follicle

The circumferential oriented layer of microfilaments in the follicle cells which form the dorsal appendages could function in a manner similar to that proposed above for the oocyte follicle. However there is no internal expansion in the appendage follicle, or at least none on the scale of the vitellogenic oocyte. Thus the filaments might generate the tension needed to reduce the diameter of, and hence elongate, the appendage follicle. A passive or elastic system would not work in this case. The microfilaments could contract, thus

deforming the follicle cells and perhaps the newly laid down appendage material. The orientation of the microfilaments and the presumably constant volume of the follicle cells will determine that the follicle elongates. The microfilamentous layer is of a comparable thickness to that in the vitellogenic oocyte follicle, although the appendage follicle has a much smaller diameter. This may reflect the need for the appendage follicle to generate all the tension, and to deform the follicle cells, while the oocyte microfilamentous layer's main function could be to resist tension created by the expanding oocyte and to control the direction of expansion.

Microfilament bundles in nurse cells

The various microfilament bundles in the nurse cells could be similar in function to the stress fibres present in mammalian cells in culture [Bragina *et al.* 1976, Goldman *et al.* 1976]. However they differ in that they insert at special attachment sites where a protrusion (or isolated microvillus) of the nurse cell membrane corresponds to an invagination of a follicle (nurse chamber follicle, anterior centripetally migrating oocyte follicle or border) cell. The nurse cells also differ considerably in shape from cultured cells and are very much larger. The bundles could perform a role in the reduction of the size of the nurse chamber by contracting and pulling the sides of the chamber inwards. It has been previously suggested that the follicle cells around the nurse chamber aid in the breakdown of the nurse cells which takes place during stages 11 and 12 [Cummings and King 1970b]. The extensions of the follicle which were observed in

stage 11 could represent a means by which they do so. Changes in the ultrastructure of the follicle cells have also been observed during the breakdown of the nurse chamber in Nasonia vitripennis (Hymenoptera: Pteromalidae) [King & Richards 1969]. The microfilament bundles in the nurse cells associated with the extensions could pull the extensions down between the cells. However no junctions other than the occasional gap junctions have been observed by which tension could be transfered from the nurse cell microfilaments to the follicle cell extensions.

The significance of the two types of microfilaments present in the nurse cells is not known. The normal 'wavy' microfilaments are often associated with membranes but also occur free in the cytoplasm. The 'highly parallel' microfilaments however normally pass through the cytoplasm, except where they enter the protrusions of the nurse cell membrane where they attach. No cross-linking has been observed between the parallel filaments unlike other parallel filaments such as horseshoe crab acrosome and sea urchin egg filament bundles [De Rosier et al. 1977].

CHAPTER 5

INDUCTION AND INHIBITION OF FOLLICULAR CONTRACTION **IN VITRO**

INTRODUCTION

This chapter provides evidence that the follicular epithelium can actively contract under certain conditions, and hence that a follicle can set up and transmit tension around the oocyte it encloses. This contractility is abolished by exposure to cytochalasin B which also disrupts the microfilamentous layer. Hence there is evidence that the circumferentially oriented microfilaments are involved in generating tension. This is consistent with the suggestion that the microfilamentous layer promotes oocyte elongation in an axial direction by limiting increase in circumference.

METHODS

Drosophila ovaries were dissected out and isolated in Drosophila phosphate buffered saline (DPBS) [Robb 1969]. Individual egg chambers were teased out with tungsten needles (see Materials and Methods chapter).

Cytochalasin B (Sigma) was dissolved in dimethyl sulphoxide (DMSO), because it will not dissolve directly in aqueous media, to produce a 1% solution. An aliquot of this solution was then added to 1ml of DPBS, to make a solution of the desired concentration. Control experiments were performed with the same DMSO concentrations, but no cytochalasin B, to assess the effect, or lack of effect, of DMSO on the egg chambers.

RESULTS

Stage 9 and 10 egg chambers isolated in DPBS often become reduced in diameter over the oocyte (plates 82 - 84) compared to egg chambers dissected in fixative. This contraction is sufficiently marked to be readily detectable using a dissecting microscope. It occurs after egg chambers are teased out from the epithelial sheath. Approximately 90% of chambers at the above stages undertake this contraction. The remainder do not change shape. The main contraction normally takes place within 5 to 15 seconds from release from the epithelial sheath. However contraction sometimes continues much more slowly after this initial period.

Various media were substituted for DPBS to examine the possibility that the contraction is caused by some simple deficiency, such as differences in osmotic strength or ionic constitution from haemolymph, in DPBS. The contraction still occurs if DPBS is replaced by 0.17M NaCl, simple Drosophila Ringer (0.17M NaCl, 5mM KCl, 2mM $CaCl_2$, pH7 [King et al. 1956]), of by Drosophila culture medium (Schneider's medium with L-glutamine (GIBCO Biocult) or the medium of Shields & Sang 1977, bicarbonate free, without serum). It also occurs if the osmotic strength or pH of the DPBS is varied (80% to 125% DPBS or pH6 to pH8). These conditions and solutions cover a large range that is likely to include a tolerable substitute for the female haemolymph. Therefore the contraction is probably not caused by a simple deficiency of the media, of the types investigated.

Effects of experimental solutions

Isolated egg chambers were treated with various solutions known to have effects on cytoskeletal systems. The contraction is not inhibited by colchicine (concentrations up to 1%), Ca^{2+} free DPBS (+1mM EGTA), simple *Drosophila* Ringer (see above) with 10 times the normal Ca^{2+} concentration, or by Na^{+} free Ringer (Na^{+} replaced by K^{+}). The only substance, apart from fixatives, which was found to prevent contraction was cytochalasin B. Therefore the effect of cytochalasin B was examined in more detail.

Concentrations of cytochalasin B in the 1 to 10 µg/ml range that inhibit microfilament function in many systems [Schroeder 1970, Wessels *et al.* 1971, Cloney 1972] have no marked effect on the contraction. At a concentration of 50 µg/ml however cytochalasin B almost completely eliminates the contraction. Egg chambers exposed to control solutions with the same DMSO concentrations as the cytochalasin B solutions contracted to the same degree as that which occurs in normal DPBS with no additives.

Many of the microfilamentous systems previously investigated show a degree of recovery when cytochalasin B is removed from the culture medium [Spooner *et al.* 1971, Wessels *et al.* 1971, Cloney 1972]. If, after 15 to 30 minutes, 50µg/ml cytochalasin B is replaced by DPBS, a rapid contraction does not occur. Instead a slow and somewhat less marked contraction occurs over a period of 15 to 60 minutes after replacement in some egg chambers (about 60%). This slow

contraction does not occur if the 50µg/ml cytochalasin B is replaced by 1 to 10µg/ml cytochalasin B solution in DPBS, or by Ca^{2+} free DPBS containing 1mM EGTA. If these solutions are in turn replaced by DPBS a slight contraction sometimes occurs in the 30min after replacement.

Electron microscopical examination of cytochalasin B treated egg chambers

The fine structure of stage 9 egg chambers was examined to assess whether 50µg/ml cytochalasin B induces any structural changes in the microfilamentous layer in the oocyte follicle. Other stages and other microfilament containing regions of stage 9 egg chambers were not examined. The microfilamentous layer is severely disrupted by cytochalasin treatment (plate 87). Microfilaments are absent in most subsurface regions of the follicular epithelium. However, small clumps of filaments and material with a microfilamentous appearance are occasionally present (plate 88). These are less common in egg chambers treated for longer periods (e.g. 30min) than those treated for 15min. The microfilaments in DMSO containing control experiments cannot be distinguished from those in egg chambers incubated in DPBS (plate 87). The ultrastructural preservation of egg chambers incubated for, up to 1h in DPBS or DPBS containing up to 1% DMSO is very good (there is little swelling of membraneous structures or loss of cytoplasmic ground substance: microfilaments and most other structures appear similar to those in egg chambers placed directly in fixative).

The microfilamentous layer is not reformed in egg chambers placed in DPBS for 1h after 15min in 50µg/ml cytochalasin B in DPBS. However, small patches of filamentous material are present (plate 89). These are not much more abundant than those found immediately following the end of the cytochalasin B treatment. If recovery takes place it is not dramatic.

DISCUSSION

The contraction of the egg chamber after it has been dissected out into DPBS or other media is almost certainly not a phenomenon that occurs *in vivo*. Some feature of the *in vitro* conditions compared with the conditions within the adult female must be responsible. *Drosophila* oocytes have never been induced to undergo vitellogenesis *in vitro* [Demal 1961]. This is even the case when they are cultured with fat body which *in vivo* synthesizes and supplies the yolk proteins that are taken up by the oocyte during vitellogenesis [J.H. Sang, personal communication]. It is possible that the contraction is caused by an immediate cessation of oocyte growth on dissection into DPBS. This will reduce tension in the follicle epithelium that is resisted by the microfilamentous layer, if this functions as proposed in chapter 4. Contraction might result from an imbalance in tensions. It is also possible that changes in the concentration of Ca^{2+} or of some other factor might interfere with a delicate control mechanism (i.e. the concentration must be kept within close limits, not represented by any of the experimental solutions). Electrophysiological factors have been shown to be involved in the cytoplasmic flow from nurse chamber to oocyte during vitellogenesis in Lepidoptera [Woodruff & Telfer 1973, 1974, De Loof 1983]. It is possible that differences between the ionic make up of DPBS and the adult female haemolymph could interfere with similar electrophysiological factors involved in *Drosophila* oogenesis, causing an imbalance in the forces involved in shape control.

The contraction occurs when the egg chambers are released from the epithelial sheath. Egg chambers are rapidly forced out of the sheath when it is ruptured so the sheath must exert some pressure on the egg chambers. Therefore it is possible that the removal of this pressure, which is exerted on the nurse chamber as well as on the oocyte chamber, results in an imbalance in the forces (e.g. expansion of oocyte, nurse chamber to oocyte cytoplasmic flow, microfilamentous layer tension) which interact to produce oocyte elongation.

The failure of some of the experimental solutions (e.g colchicine or Ca^{2+} free DPBS with 1mM EGTA) to affect the contraction could be because the contraction starts within a few seconds of the release of an egg chamber from the epithelial sheath. Drugs and ions might not have time to diffuse into the egg chambers and affect the mechanism responsible for contraction. This could also be the reason why there is a requirement for a higher concentration of cytochalasin B than is needed to inhibit the action of other microfilament associated systems [Schroeder 1970, Wessels et al. 1971, Cloney 1972]. Some evidence for this hypothesis is provided by finding that Ca^{2+} free DPBS with 1mM EGTA or low concentrations of cytochalasin B (1 - 10 µg/ml) prevent the slow contraction that sometimes occurs after 50µg/ml cytochalasin B solutions have been replaced with DPBS.

Inhibition of contraction by cytochalasin B is not an absolute indicator of a microfilamentous contractile mechanism. Cytochalasin B hs been shown to block the function of many microfilamentous systems [Schroeder 1970, Spooner et al. 1971, Wessels et al. 1971,

Cloney 1972]. The mechanism by which it does so is not well understood. It is highly soluble in lipids and the cell membrane could be its most important site of action [Spooner 1973]. Recently it has been shown to be capable of binding to an actin nucleating fragment associated with the cell membrane and of preventing filament elongation [Bray 1979]. It also affects other membrane associated systems such as glucose transport [Bloch 1973] and is generally toxic. The effect of cytochalasin B on some systems such as the alteration of the distribution of insulin receptors in adipocytes has been shown to be a result of its inhibition of glucose transport [Jarett & Smith 1979]. However the presence of microfilaments and the demonstration that they are disrupted by cytochalsin B is generally a reliable indication that the cytochalasin B is acting on microfilaments. It is perhaps unlikely that a mechanism such as glucose transport inhibition would have such a rapid effect as that demonstrated in this particular instance.

The inhibition of the contraction by cytochalasin B that occurs in this system, plus the disruption of the microfilamentous layer which occurs with it, is reasonable evidence for the involvement of the microfilamentous layer in the contraction. The existence of a contraction (reduction in diameter) is evidence for the presence of a circumferentially oriented tension producing system. The direction of tension generation must be parallel to the circumference, and therefore to the microfilaments in the layer, to produce a reduction in diameter. The microfilamentous layer is therefore probably capable of producing the tension required for the mechanism of elongation

proposed in chapter 4.

CHAPTER 6

DECORATION OF OOCYTE FOLLICLE MICROFILAMENTS WITH HEAVY MEROMYOSIN

INTRODUCTION

Decoration with heavy meromyosin (HMM) is a technique which has been used extensively to demonstrate that filaments with diameters of 5nm - 8nm have actin-like properties. HMM and light meromyosin (LMM) are the products of tryptic digestion of muscle myosin [Szent-Györgyi 1952]. HMM includes the globular head of the myosin molecule, which contains the actin binding site and the actin-dependent ATPase activity, and part of the 'tail' (the crossbridge that connects the 'head' to the body of the filament). LMM contains the portions of the 'tail' which combine with other tails to form the thick muscle filaments. Huxley [1963] demonstrated that HMM can combine with actin filaments isolated from muscle (and also *in vitro* assembled F-actin) to form a very characteristic complex. HMM side arms in an 'arrowhead' arrangement along the filaments can be detected when negatively stained preparations are examined. This technique has since been extended for use on cultured cells and intact tissues [Ishikawa *et al.* 1969, Pollard *et al.* 1970, Goldman *et al.* 1976]. Glycerol extraction is necessary to permit entry of HMM into cells.

Various control experiments can be performed to demonstrate that the binding of HMM to filaments is of the same type as the binding of myosin to F-actin in muscle. Thus decoration should not take place in the presence of Mg^{2+} and ATP, mimicing the dissociation of muscle actomyosin under these conditions. Pyrophosphate also should prevent binding of HMM to actin filaments. It is possible that the presence of HMM will induce actin molecules (G-actin) present in the cytoplasm to assemble into filaments (F-actin), which will be decorated. Therefore it is desirable to demonstrate that filaments are present in the absence of HMM in glycerol extracted control preparations. Filaments decorated by HMM, but not by HMM in the presence of ATP or pyrophosphate, are termed actin-like microfilaments.

The technique of decoration with HMM was used to investigate the nature of the microfilaments present in stage 9 egg chambers (chosen for the prominence of the microfilamentous layer). The results of these experiments are described below.

METHODS

Rabbit heavy meromyosin (HMM) was kindly supplied by Dr. J. Trinick (ARC Meat Research Institute, Bristol) as a freeze-dried powder (approximately 5% w/w HMM). This was dissolved in distilled water to a concentration of HMM of 10mg/ml and dialyzed overnight at $4^{o}C$ against standard salt solution (SSS: 0.1M KCl, 5mM $MgCl_2$, 6mM phosphate buffer, pH 7 [Ishikawa et al. 1969]).

Egg chambers were teased out of ovaries in, and then extracted for 24h at room temperature in, 50% glycerol (v/v) in SSS. The glycerol was removed gradually by replacing the 50% solution at 30 min intervals with 25%, 12.5% and finally 5% (v/v) solutions of glycerol in SSS. After a quick rinse in SSS the egg chambers were placed in the 10mg/ml HMM solution, or in a control solution, and left overnight at room temperature. The following control solutions were used: a) SSS, b) 10mg/ml HMM + 10mM ATP (disodium salt, crystalline from equine muscle, Sigma: adjusted to pH 7) dissolved in SSS, c) 2mM pyrophosphate (tetrasodium salt, Sigma, adjusted to pH 7) dissolved in SSS.

The HMM or control solution was quickly rinsed out with SSS and the egg chambers were fixed as described in the Materials and Methods chapter (Procedure C).

RESULTS

The ultrastructure of cells in egg chambers extracted in 50% glycerol for 24h at room temperature, and subsequently treated with HMM solution (following the above procedure), is highly disrupted. Much of the background cytoplasmic material is removed and the appearance of membraneous structures indicates that they have swollen up and in some cases burst. The microfilamentous layer is damaged in stage 9 egg chambers; gaps in the layer occur frequently. However regions where the layer is complete, and where the orientation of the filaments is normal, can be found. Some of the filaments are more widely separated from the plasma membrane than is normally the case. The filaments which survive are decorated (plate 90). The characteristic arrowhead pattern can be discerned in places and the filaments generally appear thicker (and fuzzier!) than they do in preparations not treated with HMM. Their appearance is typical of microfilaments decorated in intact tissues which do not normally show as clear an arrowhead pattern as filaments in cultured cells or isolated filaments which have been negatively stained. In addition, microfilaments in microvilli at the surfaces of the oocyte and follicle cells where they lie against, and on either side of, the forming vitelline membrane are decorated (plates 91 - 93). The microvilli are swollen and are separated from the vitelline membrane compared with microvilli in egg chambers which have not been glycerol extracted (plate 94). The microfilament bundles within the HMM treated microvilli remain intact (they are better preserved than in the microfilamentous layer) and the decoration is clearly detectable. The

thin filaments of the epithelial sheath muscle fibres are also decorated (plate 90).

The microfilamentous layer is preserved, but not decorated, in controls treated with buffer (SSS) instead of HMM solution (plate 95). They are however less numerous than in the chambers treated with HMM solution. The binding of HMM must help to stabilize the filaments which are presumably damaged by the glycerol extraction and rendered less able to withstand disruptive properties of the fixative (osmium tetroxide can destroy actin filaments under certain conditions and HMM is capable of protecting filaments from this destruction [Maupin-Szamier & Pollard 1978]). The microfilaments of egg chambers which were treated with 10mM ATP + 10mg/ml HMM or 2mM pyrophosphate + 10mg/ml HMM solutions are very similar to the control preparations treated with SSS (plates 96 - 98).

Other stages in the development of the egg chamber, and other microfilament containing regions of the stage 9 egg chamber were not examined.

DISCUSSION

The results clearly demonstrate that two microfilament containing regions in the oocyte follicle, the microfilamentous layer and the microvillous microfilaments, are actin-like. It is probable that other microfilaments in the egg chamber would have been found to be actin-like, had they been examined. The quality of the decoration is not as good as has been obtained for isolated actin filaments or for cultured cells which have been subjected to short (e.g. 30min in 50%) glycerol extractions. It is probable that the quality could be improved, for example by using shorter glycerol extractions (24h was chosen as a starting point to ensure penetration of HMM), or by using recently developed techniques such as the use of glutaraldehyde fixative containing tannic acid [Begg et al. 1978]. Such refinements in technique could make it practical to assess the polarity of microfilaments within the layer. This polarity can only be detected in very short lengths of the filaments in the present study, so it was not possible to discover how polarity varies within the layer. Are neighbouring filaments of opposite polarity? Only filaments of opposite polarity can actively slide past one another during myosin-mediated contraction.

Actins and actin-like proteins have not been charactrerized for Drosophila egg chambers. However two actin variants (actins II and III) have been discovered in all cell types in adult flies [Horovitch et al. 1979] and it is likely that similar cytoplasmic actins are present in the egg chamber (it has become usual to describe the

actin-like proteins as cytoplasmic actins as they become biochemically characterized). The microfilaments observed in many cell types are only sufficient to represent a small proportion of the actins found in cells [Pollard 1976]. Therefore the actin types found in adult Drosophila cells might not be typical of the proteins in microfilaments (adult or egg chamber). It has been estimated, by assuming that each filament produces a similar force to that produced per thin filament in skeletal muscle, that the numbers of filaments present are more than sufficient to supply the forces required for most observed forms of cell motility [Wolpert 1965].

Myosin has been observed in Drosophila eggs and embryos (1 to 32 cell stages) using anti-myosin immunofluorescence [Warn and Bullard 1979]. Myosin must also be present in the follicle cells in egg chambers in the ovary if the mechanism of tension generation is that generally assumed [Pollard & Weihing 1974].

The actin-like nature of the microfilaments in the microfilamentous layer provides additional support for the hypothesis that they are responsible for oocyte shape changes. As they are actin-like it is reasonable to assume that they are capable of generating tension and thus, being oriented, of spatially directing oocyte expansion as proposed in chapter 4.

APPENDIX

THE DESIGN OF TWO CYLINDRICAL TENSION-TRANSMITTING CYTOSKELETONS - PERSPECTIVES AND PROSPECTS

1. The main new information emerging from the investigations: a summary

Several new and particularly interesting findings have emerged from the investigations of a cytopharyngeal basket and an ovarian follicle. Some of these are of general interest and their significance is considered below.

The ultrastructure of the cytopharyngeal basket of Nassula was examined in two states: unstretched (nonfeeding) and stretched (during the normal ingestion of an algal filament: see chapter 1). The sheath and the fibrous and dense annuli become stretched, while the rods are not markedly changed. The sheath in particular showed interesting changes in appearance. Microtubules in the sheath are not particularly well ordered, they are not packed in a regular hexagonal array as is the case for rod and crest microtubules. Stretching causes an increase in order. The microtubules become arranged in obvious radially oriented rows (evidence for these rows was also found in

unstretched baskets when they were reexamined). Circumferentially oriented strands of material that could represent elastic links between sheath microtubules were observed. Links are generally regarded as being compact inextensible structures holding microtubules firmly together. However it is possible that this is not the case for links in deformable structures such as the sheath, suctorian tentacles and the ring of outer doublets in cilia and flagella (see Chapter 1, page 41). The nature of intermicrotubule links in the rigid rods is also considered (Chapter 2). Previously unreported bundles of microtubules were discovered splaying off the basket (Chapter 2). These could act as 'guy ropes', helping to redistribute the forces acting on the basket during feeding.

The developing oocyte and its associated follicle and nurse cells in Drosophila melanogaster were investigated to determine what, if any, cytoskeletal elements are involved in the shape changes which occur during oogenesis. A layer of microfilaments was discovered close to the outer surfaces of the oocyte follicle cells (in contact with the tunica propria) in vitellogenic egg chambers. These microfilaments were circumferentially oriented in all cells. It is proposed that this microfilamentous layer is responsible for the elongation of the oocyte that occurs during the vitellogenic stages (the size and shape changes of both the oocyte and the whole egg chamber and the changes in arrangement of follicle cells that occur throughout oogenesis are considered in chapter 3). The layer could produce elongation by controlling the increase in circumference as the oocyte expands, when it is being 'inflated' by material from the maternal haemolymph (e.g.

yolk) and the nurse cell cytoplasm which streams into the oocyte through intercellular bridges. If the layer restricts the increase in diameter the follicle and the oocyte within it will be forced to elongate. A contraction of the follicle was found to occur if the egg chamber is removed from the haemolymph and placed in PBS (and various other artificial media: see chapter 5). This contraction was inhibited by cytochalasin B. This may be an indication that the microfilaments resist the expansion by active contraction and not just by a passive elastic mechanism. The microfilaments in the layer are decorated by heavy meromyosin when it is applied to glycerol extracted egg chambers (chapter 6). This confirms that the microfilaments are actin-like. In addition, a similar layer of microfilaments was found in the follicle cells surrounding the elongating dorsal appendages; they could also cause elongation by contracting and reducing the diameter of the follicle around the forming appendages.

2. Differences, similarities and contrasts in the organization of the two tension transmitting systems

This thesis has dealt with two extremes in the great variety of configurations in which two important types of cytoskeletal elements, microtubules and microfilaments, are found. The two types of configurations are very different. This is related to the two very different cell types and situations. On the one hand the investigation has dealt with a large intracellular protozoan organelle (the cytopharyngeal basket of the ciliate Nassula) which is much bigger

than an individual eukaryote tissue cell. On the other hand the study has been concerned with a multicellular complex (oocyte + follicle cells + nurse cells) concerned with oocyte growth and shaping in an insect (Drosophila melanogaster).

What conclusions can be drawn from comparison of the two tension transmitting systems? The many differences between the systems can be divided into three overlapping categories. Firstly, there are mechanical differences; the nature of the tensions and the manner in which they are transmitted are not identical. Secondly, there are differences resulting from differences in the way the Protozoa and Metazoa are organized. Thirdly, there are differences in the organization and function of microtubular and microfilamentous structures. Finally, there are some similarities between the systems, although they may be less striking than the differences.

The cytopharyngeal basket deforms in an elastic manner during the ingestion of an algal filament which is bent into a hairpin shape as it moves through the basket. The algal filament is forced to assume this shape because a feeding Nassula normally first attaches itself to the point where it first comes into contact with the filament. It does not move along the filament to one of the ends. Such behaviour would remove the necessity of bending the filament and hence of having a deformable basket (the reason why it does not move to the end of the filament is not certain; perhaps avoiding the risk of losing the filament justifies the extra expenditure of energy). The deformation of the basket is short-lived (a few seconds). Two sets of forces are

in balance; the elastic properties of the basket resist the forces generated as cytoplasmic flow propels the 'hairpin loop' of filament though the lumen. The loop itself resists compression (this resistance may be reduced by partial digestion of the cell wall). In the Drosophila egg chamber the tension is produced by the growth of the oocyte. This is very rapid for cell growth, but still takes place over a period of about 12 hours rather than a few seconds. The oocyte is 'inflated' as material from the maternal haemolymph (e.g. yolk) and cytoplasm from the nurse cells is 'pumped in'. It has been argued that a tension transmitting system helps to control the growth of the oocyte, resisting its increase in diameter and forcing it to elongate. This system does not entirely prevent increase in diameter, it reduces the rate of increase, while the diameter increases by a factor of three or more.

A comparison of the dimensions of some of the structures involved in the two systems will help illustrate some differences in the way Protozoa and Metazoa are organized (Table A). The follicle cells, like most metazoan cells, are considerably smaller than the single cell of the protozoan. The cytopharyngeal basket is about the size of 5 follicle cells stacked on top of each other. It is interesting to note that an oocyte in the middle of its rapid growth (stage 10) has very similar dimensions to the whole ciliate. The difference is that while the ciliate performs all the functions it needs to survive within the single cell, the oocyte is a special system which is set up to perform a special function - the very rapid formation of an embryo (the syncytial blastoderm). The oocyte stores materials for this function,

Table A

Comparison of various dimensions in Nassula and Drosophila

		Width (/μm)	X	Length (/μm)
Nassula	cytopharyngeal basket	12	X	55
	whole animal	100	X	175
Drosophila	follicle cell*	10	X	10
	oocyte+ S_9 (start)	50	X	75
	S_{10} (start)	100	X	160
	S_{11} (start)	170	X	240
	mature egg	170	X	470

* The dimensions of a cuboidal follicle cell are given as an example: cuboidal and squamous cells probably have similar volumes.

+ Stages 9 to 11 (see chapter 3) include the main growth of the oocyte. They are the main vitellogenic stages.

and remains inactive until development is triggered by an external stimulus (fertilization). Much of the work done in setting up this system is not done by the oocyte itself. The follicle cells and nurse cells contribute materials, apparently help to shape the oocyte, and manufacture the specialized structures needed by the mature egg. The cytopharyngeal basket probably evolved by an elaboration of pre-

existing subcellular components (subpellicular microtubules associated with basal bodies), and is one of the most complexed of such structures found within any cell. The structures such as the micropyle and dorsal appendages formed by the follicle cells are examples of the way in which Metazoa can make a wide range of structures using the same basic unit (the epithelial cell). Neither the oocyte or its accessory cells need complicated structures for feeding: they absorb materials from the haemolymph which have been prepared elsewhere in the female Drosophila.

What similarities are there between the two systems? They share the large number of common features of all eukaryote cells. Although microtubules are more prominent in one, and actin filaments in the other, both types of cytoskeletal component are present in both systems (actin may not be organized as detectable microfilaments in Nassula, but can be assumed to be involved in the cytoplasmic streaming. This has been demonstrated in other ciliates [Metenier 1984]). Certain similarities relate to the large cell size of Nassula and the Drosophila oocyte/egg. Both have specialized nuclei (macronucleus in Nassula and nurse cell nuclei in Drosophila are both highly polyploid). Both have reinforcement (of different natures) at the cell surface (pellicle and egg shell).

3. Prospects for further investigations and analyses

What other experiments could be performed to further substantiate

the major hypotheses proposed in this thesis, and to ascertain more about the cytoskeletal elements that have been investigated? Some of the investigations could be extended. In Nassula a more detailed examination of regions of the stretched basket other than the sheath, and also of structures such as the extensions of the sheath described in chapter 2 (e.g.to discover if they insert on the pellicle) could have been made. The cytoplasmic streaming through the basket could also be examined. The heavy meromyosin decoration technique could be used to explore the involvement of actin filaments, and to ascertain whether these are associated with basket components (e.g. the lamellae). In Drosophila more stages of egg chamber development could have been examined to discover how the microfilamentous layer is set up and to see if it disappears completely as the egg shell forms. Other microfilamentous structures in the egg chamber could be similarly examined, and their actin-like nature could be confirmed by investigating whether they can be decorated by heavy meromyosin. Further confirmation of the role of the microfilamentous layer in the shaping of the oocyte could be obtained if it was found to be present in other insects. As discussed in chapter 4 (page 95) the microfilamentous system could be an adaptation permitting shape control in a rapidly expanding oocyte. If so a similar system might be found in other Diptera or in other orders with telotrophic oogenesis. However the absence of a microfilamentous layer in other insects would not prove that the layer in Drosophila is not involved in controlling oocyte shape.

It might be possible to perform some manipulative experiments to

investigate the forces involved in the stretching of the basket, and in the shaping of the oocyte. The force involved in the contraction of the microfilamentous ring during the first cleavage of sea urchin eggs has been measured using glass needles. The force involved was calculated from the amount a very fine glass needle bends [Rappaport 1967]. It would be very difficult to insert a needle into the lumen of the Nassula basket, but the force involved in the contraction described in chapter 5 could possibly be measured using Rappaport's technique. It might also be possible to measure an 'inflation pressure' for the oocyte using micropipettes.

The use of drugs such as colchicine and cytochalasin B in the study of Drosophila oogenesis could be extended if it was possible to get oocytes to undergo vitellogenesis in vitro (see page 105). Perhaps some processes such as dorsal appendage elongation might need less exacting conditions (this was tried without success, but it could be possible).

It should be possible to discover something about the composition of both systems using polyacrylamide gel electrophoresis. Attempts were made to isolate the cytopharyngeal basket for this purpose. It proved not to be practicable because no method was found by which the basket could be isolated from other components of the ciliate, even though the basket could be freed from the pellicle and other structures without visible damage by lysis with mild detergent (Triton X-100) and brief agitation (a quick shake). The basket did not band on sucrose gradients, nor was it possible to collect them individually in

sufficient numbers to run gels. If sufficient material was obtained to run gels the information gained would still be limited. Bands on the gels corresponding to the molecular weights of tubulin and actin would be expected, but it would probably not be possible to determine which of the other bands represented links or other components of the basket. Unfortunately other biochemical investigatory techniques require much more material than polyacrylamide gel electrophoresis. In the Drosophila egg chamber it would be difficult or impossible to distinguish actin, say derived from the microfilamentous layer, from actin derived from other cytoplasmic sources. However, techniques for mass collection and fractionation of different stages of Drosophila egg chambers are available [Jacobs-Lorena & Crippa 1977], so biochemical investigations would be more practicable than in Nassula; they would be likely to yield some useful information.

It is possible that much could be learnt about the composition of structures in Nassula and Drosophila using antibody labelling techniques, such as indirect immunofluorescence or ferritin binding in conjunction with electron microscopy. Antibodies to a wide range of cytoskeletal proteins (tubulin, actin, myosin, alpha-actinin etc.) are available.

Much could be learnt about the mechanisms involved in both systems if mutant strains were found which affected the systems. Any mutant strains of Nassula would have to be prepared specially and the necessary screening would make this very time consuming. There are many mutations known in Drosophila melanogaster which affect

oogenesis [Mahowald & Kambysellis 1980]. These include 'female sterile' mutations [King & Mohler 1975]. Some mutations such as tiny (ty,1-44.5) have defective follicle cell migrations and malformed eggs. The primary defect can be either in the follicle cells or in the germ line (as in tiny [Dimario & Hennen 1982]). Other mutants have endocrine defects [Postlethwait & Handler 1978] which block vitellogenesis. Mutants with reduced elongation of the dorsal appendages are known, for example fs(3)K1 [Mahowald & Kambysellis 1980]. Some information might be gained by examining these mutants even if a mutant with defective oocyte elongation could not be found.

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SYMBOLS AND ABREVIATIONS USED IN PLATE LEGENDS

LM : Light microscopy

/NIC	Nomarski interference contrast
/PC	Phase contrast
/MeBl	1um Araldite section stained with methylene blue
/Feulgen	Wholemount stained with Feulgen
/Silver	Wholemount stained with silver stain

EM : Electron microscopy

/A }
/B } FIXATION PROCEDURES
/C } SEE MATERIALS AND METHODS
/D }

/80kV High tension on electron microscope (if not specified = 60kV)

/$KMnO_4$ Stained with $KMnO_4$ in addition to standard staining

procedure

OTHER ABBREVIATIONS

DPBS	Drosophila phosphate buffered saline
DMSO	Dimethyl sulphoxide

SEE MATERIALS AND METHODS FOR DETAILS OF ABOVE

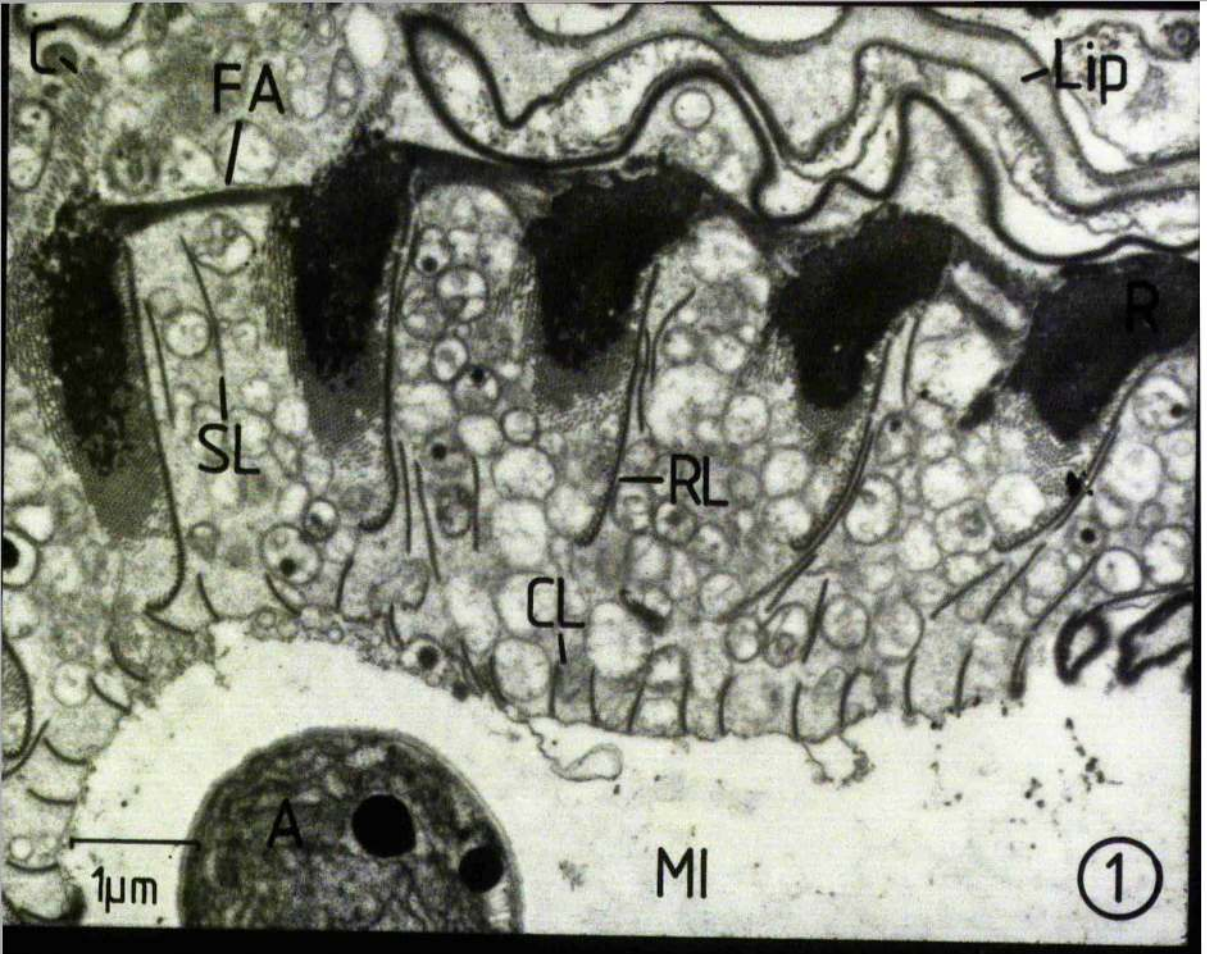
A. THE CYTOPHARYNGEAL BASKET OF NASSULA AUREA

PLATE 1

Part of a transverse section of the cytopharyngeal basket at the level of the fibrous annulus (FA), in a feeding organism. The rods (R) are further apart than in the resting basket, and the fibrous annulus is stretched. The structure is otherwise little changed. The following features can also be observed: Crests (C), lip of oral atrium (Lip), cytostomal lamellae (CL), subcytostomal lamellae (SL), rod lamellae (RL), membraneous invagination (MI) and the algal filament being ingested (A). X 12 600. EM/B.

PLATE 2

Part of a transverse section of the cytopharyngeal basket at the level of the dense annulus (DA) in a feeding organism. The appearance of the dense annulus is unchanged from the resting condition. Links of different types are present between the rod (R) and crest (C) microtubules. X 66 000. EM/B.


