EPITHELIAL ULTRASTRUCTURE IN THE VAS DEFERENS

OF THE SOW BUG, PORCELLIO SCABER (LATREILLE)

by

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TABLE OF CONTENTS

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INTRODUCTION

Histological procedures have developed over the last fifteen years to the point where it has become possible routinely to realize the very high resolving power of the electron microscope in thin sections of solid tissues. During this time a disproportionate amount of electron microscopic research has been devoted to the study of materials from vertebrate animals with a consequent neglect of the unusually interesting tissues found in some invertebrate organisms. The present study reports the results of an electron microscopic investigation of the epithelial structure in the vas deferens of the sow bug, Porcellio scaber (Latreille), with particular reference to the ultrastructure of the giant cells described by Radu (52, p. 1-14).

Although the structure of these giant cells was first described many years ago in Oniscus asellus (34, p. 77-112), there has been little effort made to capitalize on the further potential of this veritable cytologist's paradise. A careful search of the pertinent literature has revealed only one detailed optical microscope study of epithelial cytology in the isopod vas deferens (52, p. 1-14), and that originated fortuitously during a study by Radu of spermatogenesis in a species of

<u>Armadillidium</u>. According to Radu, the principal feature of interest
in this epithelium was the presence in the cytoplasm of very
numerous small bodies with dimensions between 0.5 and 5.0 microns.
These structures, which stained intensely with iron haematoxylin,
were identified as Golgi elements or "dictyosomes" by their
characteristic response to osmium impregnation techniques. Other
features of interest pointed out by Radu were the very large size
of the cells themselves, which measured as much as ninety microns
from base to apex, and the well developed brush border at the
luminal surface of the deferent canal. In addition, although Radu
scarcely mentioned nuclear structure in his presentation, the
unusually strongly condensed form of the chromatin in the inter-
phasic nuclei is readily apparent from his illustrations (see his
figures 1, 5, 7 and 8). Nichols (34, p. 77-112) had commented
briefly on these same features in her preparations of <u>Oniscus</u>
material but since her interest was in the course of spermato-
genesis in that organism she did not pursue further the study
of the epithelial structure in the vas deferens. After the work
of Radu, no accounts have appeared dealing with the cytology of
the giant cells except the very recent paper by Mathur (29, p. 9-
17) on the structure of the male reproductive tract in <u>Oniscus</u>
<u>asellus</u>. The treatment of the epithelial cell structure in this
study is disappointingly superficial, although such observations
as are included bear out the detailed description provided by the

work of Radu.

The present work was planned to exploit the peculiar advantages afforded by the giant epithelial cells for electron microscopic examination of cytoplasmic fine structure.

MATERIALS AND METHODS

Sow bugs, Porcellio scaber (Latreille), were collected as required during the late fall and winter. The animals were killed by decapitation, which simultaneously removed most of the digestive viscera. The thoracic tergites were then split along the mid-line and the divided plates folded aside to expose the reproductive tract. Removal of the intact reproductive organs was accomplished by dissecting away most of the connective tissue strands anchoring them to the thoracic wall, grasping the ejaculatory duct with fine forceps near the external opening and pulling gently. The testicular follicles were left attached to the vasa deferentia since they served as a convenient marker for identification of the anterior segment of the organs. As a rule, not more than two minutes elapsed from the decapitation of the animal until the reproductive tract was immersed in the fixative solution.

Some of the excised vasa were immediately smeared on microscope slides and examined supravitally with phase contrast optics. The remaining specimens were fixed and embedded preparatory to sectioning for light or electron microscopy.

Preparation of Specimens for Optical Microscopy

Some of the vasa were fixed in osmium tetroxide (36, p. 285-298), embedded in methacrylate (33, p. 183-189) and stained with

Feulgen reagent after sectioning at two microns. The usefulness of osmium tetroxide as a fixative for materials to be stained with the Feulgen reagent has been established (56, p. 472-476); for the present purpose it was used in preference to the more conventional Carnoy's or Farmer's fluids since it was considered desirable to use the same cells for optical and electron microscopical examination, and since osmium tetroxide has proved to be the most satisfactory general purpose fixative for electron microscopy (36, p. 285-298; 49, p. 127-141).

A second group of tissues, stained with Harris's haematoxylin after fixation in osmium tetroxide, embedding in methacrylate and sectioning at two microns, was used to establish the normal histological appearance of the vas deferens.

Still other vasa were fixed in Severinghaus's modification of Champy's fluid (59, p. 1-5) and subsequently post-osmicated for ten days at 35°C to obtain impregnation of the Golgi apparatus. These tissues were embedded either in paraffin or in araldite (54, p. 313-323) and examined both optically and electron microscopically as a check on the identity of the structures identified as Golgi bodies in the electron micrographs. Sections used for optical examination were cut two microns thick and mounted on slides without any further treatment. Several series of osmium impregnated sections obtained from Porcellio laevis (Latreille) were also examined for comparative purposes.

A fourth staining method proposed by Wigglesworth (73, p. 185-199) was tried for the optical examination of comparatively thick sections cut from the blocks prepared for electron microscopy to assist correlation of the electron optical images with those seen by direct optical examination. Generally, tissues fixed in osmium tetroxide and embedded in methacrylate or araldite have proved refractory to the usual histological staining procedures and modification of the standard methods has proved necessary (see, for example, Richardson (54, p. 313-323)); however, Wigglesworth's method of staining with tannic acid proved reliable and quite satisfactory, although the contrast obtained left something to be desired. The disadvantage of low contrast was in part offset by suitable choice of film and developer for photographic recording.

Preparation of Specimens for Electron Microscopy

Tissues to be prepared for electron microscopy were transferred immediately after excision to a drop of fixative on a narrow paper spatula which was immersed forthwith in a larger volume of the fixative solution. As a rule, not more than two vasa were treated in a milliter of fixative. Two principle methods of fixation were employed:

1. Ice cold 2% osmium tetroxide mixture buffered with veronal acetate (36, p. 285-298) to pH 7.9

and containing in addition a trace of calcium

(51, p. 153-156) and 25 mg. per ml. sucrose

(9, p. 827-830). Complete fixation was ensured

by continuing the process for one hour.

2. 3% potassium permanganate (27, p. 709-802), either

buffered to pH 7.9 or unbuffered (32, p. 761-776),

used at room temperature. Fixation in this fluid

was carried on for one to four hours.

After completion of fixation, tissues were transferred

directly to 35% methyl alcohol (1, p. 324-355) at room temperature

and rapidly dehydrated through ascending concentrations of the

alcohol. Some of the preparations were then impregnated with

monomeric methacrylate (33, p. 183-189) containing 1½% Luperco

CDB as cross-linking agent. These impregnated tissues were then

transferred to #00 gelatin capsules filled brimful with partially

polymerized methacrylate and placed in a 60°C oven for forty-eight

hours to complete the polymerization reaction (7, p. 3-14). The

polymerization inhibitor added by the manufacturer was not removed

before use of the monomer (7, p. 3-14; 45, p. 68).

For much of the work an epoxide system based on the resin,

araldite (20, p. 803; 54, p. 313-323), was used since the

methacrylate process was found to introduce an intolerably large

amount of polymerization damage, particularly in permanganate

fixed material. The low contrast associated with materials

embedded in araldite was offset by the use of permanganate as

the fixative or by staining osmium tetroxide fixed tissues with permanganate (26, p. 197-198) or heavy metal solutions (69, p. 475-478; 70, p. 727-730).

Preparation and Examination of Thin Sections

Polymerized blocks were removed from the oven, cooled and immersed in cool water for a period sufficient to soften the gelatin capsules and facilitate their removal. Embedded specimens were trimmed so as to present the region of greatest interest to the microtome knife at the apex of a truncated pyramid having an apical angle greater than 90°. The pyramid was severed from the block and remounted on a 3/8 inch length of hard commercial lucite which provided a rigid support to be grasped in the microtome chuck. Final trimming was then performed under a dissecting microscope so as to leave a block face not greater than 0.1 mm. square, except in cases where large areas were required for survey work, sometimes as much as 2.0 mm. square. The use of such a large block face inevitably was associated with production of inferior sections unsuitable for high resolution work.

Serial sections were cut using the mechanical advance of the Servall microtome (47, p. 685-712) and floated from the knife edge onto the surface of 20% acetone contained in a metal boat attached to the knife (23, p. 520-523). Collection of sections onto carbon filmed 200 mesh copper grids (67, p. 183-184) or onto

unfilmed 500 mesh copper grids was achieved by partial immersion of a grid in the acetone of the bath and adhesion of the floating ribbons to the exposed area with the aid of a very fine cat hair. When the grid was then slowly withdrawn backwards from the fluid, the sections came to lie neatly across the center. Glass knives prepared according to the method of Weiner (72, p. 175-179) and sorted for quality according to Sheldon (60, p. 621-624) or a diamond knife (18, p. 255-256) were used for the sectioning process. The preparation of sections was observed at 30X magnification under a binocular microscope with its axis inclined at thirty degrees to the axis of illumination, so as to allow use of the table of interference colors drawn up by Peachey (44, p. 233-242) for selection of sections of useful thickness. Gold or gray sections were selected according to whether the overall appearance of the tissue or the attainment of very high resolution was the primary concern. In general, no effort was made to obtain sections all of the same thickness as there proved to be a decided advantage to working with alternating thick and thin sections on any one specimen screen. Selected ribbons of sections were gently expanded by exposure to xylene vapor (58, p. 345-348) before collection onto grids in order to relieve the effects of compression resulting from the sectioning process.

Occasional thicker sections ($1\frac{1}{2}$ to $2\frac{1}{2}$ microns) were cut from the blocks used for electron microscopy to permit optical examination of structures observed electron microscopically.

These sections were removed from the fluid surface with a fragment of glass slide in the manner described by Pease (45, p. 137-141), floated onto the surface of warm water and allowed to remain for a period sufficient to eliminate compression artifacts. Finally, the sections were collected into "rafts" and transferred to albuminized slides to which they were affixed by heating overnight at 60°C. Optical examination of osmium tetroxide fixed materials could be performed with phase contrast optics or with standard conditions of illumination after staining with aqueous tannic acid or Feulgen reagent. Araldite embedded tissues proved refractory even to modified staining procedures and were, therefore, examined with the phase contrast microscope.

Thin sections were examined with an RCA EMU 2D electron microscope operating at the normal fifty kilovolt accelerating potential. The microscope used was equipped with a twenty-five micron externally centerable objective aperture located in the back focal plane of the lens, a 125 micron aperture between the objective focal planes, a 125 micron externally centerable condenser aperture and an externally adjustable electrostatic stigmator. Micrographs were taken at initial magnifications between 200X and 15,000X and enlarged photographically up to ten times. For the very low magnification work a special short specimen holder was made which allowed the objective lens to be operated at a much longer focal length than normal. This holder simultaneously reduced the basic magnification of the instrument by a factor

of about five times and produced a material gain in contrast owing to the consequent reduction of the numerical aperture of the lens. There was some loss of resolution in the image, but the loss was not critical at the low final magnifications employed under these conditions.

OBSERVATIONS AND INTERPRETATION

Anatomy of the Vas Deferens

The most striking components of the male reproductive system in Porcellio scaber are the paired vasa deferentia which extend anteriad into the thoracic cavity on either side of the gut. Near its distal extremity each of the vasa bears three elongate testicular follicles which arise from the lateral surface and extend still further into the thorax. At their proximal ends the vasa merge imperceptibly into the long narrow ejaculatory ducts which lead posteriad into the abdomen, finally turning toward the mid-line where they unite shortly before entering the single external reproductive opening. A deep constriction separates the vas itself into a posterior (proximal) and an anterior (distal) segment (figure 1). Most of the anterior segment and the testicular follicles which open into it appear milky white to grayish, whereas the section immediately adjacent to the constriction exhibits an irregular distribution of black pigment which gives this region of the organ a patchy dark gray appearance. The posterior segment from the constriction downward may be charcoal gray to black. The actual color exhibited depends on the extent to which the organ is charged with sperm, with the distended vas showing the lighter color. Tenuous strands of connective tissue envelope the reproductive organs and anchor them to the abdominal and thoracic walls.

Histology of the Vas Deferens

In optical microscope sections the wall of the vas deferens resolves into a unicellular layer of epithelium enclosed in a sheath of myoepithelial cells interspersed with melanophores.

The anterior segment contains two epithelial cell types - a very large cell up to 100 microns high, the appearance of which suggests a secretory function, and a smaller cell, of very variable dimensions but averaging about thirty microns from base to apex, which appears to play a part in the support of the larger cells (compare Radu (52, p. 1-14)). There is no apparent orderliness to the cellular disposition in the epithelial layer - in some places cells of either type may be adjacent while elsewhere the two types may alternate. In general, the large cells are more common in the distal part of the segment near the openings of the testicular follicles, while they are found only rarely near the constriction of the vas deferens. Giant cells in a state of extreme hypertrophy bulge into the lumen of the vas beyond the general epithelial level which is determined by the height of the supporting cells. In places where large and supporting cells abut, the large cells are often distended so that their lateral excursions above the basal level compress the apical portion of the adjacent supporting cells (figures 2 and 3) and give to the latter the appearance of truncated prisms (hence the name "prismatic cells" applied to these cells by Radu). Radu considered the large and supporting cells as stages in

the physiological elaboration of a single cell type with the large
cells representing the extreme of cellular hypertrophy rather than
as two distinct cellular forms. He described several identifiable
stages in a hypertrophic sequence between the extremes of the giant
and prismatic cells as well as a series of supposedly atrophic
cells. Mathur (29, p. 9-17) identifies "giant" and "epithelial"
cells in his material and implies that the two types are distinct.
Regardless of the true interpretation, it is convenient for purposes
of simplicity of description to retain the dual nomenclature and
treat the two cellular forms as different cells.