C
FA
Lip
R
SL
RL
CL
A
1µm
MI
1

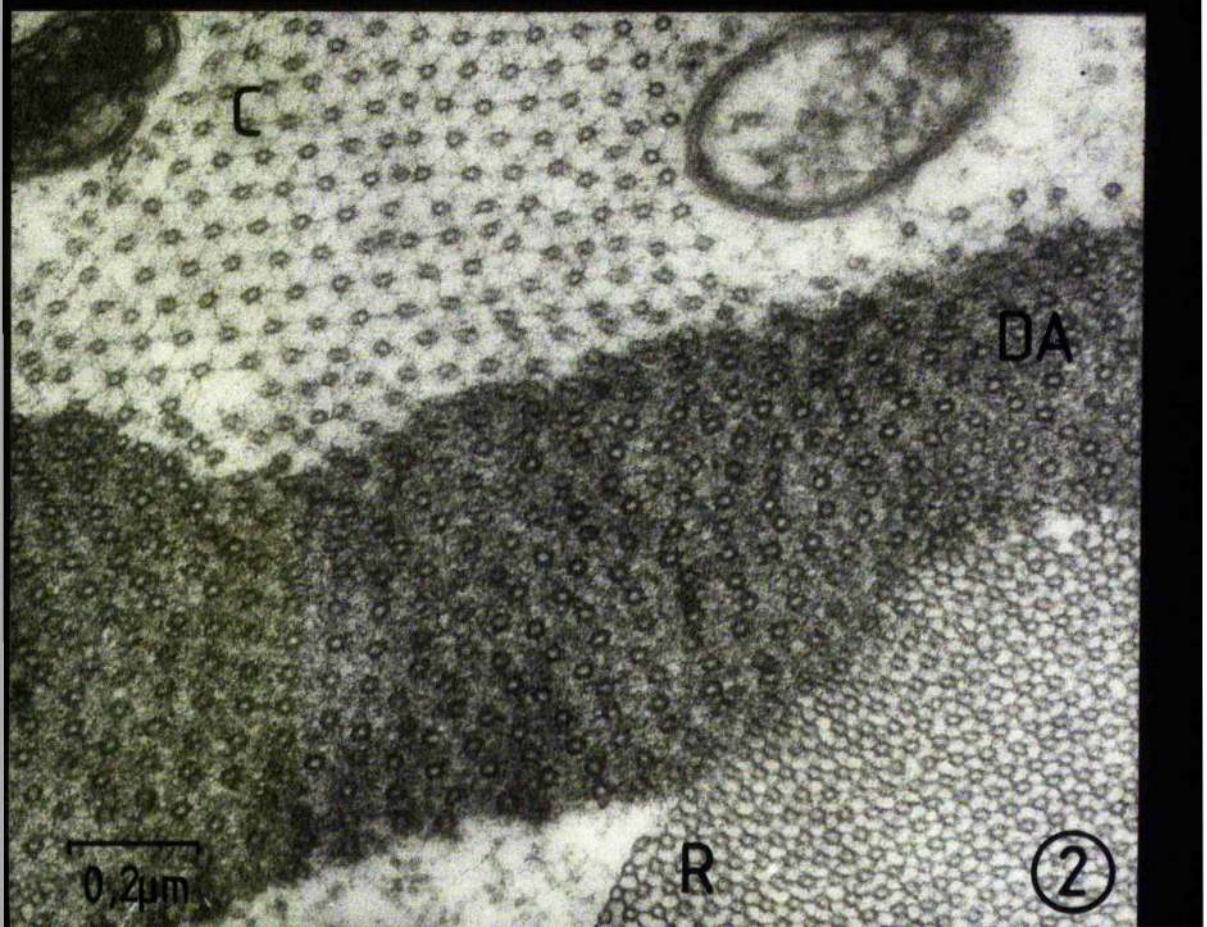

C
DA
0,2µm
R
2

PLATE 3

A transverse section of the cytopharyngeal basket of a feeding organism (10 to 20 μm beneath the dense annulus). The algal filament (A) is bent into a hairpin shape and so passes through the plane of the section twice. The presence of the hairpin loop of filament causes the basket to be considerably distended. The sheath (S) becomes stretched but the rods (R) retain their resting dimensions. X 8300. EM/B.

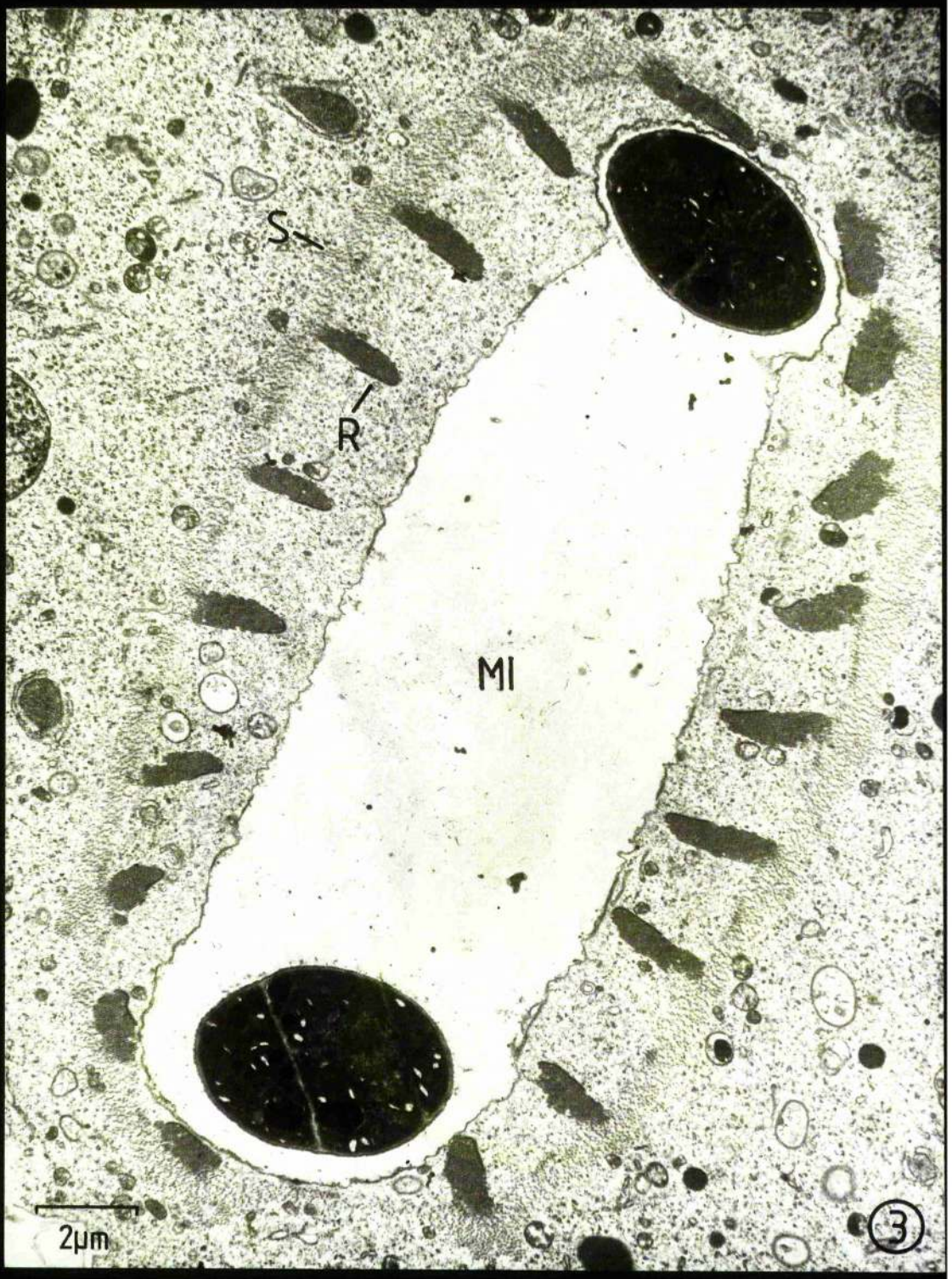
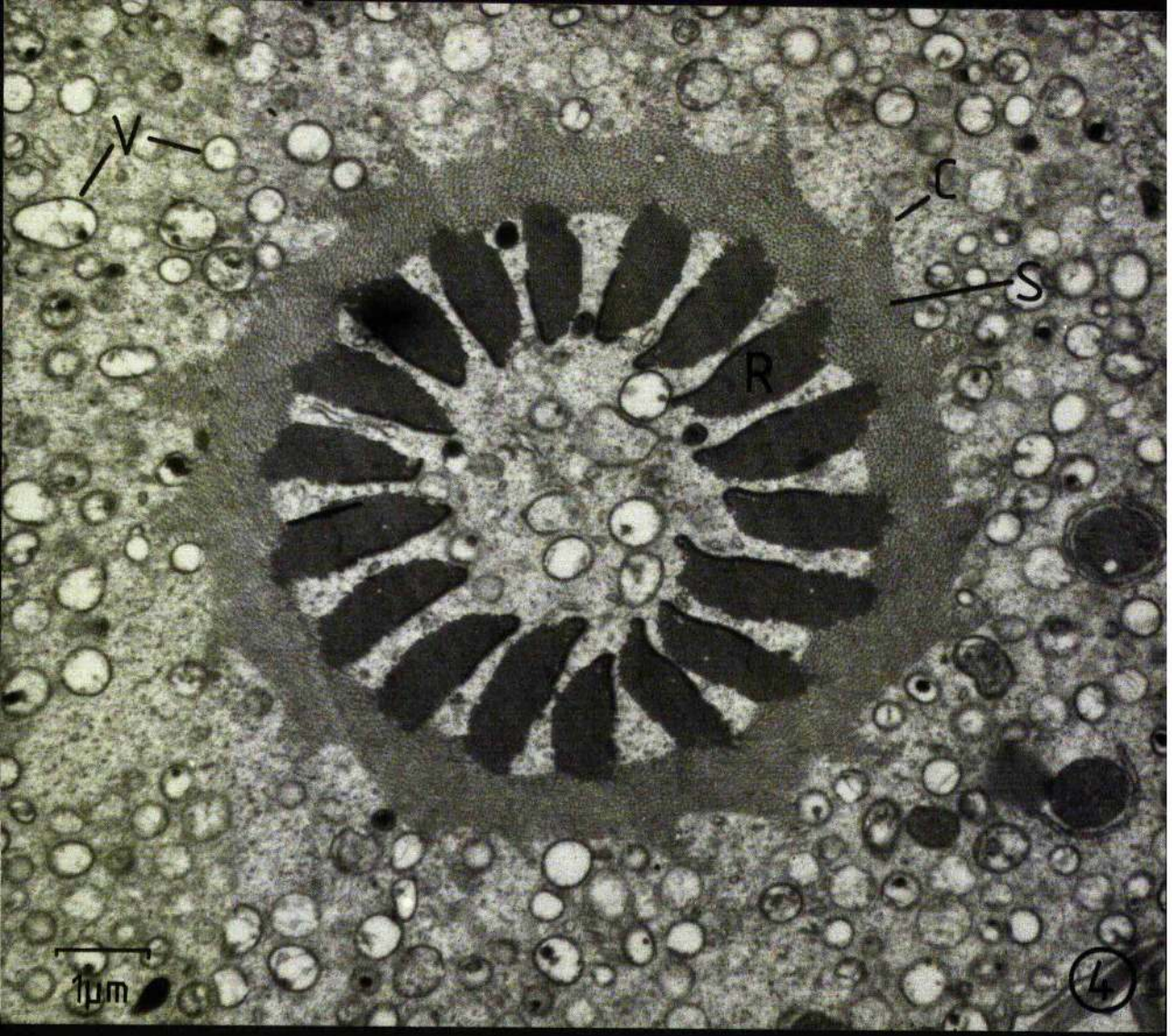

S
R
MI
2µm
3

PLATE 4

A transverse section of the cytopharyngeal basket in a nonfeeding organism (slightly closer to the dense annulus than the section illustrated in plate 3). The basket is circular in cross-section. In addition to the rods (R), crests (C) and sheath (S) of the basket, numerous cytopharyngeal vesicles (V) may be observed in the surrounding cytoplasm. X 12 000. EM/A.

PLATE 5

Part of a transverse section of the cytopharyngeal basket of a feeding organism. The crests (C) and rods (R) retain their resting appearance but the sheath (S) is stretched. Note that the microtubules in the sheath appear to be arranged in radially oriented rows.
X 54 000. EM/B.


V
C
S
R
1µm
4

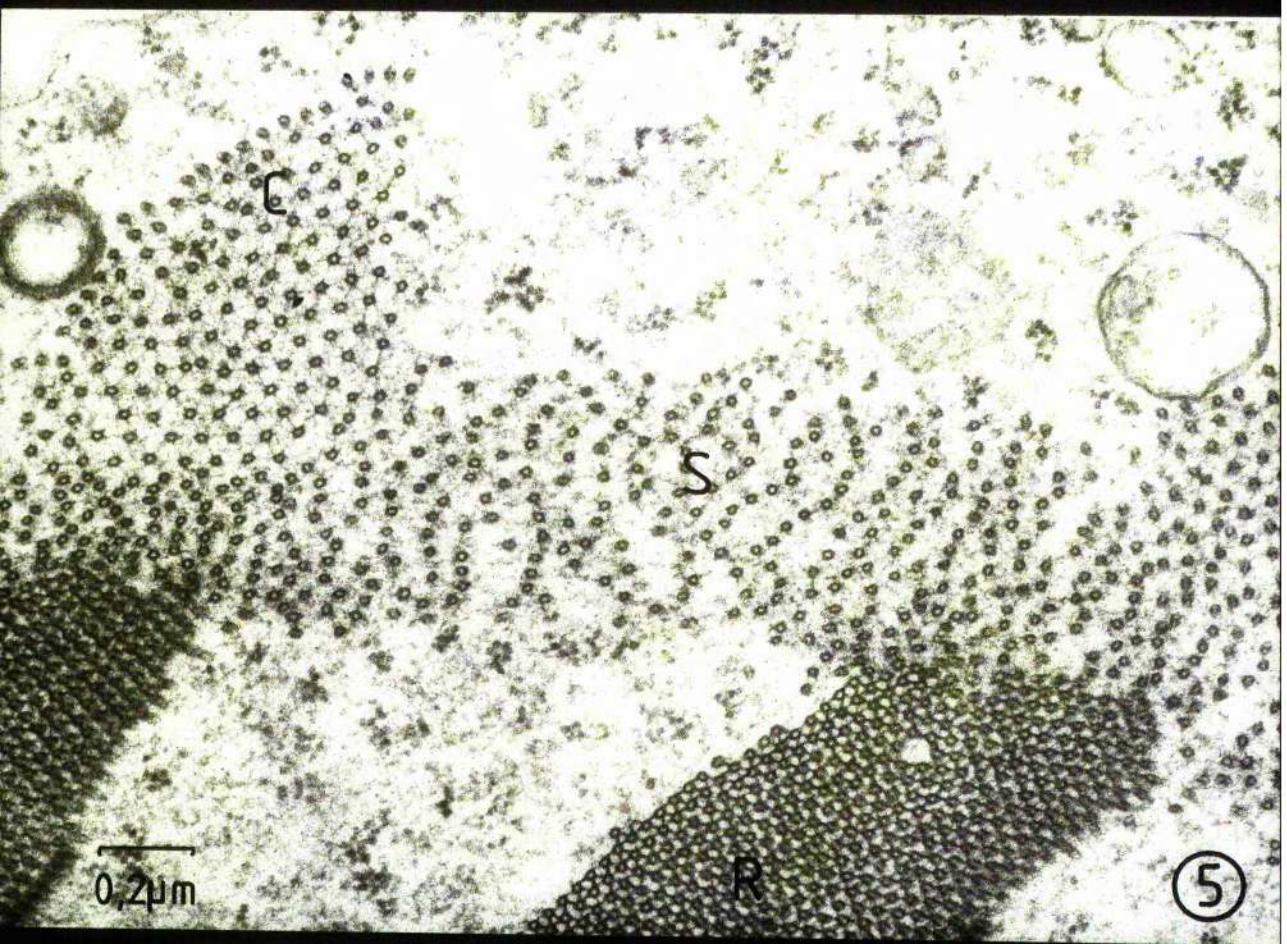

C
S
R
0,2µm
5

PLATE 6

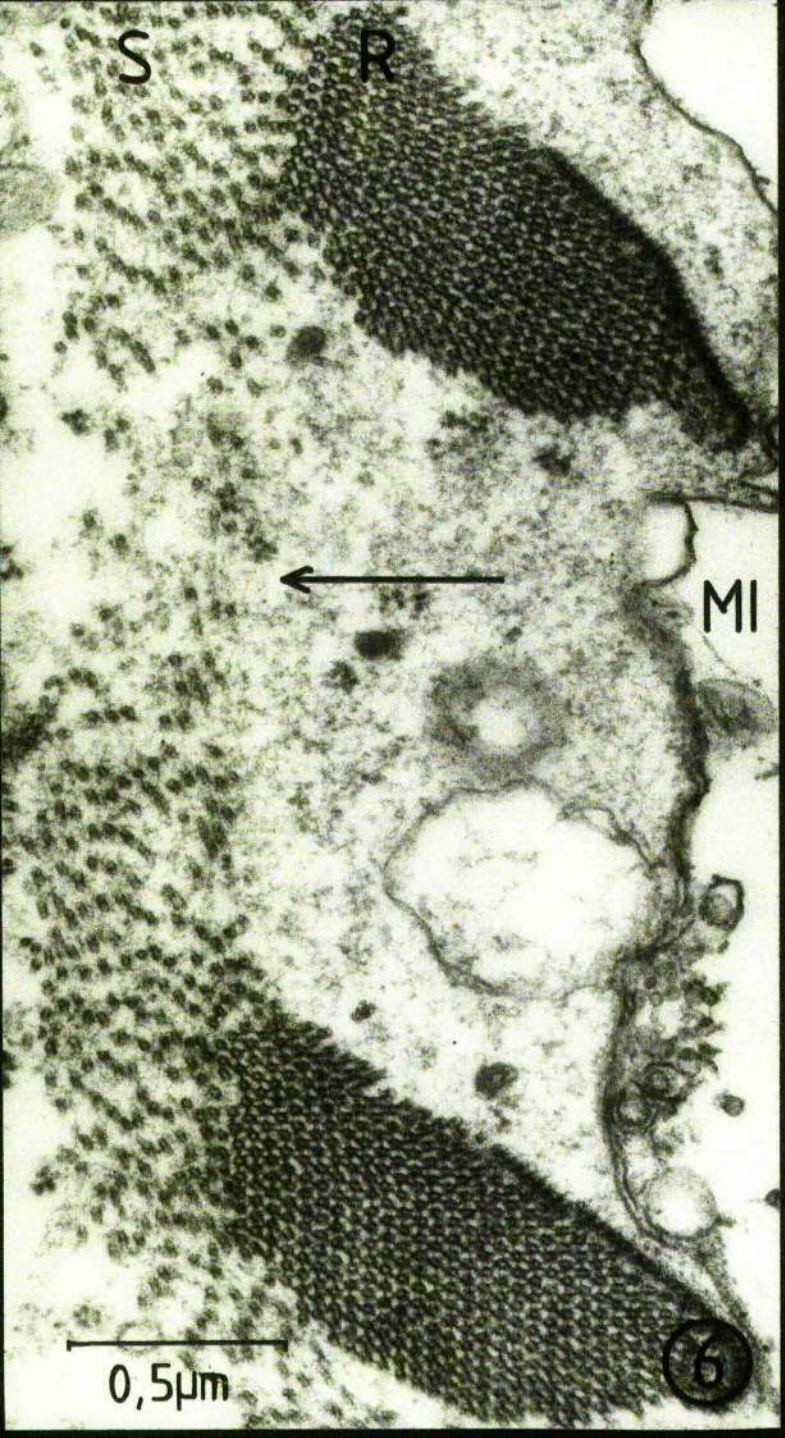
Part of a transverse section of the cytopharyngeal basket, in a feeding organism. The arrow points to a region of sheath which is much reduced in thickness (S: sheath, R: rod, MI: membraneous invagination). X 42 000. EM/B.

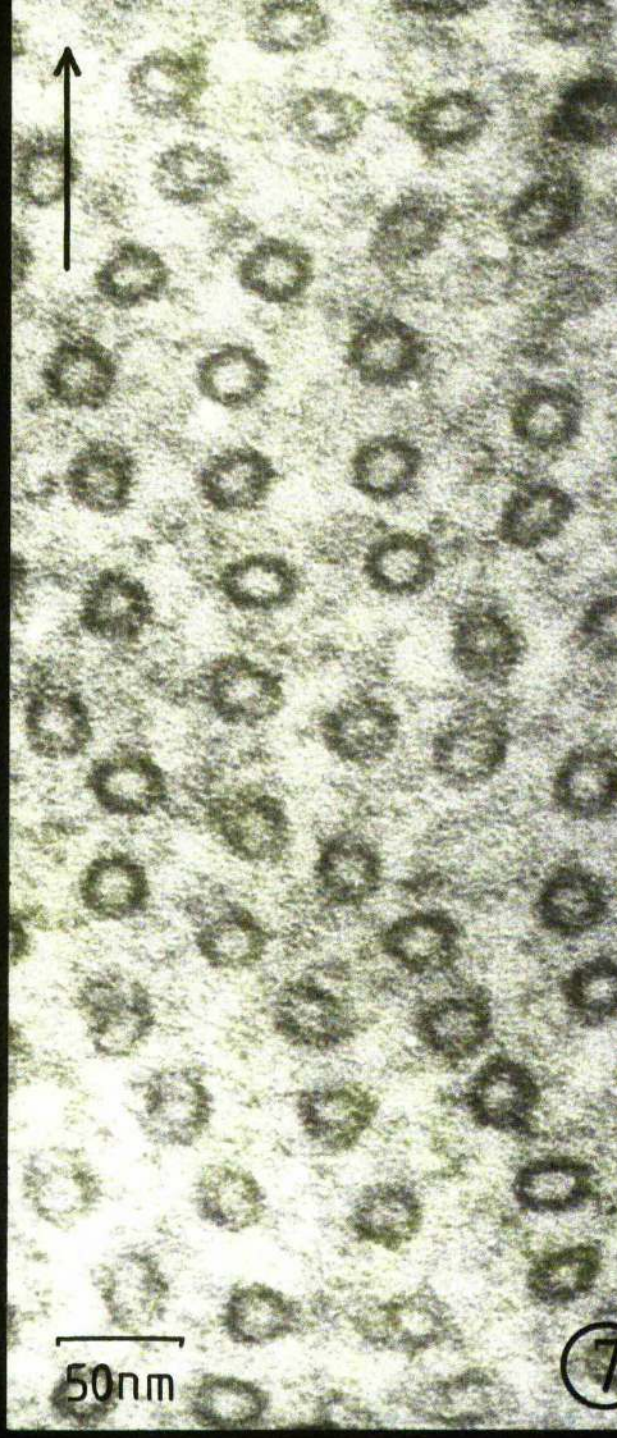
PLATE 7

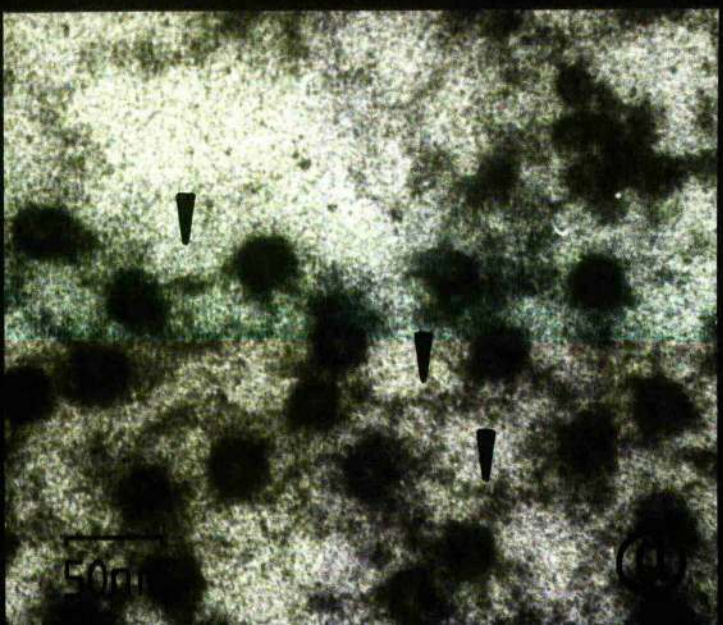
Part of a transverse section of an unstretched cytopharyngeal basket showing radially oriented rows of microtubules (arrow: radial direction). X 243 000. EM/A.

PLATE 8

Part of a transverse section of a stretched sheath. Strands of material (arrowheads) run between the radially oriented rows of material.
X 193 000. EM/B.


S
R
MI
0,5µm
6


50nm
7


50nm

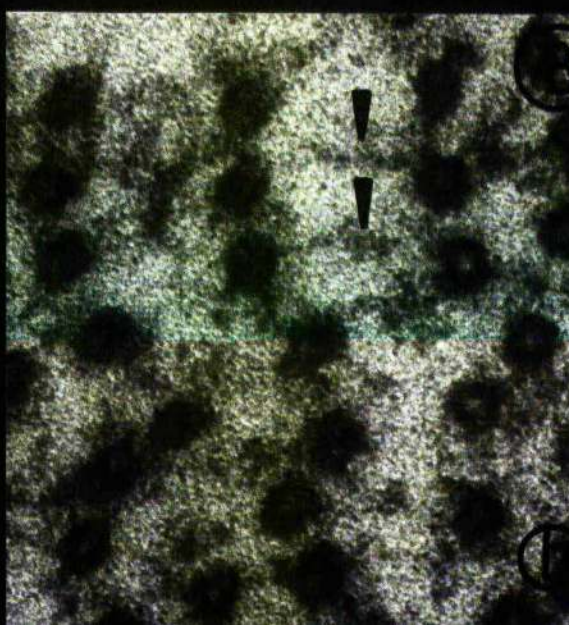


PLATE 9

Extensible models

The photographs illustrate the ways that models constructed from cork discs (representing microtubules) interconnected by flexible elastic links (rubber elastic bands) and rigid inextensible links (cross-banded matchsticks) are deformed when stretched at right angles to the side of the page by application of tension to strings attached at either end of each array. The three strings at either end of each array were attached to a wooden bar oriented at right angles to the longitudinal axes of the strings. Hence when the bars were pulled apart the same amount of tension was applied to each string during stretching. Unstretched models are shown on the left side of the page and their appearance after stretching is shown on the right. See chapter 1 for further details.

A
A'
B
B'
C
C'
D
D'
E
E'

PLATE 10

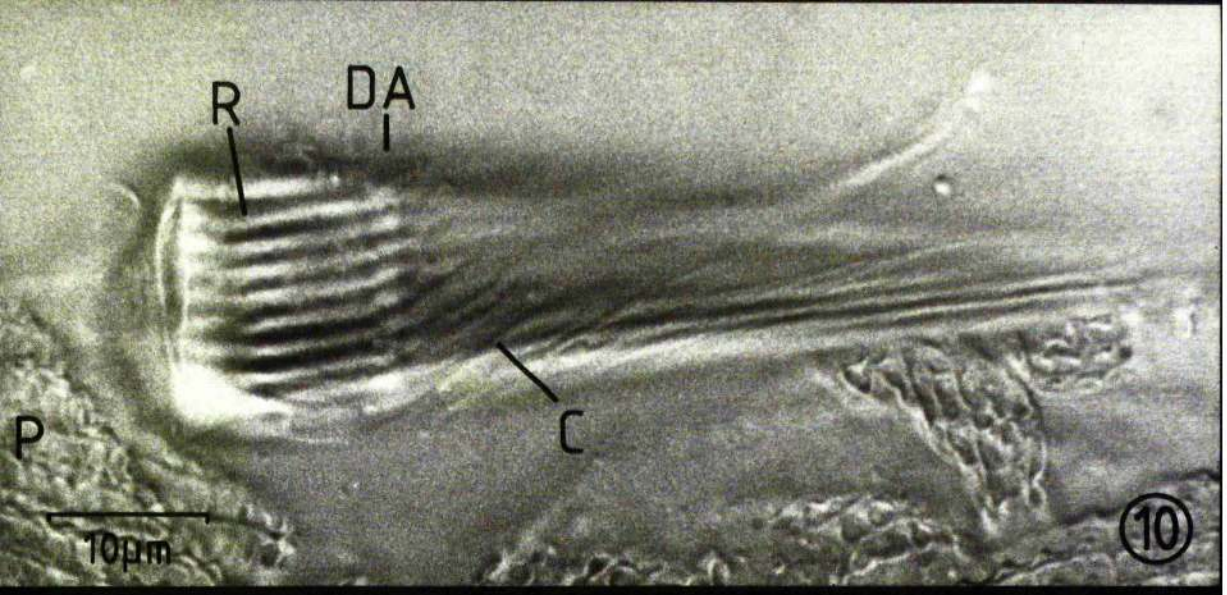
The cytopharyngeal basket of an organism lysed in 0.25% Triton X-100, 3mM $MgCl_2$, 30mM Tris-HCl, pH 7.5. The rods (R), dense annulus (DA) and crests (C) can be observed. The basket is attached to the remains of the pellicle (P). X 1900. LM/NIC.

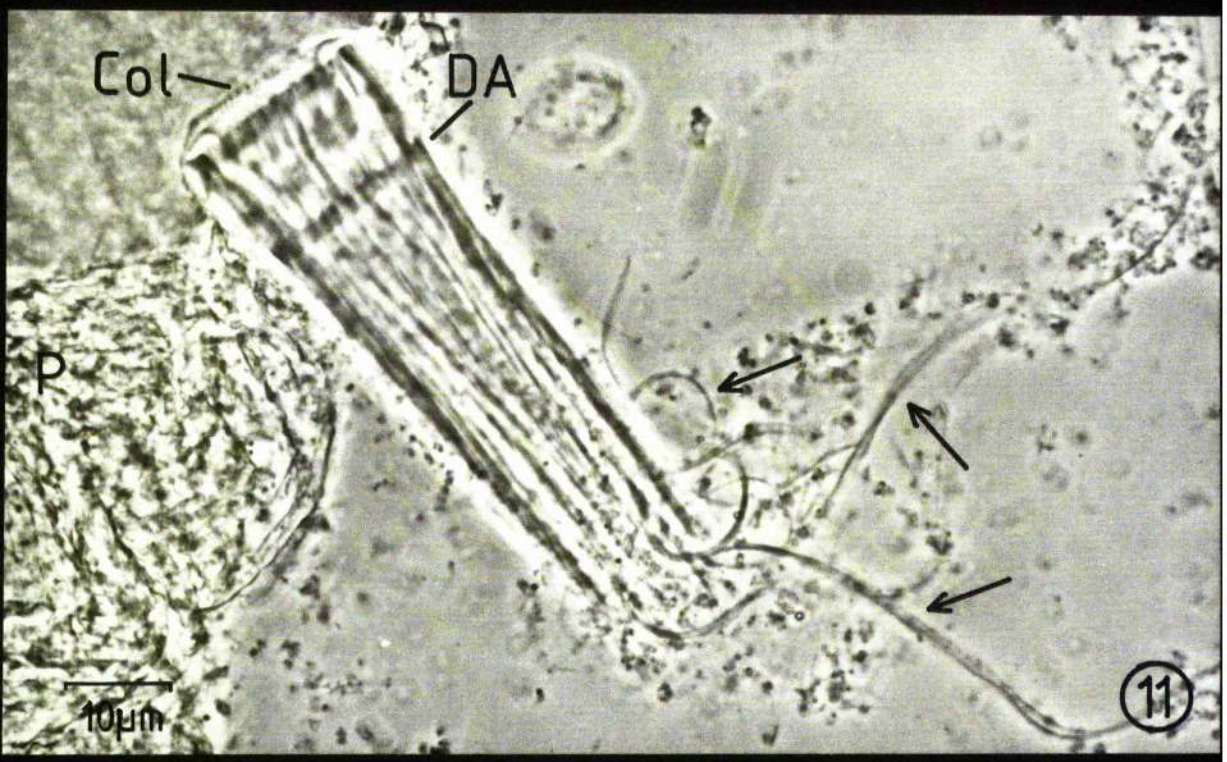
PLATE 11

A cytopharyngeal basket of a lysed organism (see legend to plate 10). The corrugations of the collar (Col), the dense annulus (DA) and extensions from the end of the basket (arrows) are illustrated. X 1300. LM/PC.

PLATE 12

Part of a cross-section of the lower region of the cytopharyngeal basket. Small bundles of microtubules (arrows) are present near the sheath (S, R: rod). X 40 000. EM/A.


R
DA
C
P
10µm
10


Col
DA
P
10µm
11

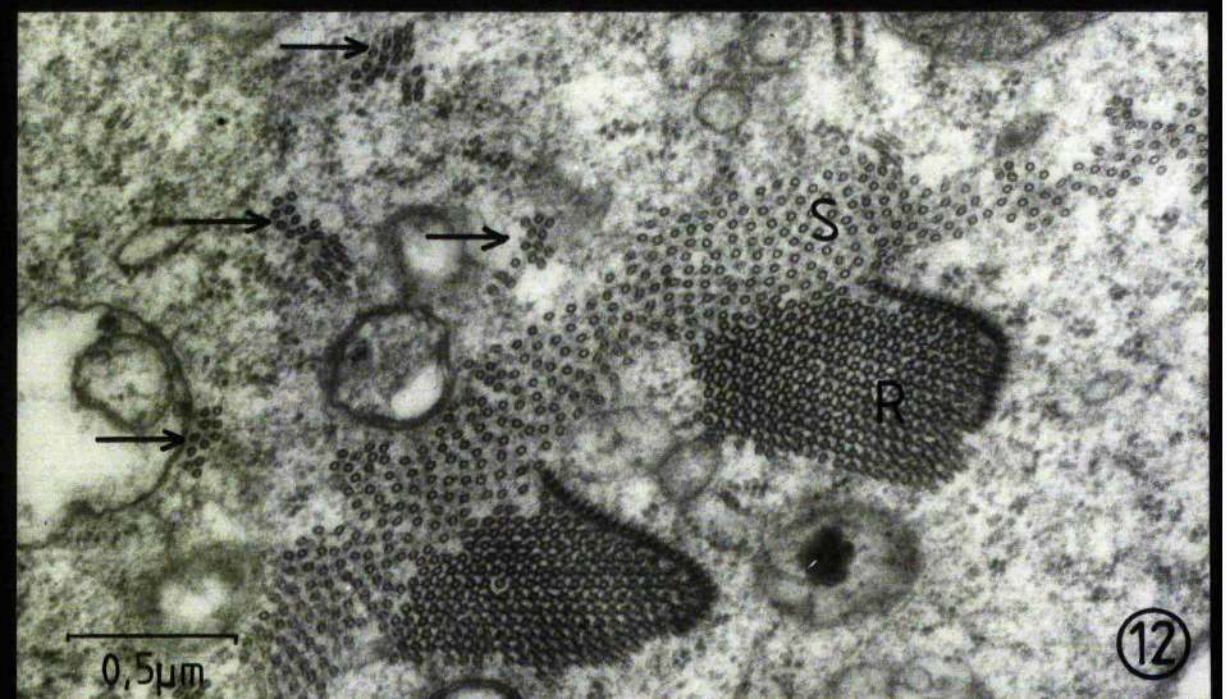
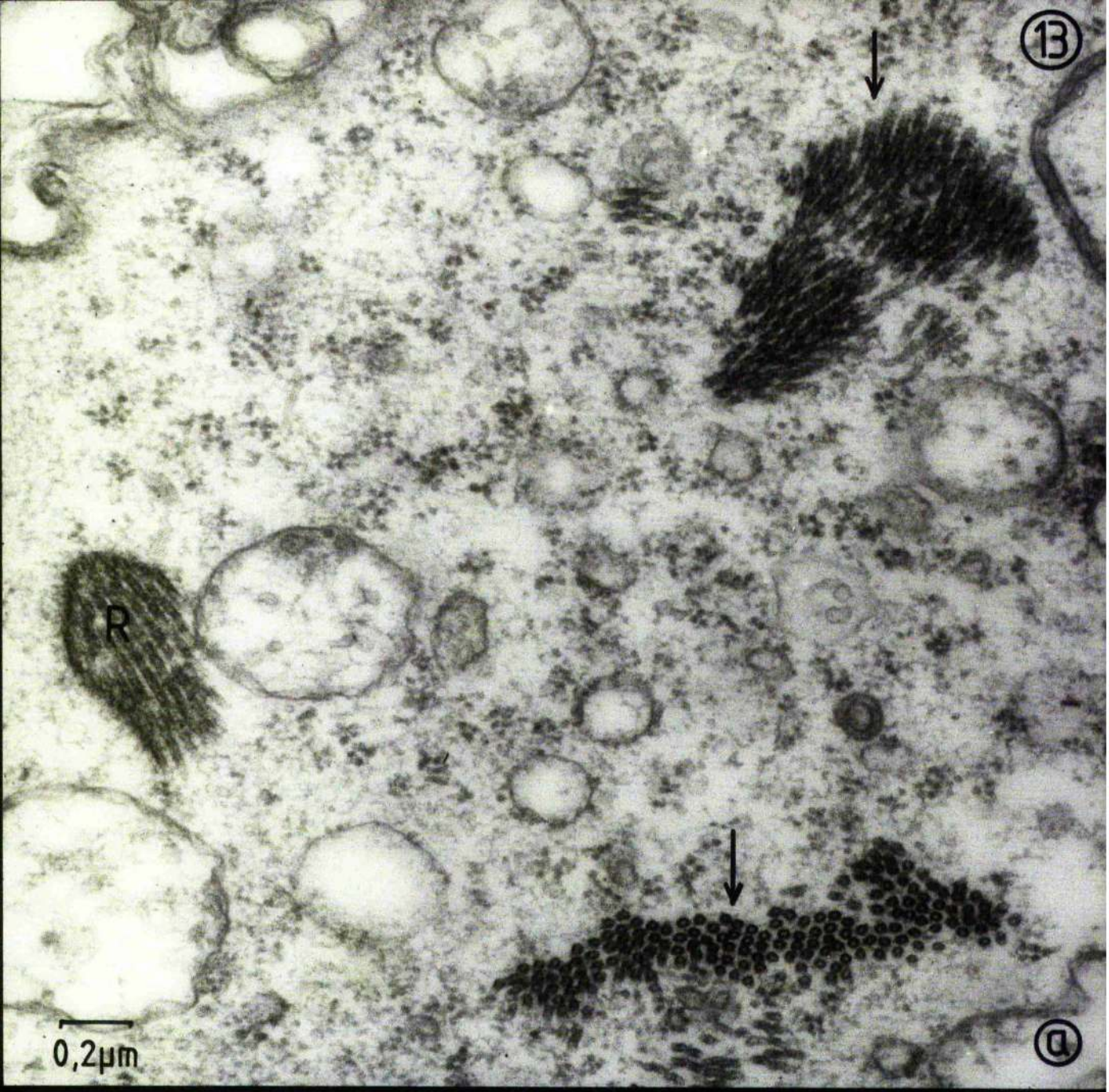

S
R
0,5µm
12

PLATE 13a

Large bundles of microtubules (arrows) present near the bottom of the basket. The sheath is not present at this level and the rods (R) are much reduced in size. X 51 000. EM/B.