The luminal surface of the giant cells is uniformly covered
with a dense brush border which may be as much as five microns high
(figure 4). In tannic acid stained sections the cellular cytoplasm
is filled with compact structures (figure 2) which may also be
discerned, though with poor contrast, upon phase microscopic
examination of smeared supravital preparations. These structures
are deeply stained by the standard osmium impregnation techniques
(figure 4) and are, therefore, to be regarded as Golgi bodies or
"dictyosomes". In the extremely hypertrophied giant cells these
Golgi structures usually appear rounded, about four microns in
diameter, and may occasionally be seen to contain a region of less
densely impregnated material which yet has a density greater than
that of the surrounding cytoplasm. In the prismatic cells of the
distal segment linear and sickle-shaped forms of the Golgi elements
predominate. The greatest abundance of Golgi bodies is to be found

in the supra-nuclear region of the cells, although they occur distributed throughout the cytoplasm. The cytoplasm itself of some of the giant cells becomes moderately stained by the osmium impregnation. Large vacuolar spaces which appear empty in the fixed and stained preparations occur in the supra-nuclear region of the giant cells but are less common in the basal area of the cytoplasm (figure 2). These vacuoles may vary in size from near the limit of resolution of the optical system to as much as ten microns. Vacuoles occur only infrequently in the prismatic cells.

The nuclei of the giant cells are very large rounded to ellipsoidal structures which lie about midway between the apical and basal surfaces of the cell (figures 2, 3 and 5). Rounded nuclei may have a diameter as great as forty microns. Within the nucleus are large numbers of heterochromatin granules between one and three microns in diameter. These granules are apparent in phase contrast images of supravitally prepared tissues but are more striking in fixed preparations stained with haematoxylin or Feulgen reagent (figures 3 and 5). Minute clear areas sometimes appear in the heterochromatic masses. Also within the nuclear envelope are a few larger, rounded granules measuring up to five microns. These granules are evidently nucleoli since they stain intensely with haematoxylin but are Feulgen negative with the exception of a narrow peripheral band.

At low optical magnifications the two cellular types in the anterior segment of the vas deferens are readily distinguished

by the disparity in their sizes. Closer examination reveals in the prismatic cells a lower, rather indistinct brush border; a lesser frequency of predominantly elongated Golgi structures which are only weakly stained by osmium impregnation; and a smaller nucleus which is usually rounded but may be highly irregular in outline. The internal nuclear structure provides a further distinction since the Feulgen positive granules of the prismatic cells are considerably less numerous, smaller and more irregular in shape than those of the giant cells (figure 5). As many as six large rounded nucleoli have been found in a single six micron section of a prismatic cell nucleus.

The epithelial cells rest on a basement membrane which, in turn, is enclosed in a sheath of myoepithelial cells. Toward the distal end of the anterior segment the myoepithelial cells are widely extended over the surface of the vas in a unicellular layer. Their nuclei are dispersed and their myofilaments concentrated in the region immediately around and beneath the nuclei so that much of the sheath consists only of the very thin extensions of single cells. Indeed, it is hard to comprehend how a structure as delicate as the anterior segment of the vas deferens can withstand as well as it does the inevitable rough handling associated with the removal of the organ. Closer to the stricture, scattered melano-phores appear in the sheath, which becomes thicker owing to overlap of the myoepithelial cells. In the constriction itself, the wall of the vas deferens consists of a number of circularly oriented

muscle fibres, usually about ten, packed in a single layer against a basement membrane upon which appears a very thin cytoplasmic layer with a sparse brush border. This cytoplasmic layer is derived from extensions of the adjacent cells of the anterior and posterior segments into the region of the constriction which never contains epithelial cell nuclei. Giant cells of the anterior segment are only rarely found in the vicinity of the constriction, and do not in any case contribute to the formation of the epithelial layer lining this area.

Optical examination of the posterior segment reveals an apparently uniform epithelium consisting of cells of a single type (figures 7 and 8). These cells, which differ in minor respects from the prismatic cells found in the anterior segment, are very variable in form. Sometimes they are more or less rectangular in section with a height of about ten microns and a length between twenty and sixty microns; sometimes they approach cuboidal with a height of about twenty microns and a length of twenty-five microns or less. The cuboidal forms are most commonly found in the lower portion of the segment and in the ejaculatory duct. Nuclei of the attenuated cells in the posterior segment are very elongate - as much as forty microns long by five microns thick - while those of the cuboidal cells are comparatively rounded with a diameter of about fifteen microns. Dense Feulgen positive granules, of a size comparable to those of the nuclei in the anterior segment, occupy much of the nuclear volume (figure 6). Rod-shaped and cuneiform

Golgi elements abound in a more or less ubiquitous distribution throughout the cytoplasm; in these cells, as in the prismatic cells, the dictyosomes are more readily demonstrated after tannic acid staining than after the usual osmium impregnation technique. The cellular surface on the luminal side is covered with a very poorly defined brush border. Radu (52, p. 1-14) differentiated the cells in this segment from the prismatic cells of the anterior segment; Mathur, on the other hand, equates the "epithelial cells" of the proximal and distal segments (29, p. 9-17). The present author, while unable to resolve the question of the possible identity of the giant and prismatic cells, feels that the similarities between the latter and the epithelial cells of the posterior segment warrant their treatment as adaptations of a single cell type, herein designated as "structural cells".

The muscular sheath of the posterior segment is considerably thicker than that of the anterior segment by virtue of extensive overlap of the myoepithelial cells comprising the sheath of the former. A nearly uniform, unicellular pigment layer covers the entire segment. Processes of the melanophores in the pigment layer extend between and even beneath the myoepithelial cells.

Ultrastructure of the Epithelium of the Vas Deferens

Nuclei. At very low electron optical magnifications the most striking features of the cells in the epithelial layer are their immense nuclei with the contained heterochromatin masses (figures 9, 10 and 11). At higher magnifications the latter appear to be quite homogeneous and finely granular (figures 12, 13 and 14). The small clear spaces seen within the heterochromatin masses in stained preparations viewed optically (p. 15) appear in electron micrographs as irregular areas unbounded by any membrane (figure 10). Presumably they represent regions within the chromosomal structure which, in life, would have been filled with some material extracted during the preparation of the tissues for microscopic examination.

Nucleoli, when demonstrable, appear in permanganate fixed tissues as regions of low density enclosed within a ring of higher density material (figure 14). Apparently the ribonucleoprotein of the nucleolus, like that of the cytoplasmic ribosomes (27, p. 799-802), is not stabilized by the action of permanganate, whereas the enveloping ring of deoxyribonucleoprotein is well preserved. Nucleoli have not been distinguishable from the heterochromatic masses in osmium fixed materials.

A nuclear membrane is apparent even at low magnifications as a 250 $\overset{\text{o}}{\text{A}}$-thick line circumscribing the nuclear outline. At higher magnifications it can be resolved into a three layered structure composed of two dense lines, each about 70 $\overset{\text{o}}{\text{A}}$ wide,

enclosing a less dense band 100 Å wide (figures 12 and 13). At

intervals the continuity of the nuclear membrane is interrupted;

at these points the outer and inner lamellae of the membrane become

confluent. Similar pore-like structures have been repeatedly

observed in cells from all plant and animal sources studied to

date, but there has been disagreement as to whether they should

properly be regarded as pores. Watson (68, p. 257-270) reported

the appearance of a very thin diaphragm apparently sealing the

mouth of pores in nuclei from a wide variety of tissues. At that

time he suggested that the diaphragms might be transient structures

occurring at a late stage in the formation of pores in a highly

labile nuclear membrane. In a later paper he concluded that the

appearance of a diaphragm was more probably the result of inclusion

of a small portion of the pore wall in one face of the section, the

thickness of which was not much less than the pore diameter (71,

p. 147-156). Barnes and Davis (3, p. 131-146) independently

arrived at the latter conclusion. Both Barnes and Davis and Watson

were of the opinion that the apparent pore structures represented

true perforations of the nuclear surface in the animal cells on

which they worked; Marinos (28, p. 328-333) reached a similar

conclusion from his observations on permanganate fixed plant

tissues. In the present work diaphragms were observed rather

frequently traversing the waist of pores in the membrane of osmium

fixed nuclei; these diaphragms were not, however, apparent in the

pores of permanganate fixed nuclei. This inconsistency is not

compatible with Watson's suggestion that apparent diaphragms in the nuclear pores represent part of the pore wall in the section since, if such were indeed the case, similar appearances would be expected in permanganate and osmium fixed tissues[1]. The conclusion to be drawn in the present case, then, is that true diaphragms do exist, at least across some of the pores in the nuclear membrane of these epithelial cells, but that the material comprising the diaphragm, unlike the remainder of the nuclear surface, is not preserved by permanganate fixation. Different situations may exist, however, in other cell types or even in these same cells under different physiological conditions. The distance between pores in the nuclear membrane of this sow bug material is very variable, with sections of nuclei showing few or no pores around much of their circumference while other areas of the same nucleus reveal pores separated by as little as 0.1 micron. The pore diameter is rather consistent at about 500 Å. At rather frequent intervals suggestions of interconnections between the outer layer of the nuclear membrane and the adjacent profiles of the endoplasmic reticulum can be found (figure 12), although no truly convincing examples have yet been found in this tissue. Such interconnections have been reported by Watson (68, p. 257-270) and have since been mentioned by other

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1. I am indebted to Dr. R. W. Merriam whose remarks during a personal visit to this laboratory in the summer of 1961 inspired the additional work on osmium fixed tissues that revealed this discrepancy.

workers on plant and animal tissues.

With the exception of the number and distribution of the included heterochromatic granules and the size and shape of the nuclei themselves, electron micrographs of nuclei from the two epithelial cell types in the vas deferens show no essential differences.

Brush Borders. The appearance of the brush border provides one of the most reliable distinctions between the different epithelial cell forms. Even at low magnifications a marked difference can be seen between the tall, orderly brush border of the giant cells and the lower, comparatively disorganized border of the structural cells (figures 9, 10 and 11). The border of the giant cells consists of densely packed, straight microvilli between three and six microns high and from fifty to ninety millimicrons in diameter (figures 15 and 16). The microvillar structure comprises an extension of the cytoplasm in the form of a long, untapered, finger-like process which is enclosed in a dense 80 $\overset{o}{A}$ membrane continuous at the base of the structure with the plasma membrane of the cell. No inclusions have been found within the structure of the microvilli in this tissue - an observation that is in accord with the findings of other authors in tissues as diverse as kidney tubules (57, p. 335-336; 65, p. 426-465), intestinal epithelium (66, p. 150-151) and gall bladder epithelium (75, p. 445-458). It should be remarked, however that the observations cited above

were recorded on vertebrate tissues; amongst the invertebrates,
Beams and his collaborators (4, p. 601-619; 6, p. 197-203) and
Meyer (31, p. 18-28) have reported finding inclusions such as fine
filaments, secretion droplets and even mitochondria in the
microvillar structure of epithelial cells from a number of
arthropod sources.

A bulbous dilation of the intervillar space, which normally
has a diameter of about twenty millimicrons, is often apparent at
the base of the brush border (figure 16). Small vesicles, of which
there are a number scattered in the cytoplasm immediately below the
brush border, may sometimes be seen to open into these dilations in
particularly well oriented sections. The vesicles are about eighty
millimicrons in diameter with a dense limiting membrane and a
content that usually has about the same density as the cytoplasmic
matrix. Massive secretion droplets occur distributed amongst the
microvilli of the brush border; however, similar droplets have not
been observed within the cellular cytoplasm.

The border of the structural cell differs from that of the
giant cell chiefly in its shorter, less abundant microvilli which
present a somewhat disorderly appearance at low magnifications
(figures 9 and 11). At higher magnifications it can be seen that
the arrangement of the microvilli is random, with some originating
normal to the epithelial surface while others originate tangentially
(figures 17 and 18). Microvilli may be bent, bifurcated (figure 17),

or even fused above the basal region (figure 18). In the structural

cells, as in the giant cells, the microvilli consist of structure-

less extensions of the cellular cytoplasm enveloped in an 80 $\overset{o}{A}$

membrane. The microvilli are comparatively widely spaced and

dilations of the intervillar space are not found at the base of

the structural cell border. Vesicular spaces open to the

extracellular region at the bases of the microvilli of the prismatic

cells and the cells of the posterior segment.

Mitochondria. The rather sparse mitochondria of the

epithelial cells in the vas deferens are similar in their rod-like

form, comparatively few and often randomly oriented cristae, and a

matrix density only slightly higher than that of the cytoplasm. In

accurately oriented transverse sections they have a diameter of

about 1.5 microns. Each mitochondrion is enclosed in a dense

membrane about 200 $\overset{o}{A}$ thick which resolves at higher magnifications

into a pair of dense lamellae 70 $\overset{o}{A}$ thick enclosing a 60 $\overset{o}{A}$ less

dense band (figures 21 to 23). The inner layer of this limiting

membrane is reflected at intervals into the interior of the

mitochondrion where it forms typical "cristae mitochondriales"

(37, p. 188-211). Both circular and elongate profiles of cristae

may appear in any one section of some mitochondria; cristae in

other of these organelles may show as transversely oriented

elongate profiles similar to the "double membranes" described

by Sjostrand and Hanzon (63, p. 393-413) in the mitochondria

of pancreatic acinar cells. It has not been determined whether the elongate profiles in the mitochondria of this vas deferens epithelium in fact represent longitudinal sections through tubular cristae or sections normal to the surface of flattened discs. Both the elongate and the circular profiles have a width around 200 $\overset{o}{A}$ comprised of 70 $\overset{o}{A}$ dense walls enclosing a 60 $\overset{o}{A}$ less dense band. The contained band is continuous with the space within the laminae of the outer limiting membrane of the mitochondrion. Mitochondria fixed in permanganate and embedded in araldite have a matrix density not greatly different than that of the ground cytoplasm; however, in osmium fixed materials, whether embedded in methacrylate or araldite, the heightened matrix density described by Palade (37, p. 188-211) is exhibited (figures 33 and 37). No doubt the difference is owing to differential extraction of the matrix during fixation in the permanganate solution (27, p. 709-802). The dense granules reported within the mitochondria of some of the cells examined by Palade (37, p. 188-211) are not in evidence in the mitochondria of the epithelial cells in the sow bug vas deferens.

Golgi Structures. Golgi bodies (dictyosomes) occur dispersed around the nuclei of the epithelial cells with some tendency to concentrate in the supra-nuclear region, particularly in the area immediately below the brush border. At low electron optical magnifications the Golgi structures of the giant cells appear as rounded or compact rod-shaped patches of increased

density within the cytoplasm (figures 9, 10 and 31). In the structural cells they appear most commonly as elongate linear or sickle-shaped forms (figures 9 and 11). Viewed at higher magnifications, the Golgi elements of both cell types prove to have a basically similar structure, with the principal difference lying in the number of membrane pairs contributing to the complex (figures 19 and 20, 23 to 29, 32, 34 and 37). In this tissue the dictyosomes can be resolved into stacks or circlets of paired parallel membranes, small vesicles clustered on either side of the membrane systems and, occasionally, vacuoles formed by wide dilation of the spaces within the membrane doublets.

The relatively constant size and shape of the Golgi elements in the hypertrophied giant cells is determined by the arrangement of the membrane doublets which occur in these cells as tightly packed whorls of as many as twenty membrane pairs (figures 19, 20, 23-26 and 32). Each lamellar pair consists of two parallel dense membranes about 70 $\overset{o}{A}$ wide separated by a band of less dense material of variable width. At exposed ends where the two membranes of a doublet are confluent the space may be dilated to as much as 1,000 $\overset{o}{A}$ (figure 25) whereas in the central region of the mass, where the doublets are particularly closely packed, the interspace may be 100 $\overset{o}{A}$ or less. Connections between the membranes of adjacent doublets have not been found in any of the Golgi structures examined.

Golgi vacuoles consisting of wide dilations of the region within a doublet (13, p. 79-84) are very seldom found in either the rounded dictyosomes of the giant cells or the more extended structures characteristic of the prismatic cells and the cells of the posterior segment. In this respect, the Golgi elements in the epithelial cells of the sow bug vas deferens resemble the structures identified by Beams and his collaborators as "acroblasts" in the developing germ cells of the cricket (5, p. 123-130). Large vacuoles occur, however, in the cytoplasm adjacent to the elongate dictyosomes of the prismatic cells. The latter vacuoles may contain a dense granule, usually only one to a vacuole, of very variable dimensions (figures 27 and 29). Similar granules, presumably arising as a result of Golgi activity, have been interpreted by Farquhar and Wellings as stages in the formation of a cellular secretion (16, p. 319-322). Present data are too limited to permit any attempt at such interpretation in this material. It is, however, singular, if these authors's interpretation should prove correct, that this evidence of activity is found only in connection with the less hypertrophied Golgi structures of the prismatic cells rather than in the giant cells which present many of the appearances characteristic of active secretion.

A cloud of small vesicles surrounds the compact membranous elements of the Golgi structures. The micrographs suggest two possible modes of formation for these vesicles - first, by

successive fragmentation of the lamellae exposed at the surfaces of the stacks (figures 23 and 25) and second, by a "budding" process from the dilated ends of the lamellar pairs (figure 32). It is, of course, difficult to verify dynamic interpretations of this nature from the static evidence furnished by the fixed and stained materials available for electron microscopy; however, examination of a very large number of Golgi structures in this study has provided no information incompatible with such modes of vesicle formation. The vesicles, which range in size from about 200 $\overset{o}{A}$ to as much as 500 $\overset{o}{A}$, consist of a 50 $\overset{o}{A}$ dense limiting membrane enclosing a mass of material of density comparable to that of the cytoplasm. A few vesicles may show a somewhat greater density (figure 32) but this appearance probably results from inclusion of entire vesicles in a single section; in this case, the overlying vesicular membrane would contribute to the density of the contents.

Minute vesicles and granules from 40 $\overset{o}{A}$ to 100 $\overset{o}{A}$ in diameter appear in association with some of the Golgi structures in permanganate fixed tissues only (figures 23, 25 and 32). Their topographical relationship to the dictyosomes indicates that they are probably not homologous to the larger vesicles. Furthermore, they have been found on occasion in situations distant from the dictyosomes (figures 20 and 36). Similar minute vesicles have been reported in permanganate fixed mammalian testicular cells but not in tissues from the same source after fixation in osmium tetroxide (32, p. 761-776).

Study of the literature shows universal agreement that there is no demonstrable association of Palade granules, or ribosomes, (38, p. 59-68) with the membranes of the Golgi elements (5, p. 123-130; 13, p. 79-84; 16, p. 319-322), even after osmium tetroxide fixation; similarly, in the present study the Golgi region has proved to be free of ribosomes (figures 27, 29, 33 and 34).

The rounded Golgi structures which predominate in the giant cells have a central region which in parasagittal sections is free of lamellae. This central region is packed with vacuolar spaces, Golgi vesicles and, sometimes, the above mentioned minute vesicles and granules (figures 23, 25 and 26). In sections passing more peripherally through the structure the outer membrane doublets appear sharply defined while the entire central region is occupied by dense material showing progressively less evidence of lamellation as the center of the structure is approached (figures 23 and 25). This appearance can be reconciled with the parasagittal sections if it is remembered that obliquely sectioned membranes will tend to lose their sharpness. Sections passing through the extreme peripheral areas of the dictyosomes show only aggregations of vesicles not enclosed in a limiting membrane and without any associated membrane lamellae (figure 30). When two or more dictyosomes lie very close together there is often continuity of their outermost membrane doublets at the point of contact (figure 25), or in some cases multiple Golgi centers may be enclosed in a few whorls of membranes (figure 23).

Whorled structures similar to the rounded dictyosomes described above have been reported from a number of sources. Haguenau (21, p. 425-483) discusses an onion-like structure which she identifies with the "ergastoplasmic nebenkern" in glandular cells such as those of the pancreatic acini. These nebenkerns differ from the Golgi elements in the sow bug vas deferens in that the former often have ribosomal particles associated with their comparatively loosely packed membranes. Vesicles and vacuoles do not occur in numbers around the nebenkern. Palay and Palade (43, p. 69-88) described whorled structures in sympathetic neurons which they suggested might prove to be identifiable with the Golgi elements of other cells. The membranes of these whorled structures lack granules. Beams et al. (5, p. 123-130) found that at certain stages in the development of the acrosome from the fused dictyosomes of the grasshopper spermatocyte the acroblast assumes a spherical structure composed of whorls of agranular membranes. These observations lend support to the identification of the rounded structures as Golgi elements in the present work.

The elongate Golgi structures found in the prismatic cells and in the cells of the proximal segment differ in only minor respects from the rounded structures in the giant cells. The principal differences lie in the lesser number of membrane doublets and in their being extended structures rather than curled around to a horseshoe or circlet (figures 27 to 29,

34 and 37). There are rarely more than ten membrane pairs in these extended organelles. The associated vesicles occur distributed along the sides of the membrane stacks and in clusters at the free ends of the lamellae.

The distribution of osmium deposits in the dictyosomes of heavily impregnated cells from various sources has been investigated electron microscopically by Dalton and Felix in a series of papers (11, p. 277-306; 12, p. 171-208; 13, p. 79-84). According to these authors, either vacuoles, vesicles or the space between membrane doublets may be the site of maximum deposition of osmium and its lower oxides; the site, however, is fairly specific for any particular cell type. In the giant cells of the isopod vas deferens, the "osmiophile substance" is apparently identified with the interlamellar spaces of the dictyosomes; in the structural cells with the vesicular component.

Endoplasmic Reticulum. Perhaps the most striking variation in cellular fine structure is that exhibited by the elements of the endoplasmic reticulum. These are made up of the widely reported tubular and cisternal structures first observed by Porter and his associates (48, p. 233-246) in micrographs of the thin region of tissue cultured cells examined entire. The endoplasmic reticulum has subsequently been described in great detail in sectioned material from many sources (21, p. 425-483; 40, p. 567-582; 41, p. 85-98; 42, p. 641-656; 46, p. 175-228; 50, p. 167-180;

61, p. 301-318). Fundamentally, this cellular component is a system
of interconnected tubules and/or flattened sacs ("cisternae") which
may or may not be associated with ribosomal particles, according to
the cell type under consideration and its physiological state at
the time of fixation. In the present material the degree of
development of the system in both cell types is startling, with
the entire cytoplasm being permeated with profiles. At low electron
optical magnifications the extreme cisternal dilation found in the
giant cells gives their cytoplasm a frothy appearance (figure 9)
not unlike the alveolar structure visualized by Bütschli (8, p. 14-
20) for the essential cytoplasmic structure; in many cases, dilation
of the cisternae is such that there is little cytoplasm remaining
between the walls of adjacent profiles (figures 19, 20, 30 and 31).
It seems probable that the rather general cytoplasmic staining
observed in some giant cells after osmium impregnation (see p. 15)
may be the result of uptake of osmium by the cisternal contents.
The prismatic cells and the cells of the proximal segment are
likewise well permeated with reticular elements, but the cisternal
dilation is much less, so that considerable amounts of cytoplasmic
matrix appear between cisternal limiting membranes (figures 12, 17
and 35). Tubular profiles - a rarity in the giant cells - are
common in the prismatic cells and the cells of the posterior
segment of the vas deferens.

In limited areas of the basal regions of the giant cells
there is some tendency toward orientation of the profiles of the

endoplasmic reticulum into arrays of parallel flattened cisternal
sacs (figure 9) similar to those described in actively secreting
cells of the pancreatic acini (41, p. 85-98; 63, p. 393-414),
liver (41, p. 85-98) and thyroid gland (15, p. 171-173). In
osmium fixed tissues Palade granules, or "ribosomes", (38,
p. 59-68) occur distributed along the membranes of the reticulum
in both the giant and the structural cells (figures 18, 27, 29,
33 and 34). These granules appear as very dense particles with
a diameter between 100 $\overset{o}{A}$ and 200 $\overset{o}{A}$. In sections tangential to
the surface of the reticulum elements the granules may sometimes
be seen to be arranged in rosettes (figures 18 and 33).

Apparent continuities of the endoplasmic reticulum with
the outer layer of the nuclear membrane can readily be found
(figure 12), although a really convincing demonstration of such
a connection has yet to be shown in this tissue; actual connections
with the Golgi lamellae, such as have been reported by Palay and
Palade (43, p. 69-88), have not been found. However, a very close
association of the membranes of adjacent cisternae with the outer
lamellae of the Golgi elements was often apparent in giant cells,
sometimes to the extent that the cisternal membranes appeared to
form an integral part of the membrane stack (figures 24, 25 and
32). The vacuolar spaces filling the concavity of the cup-shaped
Golgi structures were in a few cases found to be in continuity
with adjacent cisternae (figure 32). In view of this observation
and the marked similarity in appearance of the cisternal spaces

and the "vacuolar" spaces within the Golgi elements it seems highly probable that the latter are, in fact, merely cisternae. It may be speculatively proposed that cisternae closely associated with an initially straight stack of Golgi membranes become included within the Golgi structure when rounding occurs. Connections of the reticulum with the cellular surface have not been demonstrated in spite of a rather careful search prompted by claims of such relations by Palade (39, p. 445). If, in fact, such connections do exist, this material should prove ideal for further investigation of the situation, since the cytoplasmic structure of the giant cells is not complicated by the presence of numerous small circular profiles whose interpretation is always doubtful. The close apposition of the cisternal membranes to the cellular surface should make detection of connections comparatively easy.

Intercellular Boundaries. The surfaces of adjacent cells are closely applied with only about 200 Å of separation between them (figures 35 and 37). Attachment specializations of the type described in a wide variety of epithelial tissues (22, p. 575-578; 24, p. 348-362; 35, p. 529-538; 53, p. 328-329; 74, p. 445-458) have not been found in this epithelium. Occasional vesicular dilations of the intercellular space are evident (figure 35), usually in the area immediately below the brush border, but have not been found associated with any cytoplasmic specializations such as the filaments demonstrated in connection with terminal

bars and desmosomes (22, p. 575-578; 24, p. 348-362). Near the basal region of the intercellular boundary much wider dilations of the intermembranous space are sometimes demonstrable (figure 36). These areas contain a system of interconnected tubular and dilated components embedded in a matrix continuous with the intercellular medium and of similar density. These aggregations are not bounded by any membrane other than those of the adjacent cells. Similar unenclosed agglomerations of tubular and dilated structures occur between the basal cell surface and the basement membrane. Although insufficient numbers of these structures have been studied to permit positive interpretation, it seems probable that they are composed of finger-like extensions of the adjacent cells into the intercellular or subcellular space. Direct evidence in the form of unmistakeable connections of the cellular surface with the profiles is lacking in the micrographs. If the interpretation is correct, these areas may represent regions of the cellular surface specialized for cell attachment. Further stability is provided for the epithelium by extensive infolding of the adjacent cellular surfaces (figure 35).

Occasionally, clusters of small vesicles 60 Å to 150 Å in diameter can be found in the cytoplasm between cisternae of giant cells fixed in permanganate. These clusters are usually enclosed in a tenuous membrane (figures 19, 20, 25, 30 and 35), although some of the large structures are incompletely enclosed by the membrane (figures 19 and 35). Similar "multivesicular bodies" have been demonstrated in spermatogenic cells of rat testis in

which they can be shown in great numbers after fixation in permanganate (32, p. 761-776). Like Mollenhauer and Zebrun, this writer cannot ascribe a role in cellular physiology to these structures.

GENERAL COMMENTS

Relationship and Function of Epithelial Cell Types

In the light of the optical and electron optical observations, this author agrees with Mathur (29, p. 9-17) that the epithelial cells in the proximal segment of the sow bug vas deferens are identical with the prismatic cells of the distal segment. The differences between these cells are primarily a matter of shape, which can be attributed to purely physical causes; furthermore, there is often more difference in appearance between two prismatic cells, for example, than between the prismatic cells and the cells of the posterior segment. The similarities in nuclear and cytoplasmic details are particularly evident in electron micrographs. The question of the identity of the giant cells with the structural cells is less satisfactorily resolved and must be left unsettled pending further investigation by other means; it is, however, the opinion of this author that the giant cells differ sufficiently from the other epithelial cells that a separate classification is tentatively justifiable.