PLATE 13b

Enlargement of the lower part of plate 13a. Thin links connect some of the microtubules (arrowheads) in the bundle. X 113 000. EM/B.


13
R
0,2µm
a

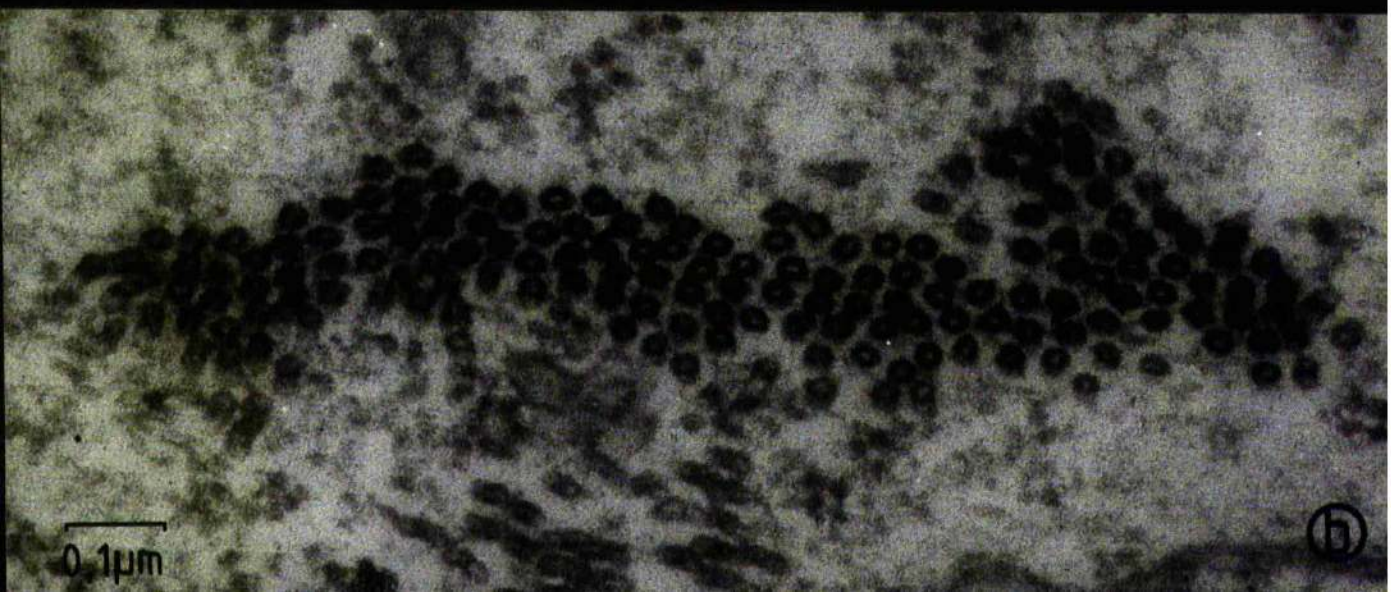

0,1µm
b

PLATE 14

Bundles of microtubules (arrows) close to the pellicle. Microtubules occasionally insert on the pellicle (arrowhead). X 43 000. EM/A.

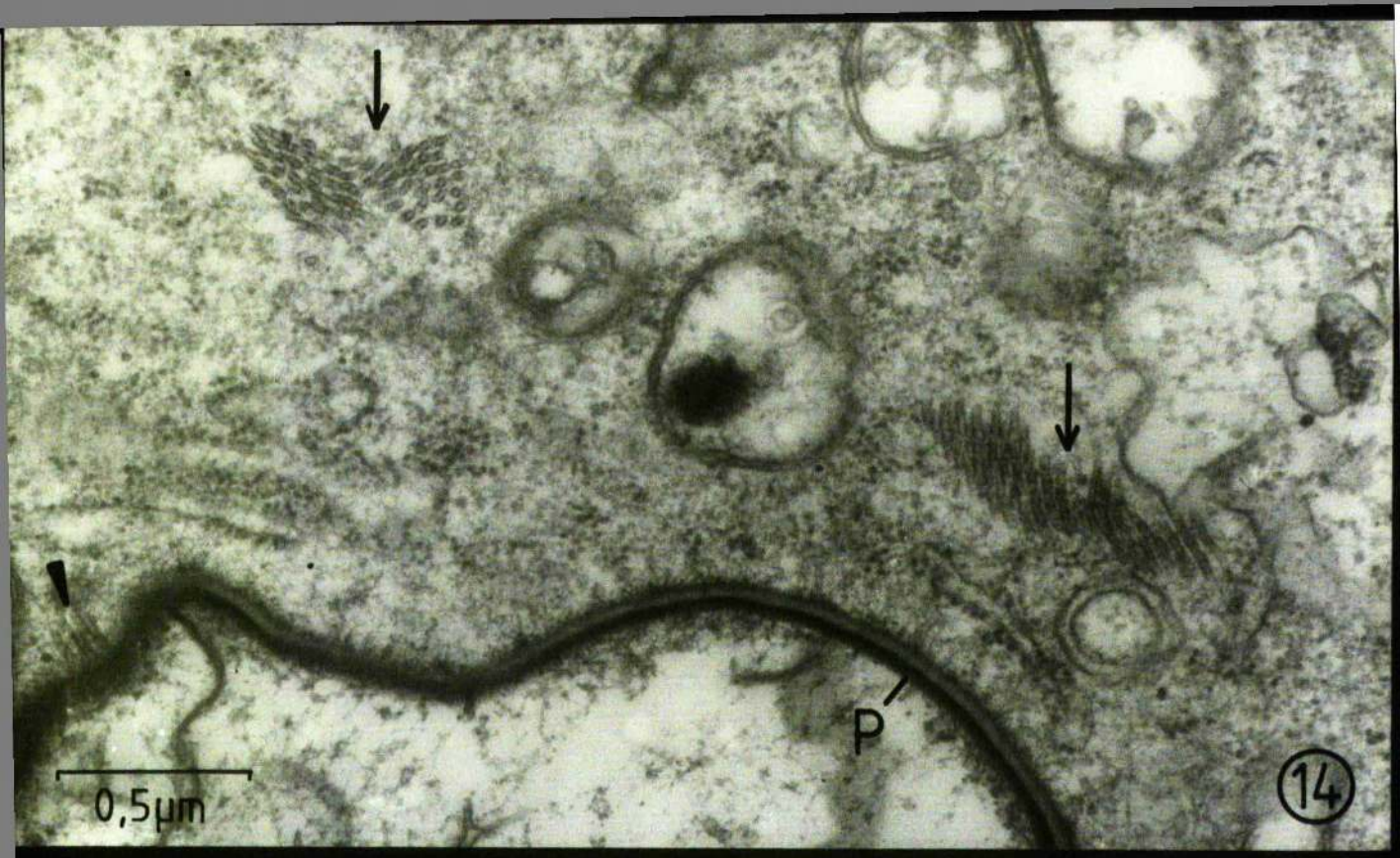
PLATE 15

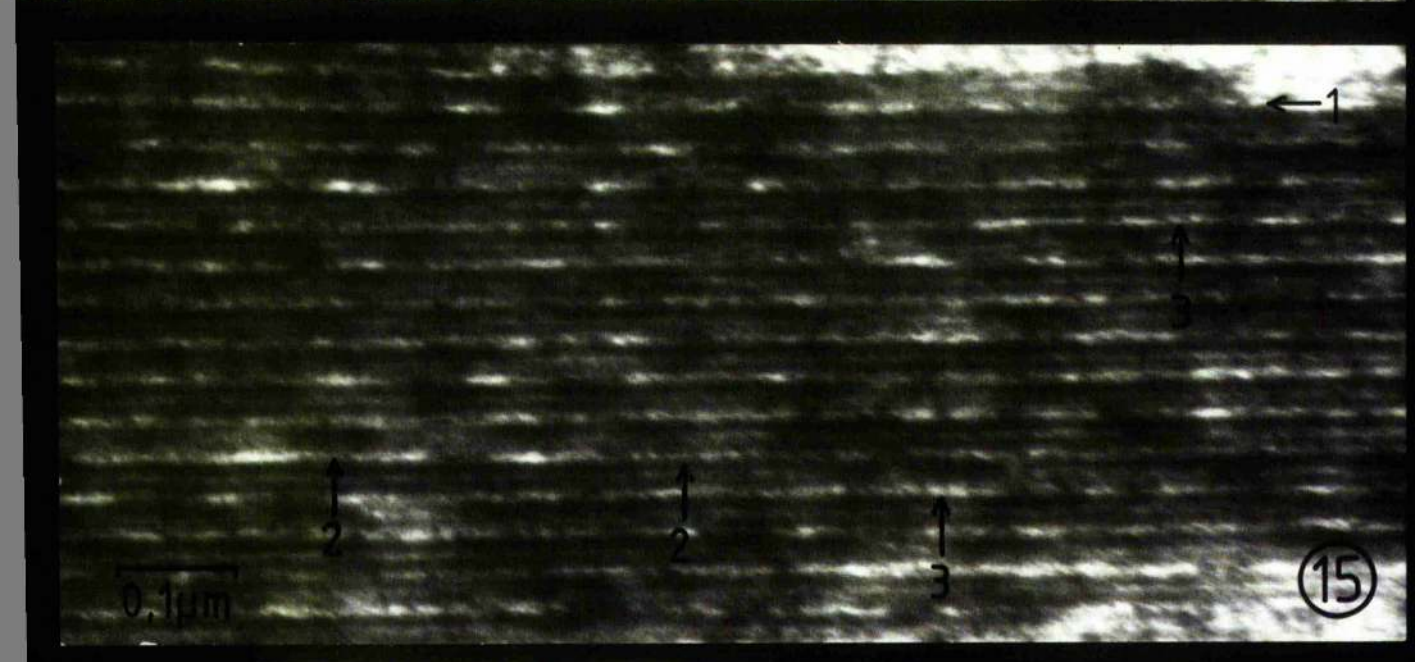
A longitudinal section of a rod stained with $KMnO_4$. Link-like structures can be observed:

1. A row of patches of dense and less dense material between two microtubules, giving the appearance of broad links (cf. figure 2.3a).
2. Regions where the dense patches appear to be composed of several small links (cf. figure 2.3b).
3. Regions where the less dense patches appear to be composed of small links (cf. figures 2.3c and 2.3d). X 133 000. EM/A/$KMmO_4$.

PLATE 16

Rod microtubules stained with $KMnO_4$ passing out of the plane of section. There appear to be small links between the microtubules (arrows). X 133 000. EM/A/$KMnO_4$.


P
0,5µm
14


1
3
2
2
3
0,1µm
15

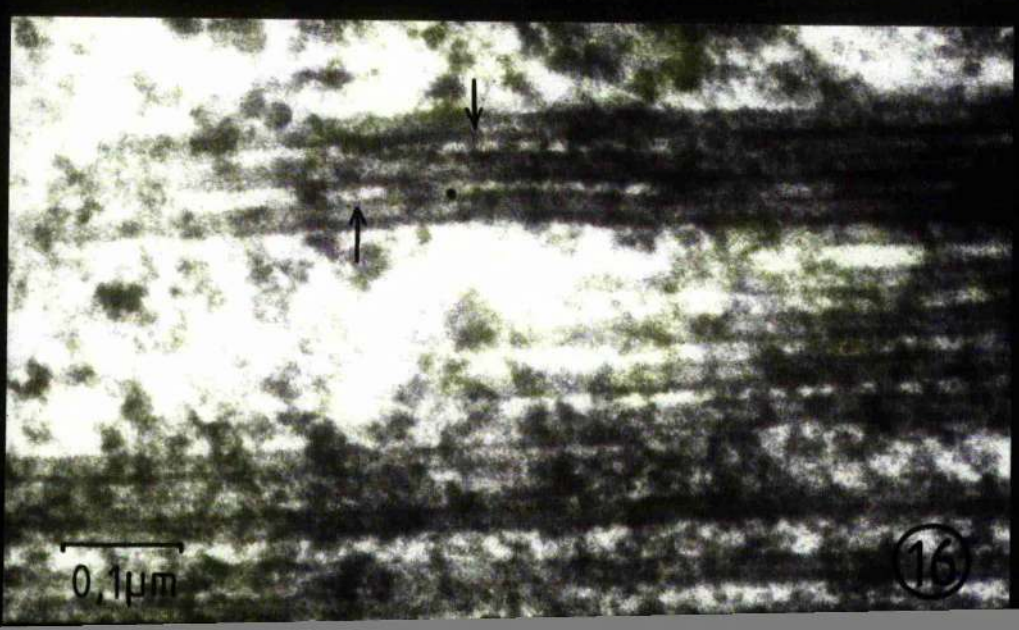

0,1µm
16

PLATE 17

A longitudinal section of the upper region of a cytopharyngeal basket in an organism forming a cytoplasmic extrusion. X 6500. EM/A.

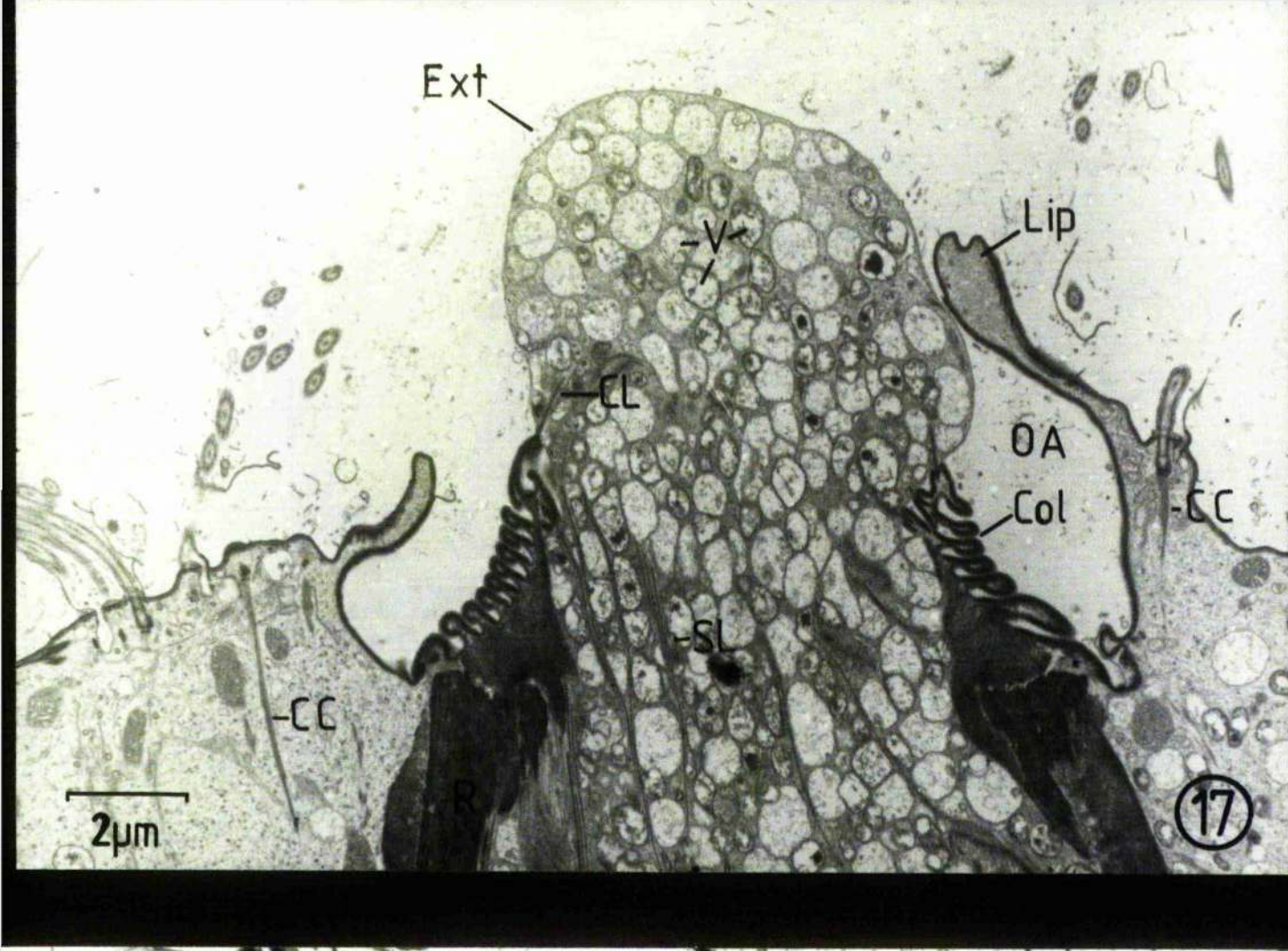
PLATE 18

A similar section to that in plate 17, but in a resting organism. The arrow points in the direction of the opening of the oral atrium (out of plane of section). X 9200. EM/A.

SYMBOLS USED ON PLATES 17 AND 18

CC	Ciliary connectives	OA	Oral atrium
CL	Cytostomal lamellae	OM	Oral membrane
Col	Collar	R	Rod
Ext	Cytoplasmic extrusion	SL	Subcytostomal lamella
Lip	Lip of oral atrium	V	Cytopharyngeal vesicles

The lip of the oral atrium, the collar and the cytopharyngeal lamellae are raised in the organism forming the extrusion (plate 17). The extrusion contains a large number of cytopharyngeal vesicles.


Ext
V
Lip
CL
OA
Col
CC
SL
CC
R
2µm
17

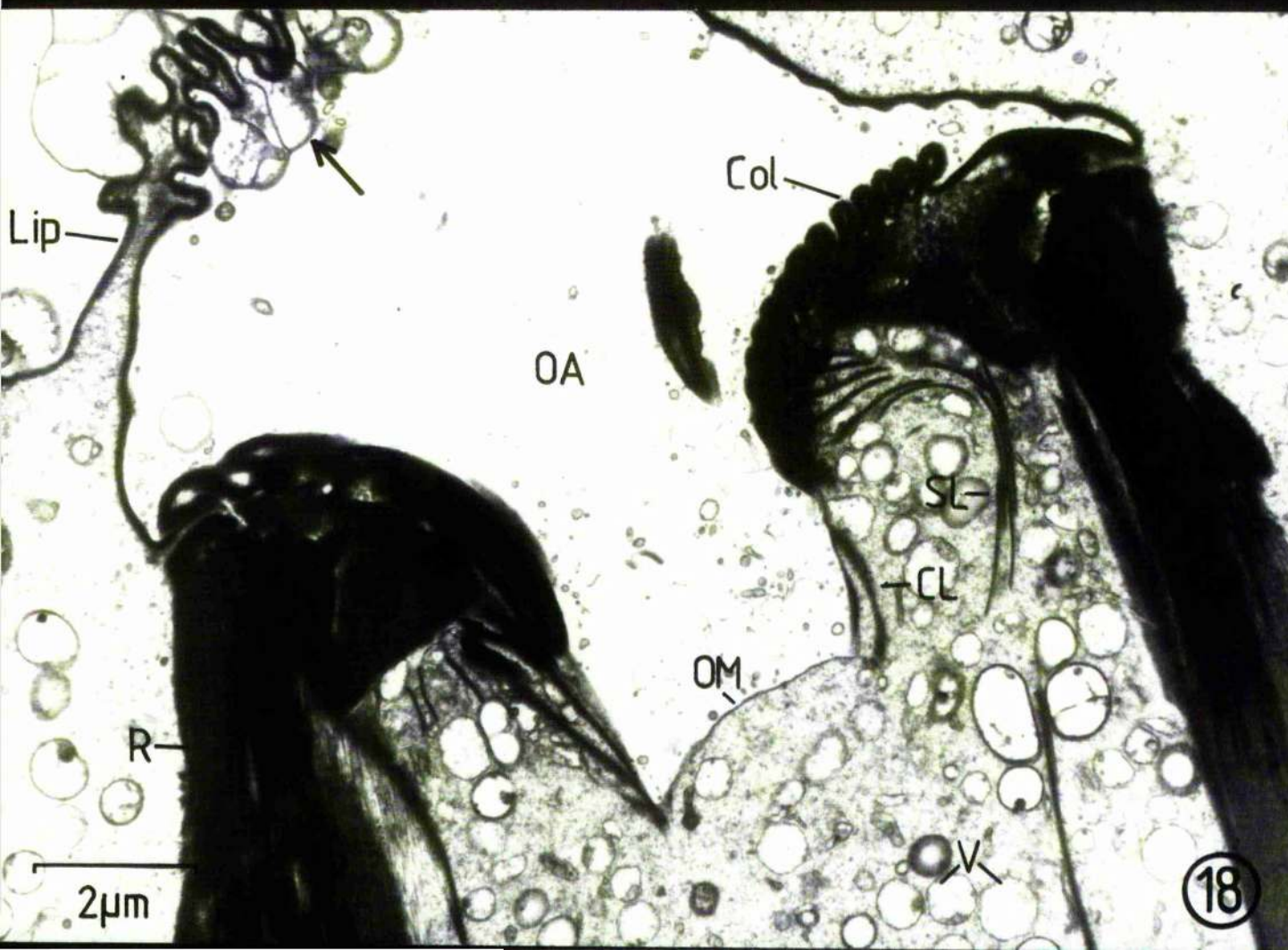
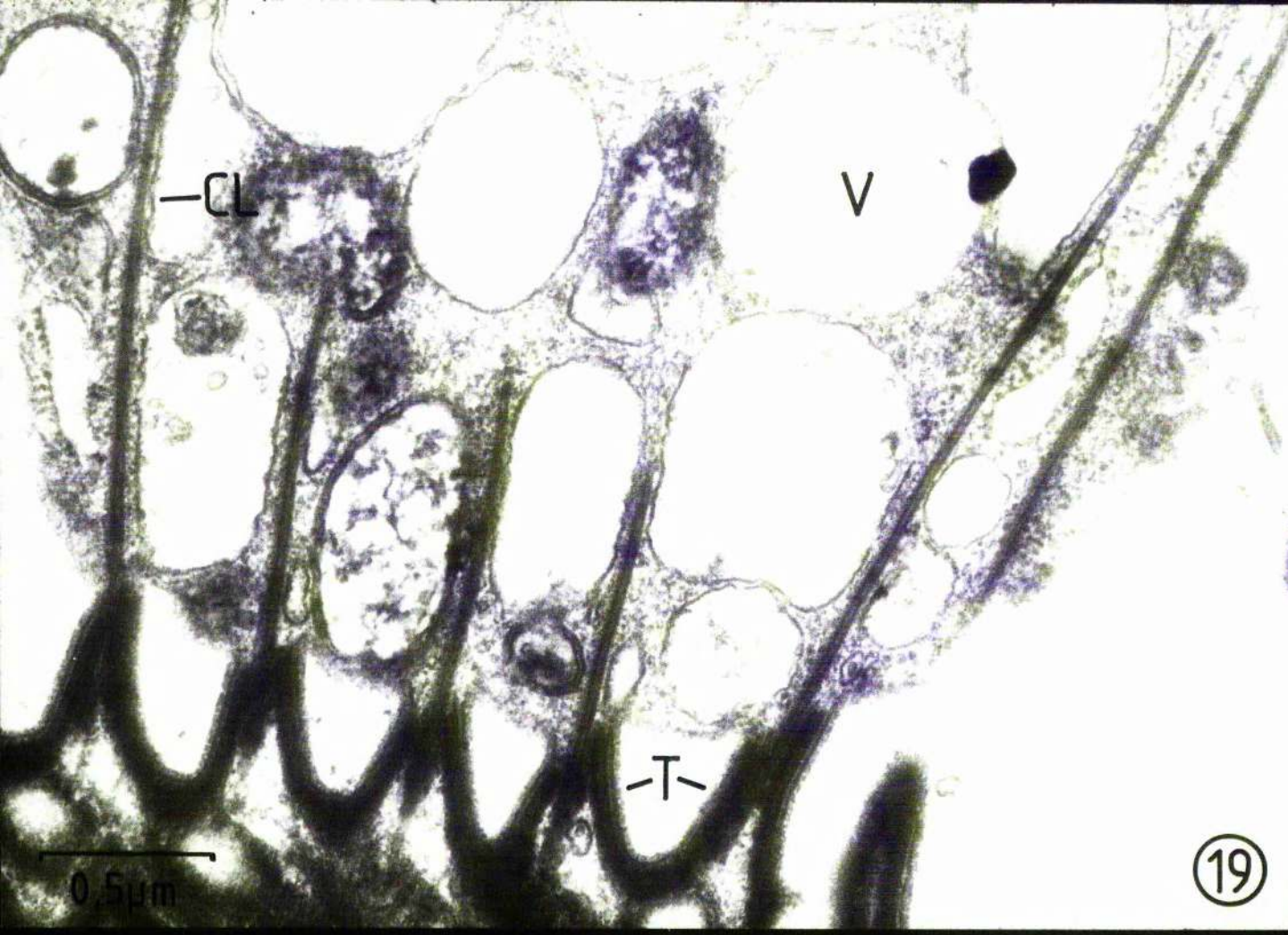

Col
Lip
OA
SL
CL
OM
R
V
2µm
18

PLATE 19

A section through the cytoplasmic extrusion in the same organism as plate 17. This section grazes accross the thickened corrugations (T) of the collar. The cytopharyngeal lamellae (CL) point upwards from the thickened corrugations. The extrusion contains many cytopharyngeal vesicles (V). X 40 000. EM/A/$KMnO_4$.

PLATE 20

The boundary between the region of cytoplasm around the cytopharyngeal basket which contains a large number of cytopharyngeal vesicles (V, bottom right) and the neighbouring cytoplasm (top left) which does not. The cytoplasm between the vesicles (arrows) appears to contain large numbers of fine granules. X 46 000. EM/A/$KMnO_4$.


CL
V
T
0,5µm
19

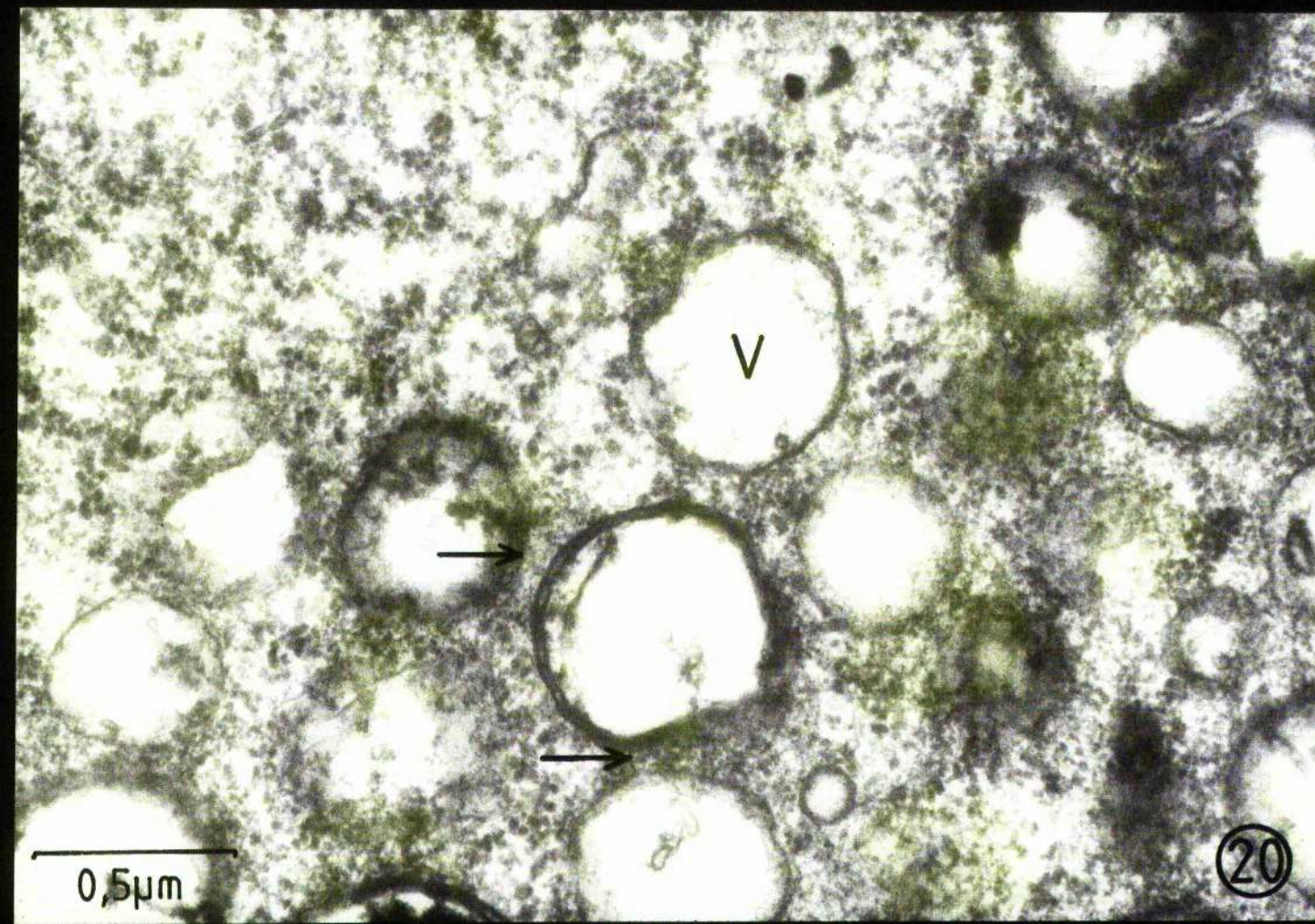

V
0,5µm
20

PLATE 21

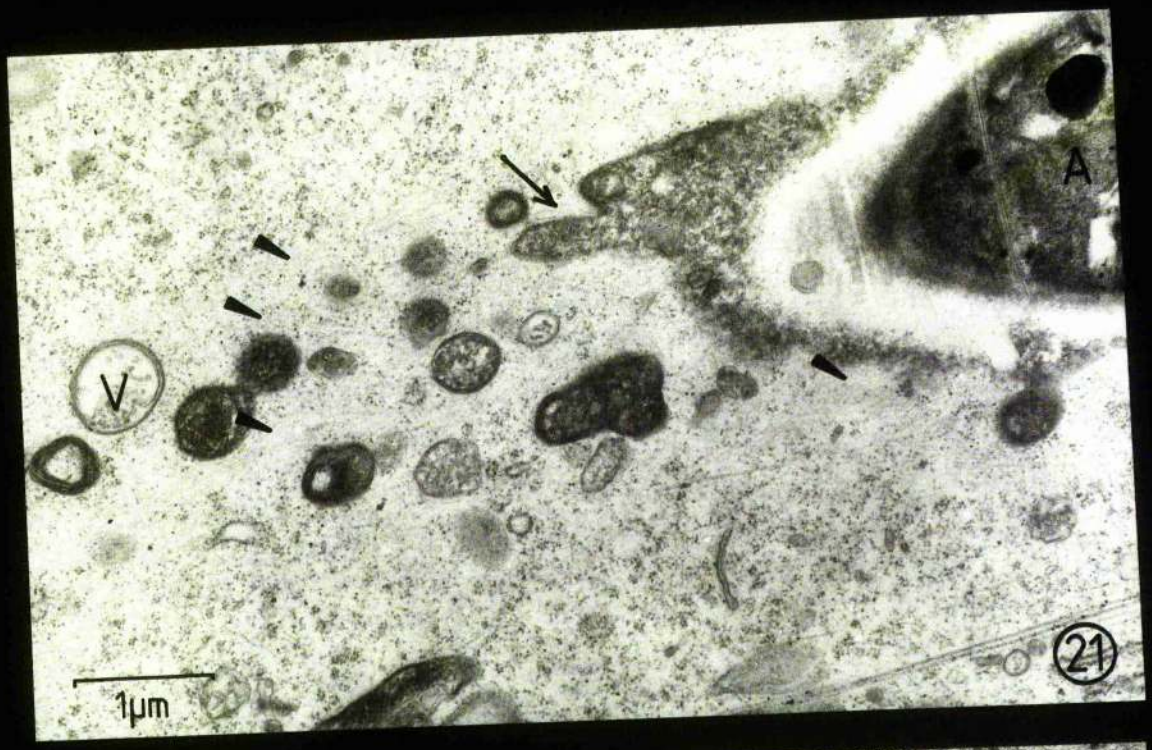
A section grazing accross the ingested algal filament (A) after it has passed out of the end of the cytopharyngeal basket. Cytopharyngeal vesicles (V) fuse with the food vacuole membrane in this region (arrow). The cytoplasm between the vesicles has a fibrous appearance (arrowheads). X 19 000. EM/B.

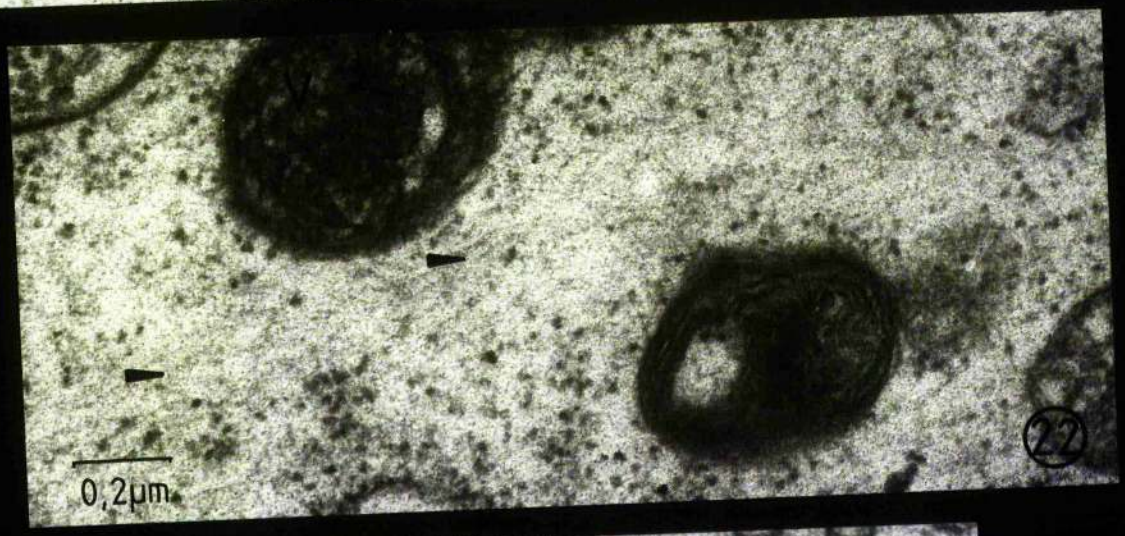
PLATE 22

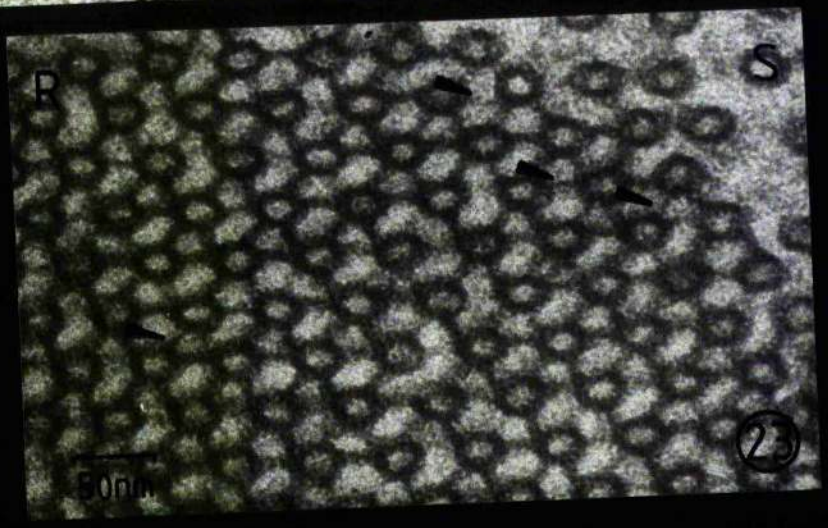
As plate 21 but at a higher magnification. The cytoplasm appears fibrous in places (arrowheads). X 66 000. EM/B.

PLATE 23

A transverse section of a rod. The links between the microtubules normally appear to be about 6nm thick, but some (arrowheads) are only 3nm thick. The thicker appearance could arise because several 3nm links, which are not in perfect register, are superimposed within the thickness (50 nm approximately) of the section. X 220 000. EM/A.


A
V
1µm
21


0,2µm
22


R
S
50nm
23

nurse chamber (NC). The oocyte (Ooc) contains some yolk. The follicle (Fol) is thickest over the posterior end of the oocyte and is very thin over the anterior end of the nurse chamber. This change in thickness is gradual. X 595. LM/NIC.

PLATE 26

A stage 10 egg chamber. The oocyte (Ooc) occupies nearly half of the chamber. The oocyte follicle (OF) is clearly visible and part of it, the anterior inwardly migrating follicle (AMF), has started to penetrate between the oocyte and the nurse chamber (NC). The follicle over the nurse chamber has become too thin to observe with light microscopy. X 230. LM/NIC.