The abundant Golgi structures, the hypertrophied nuclei, the conspicuous brush border and the highly elaborated endoplasmic reticulum of the giant cells all bespeak cells engaged in an active metabolic process, presumably one of secretion. Mathur (loc. sit.) found that the cytoplasm of the giant cells was positive to the

periodic acid Schiff (P.A.S.) reagent, as were also the droplets found amongst the microvilli of the brush border and in the lumen of the deferent canal. The sperm themselves were bound in packets by a sheath of similarly P.A.S. positive material. On the basis of these findings, he suggested that the function of the giant cells is elaboration of a mocopolysaccharide cementing material. In the present study, droplets presumed to be secretions were found in abundance in the lumen of the vas deferens; however, intracellular secretion droplets were only very rarely found, and then more frequently in the prismatic cells than in the giant cells. In view of these observations, it is quite evident that the present study can add nothing to understanding of the secretion processes in these cells; further work of a cytochemical nature is required on this point.

The possibility of seasonal differences in the structure of the epithelium of the vas deferens has not been examined, as all the specimens were prepared at the same time. Seasonal variation might be invoked to account for the discrepancies between the inter-pretations of Mathur (29, p. 9-17) and Radu (52, p. 1-14) and the data presented in the present work. The possibility of generic differences also exists.

Interrelationship of Cellular Components

Of particular interest in this study is the demonstration of the intimacy of the morphological interrelationships between different cellular constituents. In this connection may be cited the apparent direct connection of the cisternae of the endoplasmic reticulum with the perinuclear space. The interrelationship of these two membranous components of the cell has been further emphasized in recent years by the demonstration of the manner of the nuclear membrane's reformation following mitosis, this occurring through accumulation and coalescence of large flattened sacs presumably derived from the cisternae of the endoplasmic reticulum (2, p. 179-182; 10, p. 902-904; 30, p. 607; 50, p. 167-180). There is also the very close association demonstrated between the Golgi elements and the reticulum, and between the cell surface and the reticulum. The fact that actual direct connections of the reticulum to cell components other than the nuclear membrane have not been found in the micrographs available does not necessarily belie their existence. Since, owing to the rather wide scope of this study, it was not possible to examine any one aspect of cellular structure in great detail, fine interconnections of this nature might well have been overlooked. The close morphological association of the endoplasmic reticulum with the membrane stacks of the Golgi structures is certainly suggestive of functional interaction between these cellular constituents; it is even tempting to

suggest that the increase in size of the lamellar stacks may occur by appressing of flattened cisternae to their surfaces or, conversely, that the Golgi bodies may serve as "reservoirs" from which new endoplasmic reticulum may be derived.

Regardless of whether or not direct connections between the cytoplasmic organelles and the endoplasmic reticulum are finally demonstrated - and it is the author's intention to pursue the question further in this material at a later date - it is probable, in view of the very close association of the endoplasmic reticulum with the different inclusions, that the cell should be regarded as a complexly interrelated system of organelles specialized in local areas for particular metabolic roles. This point of view has been expressed particularly well by the workers at the Rockefeller Laboratories in New York (41, p. 85-98; 46, p. 175-228) who apparently feel that all the membranous structures within the cell may be regarded as regional specializations of an ubiquitous and all-important endoplasmic reticulum.

Reliability of Structural Detail

Much has been written concerning the validity of descriptions of tissue structure that are based on examination of the fixed and stained materials available for close scrutiny by light and electron microscopy. In the case of data derived from electron microscopy, the situation is further complicated by the fact that

materials suitable for this type of work cannot be examined with equivalent resolution, either in the fixed or living state, by any other means. For the present purpose it should suffice to observe that a wide variety of cells show similar structures in electron micrographs whether fixed by formalin (1, p. 342-355), osmium tetroxide (36, p. 285-298), permanganate (27, p. 709-802), or prepared physically by freeze-drying (62, p. 239-246). This uniformity of cellular structure augurs well for the assumption, tacit in all electron microscopy, that the biochemical constituents of the living cell are arranged in an orderly fashion predisposing them to precipitate in a certain way, and that the manner of their precipitation is more specific for the basic arrangement than for the action of any particular fixation method. The way in which these constituents are precipitated then gives rise to the amazingly detailed images seen in electron micrographs of "well preserved" tissues. Preservation of the basic pattern appears to be comparatively independent of the post-fixation treatment except as obvious mechanical damage is caused by such variables as shrinkage or "explosion damage" (1, p. 342-355; 7, p. 3-14). Further and more direct evidence for the validity of electron microscopic images comes from comparison of phase contrast (17, p. 135-142; 55, p. 423-430) and polarization microscope (14, p. 324-325; 25, p. 596-599) images of living cells with low magnification electron micrographs of similar cells. At a higher resolution level, x-ray diffraction studies of supra-vital

preparations of myelin sheath yield spacings in the diffraction
image that correspond to those obtained after fixation of the
material in osmium tetroxide or permanganate (19, p. 725-747)
if due allowance is made for the volume changes known to occur
during these treatments (1, p. 342-355). These spacings are also
very similar to the measured density distributions in electron
micrographs of the same material after fixation and embedding in
methacrylate (19, p. 725-747). It is, then, not unreasonable to
conclude that images of density distributions seen in electron
micrographs are realistic representations of the distributions
within the living cell of some of its major biochemical
constituents.

SUMMARY

The structure of the epithelium in the vas deferens of the sow bug, (Porcellio scaber Latreille), has been investigated using standard histological and electron microscopical methods.

On the basis of anatomy and histology, the vas deferens is divided into an anterior and a posterior segment. The anterior segment is remarkable for the giant cells which constitute a large part of its epithelial surface, while the posterior segment has a uniform epithelium comprised of "structural" cells having a less hypertrophied appearance. Small prismatic cells are also distributed among the giant cells of the anterior segment, and these are apparently identical with the cells characterizing the posterior segment.

The epithelial cells are remarkable for the abundance of their inclusions. The giant cells, in particular, are notable for the extraordinary development of the Golgi elements and endoplasmic reticulum, as well as for their large, polyploid nuclei. The fine structure of these cellular structures and of the mitochondria, brush border and intercellular boundaries is discussed in some detail and the differences between the giant and structural cells in respect to the appearance of their organelles is considered in the light of the electron microscopic observations. The most striking differences are to be found in the Golgi elements, which in the giant cells are most commonly rounded and contain up to twenty

membrane pairs compared to the three to ten pairs of the corresponding structural cell organelles. The cisternae of the endoplasmic reticulum almost completely fill the cytoplasm of the giant cells whereas those of the structural cells more closely conform to the classical picture of endoplasmic reticulum. A curious structure containing numbers of wide and narrow tubules is present between the surfaces of adjacent cells; similar tubular aggregations are found between the basal surface of the cell and the basement membrane. It is suggested that these agglomerations are formed by interlocking evaginations from the adjacent cellular surfaces, and that they play a part in intercellular attachment.

The homological relationships of the epithelial cell types are discussed, and the functions of these apparently metabolically highly active cells in the vas deferens are considered. It is pointed out that the present purely morphological data permit only inferences in relation to cellular function. Particular emphasis is laid on the importance of recognizing the non-autonomous nature of the different cellular organelles as indicated by their very intimate association with the endoplasmic reticulum; the probable vital importance of the endoplasmic reticulum itself in intracellular coordination is recognized.

Finally, some remarks have been directed toward the validation of data obtained from the fixed and stained materials used in electron microscopy; it was pointed out that information derived from other microscopic methods has served to increase

confidence in the realistic nature of the electron microscopic

descriptions of tissue structure.

PLATE I

Figure 1. Outline drawing of the left vas deferens and testicular
follicles of <u>Porcellio</u> <u>scaber</u> (Latreille).
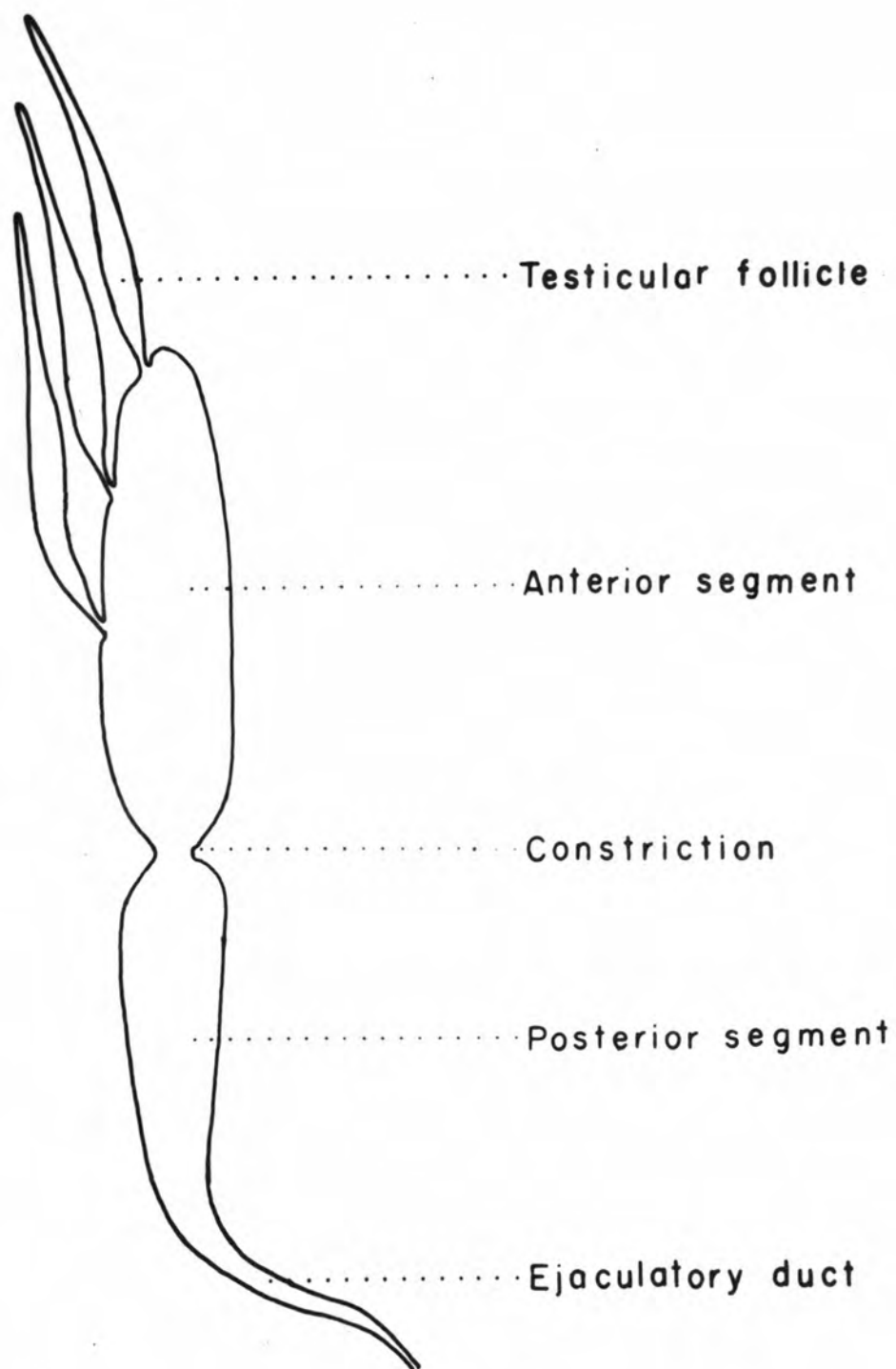
Dorsal view

x 30

PLATE I

Figure I

PLATE II

Figure 2.   Giant and prismatic cells in a typical photomicrograph of
            the anterior segment of the vas deferens. The two cell
            types are readily distinguishable by the disparity in their
            sizes and by the different appearance of their
            dictyosomes.

            Osmium tetroxide fixation; methacrylate embedding.
            Tannic acid stain.
            Sectioned two microns thick.

                                X 700

Figure 3.   A region of the anterior segment similar to that
            illustrated in figure 2, but prepared by standard
            histological techniques.

            Osmium tetroxide fixation; methacrylate embedding.
            Harris's haematoxylin stain.
            Sectioned two microns thick.

                                X 700

ABBREVIATIONS

b . . . . brush border
chr . . . . heterochromatic granules
d . . . . dictyosome
M . . . . myoepithelial sheath
NG . . . . giant cell nucleus
NP . . . . prismatic cell nucleus
V . . . . vacuole
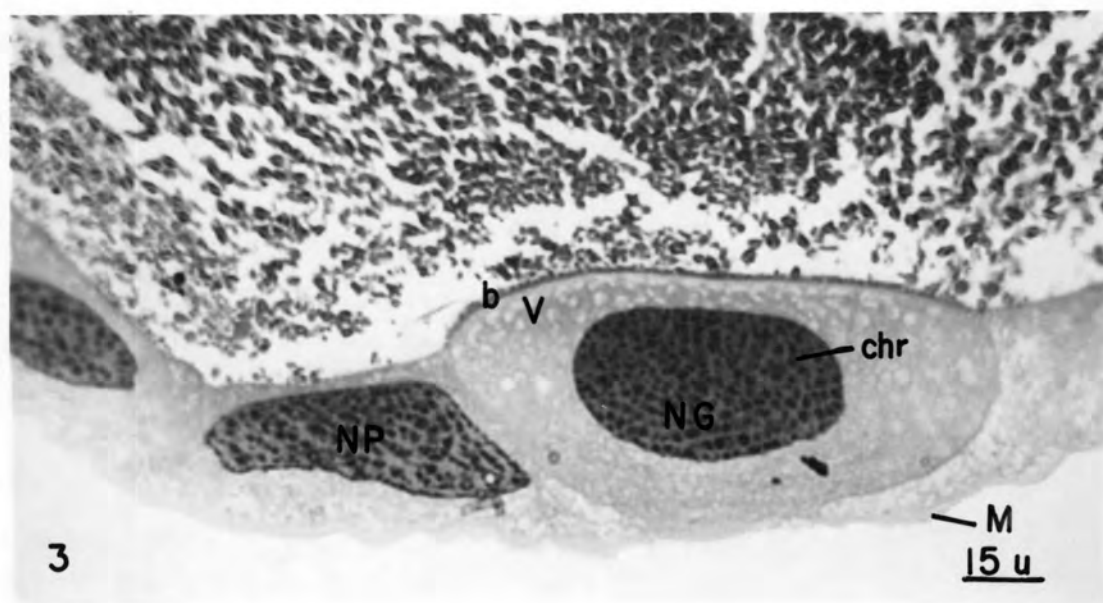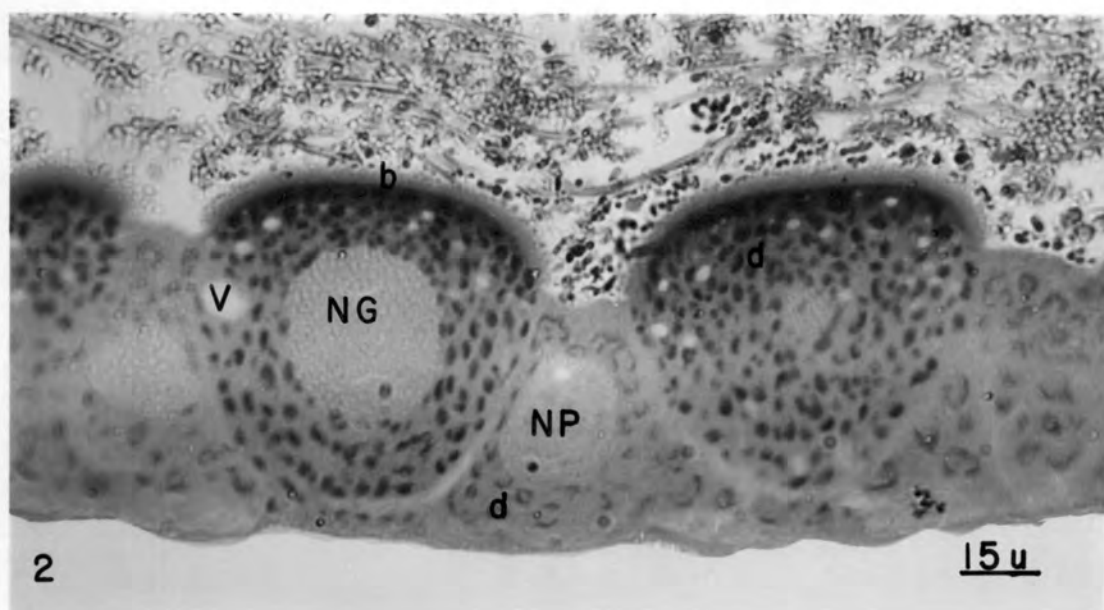
PLATE II

2   15 u

3   15 u

PLATE III

Figure 4. Giant cells heavily impregnated with osmium tetroxide. Most of the dictyosomes in this micrograph exhibit the rounded appearance characteristic of these organelles in the giant cells.

Severinghaus's fixation; impregnated with 2% osmium tetroxide for ten days at 35°C.
Sectioned five microns thick.

X 700

ABBREVIATIONS

b . . . . brush border
d . . . . dictyosome
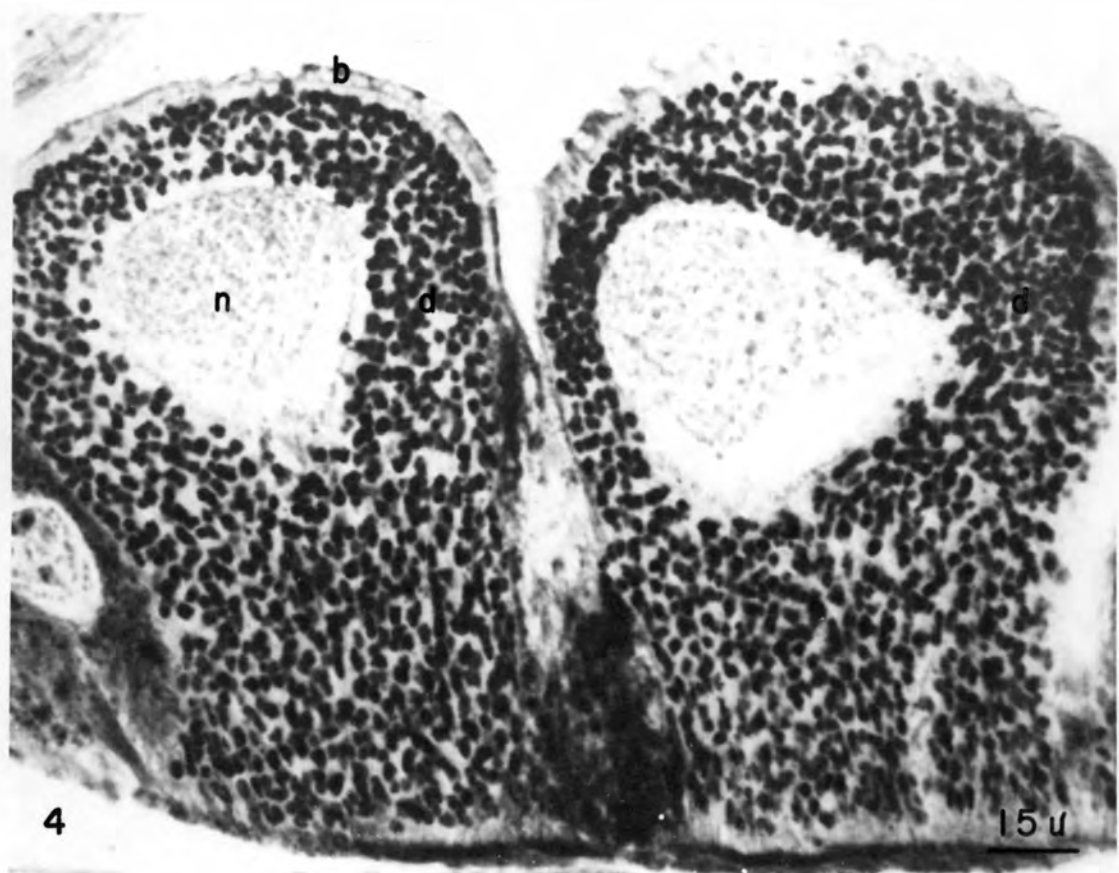n . . . . nucleus

PLATE III

PLATE IV

Figure 5. Giant and prismatic cell nuclei in the anterior segment
of the vas deferens.

Osmium tetroxide fixation; methacrylate embedding.
Feulgen stain.
Sectioned two microns thick.

X 700

Figure 6. Nuclei in the epithelial cells of the posterior segment
of the vas for comparison of the nuclear appearance of
these cells with that of the cells in the anterior
segment.

Osmium tetroxide fixation; methacrylate embedding.
Feulgen stain.
Sectioned two microns thick.

X 700

ABBREVIATIONS

chr . . . . heterochromatin granules
NG . . . . giant cell nucleus
NP . . . . prismatic cell nucleus
nuc . . . . nucleolus
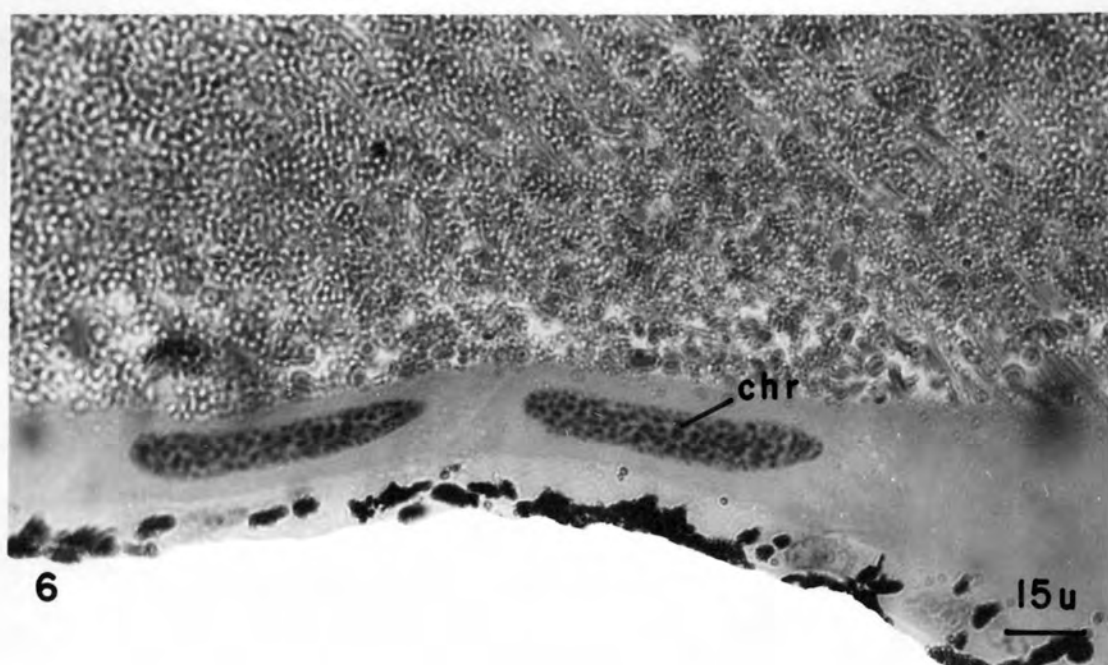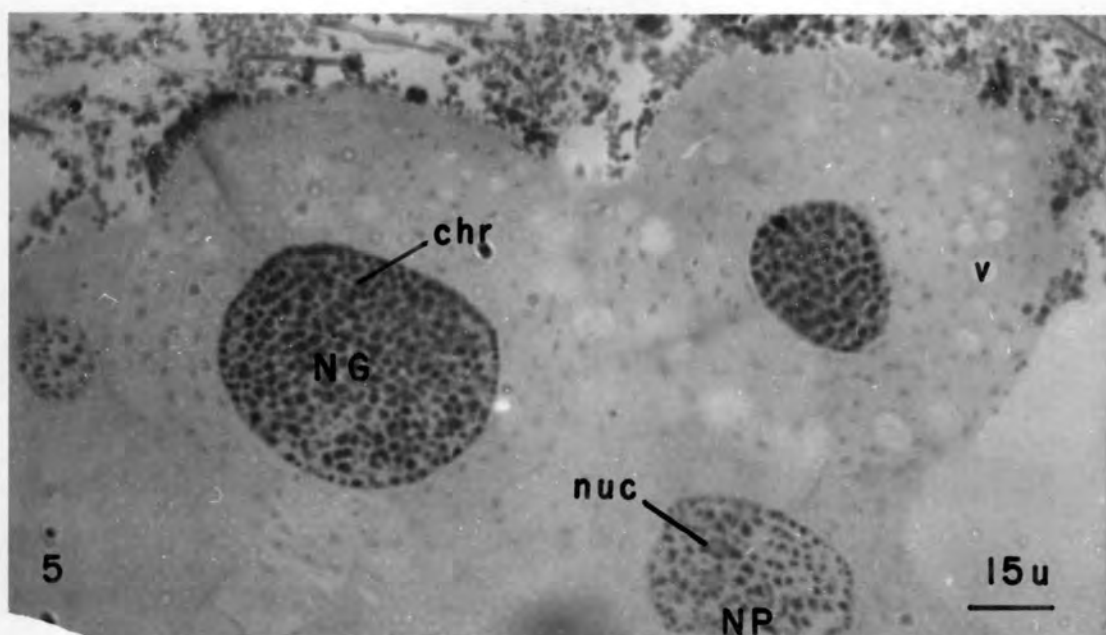V . . . . vacuole

PLATE IV

PLATE V

Figure 7.   Routine histological preparation of part of the
posterior segment wall illustrating the distribution
of basophilic granules in the nuclei of the epithelial
cells.

Osmium tetroxide fixation; methacrylate embedding.
Harris's haematoxylin stain.
Sectioned two microns thick.

X 700

Figure 8.   Tannic acid stained preparation of cells in the posterior
segment. The thick myoepithelial sheath of this segment
is shown.

Osmium tetroxide fixation; tannic acid stain.
Sectioned two microns thick.

X 700

ABBREVIATIONS

chr . . . . heterochromatic granules
d . . . . dictyosome
M . . . . myoepithelial sheath
n . . . . nucleus