B. OOGENESIS IN *DROSOPHILA MELANOGASTER*

STAGES OF EGG CHAMBER DEVELOPMENT

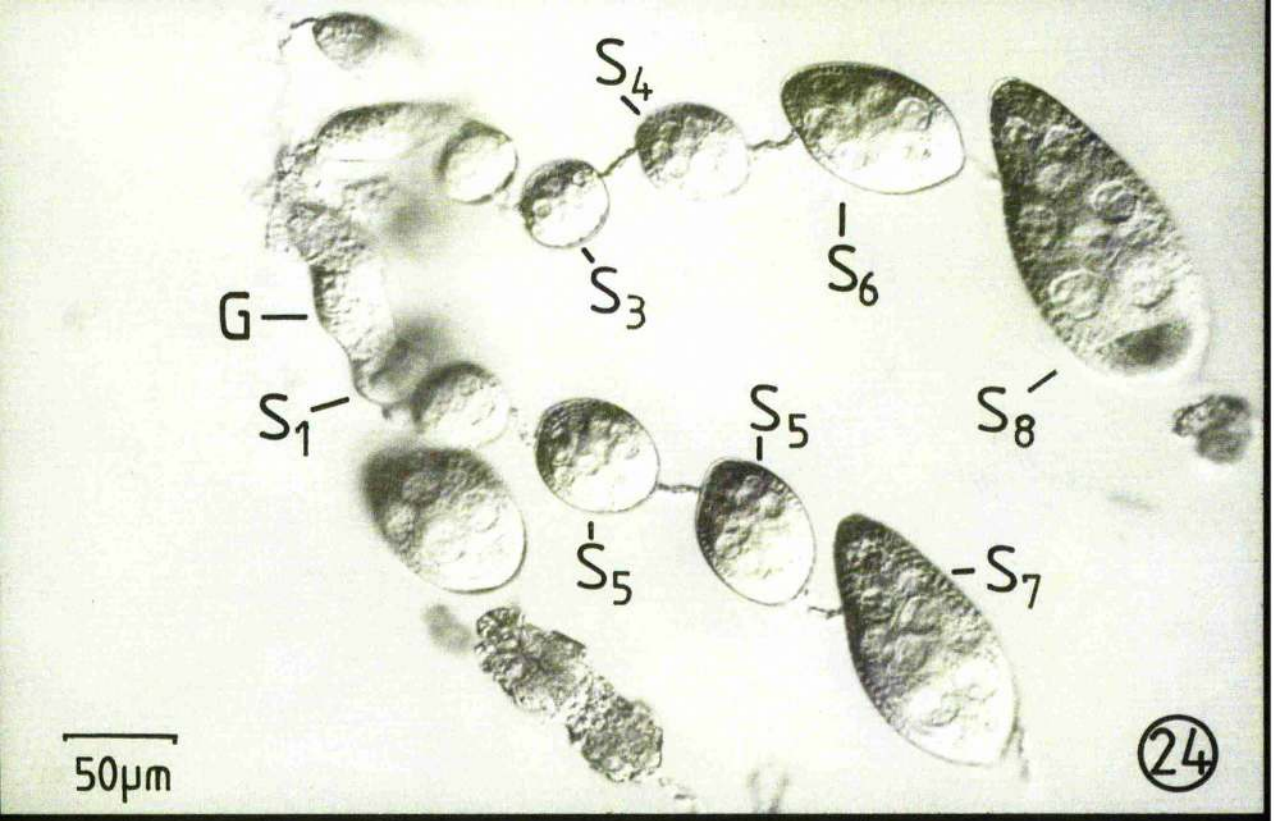
The symbol S_x, where the subscript x is the stage (1-14), is used to represent the stage of development of egg chambers in several of the micrographs below. The staging convention of King *et al.* 1956 is followed (see chapter 3).

PLATE 24

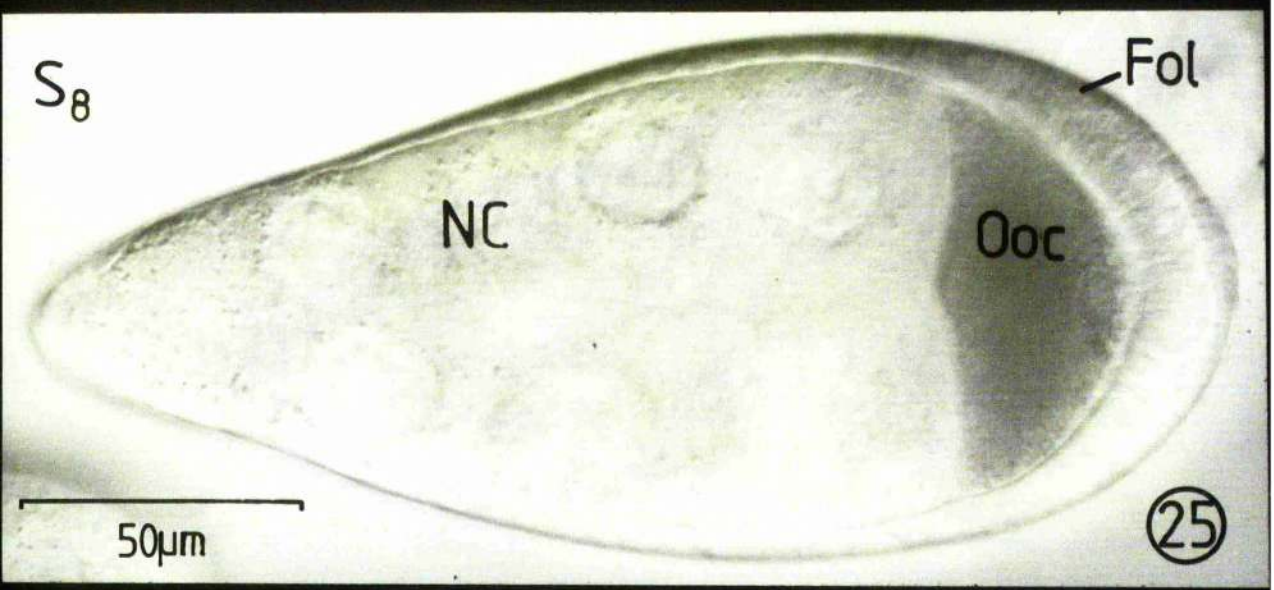
The early stages of development of the egg chamber. The egg chambers from two ovarioles have been released from their ovariole sheaths. Each ovariole contained a germarium (G) and several egg chambers (S_{1-8}) connected by interfollicular stalks. The earliest stages are slightly elongate ellipsoids, stage 6 is more elongated. Stages 7 and 8 are markedly elongated and the anterior ends have a smaller diameter than the posterior (i.e. the chambers are pear shaped). The oocyte is small in these stages with yolk (which appears dark) only detectable in stage 8. X 230. LM/NIC.

PLATE 25

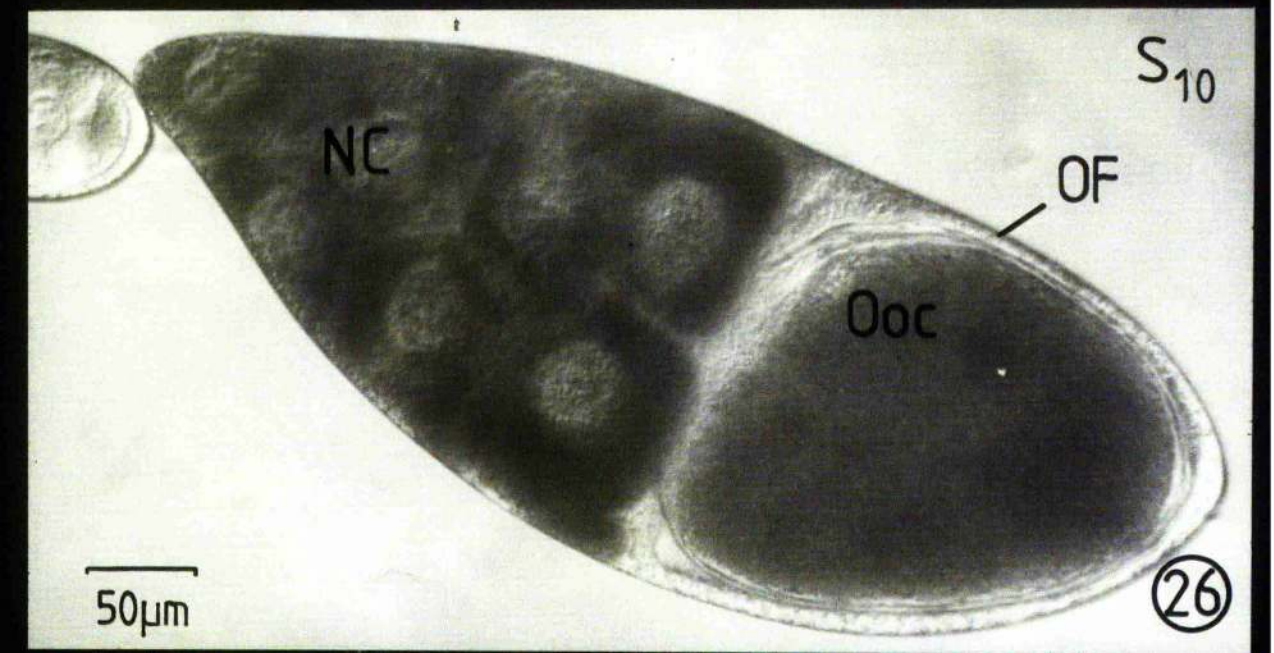
A stage 8 egg chamber. Most of the chamber is taken up by the



24



25



26

PLATE 27

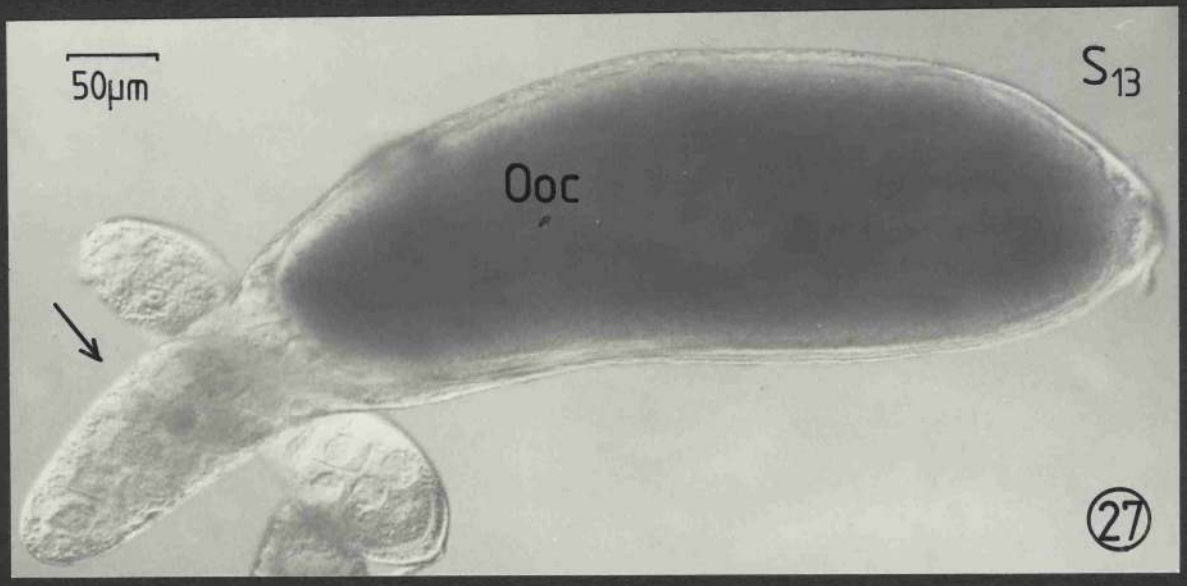
A stage 13 egg chamber. The oocyte has grown to close to its final size and shape. The nurse chamber has disappeared. The dorsal appendages are developing within an anterior region of the follicle (arrow) which has developed from part of the anterior inwards migrating follicle of stage 10. The egg shell is forming beween the oocyte follicle and the oocyte. X 230. LM/NIC.

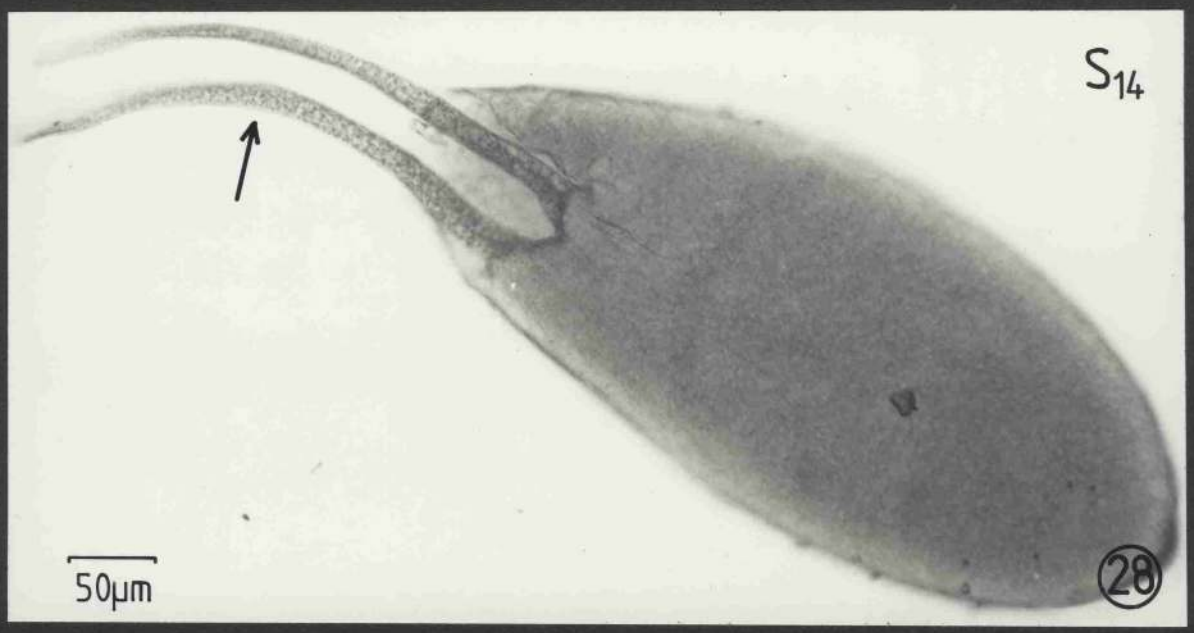
PLATE 28

Stage 14: the mature egg, ready to be layed. The follicle has sloughed off. The dorsal appendages (arrow) are fully formed. X 230. LM/NIC.

PLATE 29

A ring canal (arrow) between two nurse cells. The nurse cell cytoplasm contains many lipid droplets. X 1400. LM/NIC.


50µm
S13
Ooc
27


S14
50µm
28


10µm
29

PLATES 30, 32 - 36

These plates illustrate the arrangement of follicle cells at various stages in the development of the egg chamber. In particular they demonstrate the setting up of a boundary between the oocyte follicle and the nurse chamber follicle during stage 9. Follicle cell positions are revealed by silver staining of cell boundaries (plates 30, 32, 34) or by Feulgen staining of their nuclei (plates 33 and 35). During the early stages the follicle cells are evenly spread over the surface of the chamber (plate 30). During early stage 9 there is a gradual transition from thin squamous cells over the nurse chamber to thicker cuboidal cells over the oocyte (plates 32 and 33). Towards the end of stage 9 a sharp boundary has been set up (arrows in plates 34 and 35). The follicle cells over the nurse chamber have become too thin for their cell boundaries to be detected in silver stained preparations (plate 34) and their position must be judged from the position of their nuclei (plate 35). Plate 36 (next page) illustrates the boundary (arrow) in a stage 10 chamber using Nomarski interference contrast microscopy: the nuclei and cell boundaries of the oocyte follicle are clearly defined (righthand side) but they cannot be made out over the nurse chamber.

PLATE 30

Stage 5 and stage 6 egg chambers. X 770. LM/Silver.

PLATE 31

Dorsal appendage forming follicle of late stage 13 egg chamber. See description below. X 290. LM/Feulgen.

PLATE 32

Early stage 9 egg chamber. X 483. LM/Silver.

PLATE 33

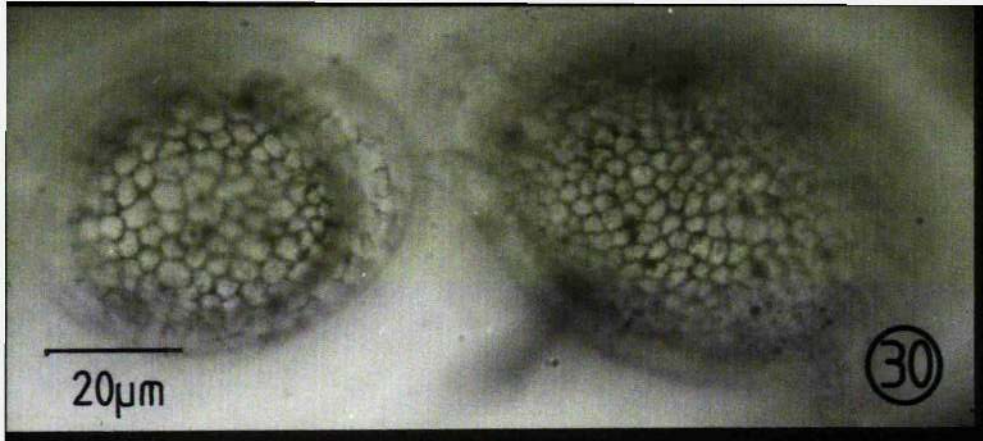
Early stage 9 egg chamber. X 440. LM/Feulgen.

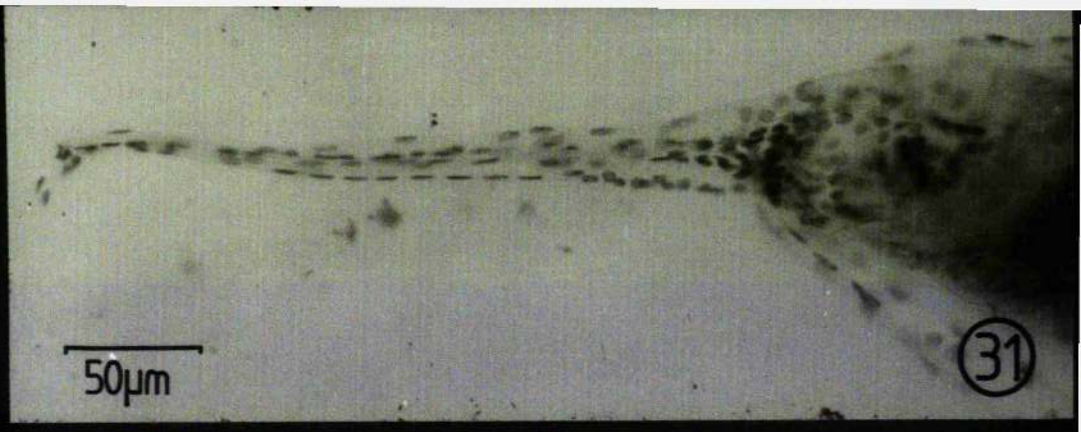
PLATE 34

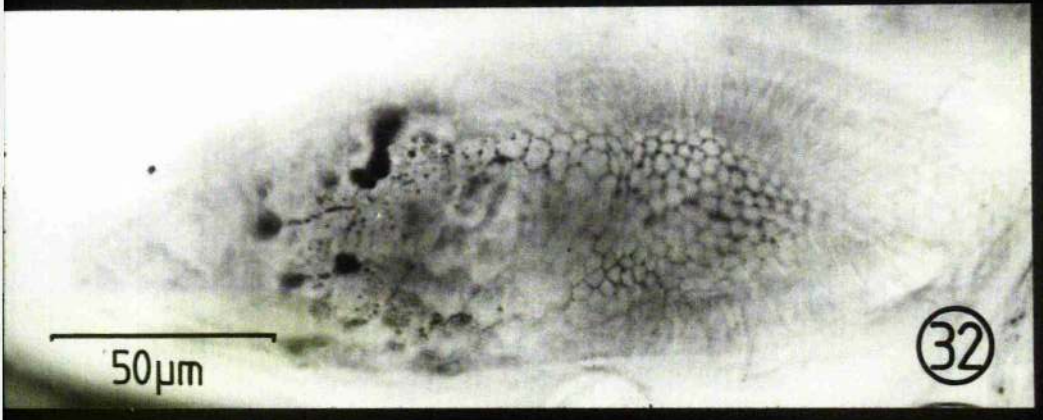
Late stage 9 egg chamber. X 290. LM/Silver.

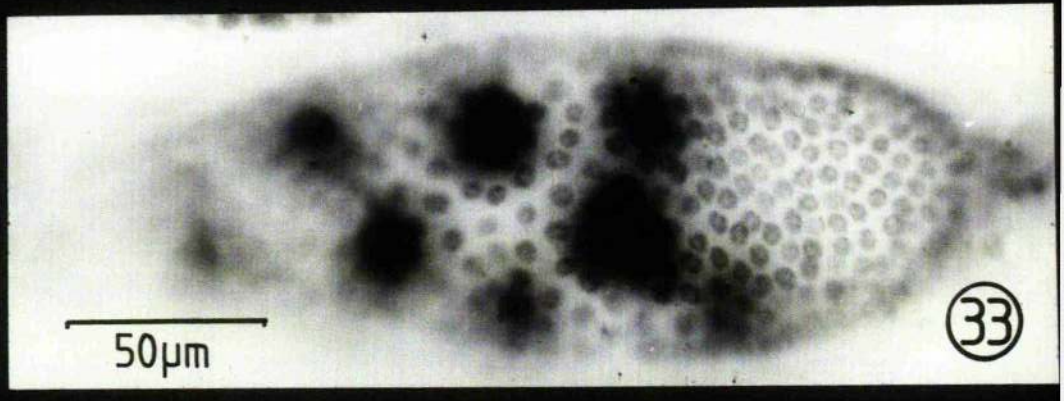
PLATE 35

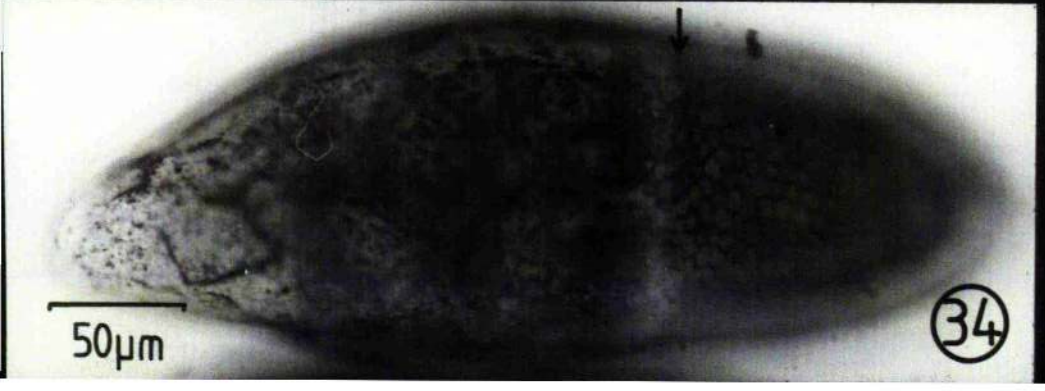
Late stage 9 egg chamber. X 290. LM/Feulgen.

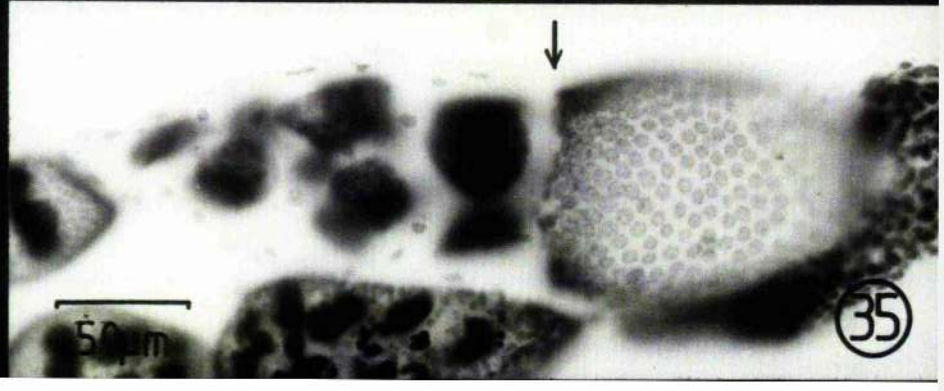

20µm
30


50µm
31


50µm
32


50µm
33


50µm
34


50µm
35

PLATES 31, 38 - 40

These plates demonstrate that the cells in the follicle which forms the dorsal appendages during stage 13 are not markedly elongated. Their nuclei are fairly evenly distributed along the appendage (plate 31) and the distance between them is only slightly greater in the anterior-posterior direction than in the circumferential direction. Where cell boundaries can be made out (plates 38, 39 and 40) the cells do not appear particularily elongated.

PLATE 36

Boundary (arrow) between oocyte (righthand side) and nurse chamber follicles in a stage 10 egg chamber. X 1140. LM/NIC.

PLATE 37

A stage 11 egg chamber (1μm section). The oocyte (Ooc) fills most of the egg chamber. The anterior inwardly migrating follicle (AMF) separates the nurse chamber (NC) from the oocyte except at the anterior tip of the oocyte where the border cells (BC), which will form the micropyle, are situated. X 290. LM/MeBl.

PLATE 38

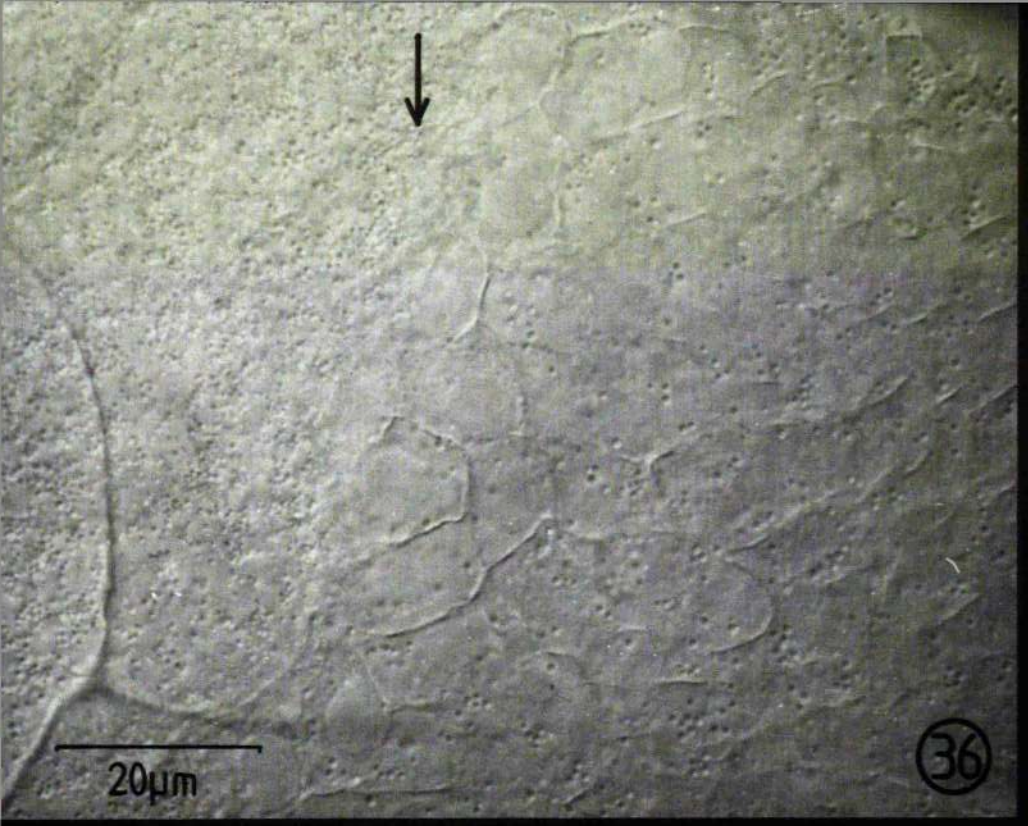
Dorsal appendage forming follicle of stage 13 egg chamber. X 483. LM/NIC.

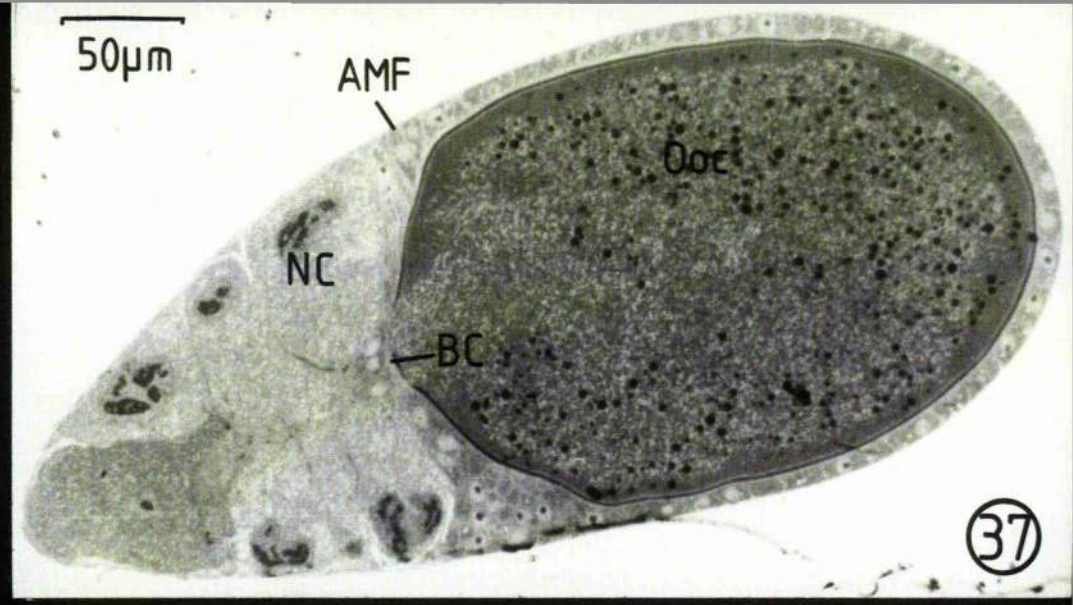
PLATE 39

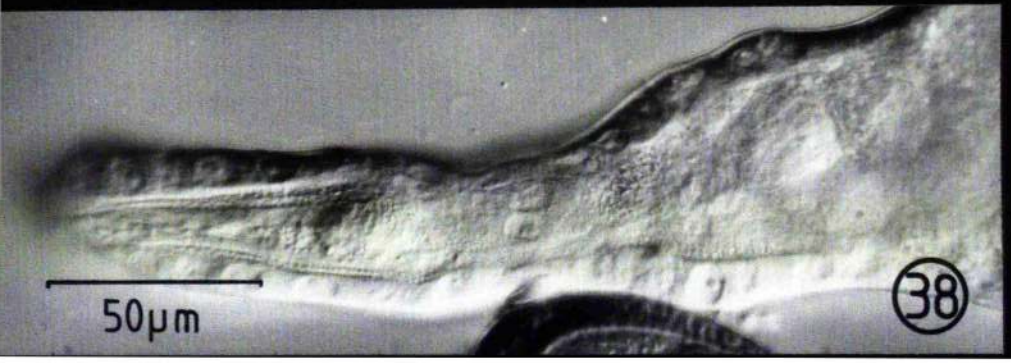
Late stage 13 or early stage 14 dorsal appendage with surrounding follicle sectioned (1μm) transversely at various levels: a) near tip, b) about 50μm from tip where the end of the appendages are club shaped, c) in mid appendage. X 720. LM/MeBl.

PLATE 40

Late stage 13 or early stage 14 appendage sectioned (1μm) longitudinally. X 180. LM/MeBl.

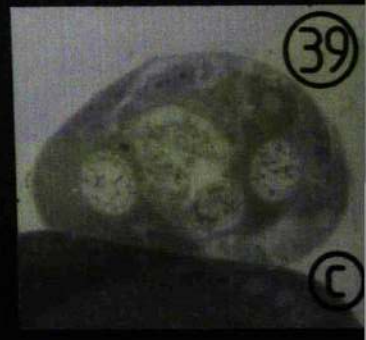

20µm
36


50µm
AMF
Ooc
NC
BC
37


50µm
38


20µm
a


b


39
c

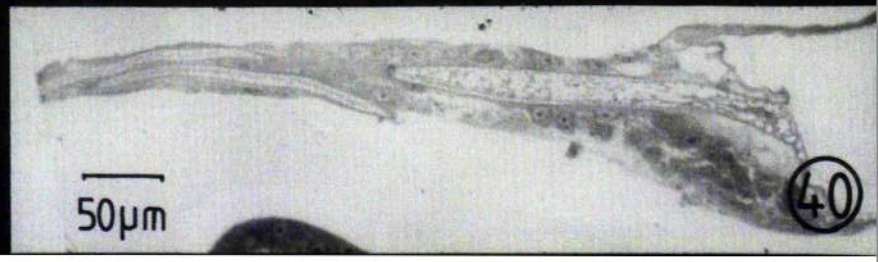

50µm
40

PLATE 41

Cross-section of a stage 7 egg chamber. Circumferentially oriented microtubules (Mt) are present close to the outer plasma membrane (PM) of some follicle cells. The tunica propria (TP) covers the follicle cells. X 46 000. EM/B.

PLATE 42

Longitudinal section of a stage 6 egg chamber. Small patches of filamentous material (Mf) are occasionally found close to the outer plasma membrane (PM) of the follicle cells (TP: tunica propria). X 73 000. EM/B.

PLATE 43

The belt desmosome (BD) at the base of a stage 8 follicle cell (tangental section). Filamentous material (Mf) is associated with the desmosome. X 45 000. EM/B.

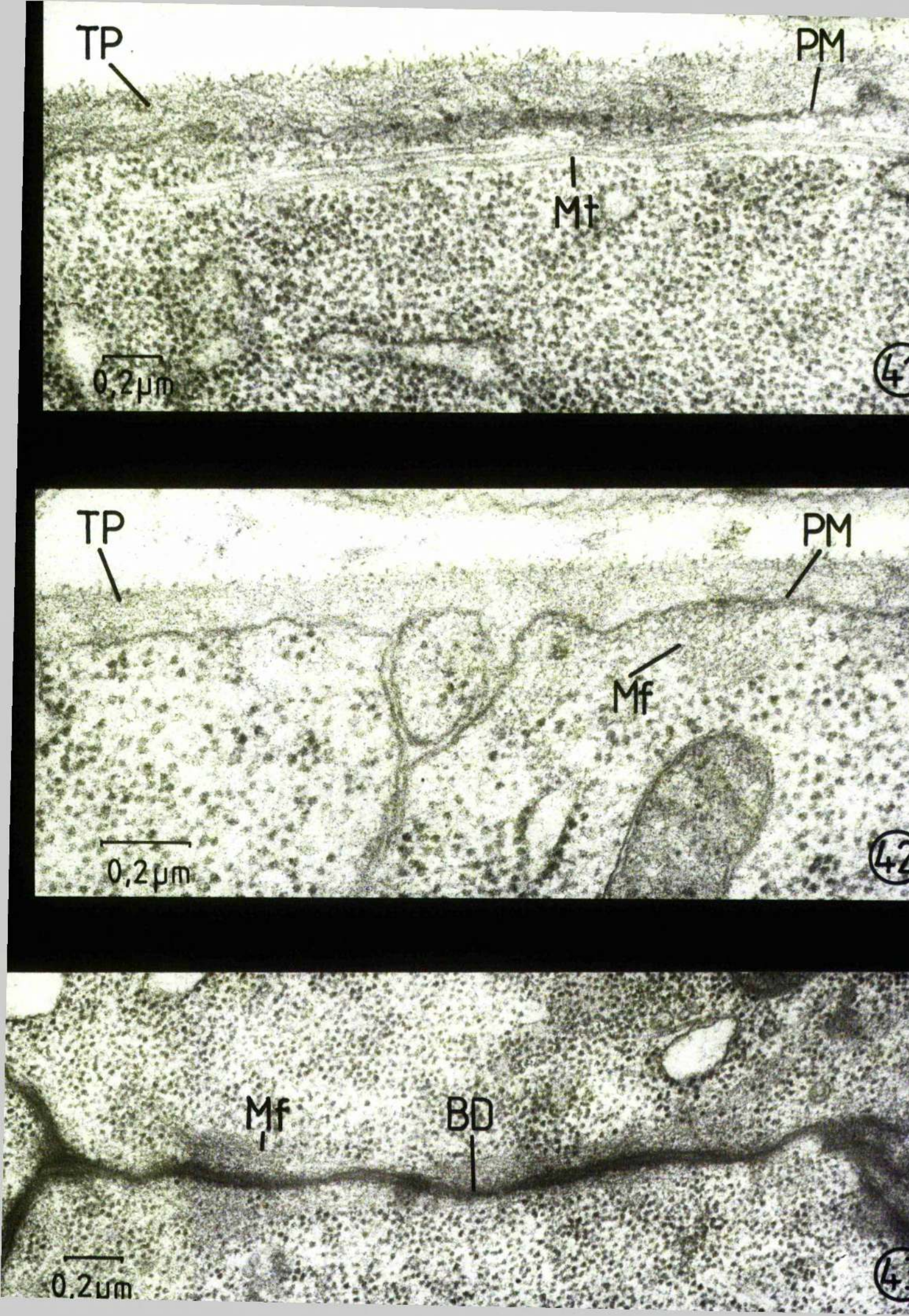

TP
PM
Mt
0,2µm
TP
PM
Mf
0,2µm
Mf
BD
0.2µm

PLATE 44

The belt desmosome (BD) of a stage 11 oocyte follicle cell. Microtubules (Mt) are associated with the desmosome. X 51 000. EM/C.

PLATE 45

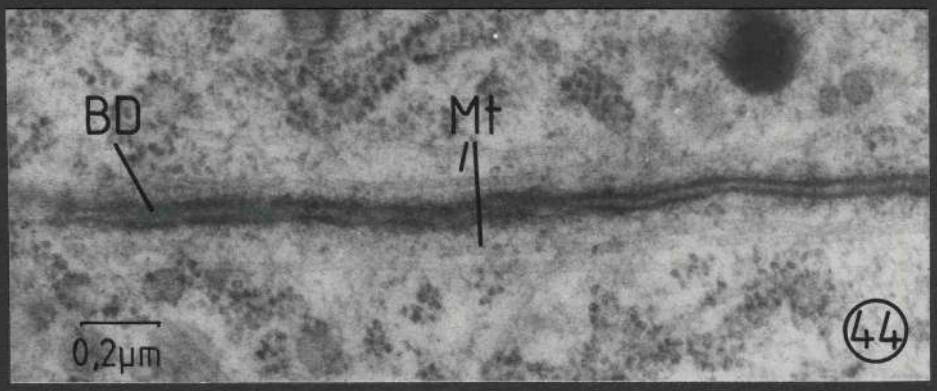
The boundary between a follicle cell (Fol) and the oocyte of a stage 8 egg chamber. Microvilli (Mv) are present. They contain microfilaments (Mf). Microtubules (Mt) run into the region containing the microvilli. X 46 000. EM/B.