PLATE V

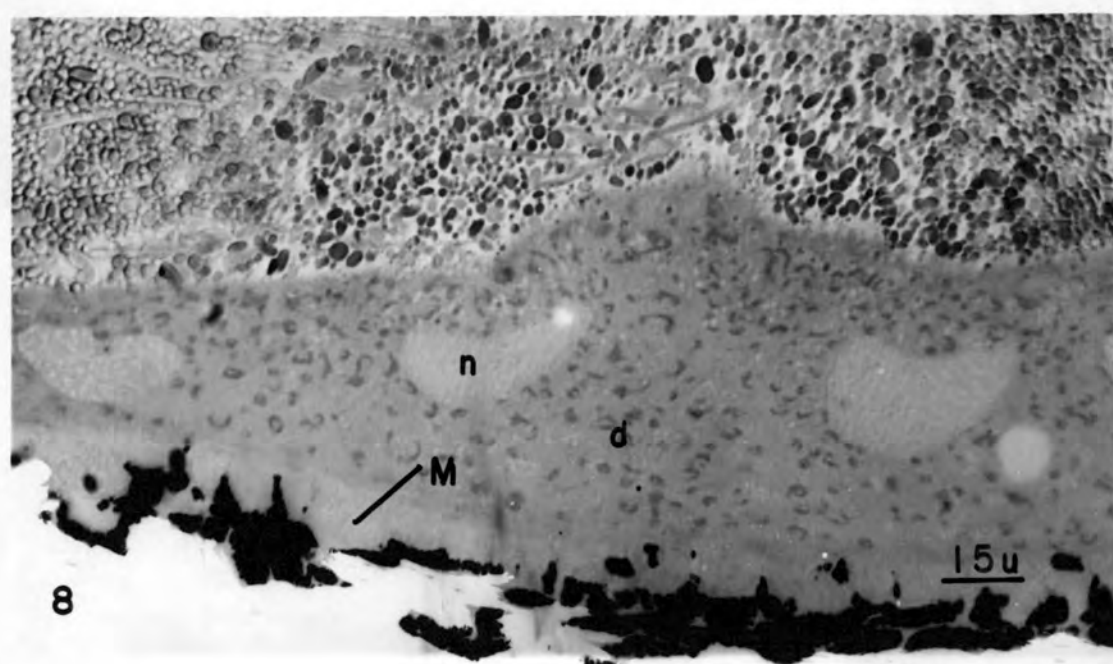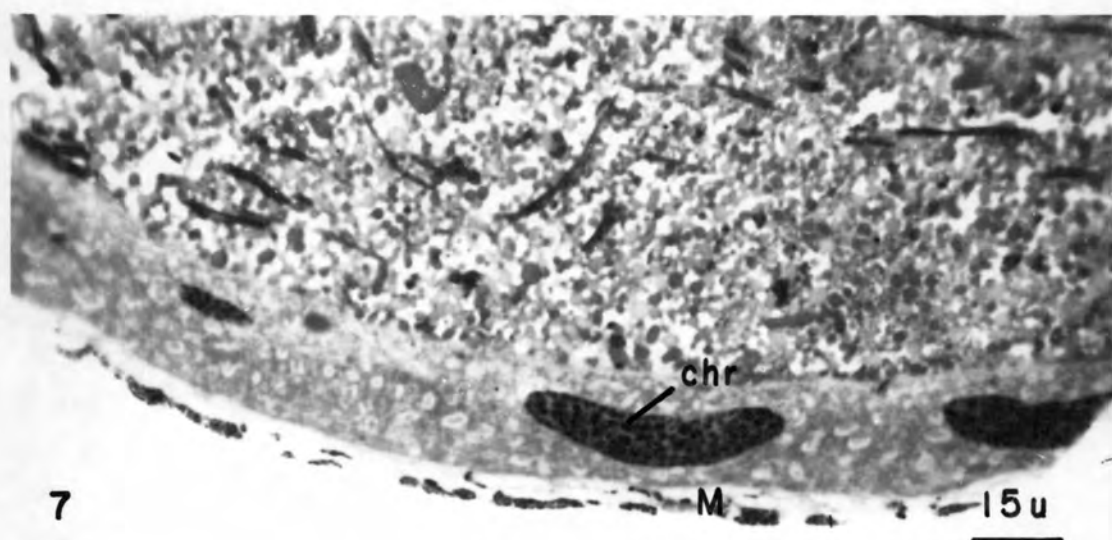

7

chr

M

15 u

8

n

d

M

15 u

PLATE VI

Figure 9. Low power electron micrograph of giant and prismatic
cells in the anterior segment of the vas deferens. The
two cell forms are readily distinguished by the disparity
in their sizes and by the different character of their
dictyosomes and brush borders. The prismatic cell in
this micrograph shows the characteristic shape of a
truncated pyramid. The arrows draw attention to a region
where the endoplasmic reticulum is ordered in parallel
arrays of flattened cisternae.

Osmium tetroxide fixation; methacrylate embedding.

X 2,000

ABBREVIATIONS

d	dictyosome
er	profiles of the endoplasmic reticulum
G	giant cell
M	myoepithelial sheath
mv	microvilli of brush border
NG	giant cell nucleus
NP	prismatic cell nucleus
nuc	nucleolus
P	prismatic cell
V	vacuole

PLATE VI

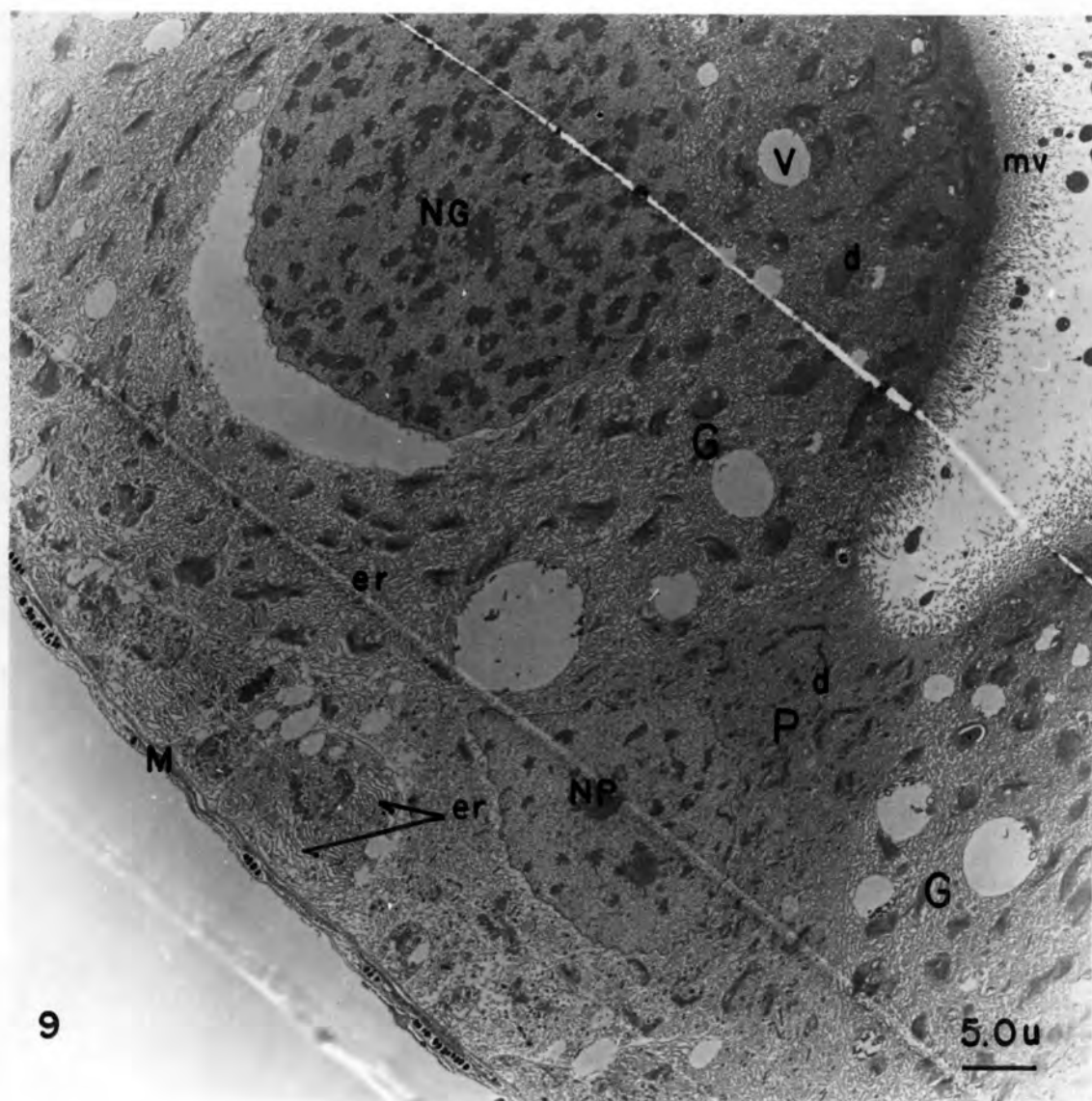9   5.0u

PLATE VII

Figure 10. A portion of a giant cell. The rounded dictyosomes, the high, dense, well ordered brush border, the heterochromatic masses and the all-pervading cisternae of the endoplasmic reticulum are well illustrated in this electron micrograph.

Osmium tetroxide fixation; methacrylate embedding.

X 5,000

ABBREVIATIONS

chr . . . . heterochromatic mass
cm . . . . cell membrane
d . . . . dictyosome
er . . . . endoplasmic reticulum
mv . . . . microvilli of brush border
nm . . . . nuclear membrane
nv . . . . space in chromosomal structure
s . . . . secretion droplet
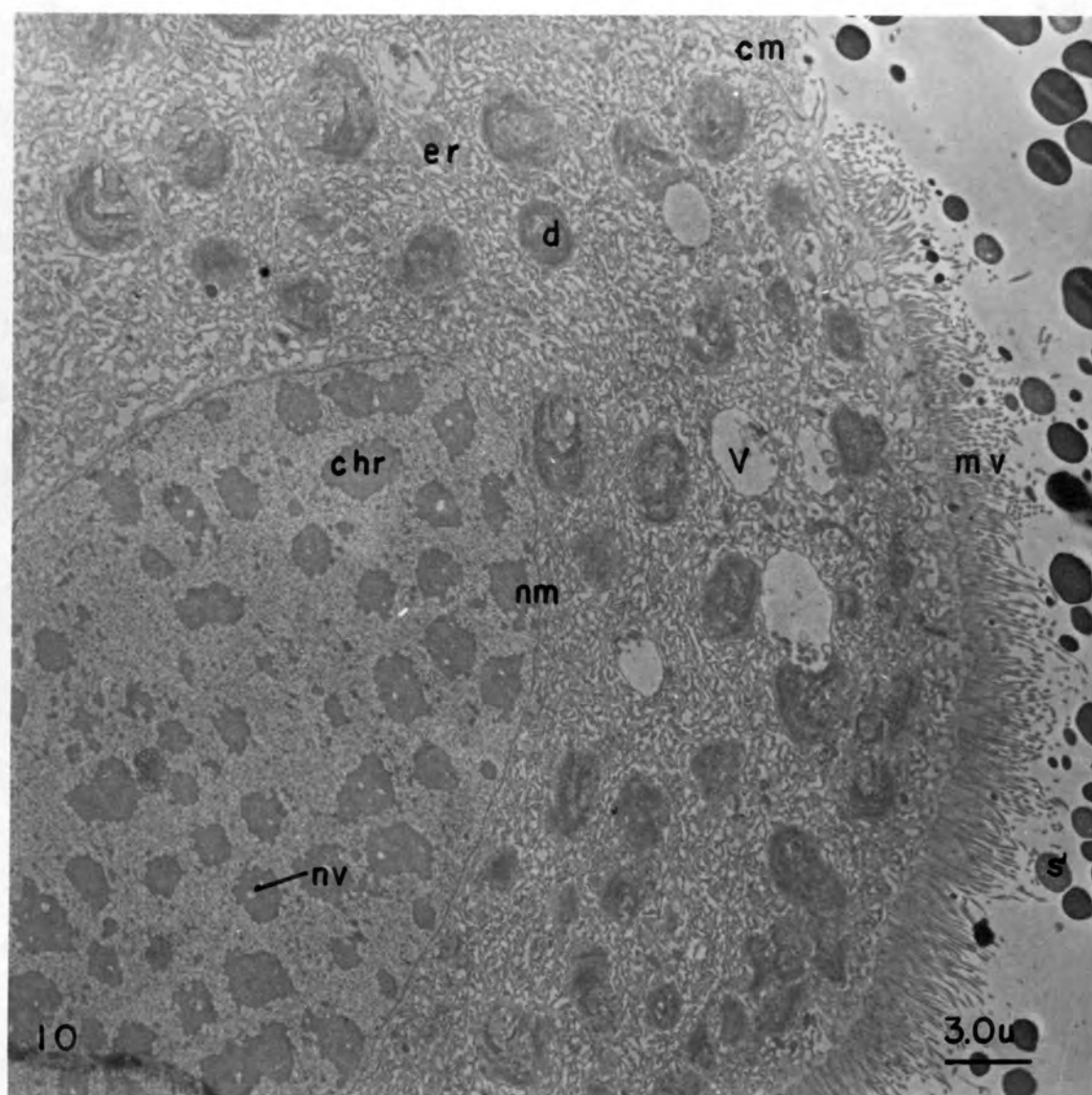V . . . . vacuole

PLATE VII

PLATE VIII

Figure 11. A cell of the posterior segment of the vas deferens.
The poorly organized, sparse brush border, the plentiful
vacuoles, elongate dictyosomes and nucleus are clearly
shown. The arrow draws attention to a region where the
endoplasmic reticulum is organized into parallel arrays
of cristae.

Osmium tetroxide fixation; methacrylate embedding.

X 4,500

ABBREVIATIONS

b . . . . basement membrane
d . . . . dictyosome
er . . . . endoplasmic reticulum
M . . . . myoepithelial sheath
mv . . . . microvilli
N . . . . epithelial cell nucleus
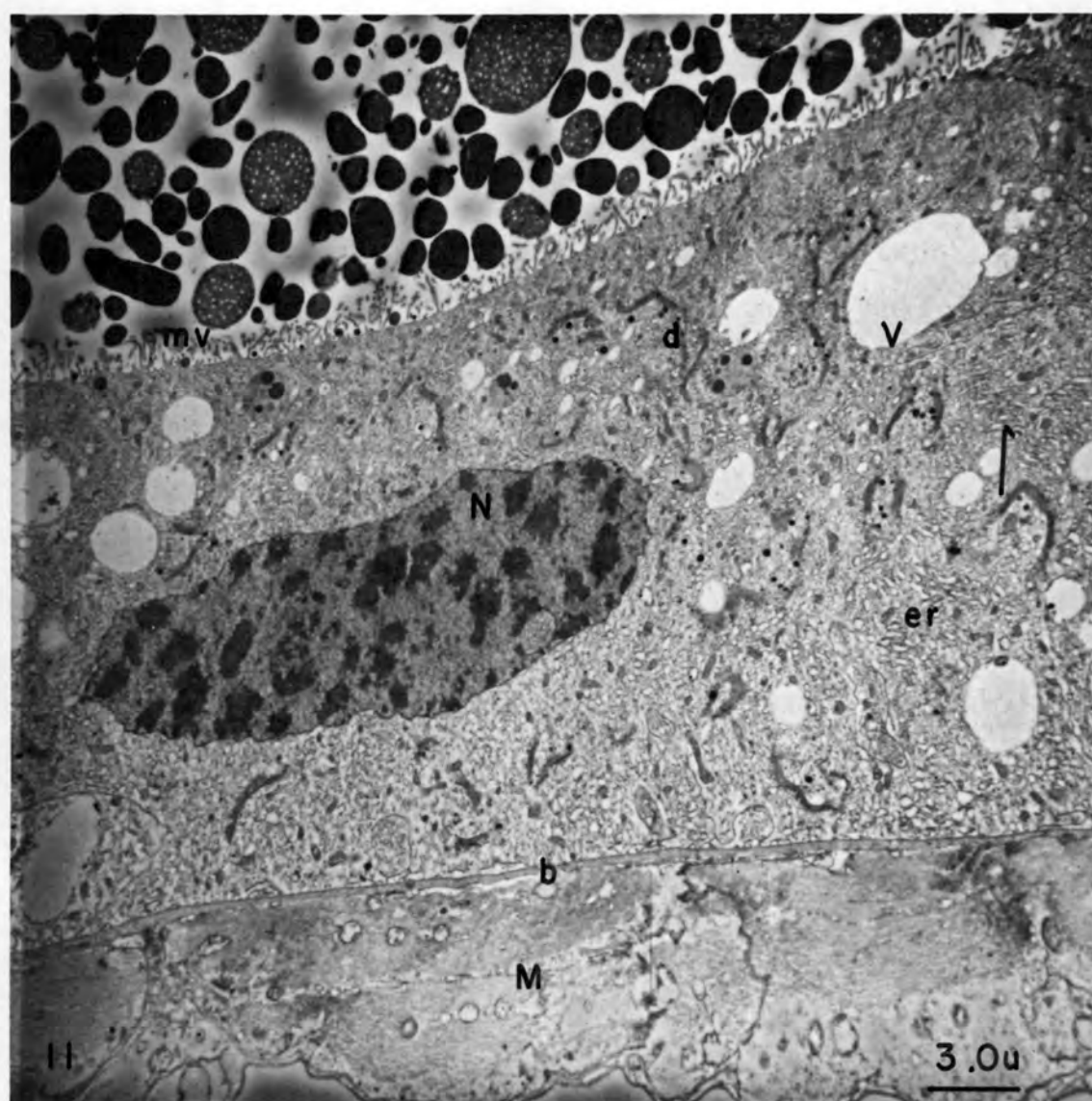
PLATE VIII