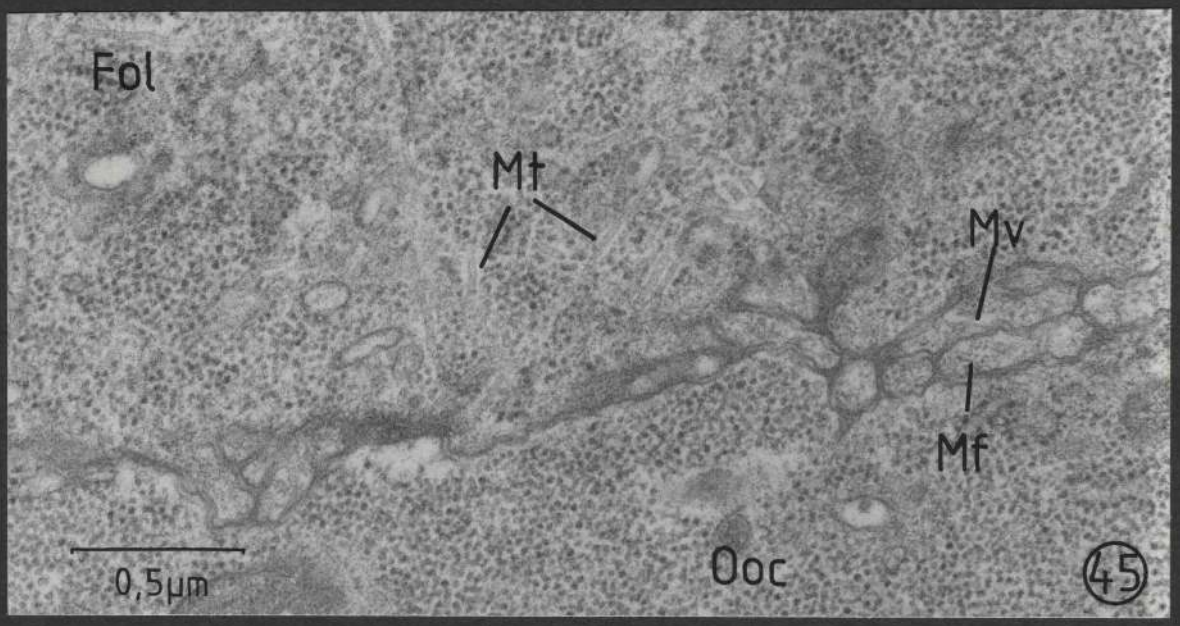
PLATE 46

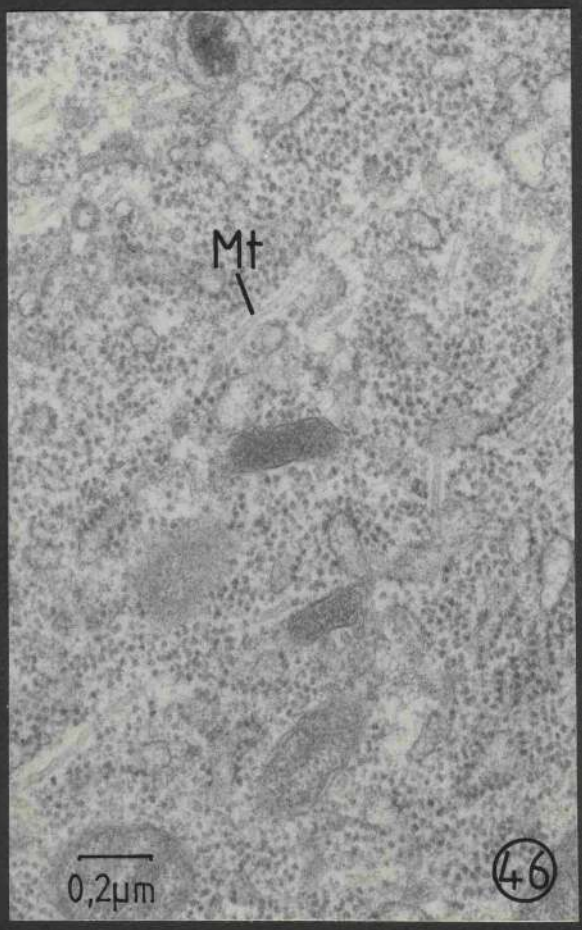
The cytoplasm of a stage 8 oocyte. A few microtubules (Mt) are present. X 51 000. EM/B.

PLATE 47

The oocyte (Ooc) of one stage 8 egg chamber contained a large number of microtubules (Mt). Fol: follicle. X 14 000. EM/B.


BD
Mt
0,2µm
44


Fol
Mt
Mv
Mf
0,5µm
Ooc
45


Mt
0,2µm
46

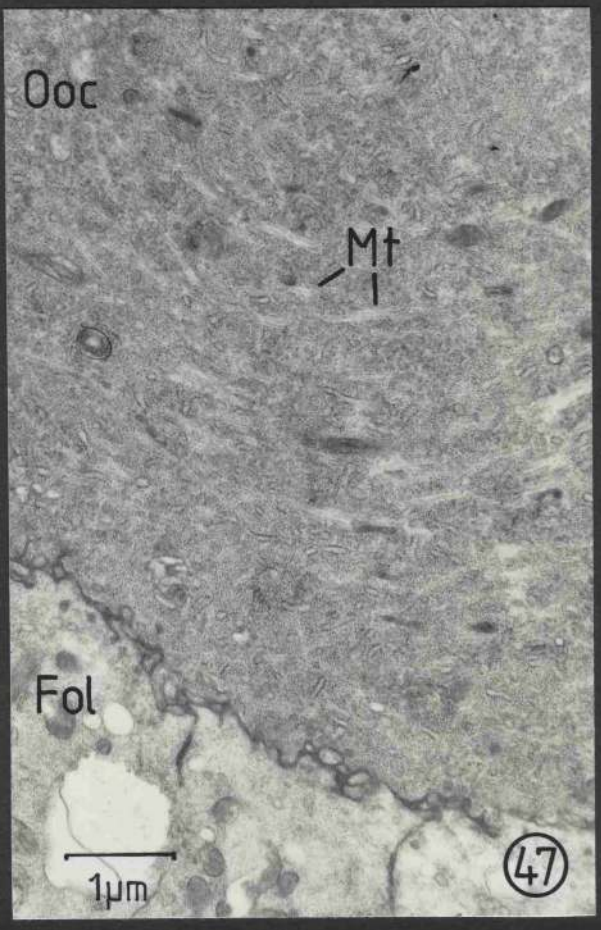
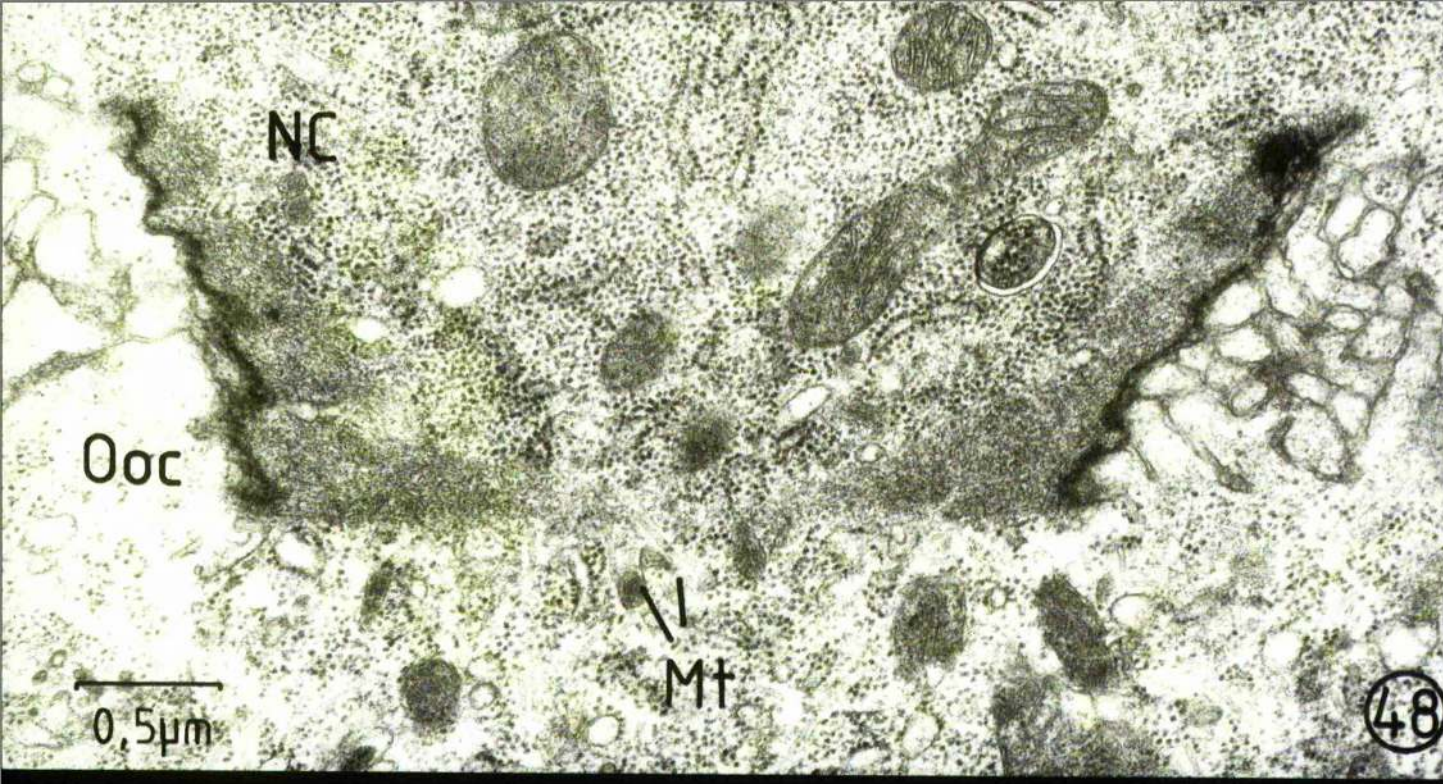

Ooc
Mt
Fol
1µm
47

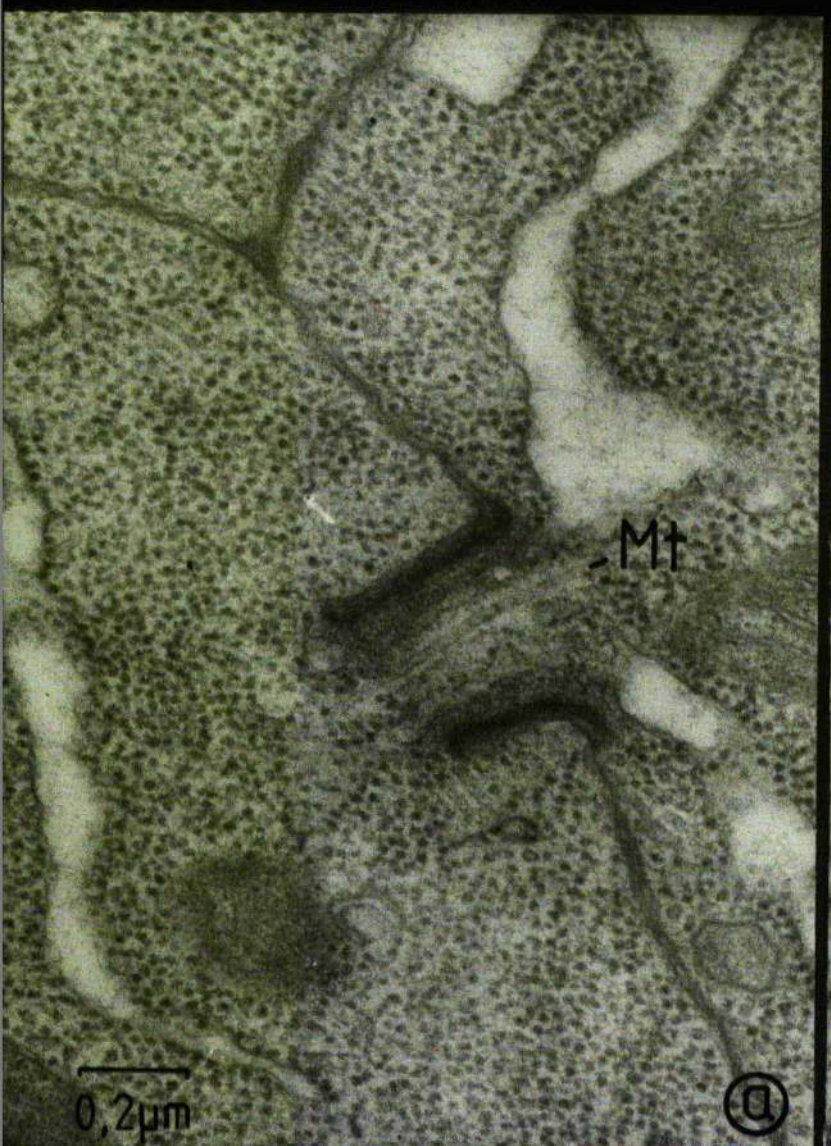
PLATE 48

A section grazing across the rim of a ring canal between a nurse cell (NC) and the oocyte (Ooc) in a stage 8 egg chamber. A few microtubules (Mt) insert on the rim. X 31 000. EM/B.

PLATE 49

Intercellular bridges between follicle cells sectioned a) longitudinally and b) transversely. They contain microtubules (Mt). a) stage 9, b) stage 7. X 54 000. EM/B.


NC
Ooc
Mt
0,5µm
48


Mt
0,2µm
a

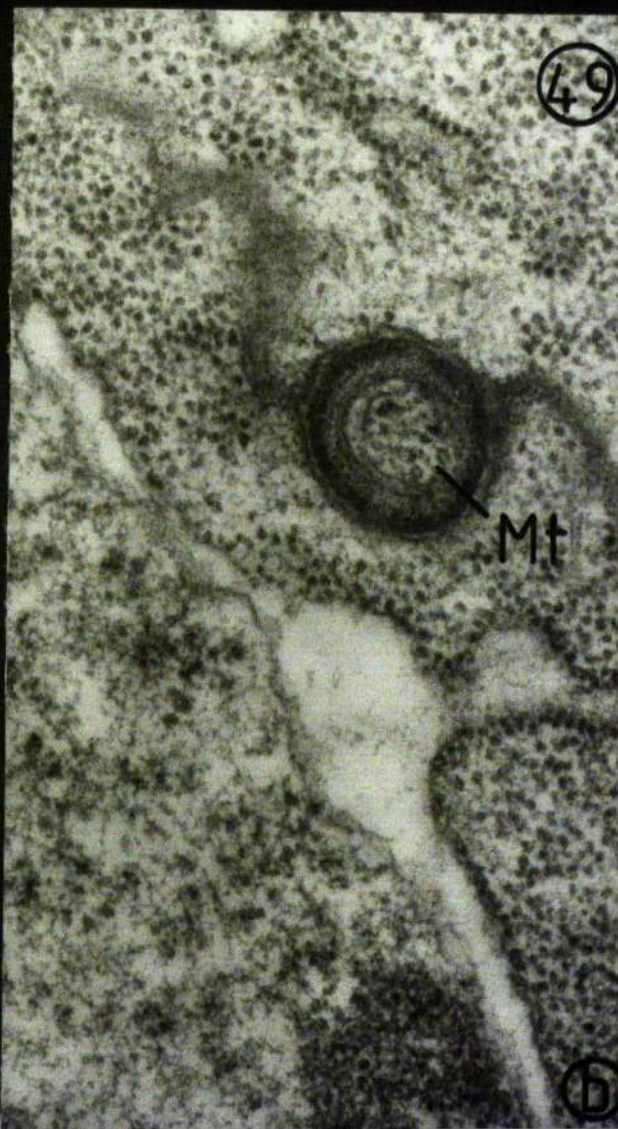

49
Mt
b

PLATE 50

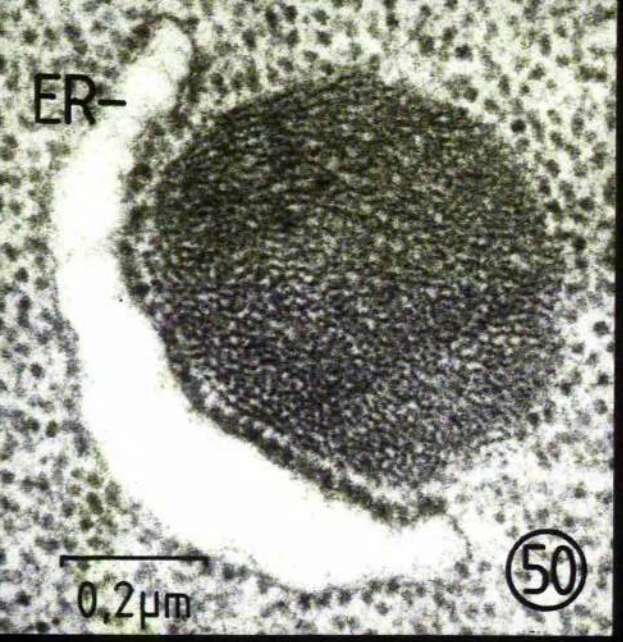
A cytoplasmic inclusion with a fibrous appearance in a stage 9 nurse cell. The inclusion is associated with endoplasmic reticulum (ER). The filaments are sectioned transversely. X 79 000. EM/B.

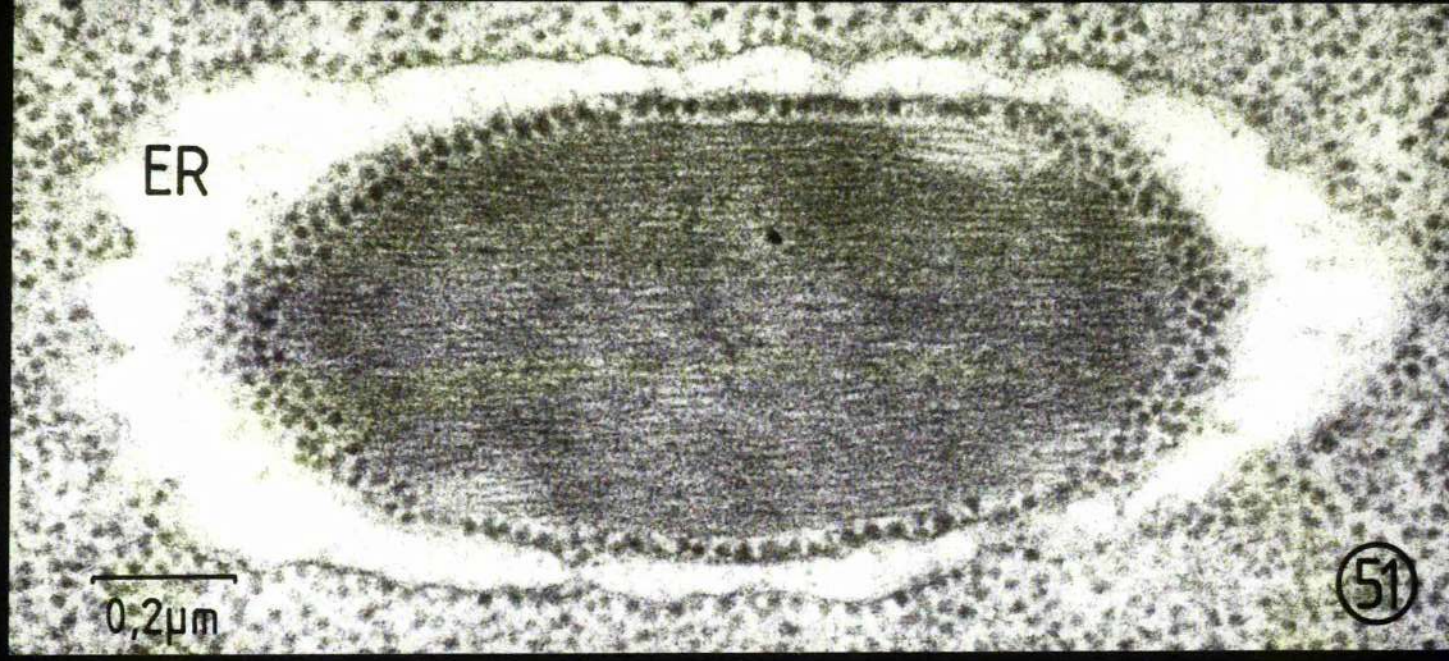
PLATE 51

As plate 50 but the filaments are sectioned longitudinally (stage 8). X 79 000. EM/B.

PLATE 52

Another fibrous inclusion sectioned longitudinally in a stage 8 nurse cell. This one is at least 8.5µm long. X 22 000. EM/B.


ER-
0,2µm
50


ER
0,2µm
51

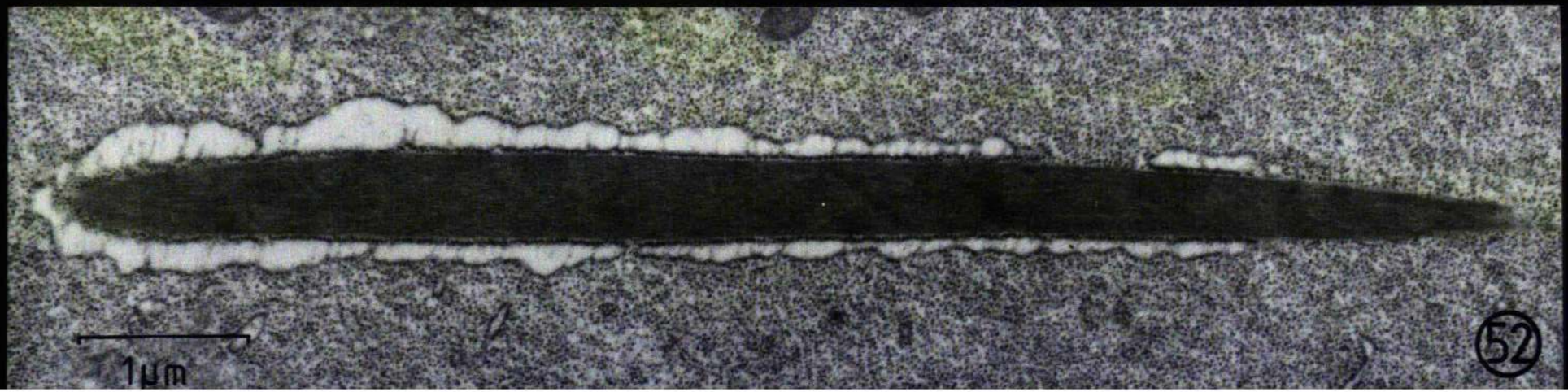

1µm
52

PLATE 53

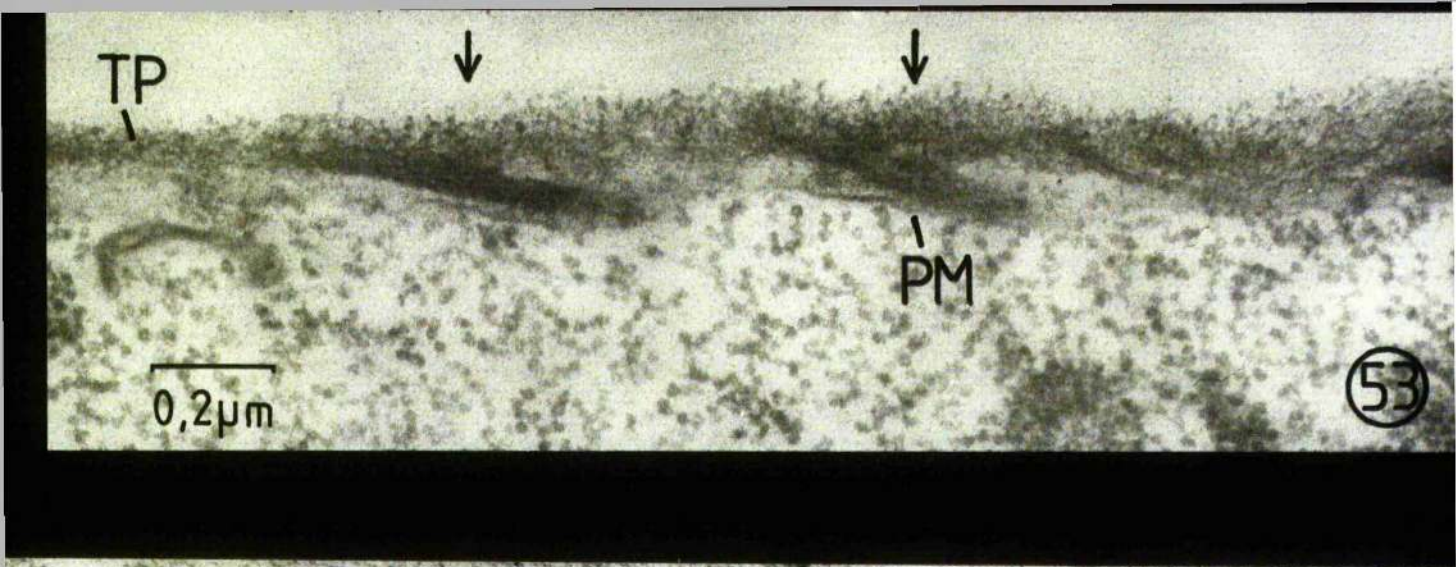
The tunica propria (TP) close to the anterior of a stage 8 egg chamber (glycerol extracted). Ridges (arrows) are present on the inner surface, outside the plasma membrane (PM). Longitudinal section. X 64 000. EM/C.

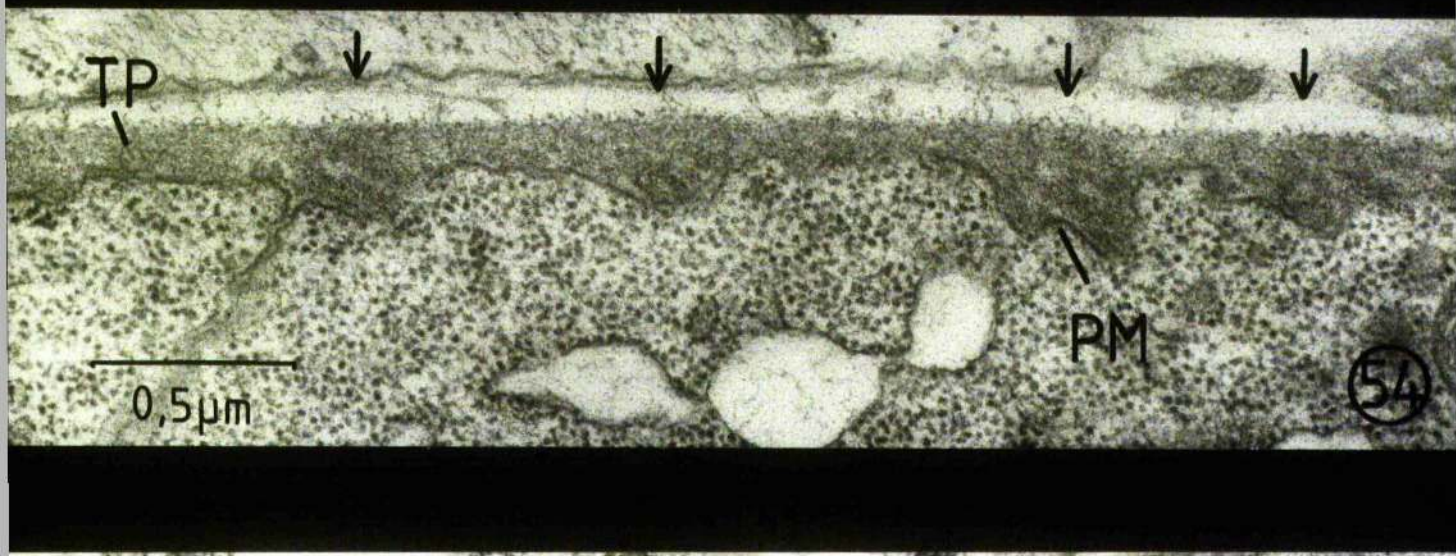
PLATE 54

Ridges (arrows) on the inner surface of the tunica propria (TP) in a more median longitudinal section than plate 53 (stage 8). X 42 000. EM/B.

PLATE 55

A non-median longitudinal section (arrow: anterior-posterior axis) of the anterior end of a stage 8 egg chamber. The ridges are circumferentially oriented. X 12 000. EM/B.


TP
PM
0,2µm
53


TP
PM
0,5µm
54

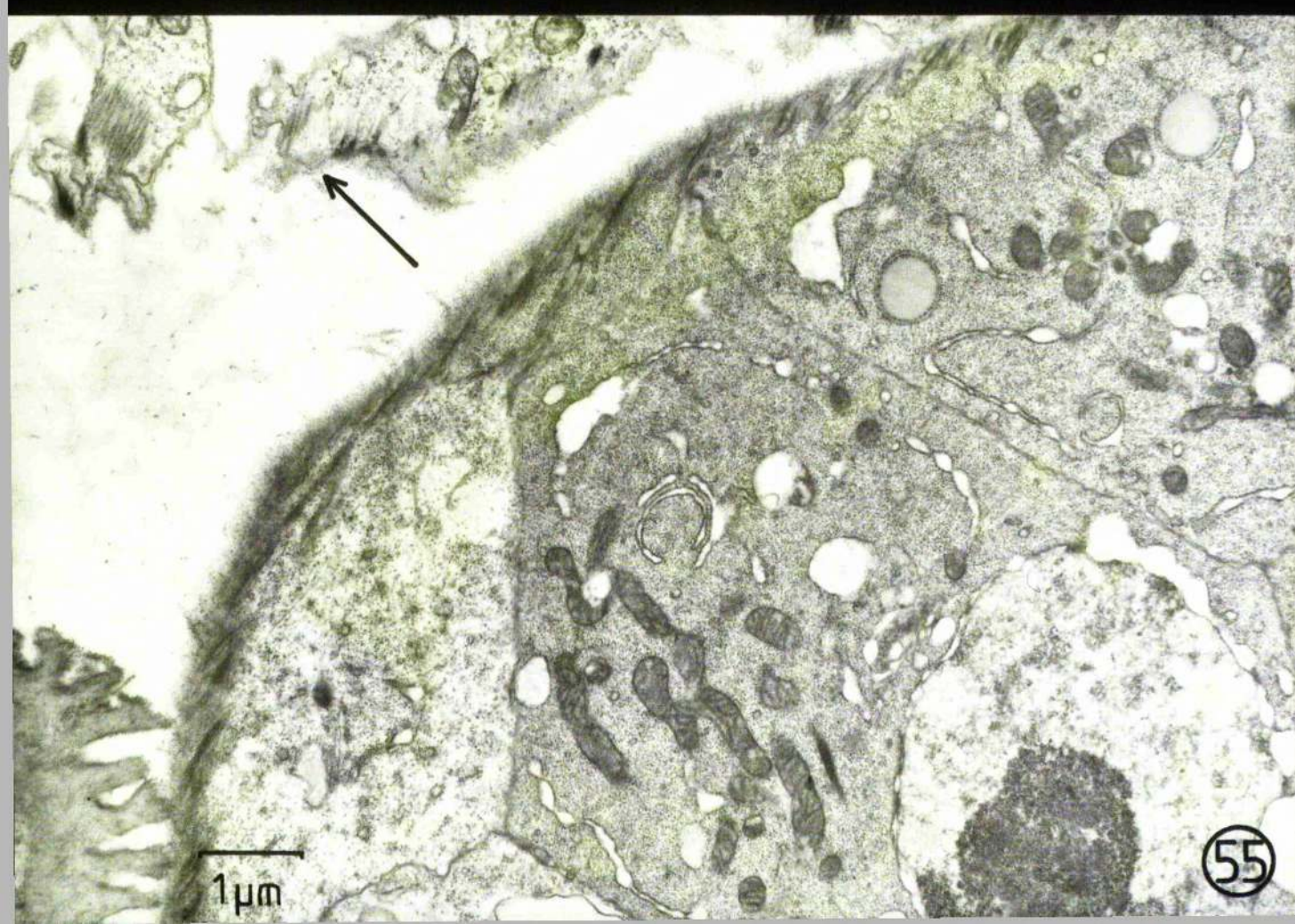

1µm
55

PLATE 56

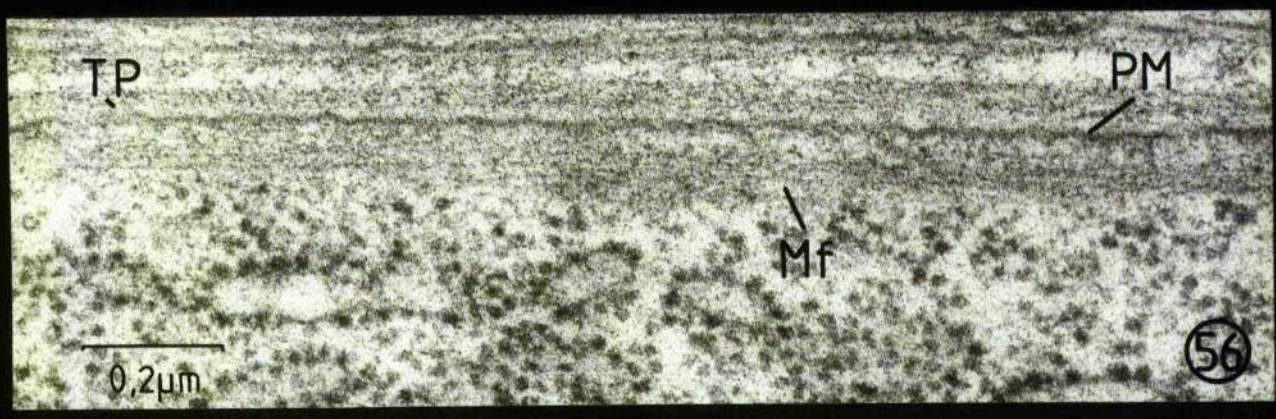
Part of an oocyte follicle cell in a stage 9 egg chamber which has been sectioned transversely. Microfilaments (Mf) are present in a layer underneath the outer plasma membrane (PM). The microfilaments are oriented parallel to the circumference of the egg chamber (TP: tunica propria). X 93 000. EM/B(80kV).

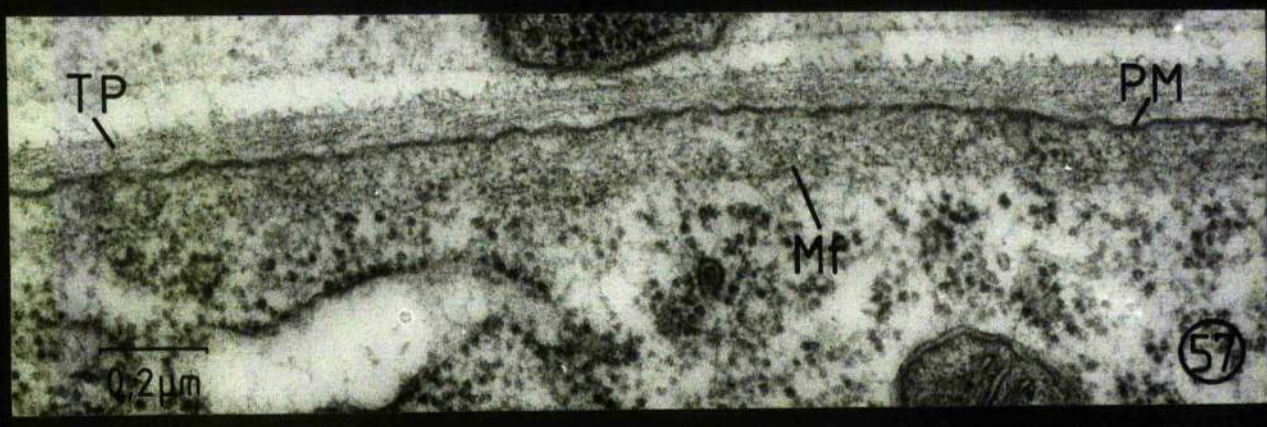
PLATE 57

The microfilamentous layer (Mf) in a stage 10 egg chamber in median longitudinal section (TP: tunica propria, PM: plasma membrane). The microfilaments are in cross-section. X 69 000. EM/B.

PLATE 58

A grazing longitudinal section of the anterior oocyte follicle of a stage 13 egg chamber. Circumferentially oriented microfilaments (Mf) are present beneath the tunica propria (TP). The plasma membrane can not be distinguished when sectioned at this angle. An occasional microtubule (Mt) runs parallel to the microfilaments. X 61 000. EM/B.


TP
PM
Mf
0,2µm
56


TP
PM
Mf
0,2µm
57

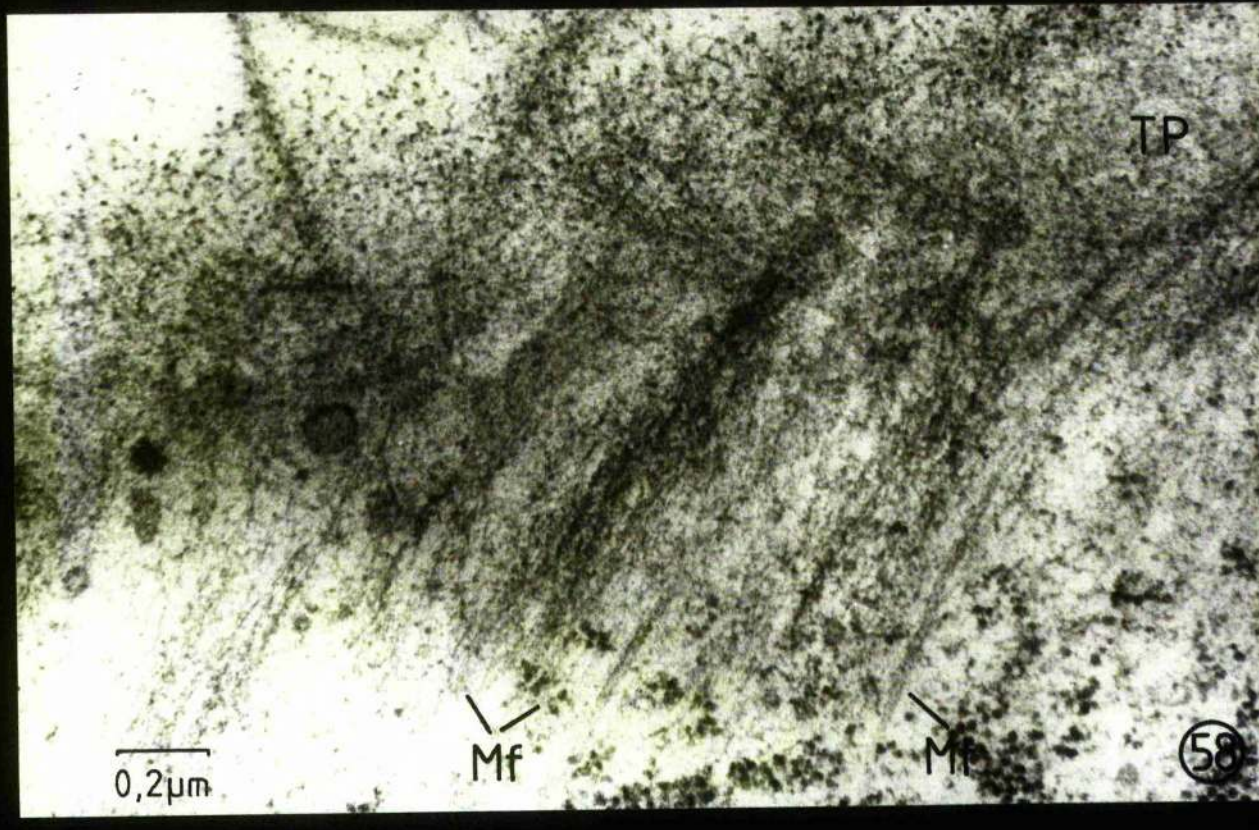

TP
Mf
Mf
0,2µm
58

PLATE 59

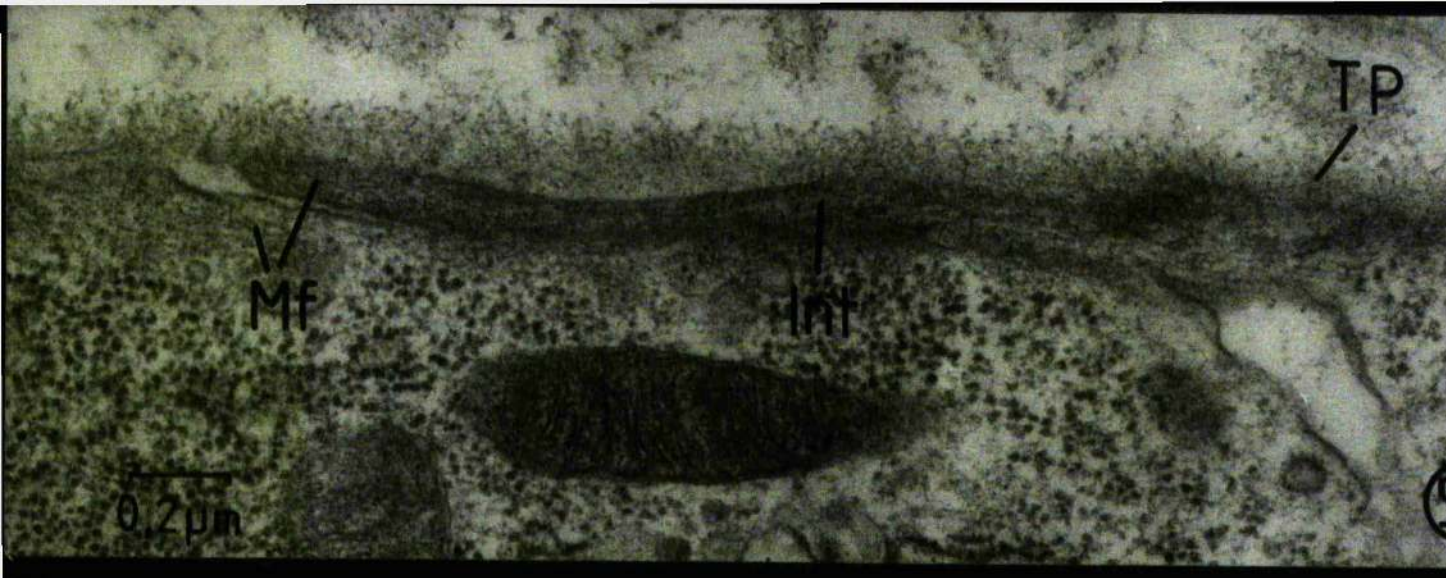
An interdigitation (Int) between two follicle cells (beneath the tunica propria: TP). The interdigitation contains microfilaments (Mf) which appear to be continuous with the microfilamentous layer. X 51 000. EM/B.

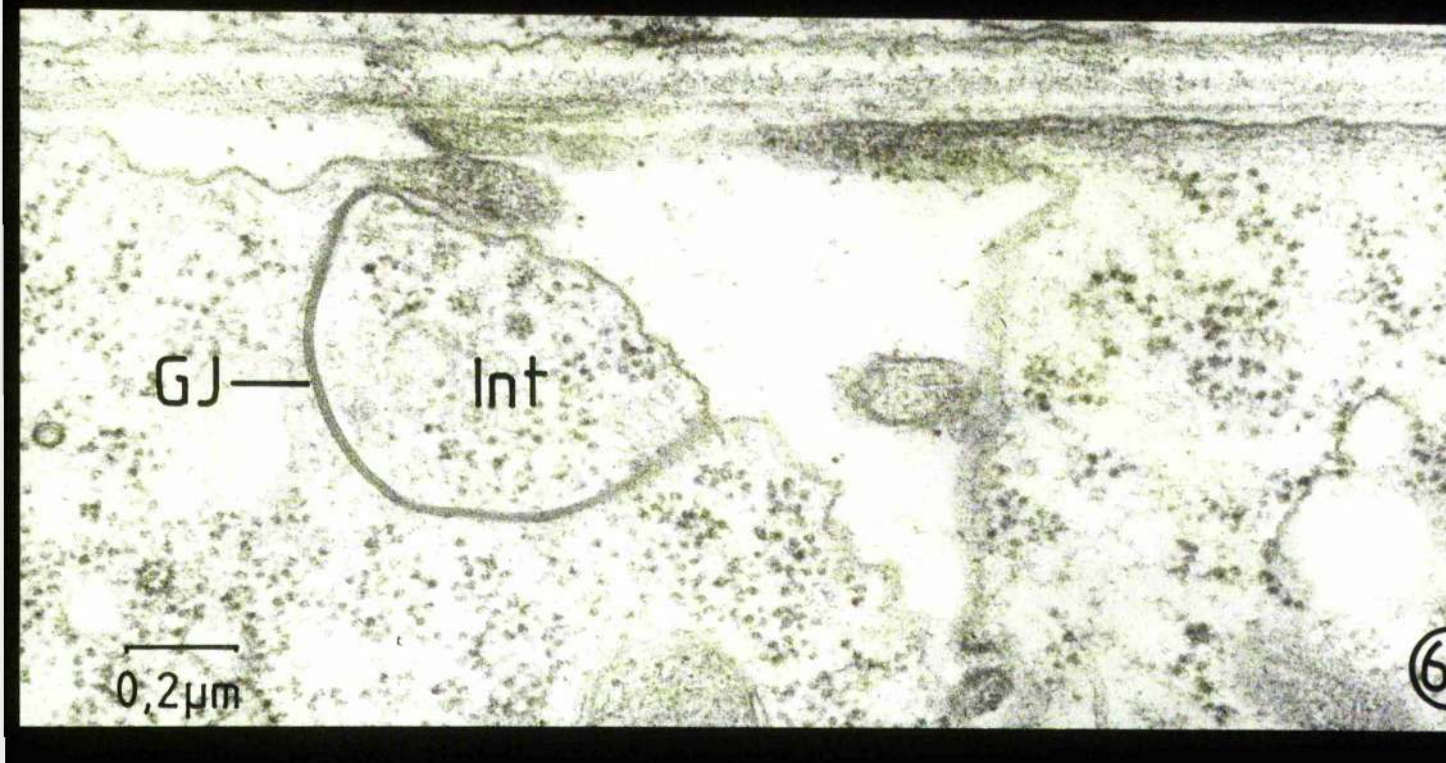
PLATE 60

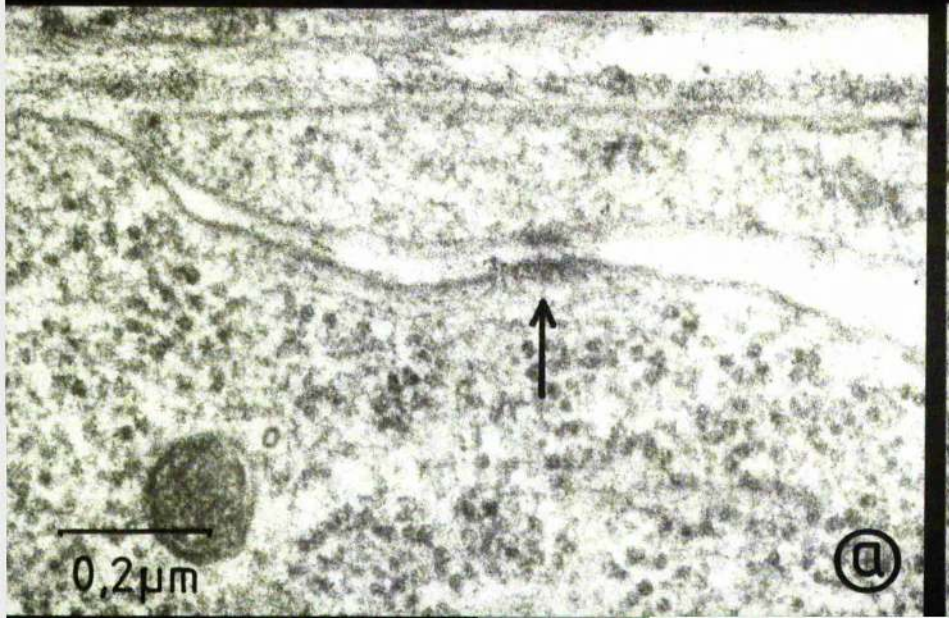
A cross-section of an interdigitation (Int) between two follicle cells. A gap junction (GJ) surrounds much of the interdigitation. X 57 000. EM/B.

PLATE 61

Possible junctions between follicle cells. Structures which resemble small spot desmosomes are occasionally observed between follicle cells (arrows). There is only a slight increase in density on the cytoplasmic side of the junctional membrane. There is a slight concentration of extracellular material between the opposing membranes. a) X 74 000. EM/C. b) X 53 000. EM/B.


TP
Mf
Int
0,2µm


GJ
Int
0,2µm


0,2µm


0,2µm

PLATE 62

Regions of closely opposed densely stained membrane are present between follicle cells during all stages of oocyte development. The 'unit membrane' trilaminar structure is clearly defined. The arrangement of these structures varies considerably. a) Stage 8, b) Stage 4, c) Stage 5-6, d) Stage 8. X 82 000. EM/B.

PLATE 63

The inner end of a centripetally migrating anterior oocyte follicle cell in a median longitudinal section of a stage 10 egg chamber. Vitelline bodies (VB) are present between the oocyte and the follicle cell. These will later become incorporated in the vitelline membrane. Filamentous material is present in this region (arrows). Microtubules (Mt) are present in considerable numbers within the cell. Throughout most of the cell they are oriented parallel to the longitudinal axis of the cell but close to the oocyte they are frequently found in cross-section. The latter are arranged in a ring around the anterior end of the oocyte. X 39 000. EM/B.

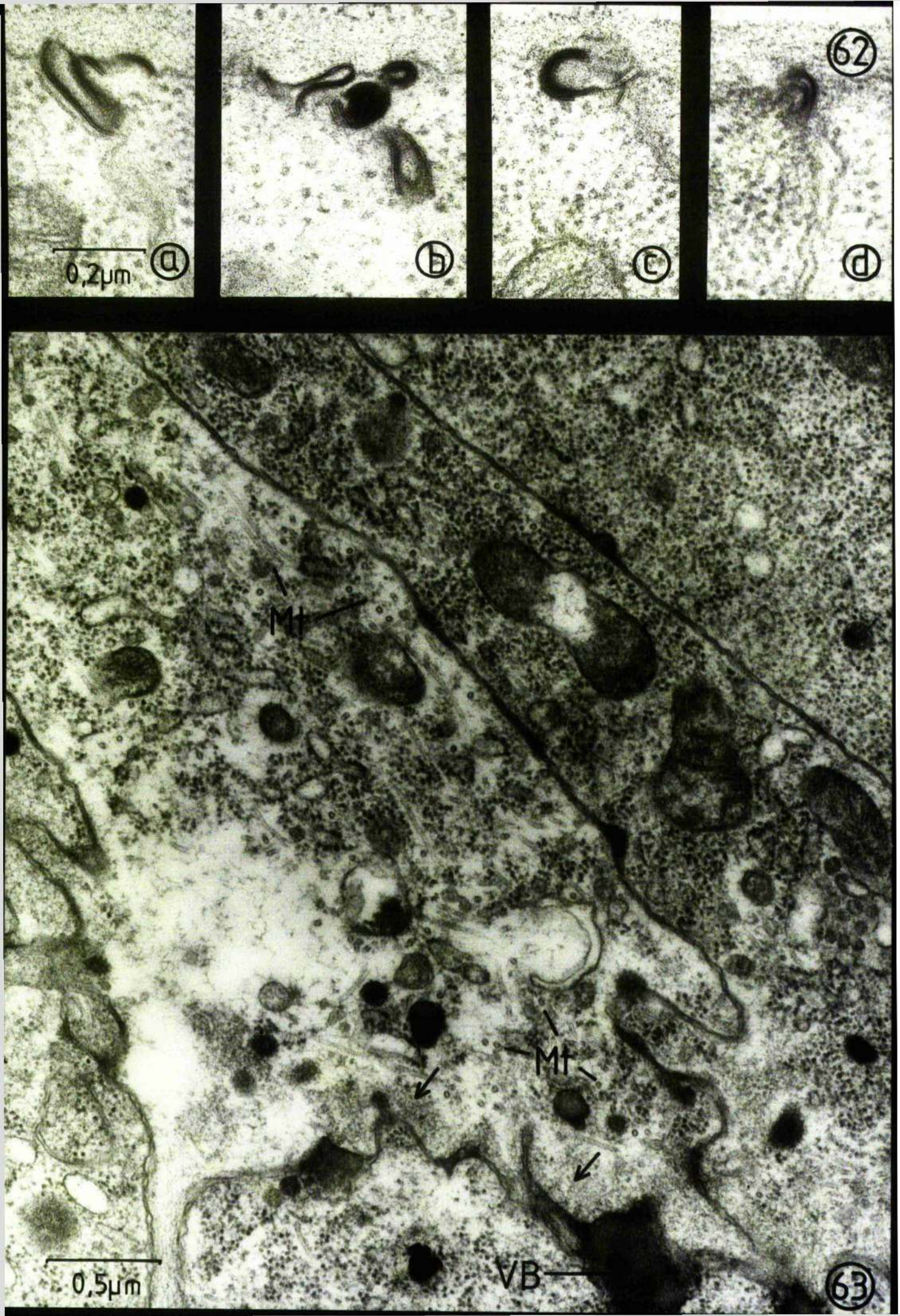
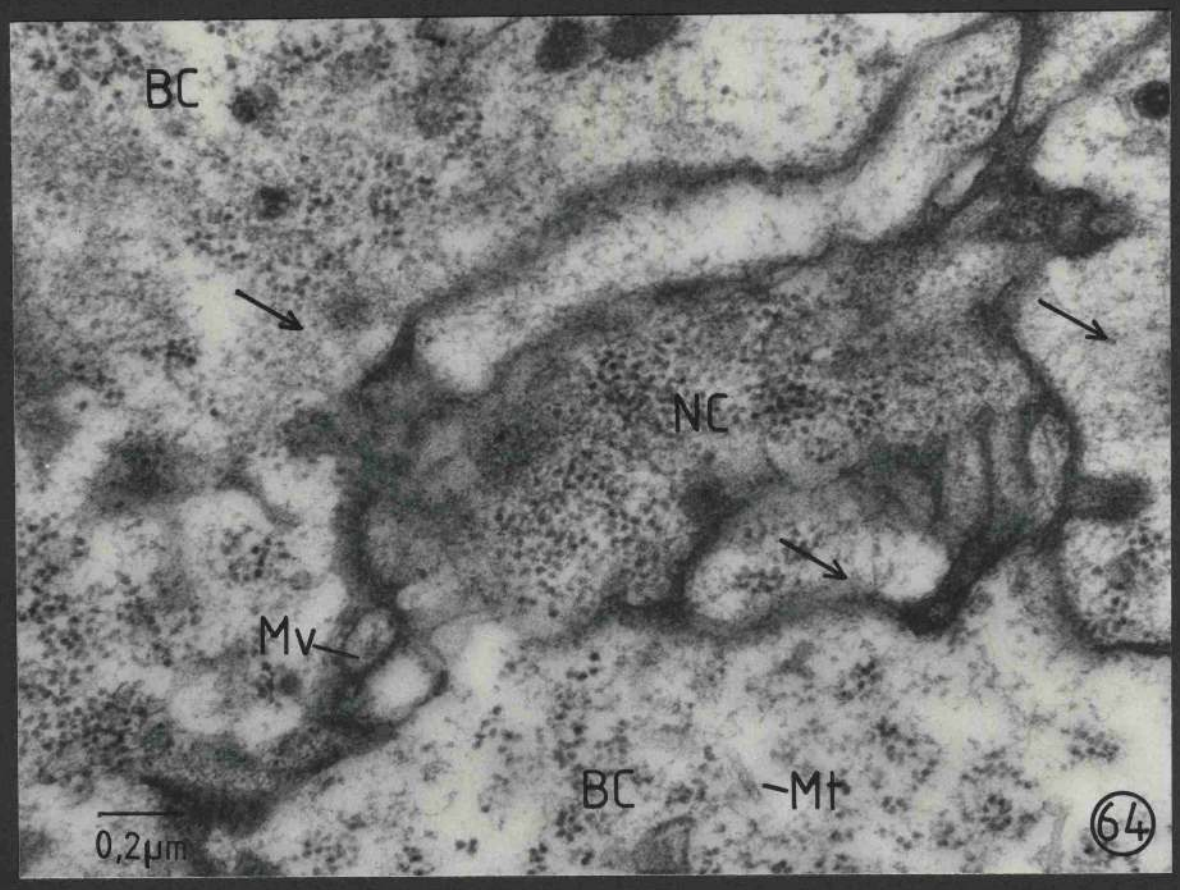

62
0,2µm
a
b
c
d
Mt
Mt
0,5µm
VB
63

PLATE 64

Part of a cross-section of an early stage 9 egg chamber. This section grazes accross the anterior end of two border cells which are migrating through the nurse chamber. Microvilli (Mv) are present at the border cell/nurse cell boundary. Microtubules (Mt) are common in the border cells close to the boundary and filamentous material may be present (arrows). BC: Border cell, NC: Nurse cell. X 56 000. EM/B.

PLATE 65

The posterior (nurse chamber) end of a border cell (BC). Bundles of microfilaments (arrows) in the nurse cell (NC) cytoplasm insert into pits in the border cell membrane. Occasionally bundles in which the filaments are unusually straight and are parallel to one another can be observed (arrowheads). X 62 000. EM/B.


BC
NC
Mv
BC
Mt
0,2µm
64

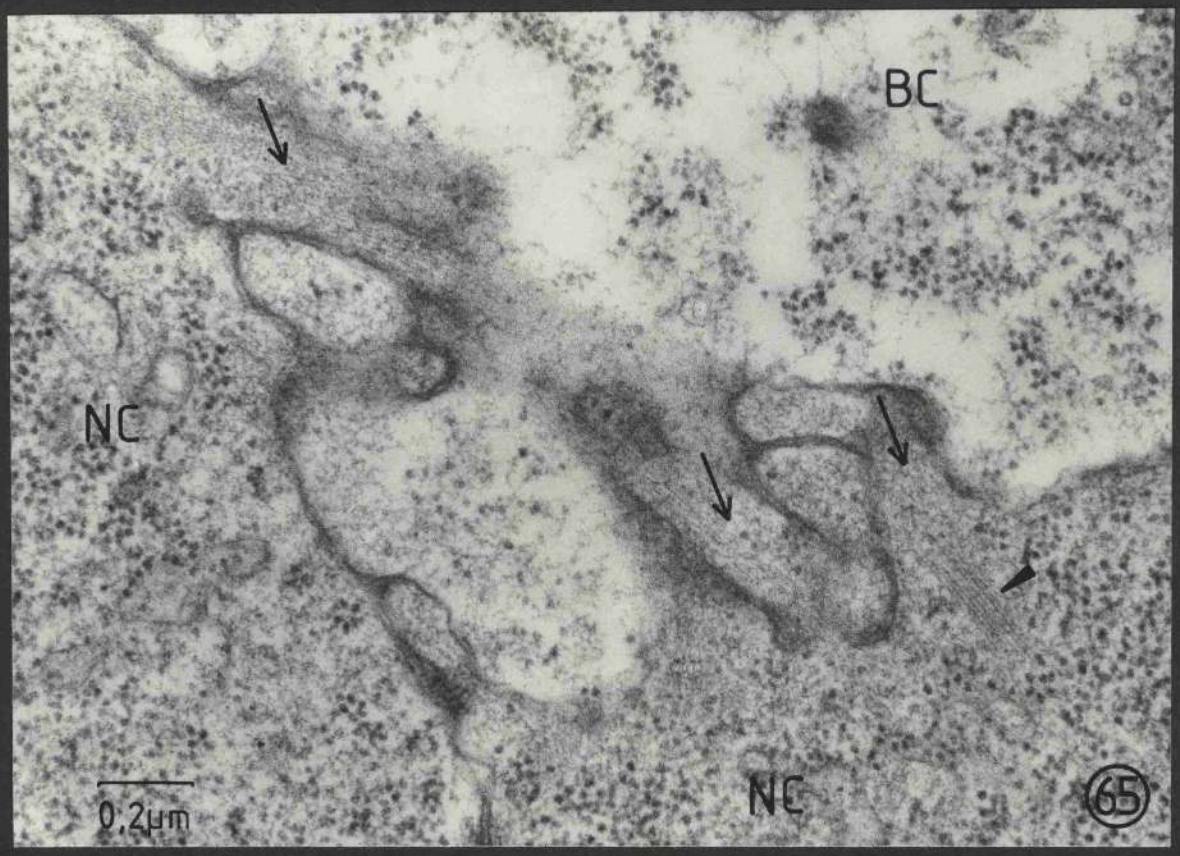
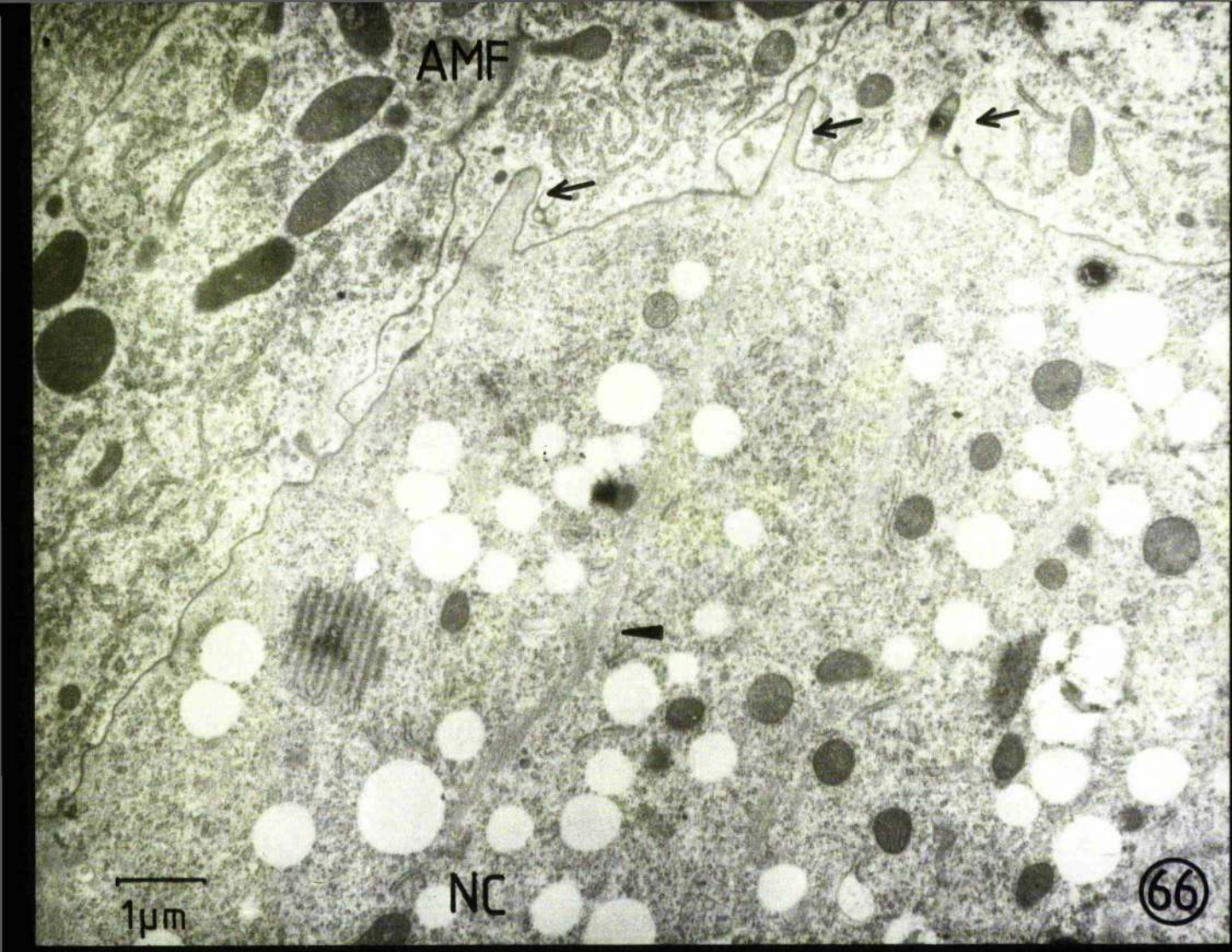

BC
NC
NC
0,2µm
65

PLATE 66

The anterior centripetally migrating follicle (AMF) and the adjacent nurse cell (NC) of a stage 11 egg chamber in longitudinal section. Bundles of microfilaments are present in the nurse cell cytoplasm. These insert into pits in the follicle membrane (arrows). One (arrowhead) extends for at least 7µm through the nurse cell cytoplasm. X 11 000. EM/C.

PLATE 67

The nurse chamber follicle (NF) of a stage 10 egg chamber. The tunica propria (TP) is of simailar thickness and appearance to that of the oocyte chamber. The occasional microtubule (Mt) is present in the follicle cell. Microvilli (Mv) are common between the follicle and nurse cell (NC). X 54 000. EM/B.


AMF
1µm
NC
66

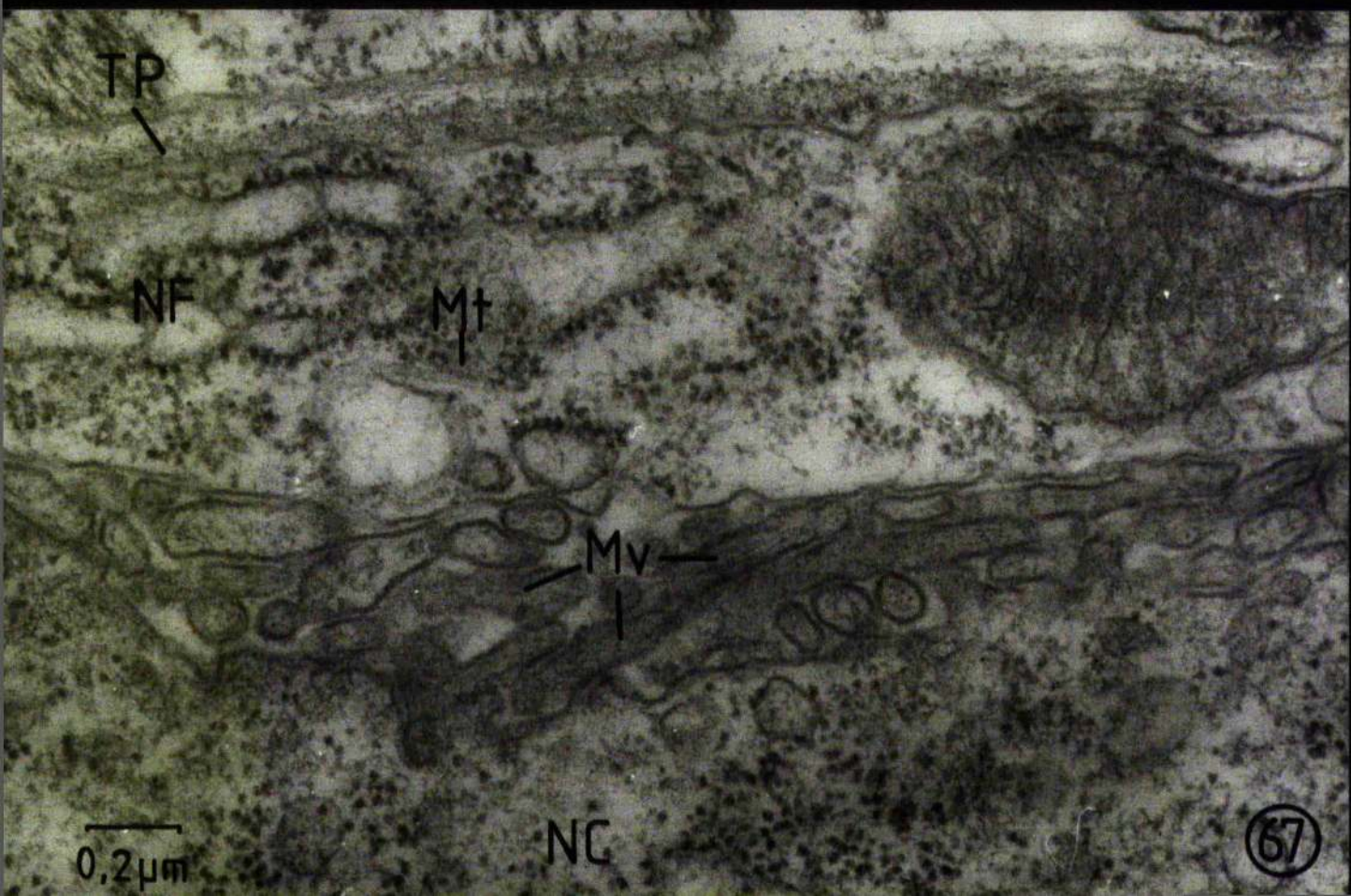

TP
NF
Mt
Mv
0,2µm
NC
67

PLATE 68

A nurse follicle cell (NF) of a stage 11 egg chamber. A bundle of microfilaments can be seen to insert into a microvillus (arrow) which is engaged in a pit in the follicle cell membrane. TP: tunica propria. X 44 000. EM/C.

PLATE 69

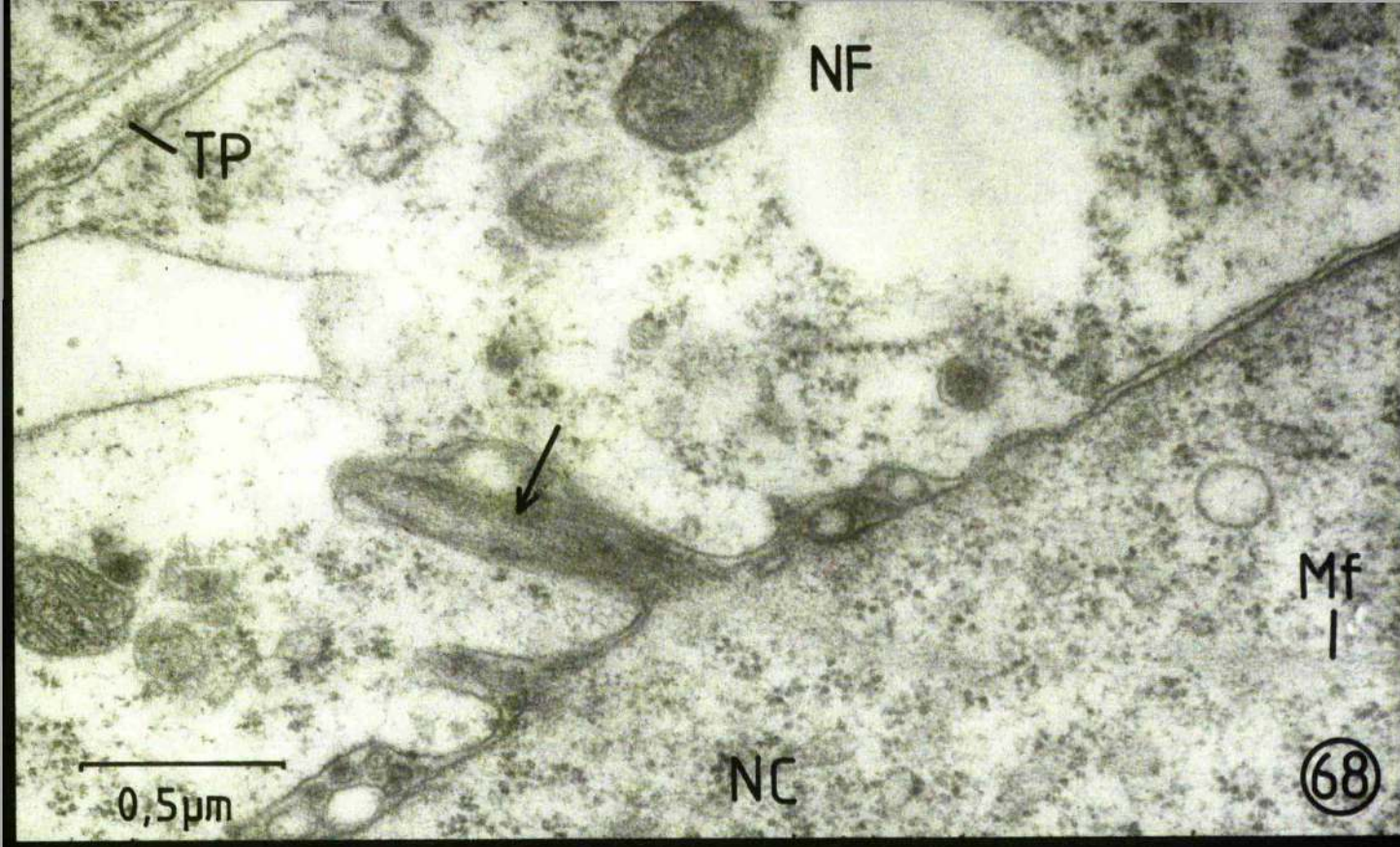
The microfilamentous layer (Mf) in the oocyte follicle of a stage 11 egg chamber in longitudinal section. TP: tunica propria. X 65 000. EM/C.

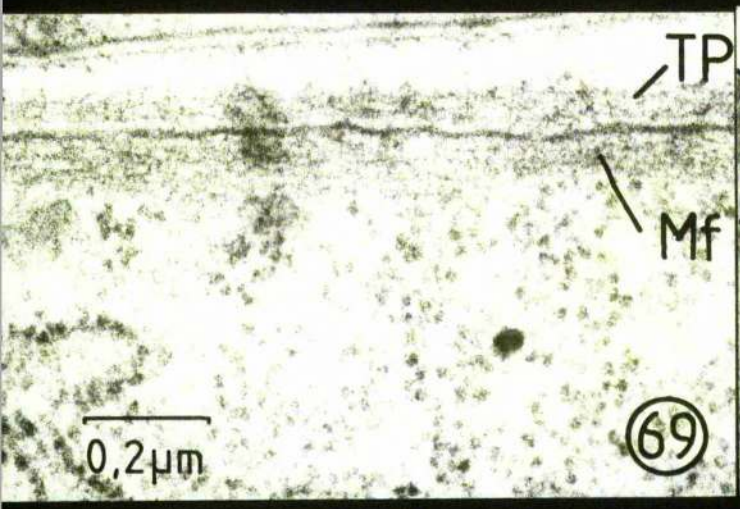
PLATE 70

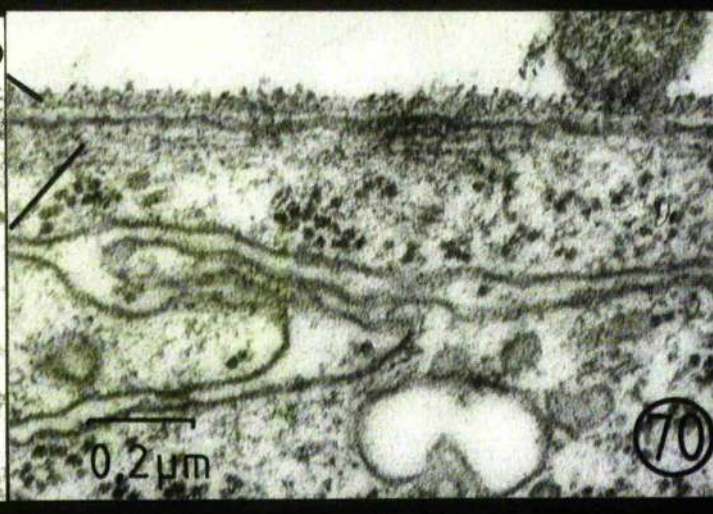
The microfilamentous layer (Mf) in the oocyte follicle of a stage 12 egg chamber in transverse section. TP: tunica propria. X 56 000. EM/B.

PLATE 71

Part of an extension of a nurse follicle cell (NFE) present between two nurse cells (NC). A gap junction (GJ) is present between the extension and a nurse cell. X 74 000. EM/D.