PLATE IX

Figure 12. Portion of the nucleus and adjacent cytoplasm of a
prismatic cell in the anterior segment of the vas
deferens. The bilaminar structure of the nuclear
membrane is quite evident. Pores which appear to
lack sealing diaphragms appear at intervals, and
sites of possible connection of the outer layer of
the nuclear membrane to the adjacent endoplasmic
reticulum are indicated by arrows. The boundary
between two adjacent cells appears at lower right
with an area possibly specialized for intercellular
attachment included between the two cell surfaces.

Potassium permanganate fixation; araldite embedding.
Stained with uranium acetate.

X 20,000

ABBREVIATIONS

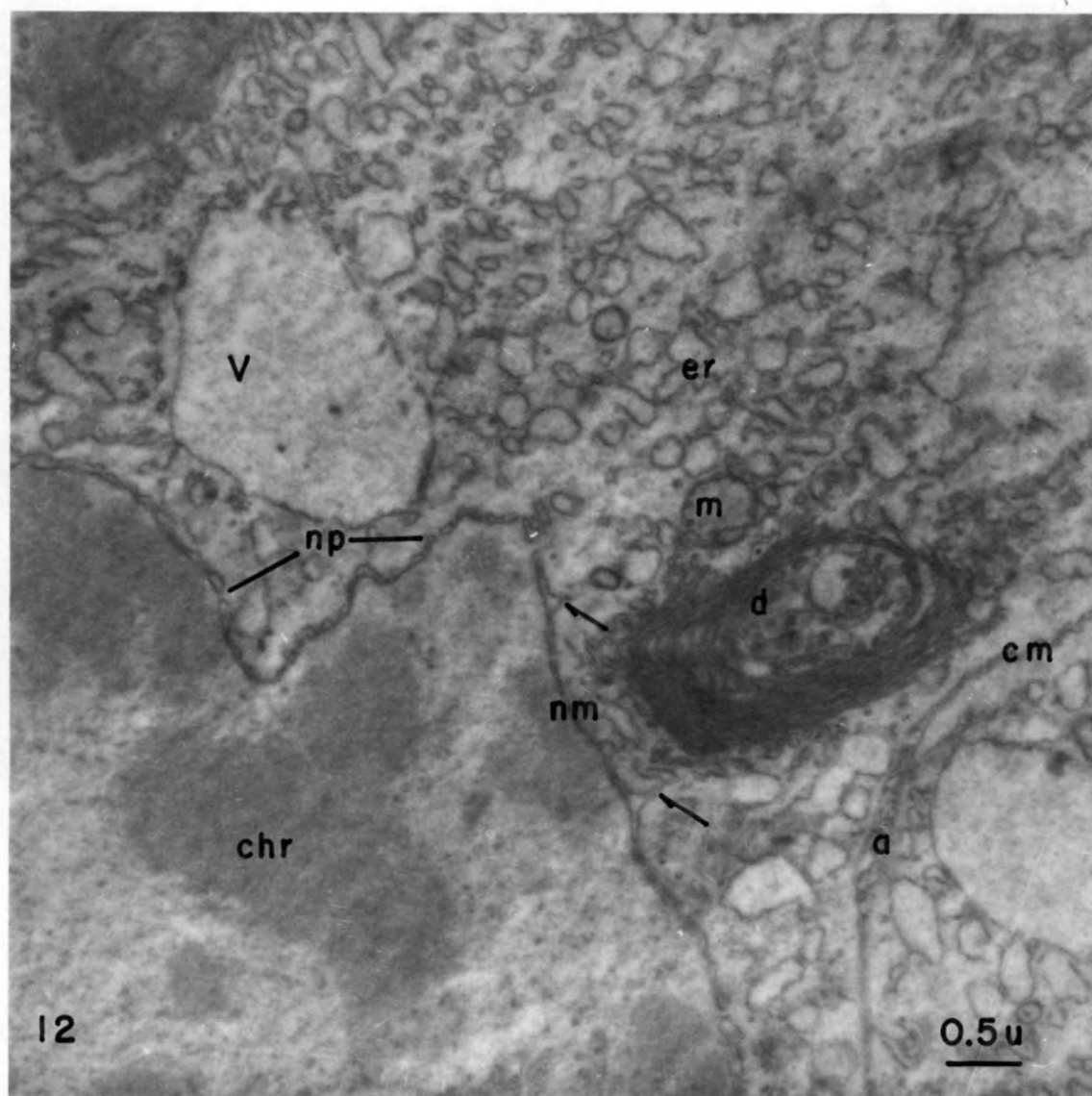|	
a	attachment area
cm	cell membrane
chr	heterochromatic mass
d	dictyosome
er	endoplasmic reticulum
m	mitochondrion
nm	nuclear membrane
np	pores in nuclear membrane
V	vacuole

PLATE IX

PLATE X

Figure 13. Portion of nucleus and adjacent cytoplasm of a giant
cell in the anterior segment of the vas. Here, too,
the bilaminar nuclear membrane, interrupted at
intervals by pores, is apparent. In this case,
however, a diaphragm can be discerned traversing
the waist of the pores.

Osmium tetroxide fixation; methacrylate embedding.

X 45,000

ABBREVIATIONS

chr . . . . heterchromatic mass
d . . . . dictyosome
er . . . . endoplasmic reticulum
m . . . . mitochondrion
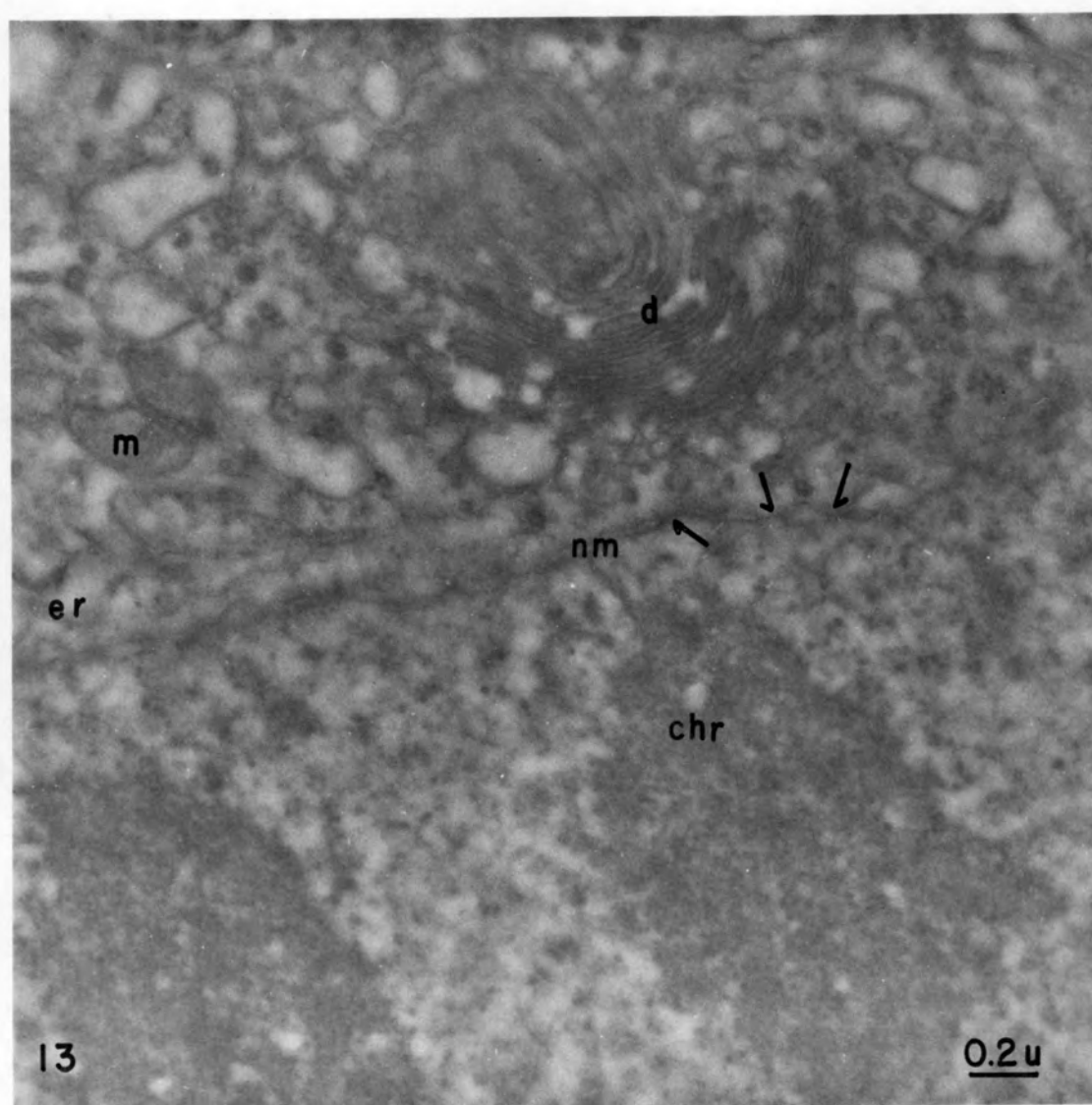nm . . . . nuclear membrane

PLATE X

PLATE XI

Figure 14.   Portion of the nucleus of a prismatic cell showing
heterochromatic masses and a small nucleolus. Small
clear spaces within the heterochromatin are evident
in this electron micrograph, though less obvious than
in the osmium fixed, methacrylate embedded tissues
(figure 10).

Potassium permanganate fixation; araldite embedding.

X 20,000

ABBREVIATIONS

chr . . . . heterochromatin
cv . . . . space in heterochromatin
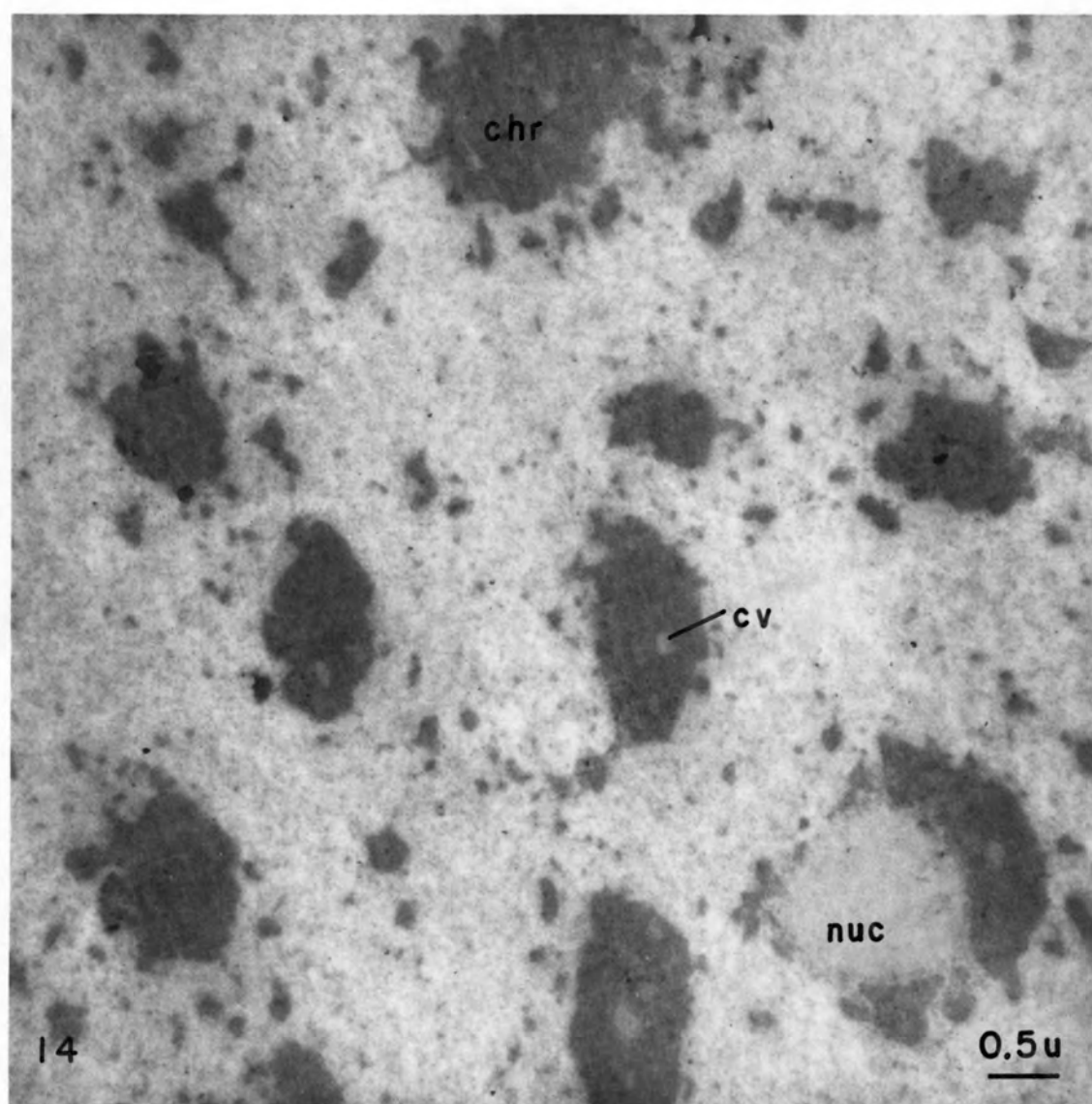nuc . . . . nucleolus

PLATE XI

PLATE XII

Figure 15. Micrograph of the luminal surface of a giant cell
sectioned tangentially to the cell surface. Most
of the microvilli are seen in cross section.