NF
TP
Mf
NC
0,5µm
68


TP
Mf
0,2µm
69


0,2µm
70

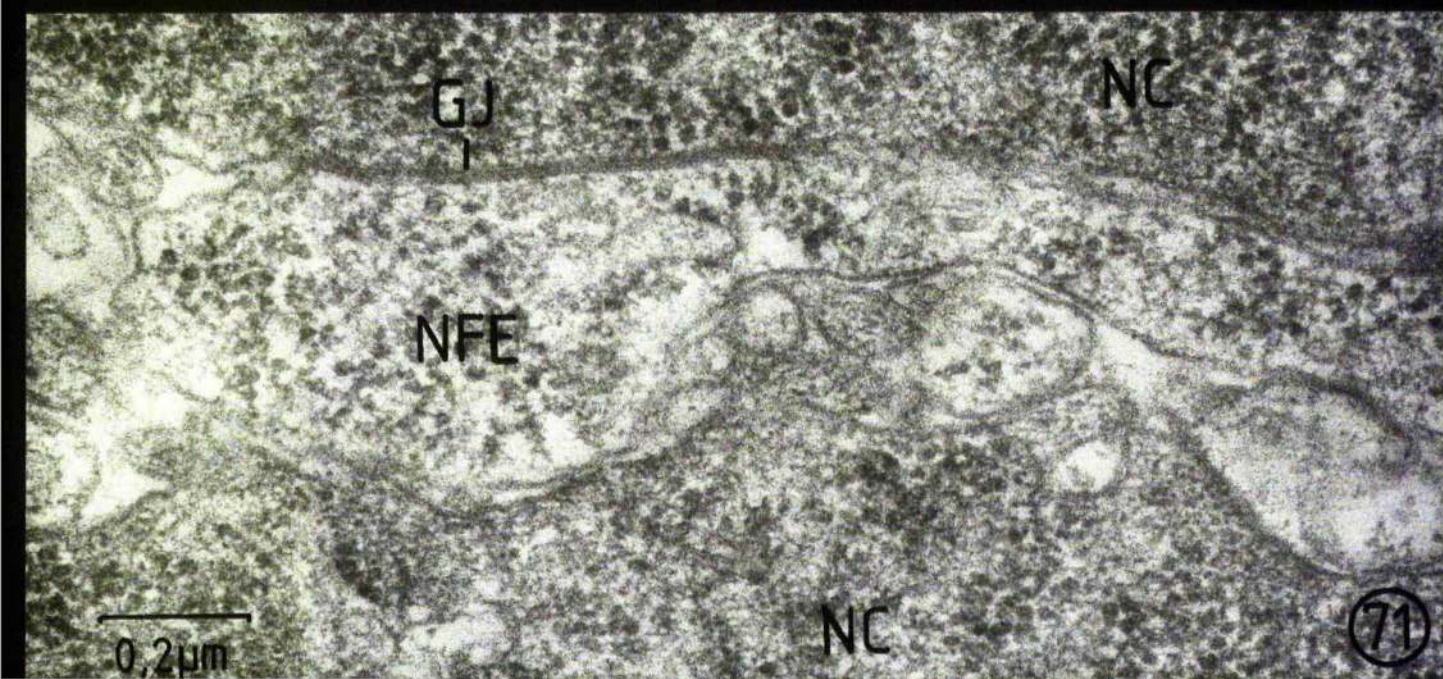
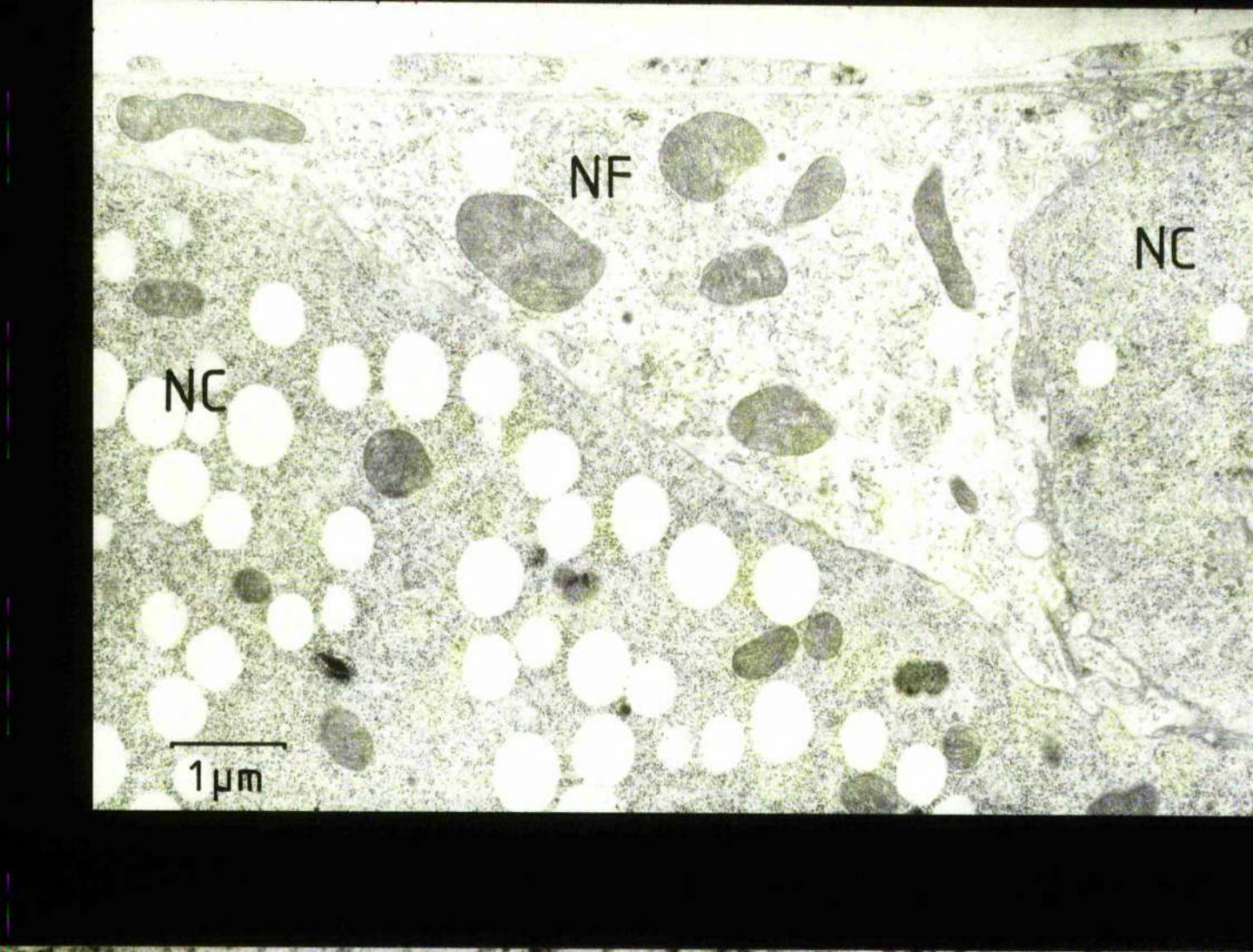

GJ
NC
NFE
NC
0,2µm
71

PLATE 72

A nurse chamber follicle cell (NF) with an extension (arrow) which passes between two nurse cells (NC). Stage 11. X 13 000. EM/C.

PLATE 73

Microfilaments (Mf) present in the nurse cell cytoplasm (NC) close to an extension of a nurse chamber follicle cell (NFE). Some (PMf) are of the straight, parallel, type. X 91 000. EM/B.


NF
NC
NC
1µm

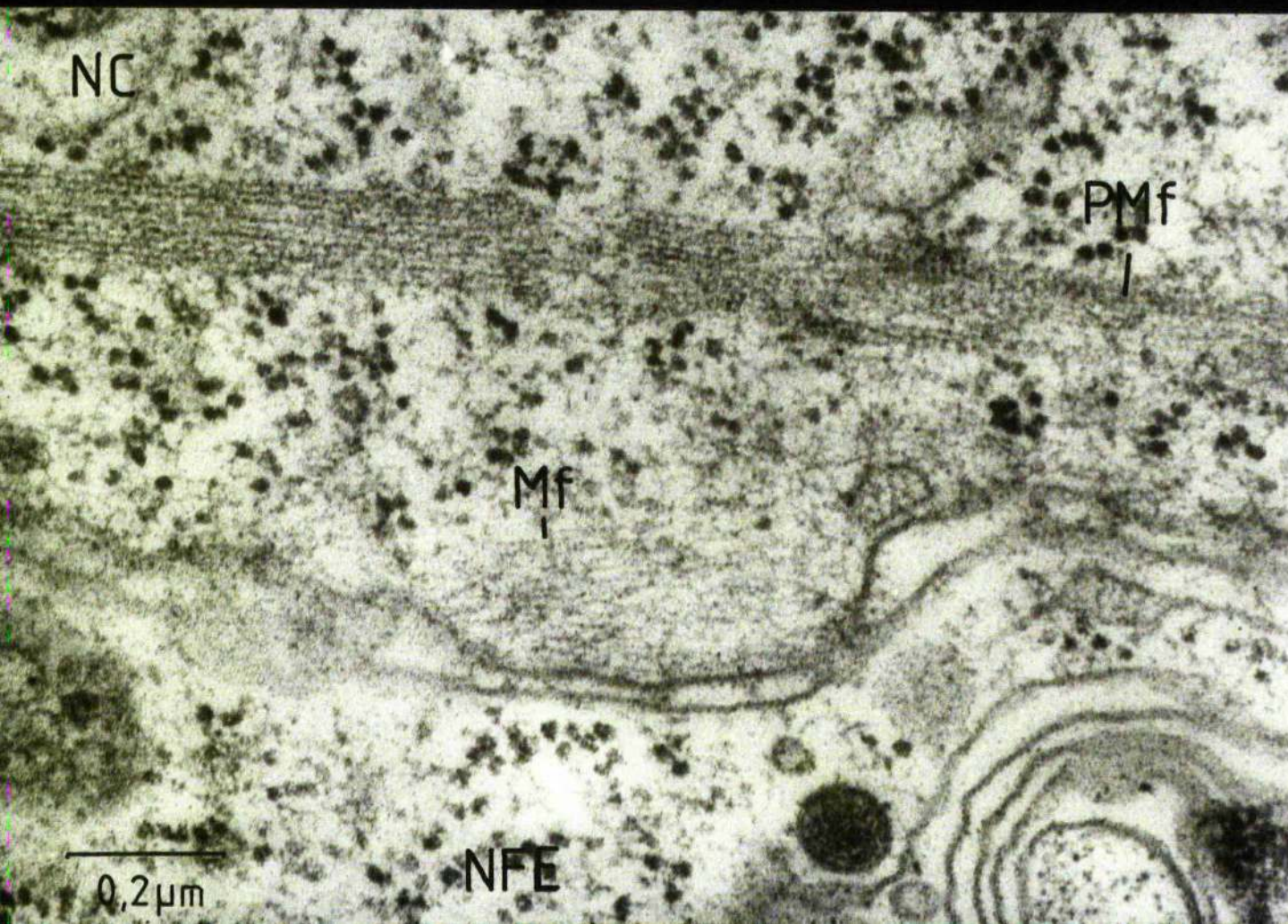
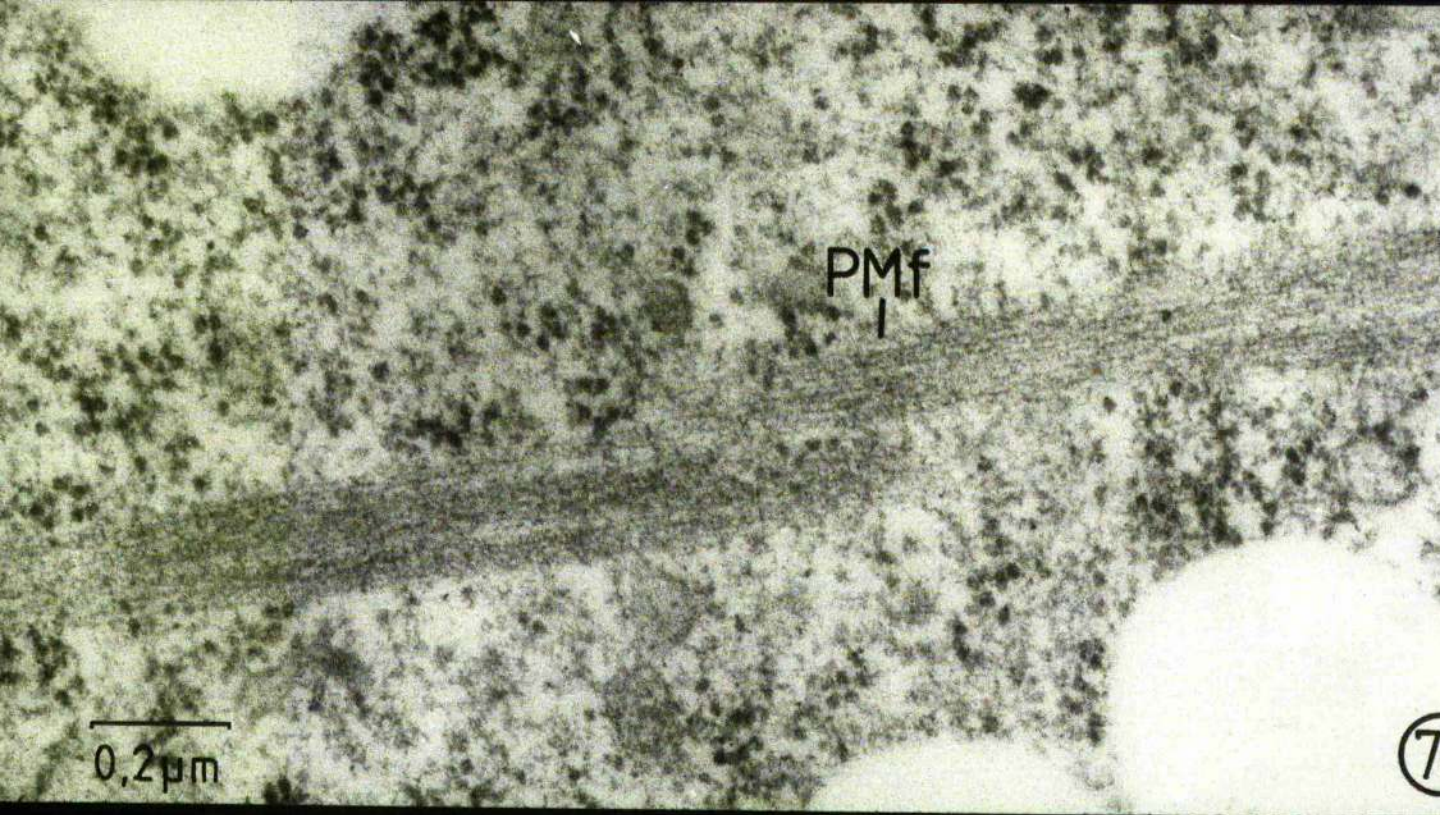

NC
PMf
Mf
NFE
0,2µm

PLATE 74

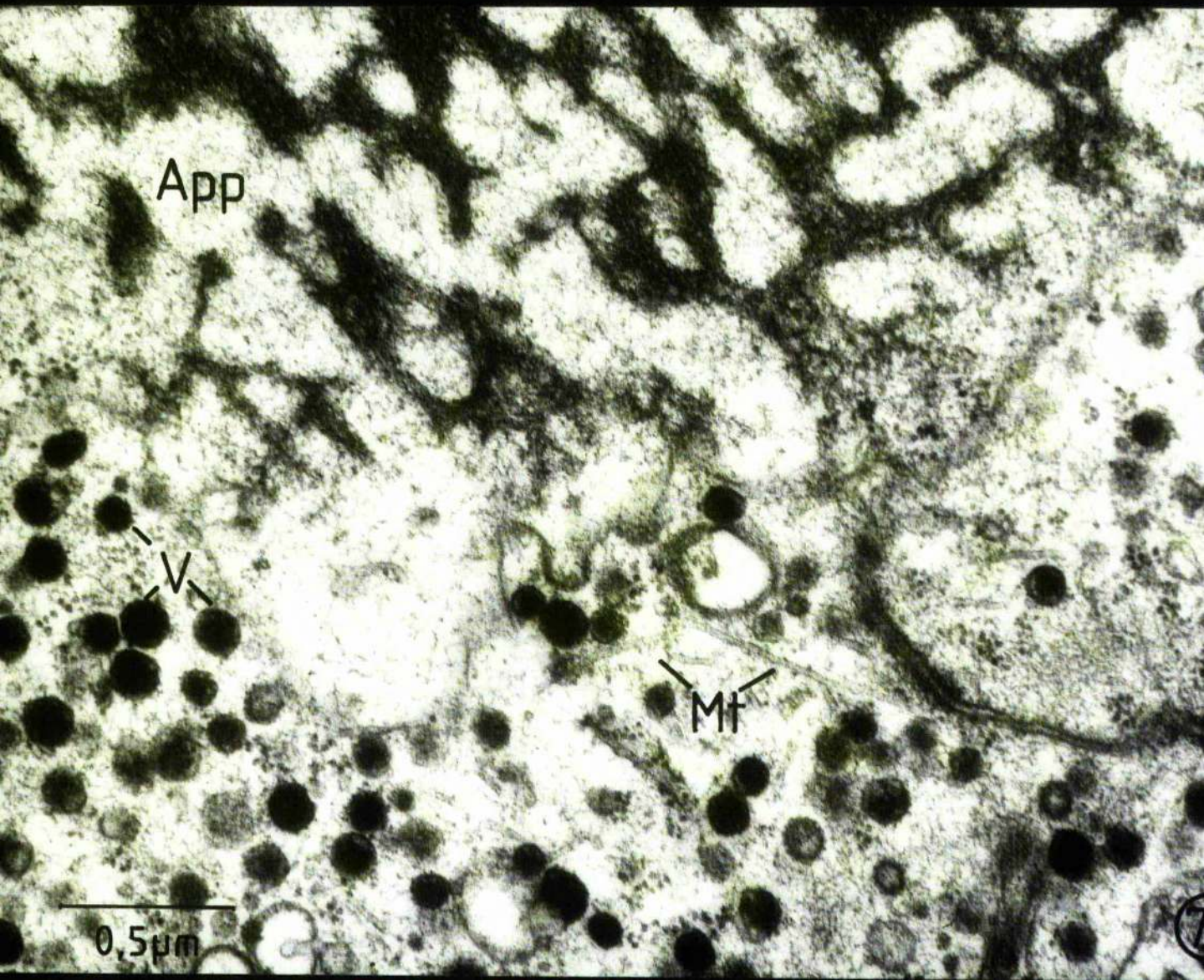
A microfilament bundle of the straight, parallel, type (PMf) passing through the cytoplasm of a nurse cell, away from cell boundaries. Stage 11. X 67 000. EM/C.

PLATE 75

The region in which a dorsal appendage (App) is being formed in a stage 12 egg chamber. Microtubules (Mt) are common. The densely staining material within the numerous vesicles (V) is presumably precursor material for the formation of the appendages. X 42 000. EM/B.



7



7

PLATE 76

A longitudinal section of a stage 13 egg chamber close to the plane of the developing dorsal appendage. Microtubules (Mt) run parallel to the axis of the appendage. Numerous vesicles (V) are present which probably contain precursor material for the formation of the appendage. X 46 000. EM/B.

PLATE 77

Septate junctions (SJ) are common between the follicle cells which form the dorsal appendages. When these junctions are sectioned parallel to the plane of the junctional membranes the septae can be observed to be of the pleated sheet type (SJP). Microtubules (Mt) are pesent as in plate 76. X 41 000. EM/B.

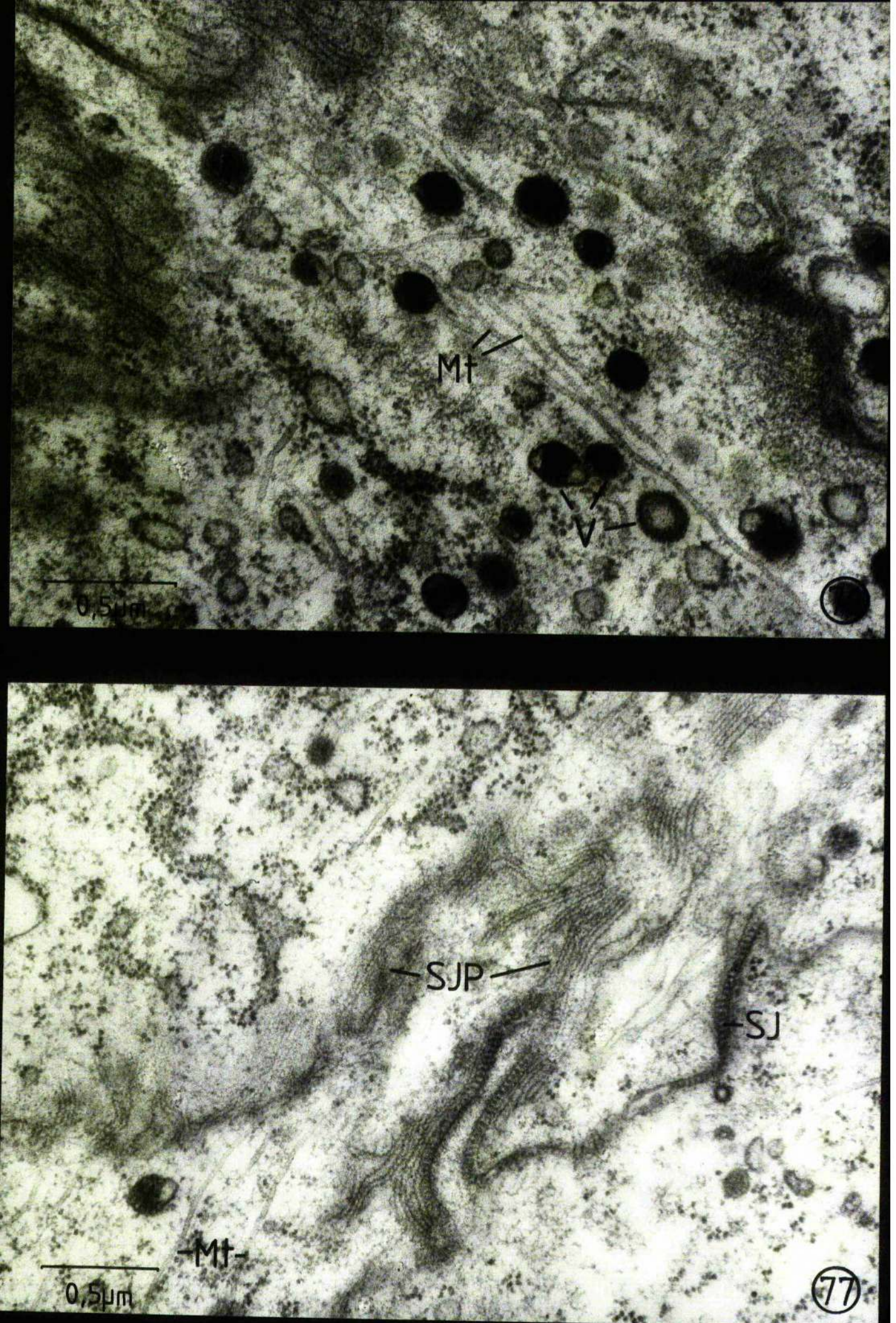
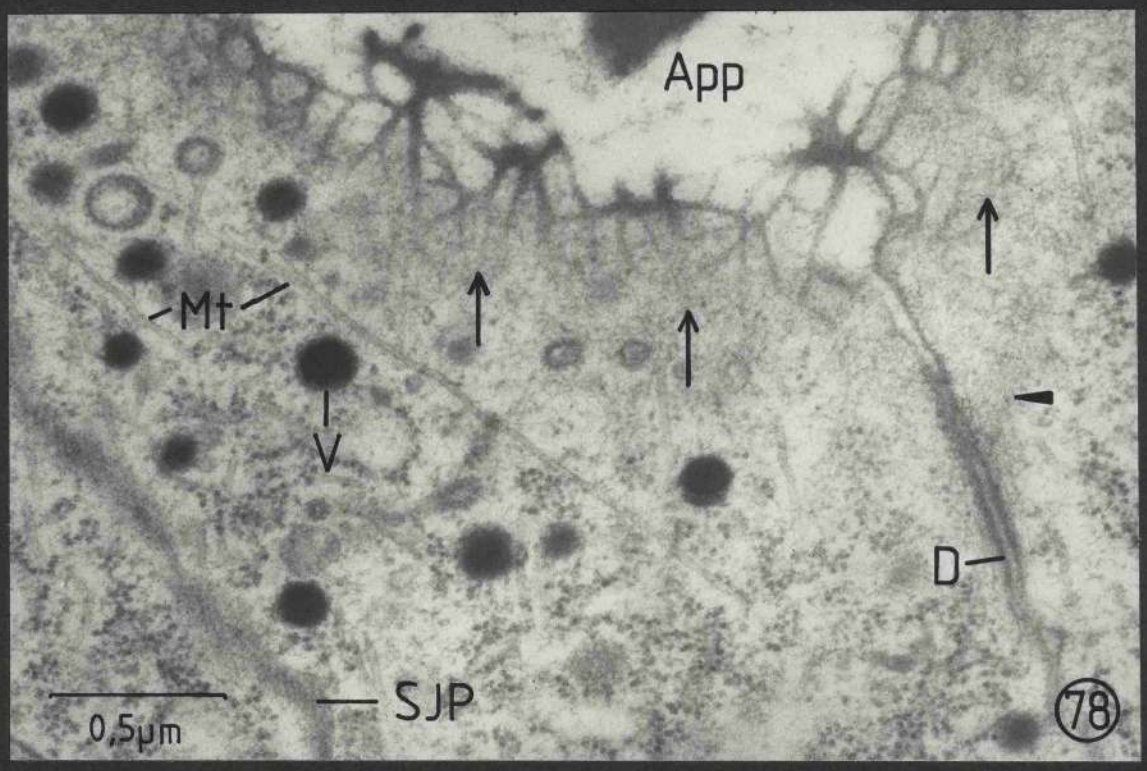

Mt
V
0,5µm
SJP
SJ
Mt
0,5µm
77

PLATE 78

A section grazing accross the forming dorsal appendage in a stage 13 egg chamber. The cytoplasm surrounding the appendage has a somewhat filamentous appearance (arrows). Filamentous material (arrowhead) also appears to be associated with a desmosome (D). Microtubules (Mt), precursor vesicles (V) and septate junctions (sectioned obliquely, showing pleated sheets: SJP) are also present: see legends to plates 75 to 77. X 47 000. EM/C.

PLATE 79

A transverse section of the dorsal appendage forming follicle of a stage 13 oocyte. A layer of microfilaments (Mf) is present beneath the outer plasma membrane (PM). These microfilaments are observed in longitudinal profile as are the few microtubules (Mt) present: both are circumferentially oriented. TP: tunica propria. X 68 000. EM/C.


App
Mt
V
D
SJP
0,5µm
78

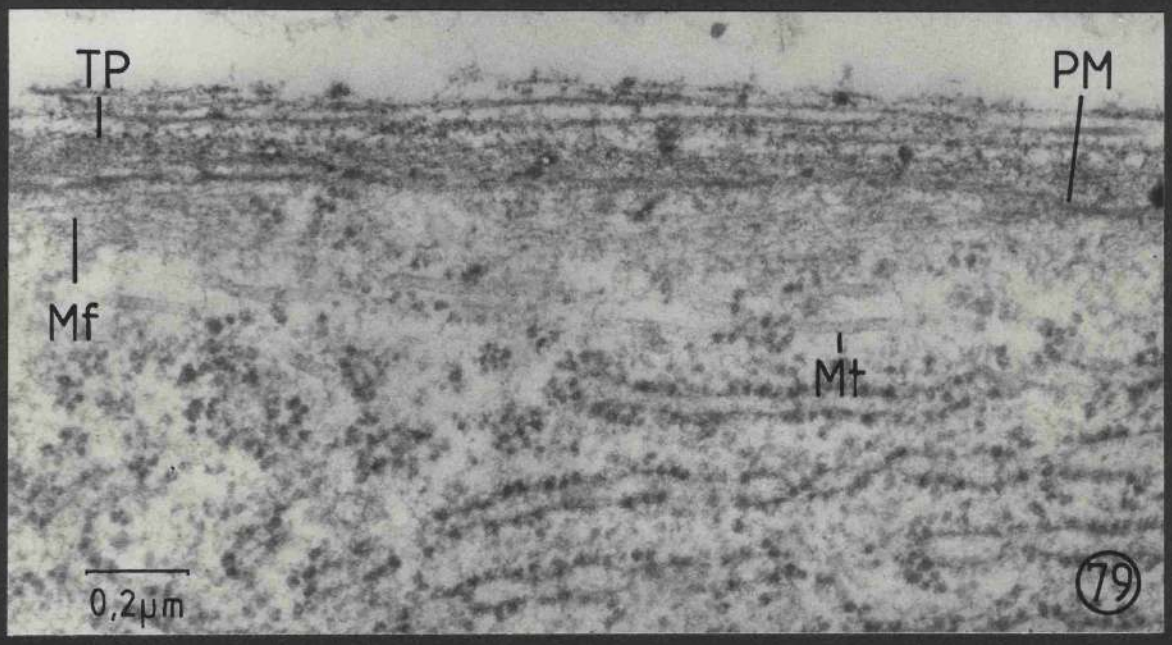
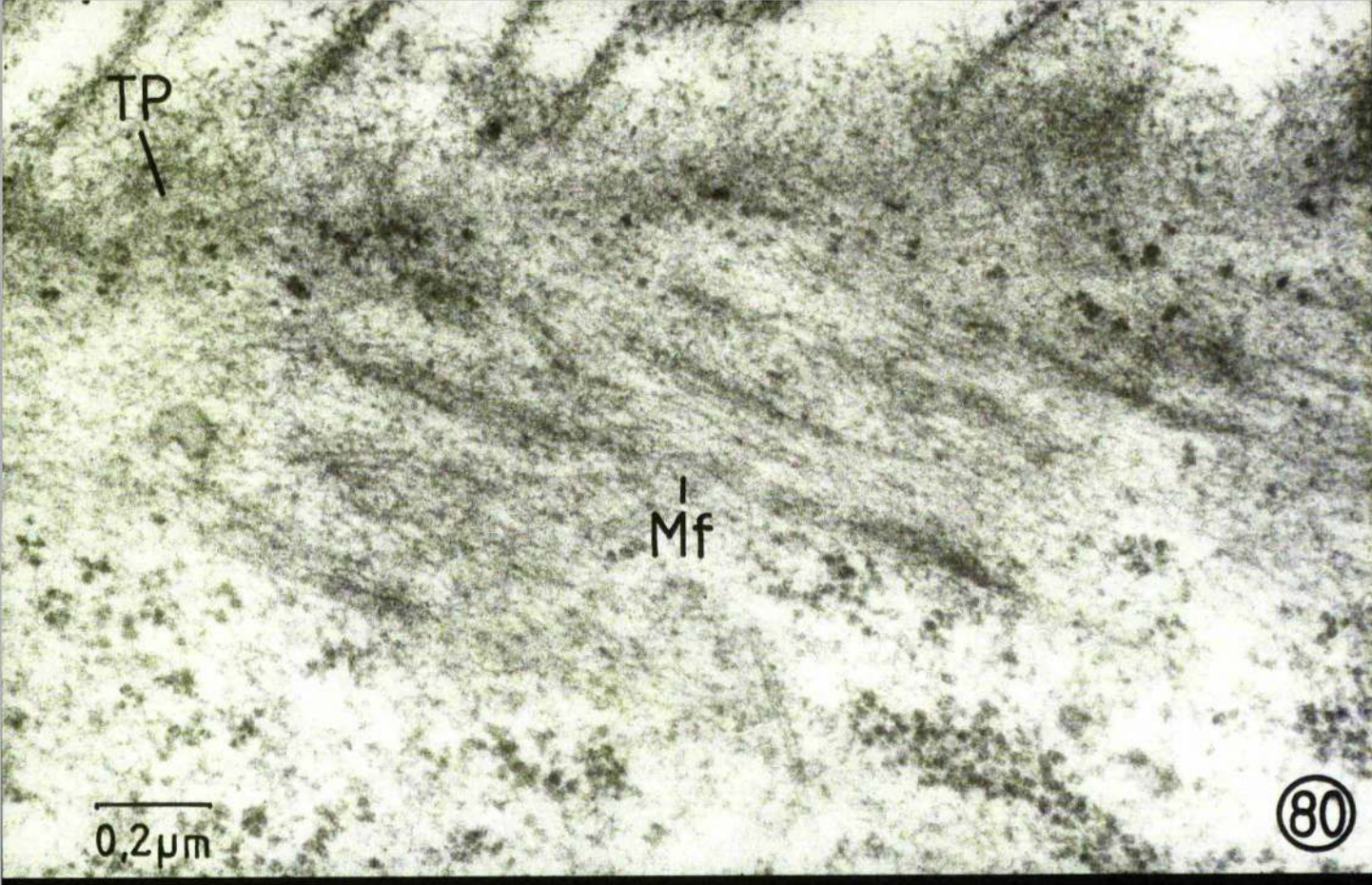

TP
PM
Mf
Mt
0,2µm
79

PLATE 80

A longitudinal section of a stage 13 egg chamber grazing across the surface of the appendage forming follicle. Numerous microfilaments (Mf) are present. These are probably circumferentially oriented (the cell surface is not parallel to the axis of the follicle in this section. TP: tunica propria). X 64 000. EM/C.

PLATE 81

A gap junction (GJ) between two follicle cells in a stage 10 egg chamber which was fixed using a fixative with double the normal buffer concentration. The gap junction (GJ) remains intact although the cells have pulled apart and therefore may be able to transmit a degree of tension. X 36 000. EM/D.


TP
Mf
0,2µm
80

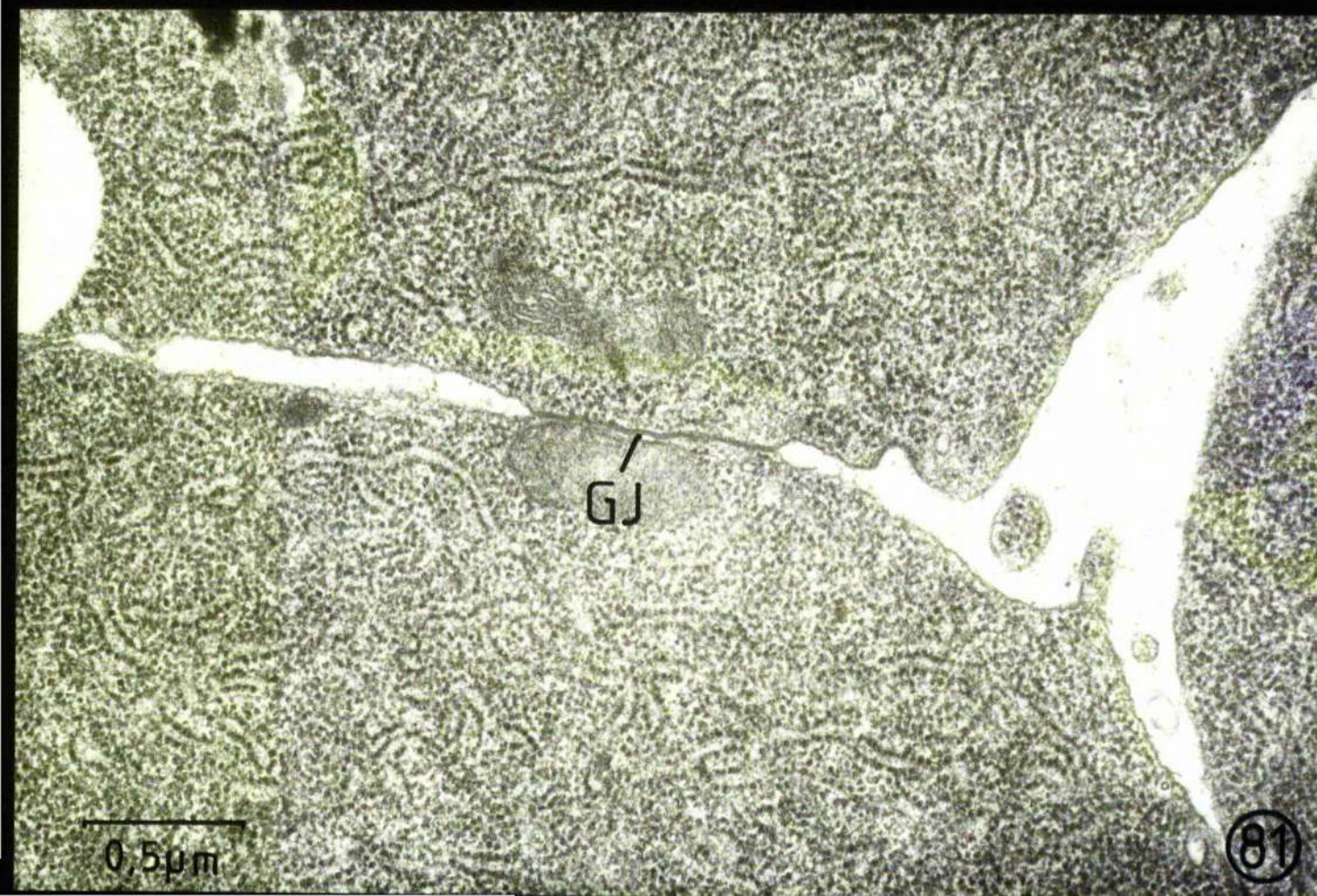

GJ
0,5µm
81

PLATE 82

An early stage 9 egg chamber which has been teased out of the epithelial sheath into DPBS. The oocyte chamber has become reduced in diameter (having had the same diameter as the nurse chamber before release from the epithelial sheath). The boundary between the nurse and oocyte follicles is more gradual at the beginning of stage 9 than it is in later stages of development. In this egg chamber the short length of collumnar follicle over the nurse chamber also appears to be contracted. X 290. LM/NIC.

PLATE 83

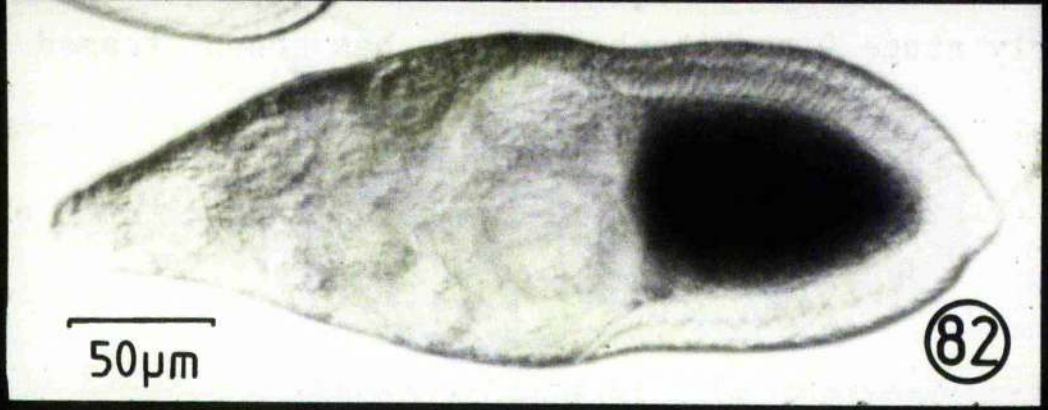
A late stage 9 egg chamber released into DPBS. The contracted region corresponds more closely with the oocyte chamber than in the case in the egg chamber in plate 82. X 290. LM/NIC.

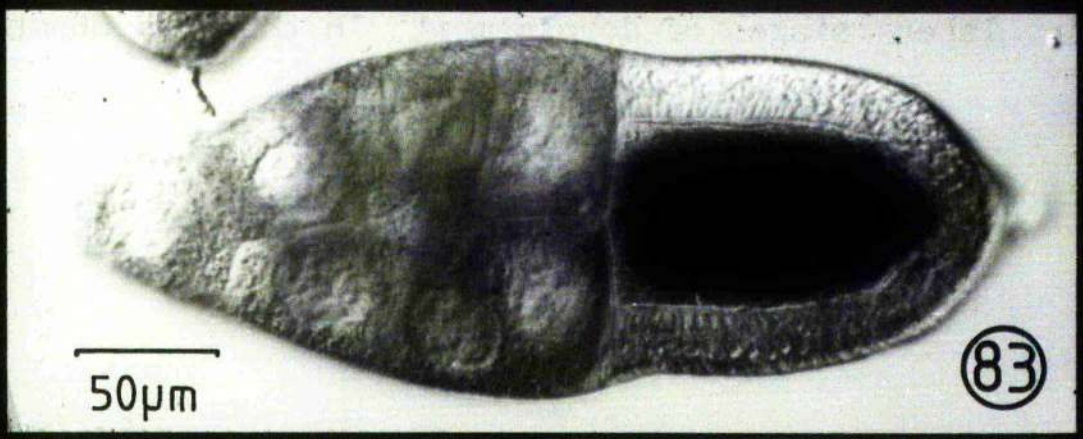
PLATE 84

A stage 10 egg chamber released into DPBS. The oocyte chamber has contracted, but not to such an extent as occurs with stage 9 chambers. X 290. LM/NIC.

PLATE 85

A stage 10 egg chamber in DPBS which has been pricked with a glass needle close to the posterior end. Ooplasm has been expelled from the oocyte and the oocyte chamber has contracted to a much greater extent than occurs in intact chambers. This suggests that the pressure in the oocyte normally resists the force of contraction of the follicle. X 290. LM/NIC.


50µm
82


50µm
83


50µm
84

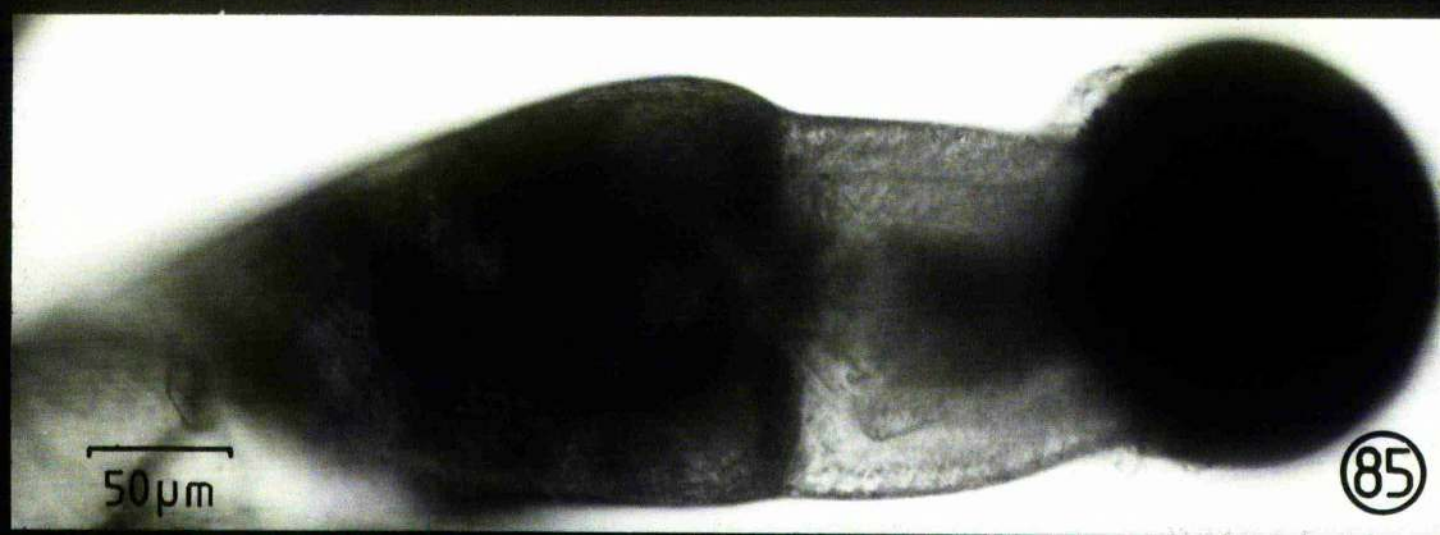

50µm
85

PLATE 86

Part of the outer surface of a stage 10 oocyte follicle cell from a transverse section of an egg chamber which has been incubated in 1% DMSO in DPBS for 15min. The filamentous layer (Mf) is still present (TP: tunica propria, PM: plasma membrane). X 74 000. EM/C.

PLATE 87

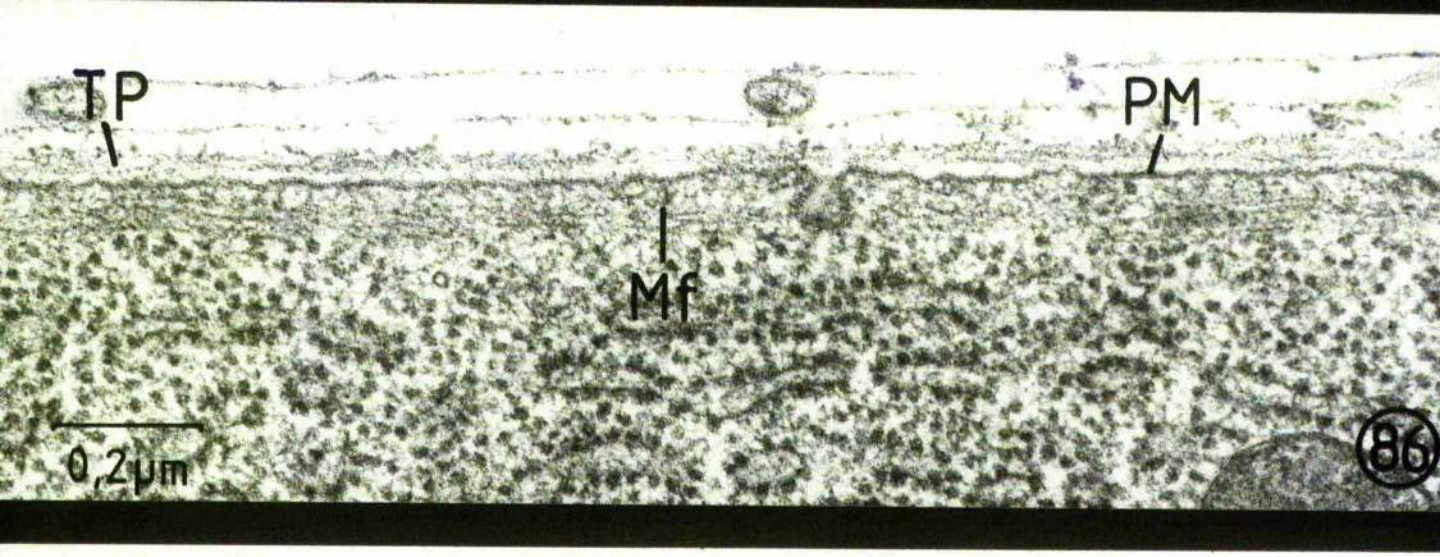
As above but from an egg chamber treated with 50µg/ml cytochalasin B, 0.5% DMSO in DPBS for 30min. The microfilamentous layer is absent. X 65 000. EM/C.

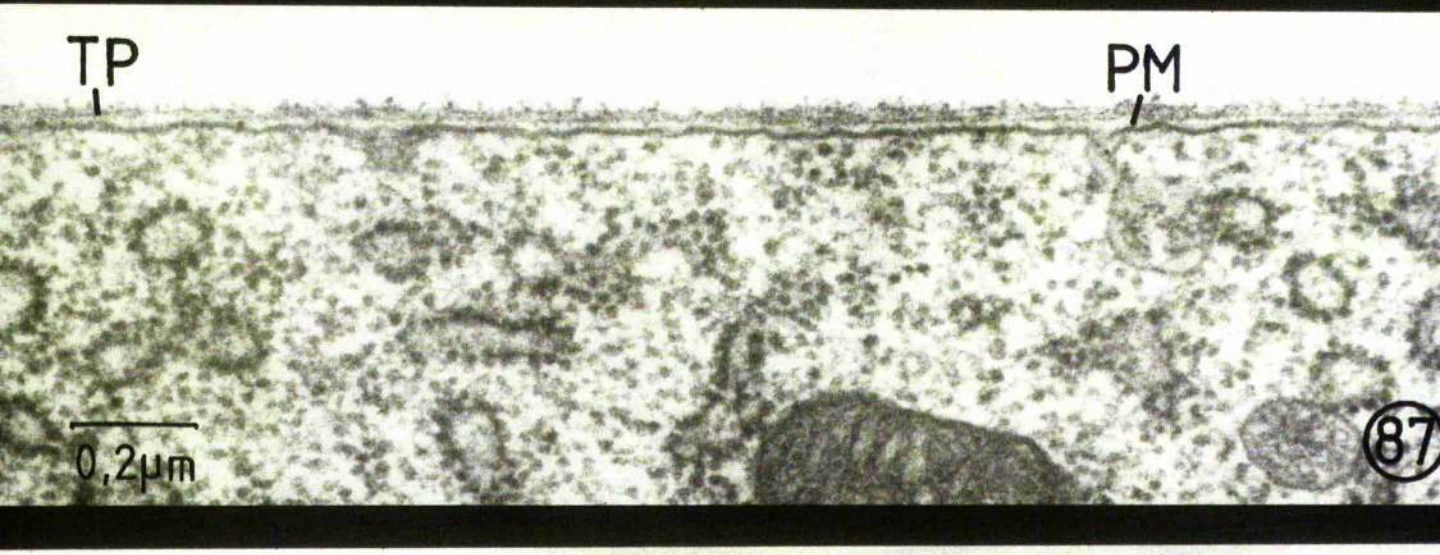
PLATE 88

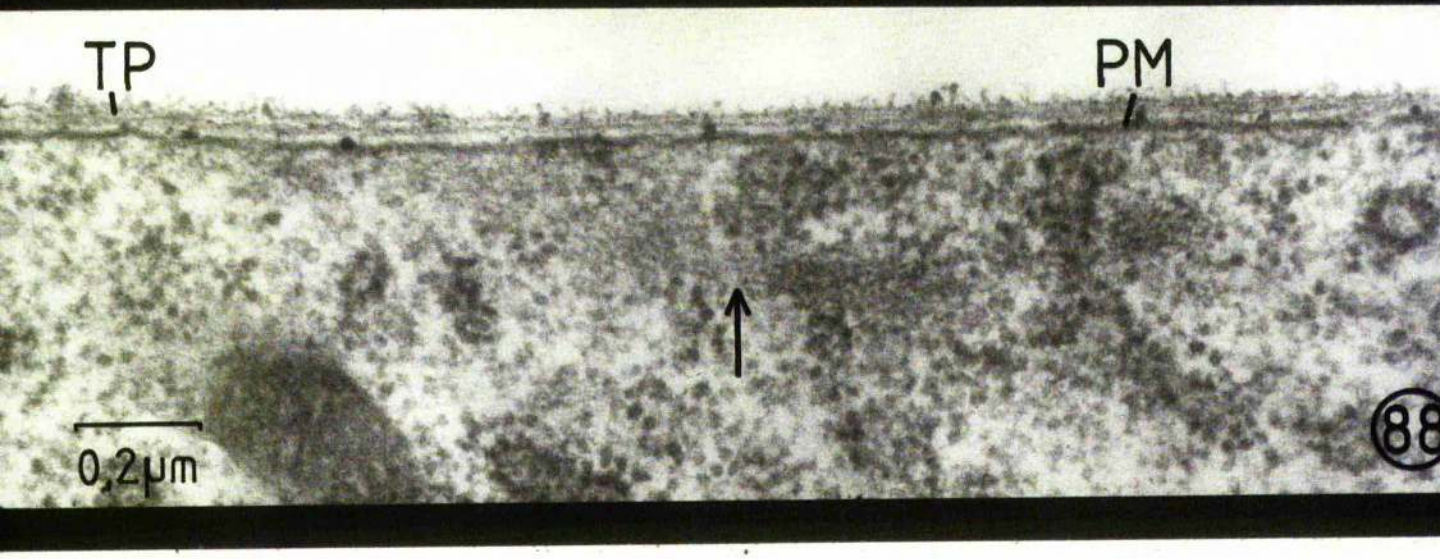
The same egg chamber as plate 87, but another region of the follicle surface. A patch (arrow) of filamentous material is present. X 65 000. EM/C.

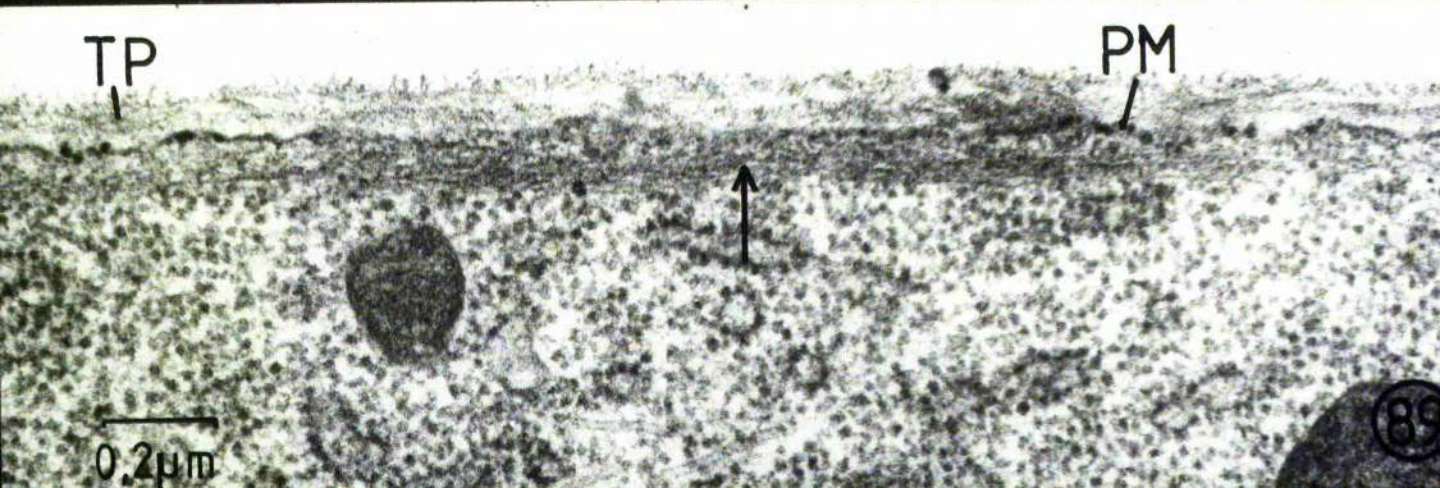
PLATE 89

Part of the outer surface of a stage 9 oocyte follicle cell treated with 50µg/ml cytochalasin B for 30min (as were the chambers in plates 87 and 88), but which was then stood in normal DPBS for 1h after the cytochalasin B had been rinsed out. Some microfilaments (arrow) are present, but the microfilamentous layer is not completely reformed. X 58 000. EM/C.


TP
PM
Mf
0,2µm
86


TP
PM
0,2µm
87


TP
PM
0,2µm
88


TP
PM
0,2µm
89

PLATES 90 - 93

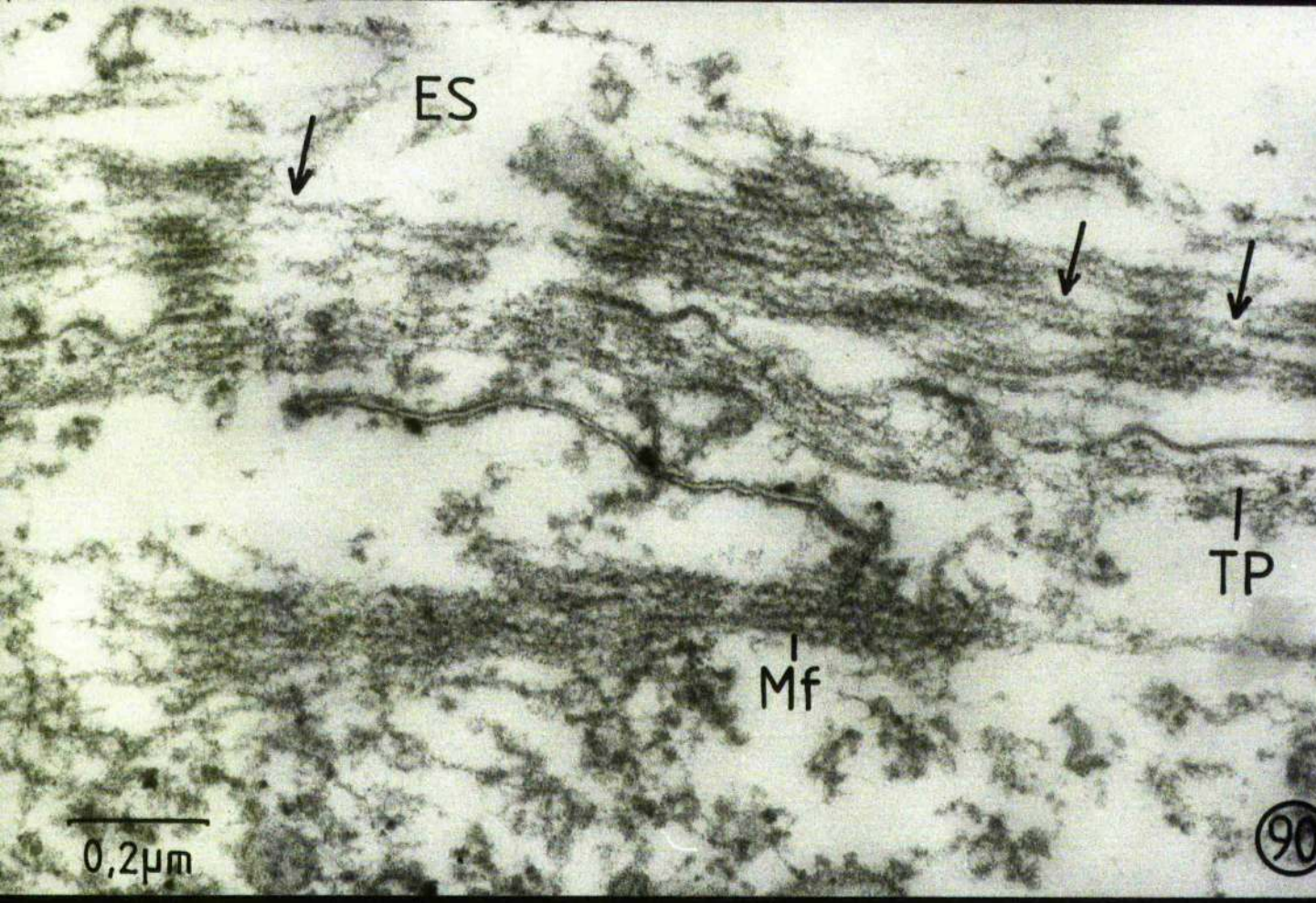
These plates demonstrate decoration of microfilaments by heavy meromyosin (HMM) in egg chambers that have been glycerol extracted and subsequently placed in 10mg/ml HMM solution, and left overnight at room temperature: see methods section in chapter 6.

PLATE 90

Decorated microfilaments (Mf) of the microfilamentous layer in a stage 9 egg chamber. The thin filaments (arrows) of the epithelial sheath (ES) muscle fibres are also decorated (TP: tunica propria). X 80 000. EM/C.

PLATE 91

The microvilli (Mv) of the vitelline membrane (VM) surface of the oocyte (Ooc) contain decorated microfilaments (arrow). Stage 9. Compare with the undecorated microvilli in plate 94. X 60 000. EM/C.


ES
TP
Mf
0,2µm
90

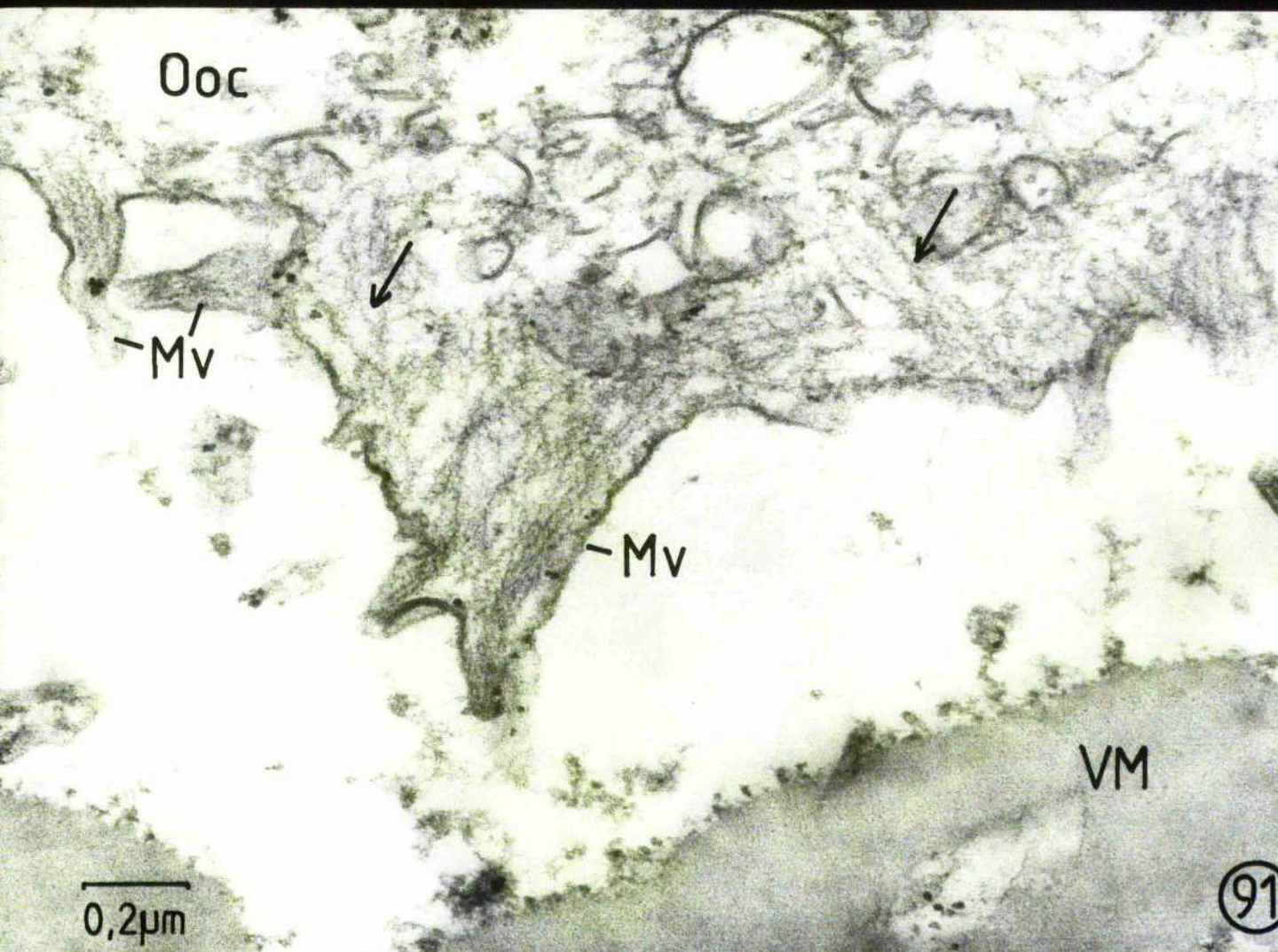

Ooc
Mv
Mv
VM
0,2µm
91

PLATE 92

The follicle cell (Fol) microvilli on the opposite side of the vitelline membrane to those in plate 91 are also decorated. The microfilaments of the terminal web (arrow) can clearly be seen to be decorated in this micrograph . Stage 9. X 66 000. EM/C.

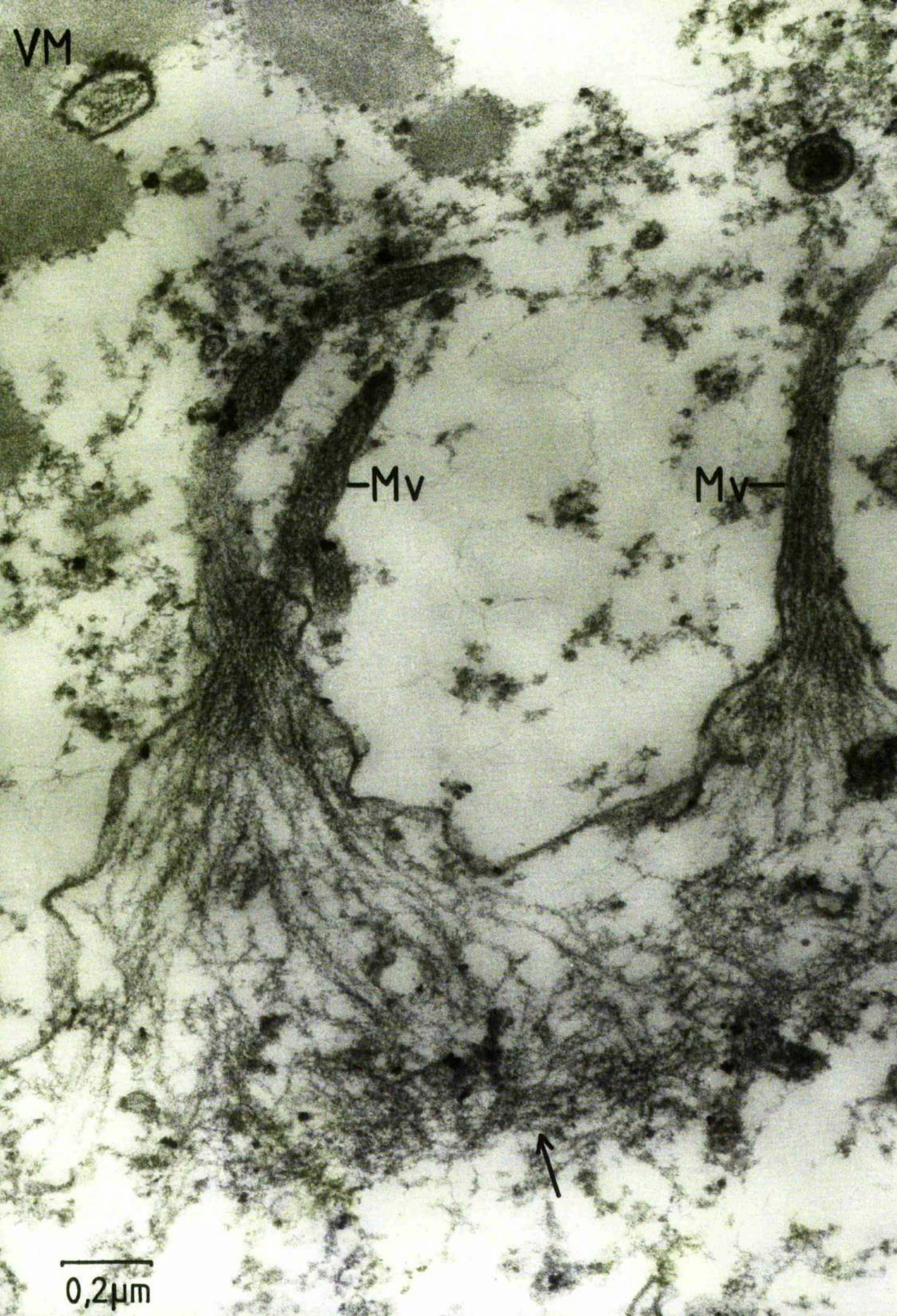
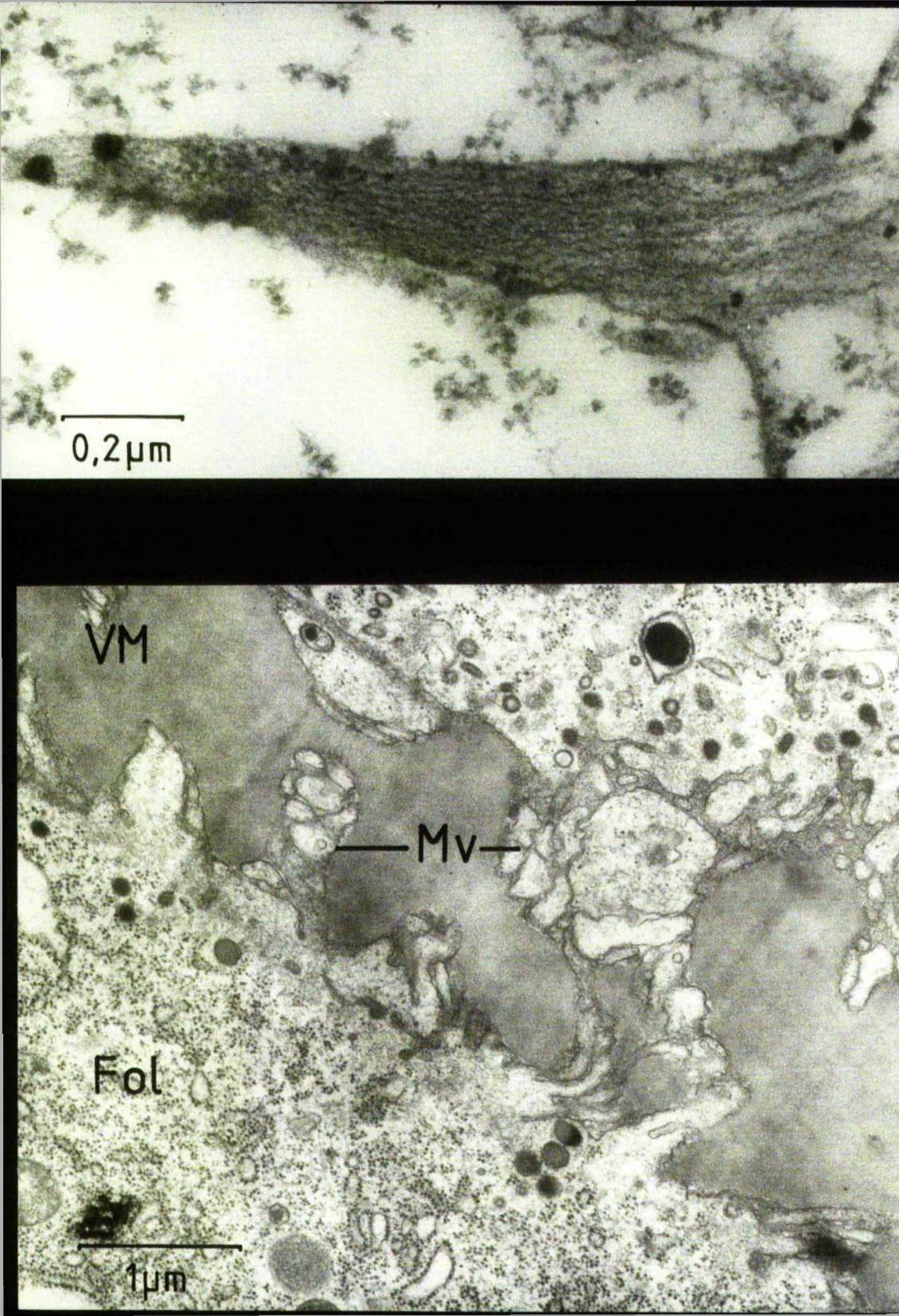

VM
Mv
Mv
0,2µm

PLATE 93

Decorated microfilaments in a microvillus of a stage 10 follicle cell. 20mM $MgCl_2$ (instead of 5mM) in all solutions. X 77 000. EM/C.

PLATE 94

The boundary between the oocyte (Ooc) and a follicle cell (Fol) in a stage 10 egg chamber (that has been fixed conventionally) for comparison with plates 91 to 93. The vitelline membrane (VM) is incomplete and is crossed by microvilli (Mv). X 21 000. EM/B.


0,2µm
VM
Mv
Fol
1µm

PLATES 95 - 98

These plates demonstrate the presence of undecorated microfilaments in stage 9 egg chambers which have been treated with control solutions after glycerol extraction identical to that given to the egg chambers in the experiments illustrated in plates 90 - 92. TP: tunica propria, PM: plasma membrane, Mf: Microfilaments. X 77 000. EM/C.

PLATE 95

Treatment with standard salt solution (SSS), no HMM. The remnants of the microfilamentous layer (Mf) can be observed.

PLATE 96

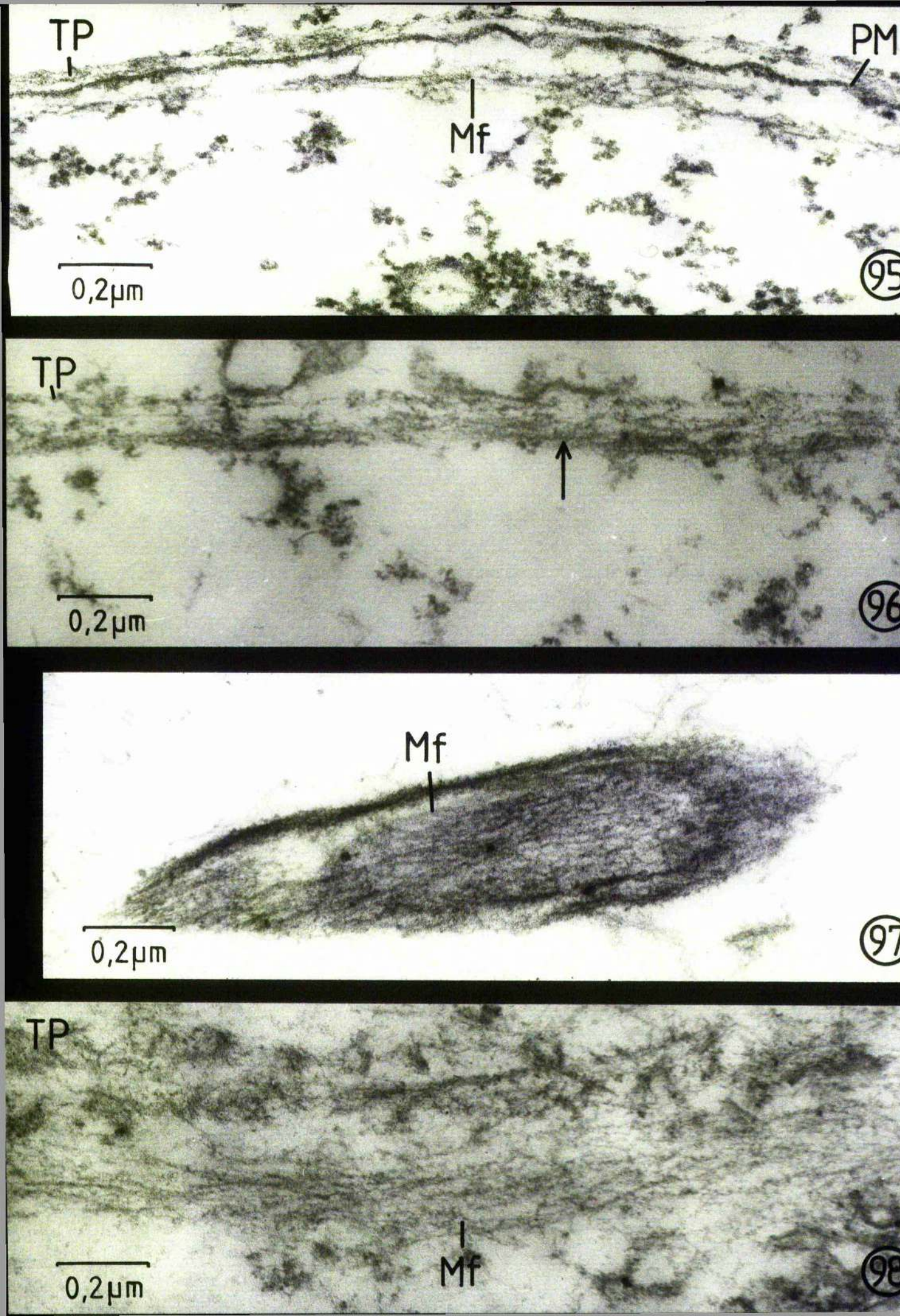
Treatment with 10mg/ml HMM and 10mM ATP in SSS. The microfilamentous layer (arrow) is present beneath the thin tunica propria (TP) which has about the same thickness as in plate 95. The layer is very poorly preserved and the individual filaments can hardly be made out.

PLATE 97

Treatment as in plate 97. The microfilaments (Mf) in an interdigitation are better preserved than those in the microfilamentous layer. They are clearly not decorated.

PLATE 98

Treatment with 2mM pyrophosphate, 10mg/ml HMM in SSS. The microfilamentous layer (Mf) is disrupted. The filaments are not decorated.


TP
PM
Mf
0,2µm
95
TP
0,2µm
96
Mf
0,2µm
97
TP
Mf
0,2µm
98