Osmium tetroxide fixation; methacrylate embedding.

X 20,000

ABBREVIATIONS

d . . . . dictyosome
er . . . . endoplasmic reticulum
mv . . . . microvilli
r . . . . ribosomes
s . . . . secretion droplet

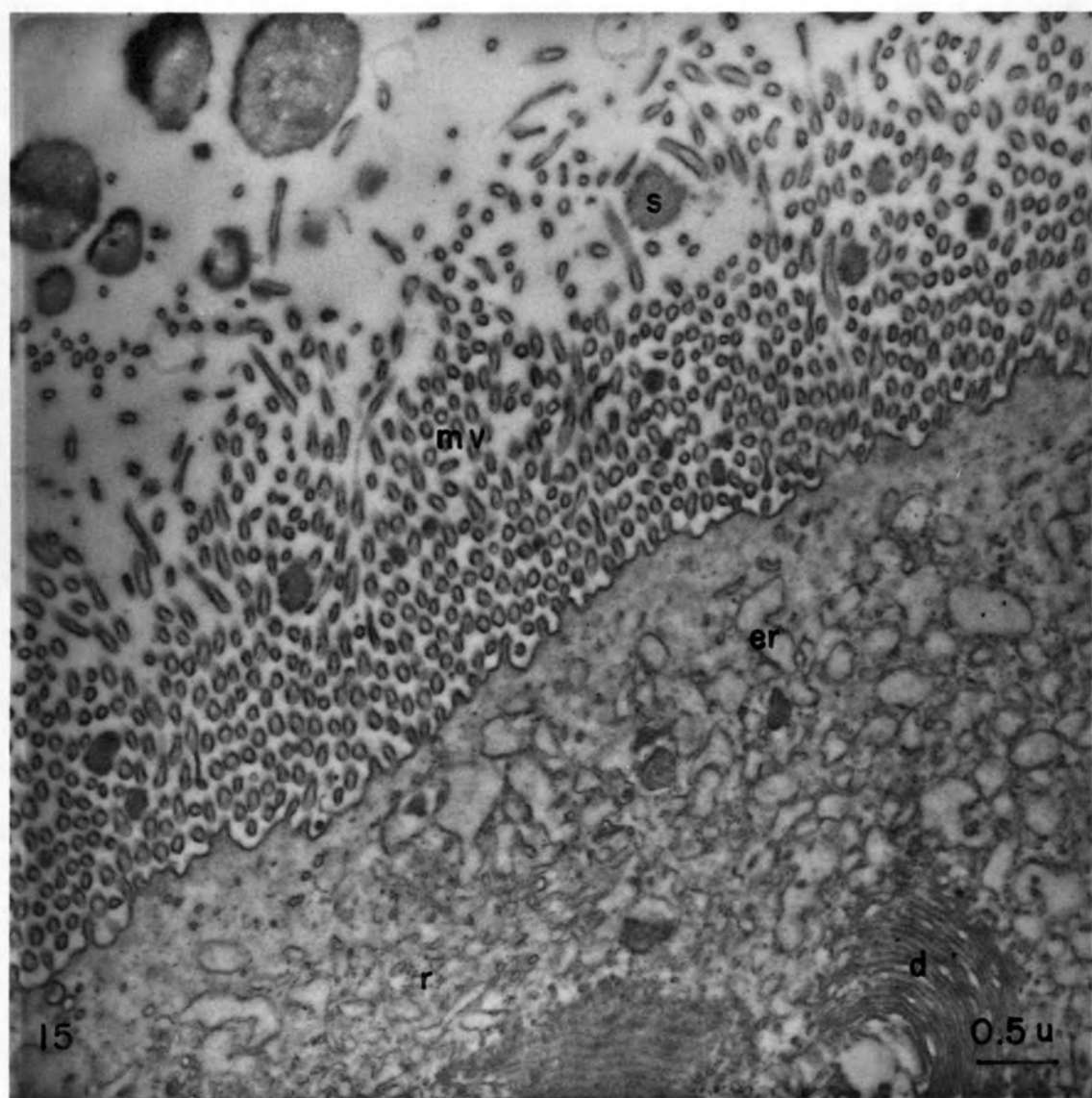PLATE XII

PLATE XIII

Figure 16.   Section of part of the luminal surface of a giant cell
             cut nearly normal to the surface. most of the
             microvilli are longitudinally sectioned. The arrows
             denote points at which sub-surface vesicles appear to
             open into the expanded regions between the bases of
             the microvilli.

             Osmium tetroxide fixation; methacrylate embedding.

                          X 45,000

                       ABBREVIATIONS

                mv . . . . microvilli
                s . . . . secretion droplet
                ves . . . . vesicles
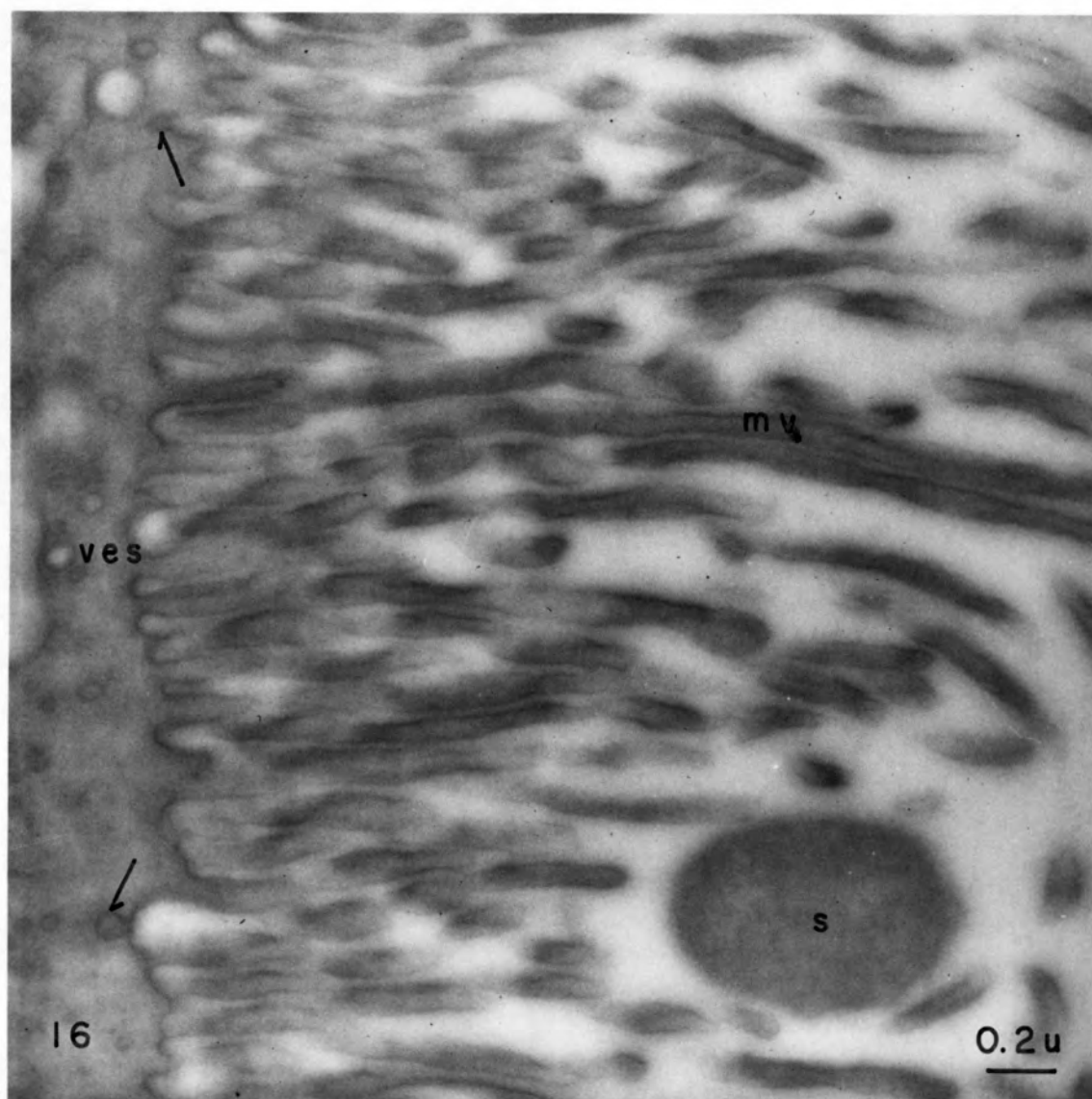
PLATE XIII

PLATE XIV

Figure 17. Section of part of the luminal surface of a prismatic
cell cut nearly normal to the surface. Microvilli are
cut in various planes, indicating that their origins
were not parallel. The arrows denote respectively:
1. bifurcation of a single microvillus
2. sub-surface vesicle apparently opening
into the extracellular space between
the bases of the microvilli.

Osmium tetroxide fixation; araldite embedding;
uranium acetate stain.

X 45,000

ABBREVIATIONS

cm . . . . cell membrane
d . . . . dictyosome
er . . . . endoplasmic reticulum
m . . . . mitochondria
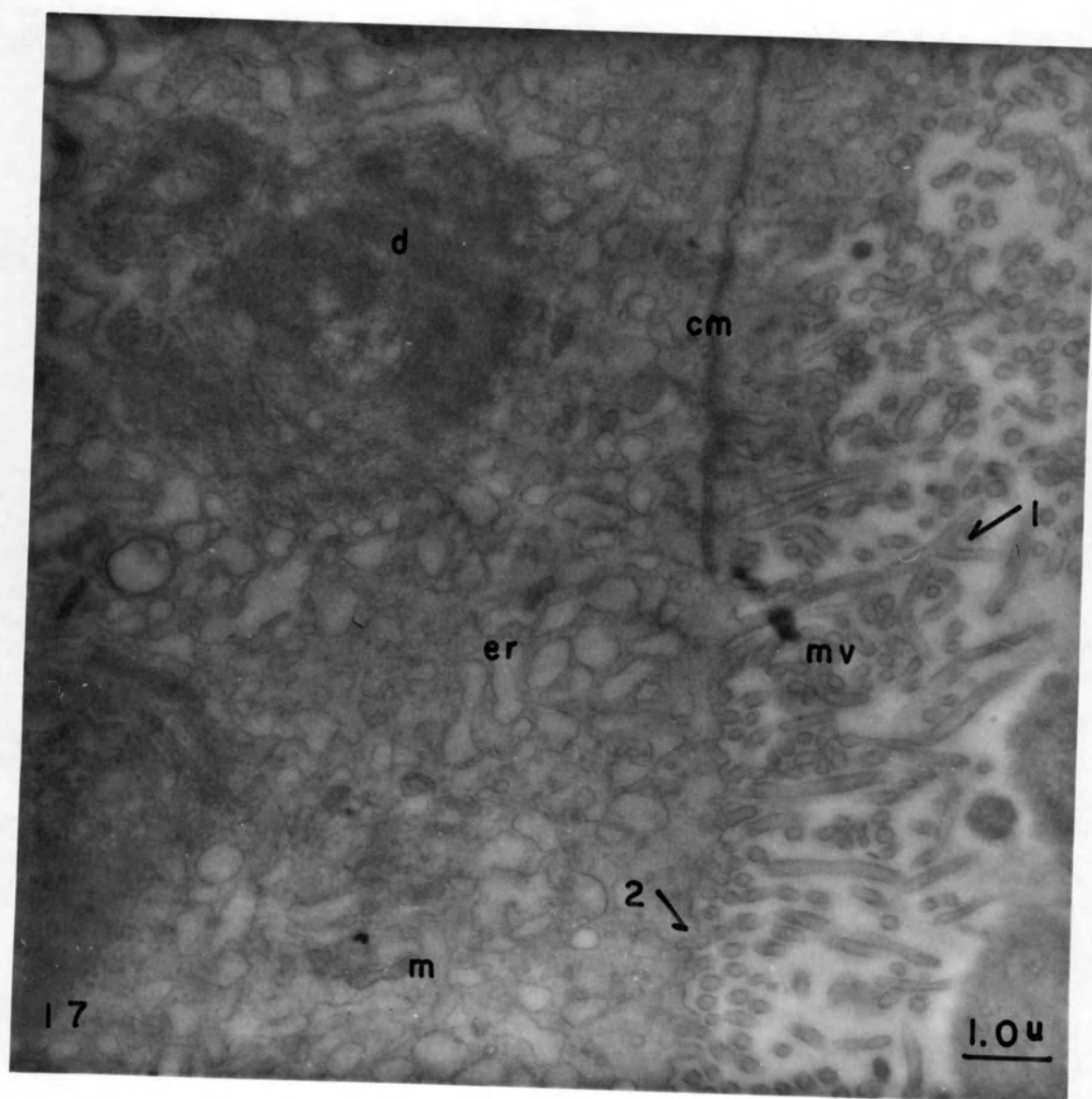mv . . . . microvilli

PLATE XIV

PLATE XV

Figure 18. Section of part of the luminal surface of a structural
cell in the posterior segment of the vas deferens. The
haphazard arrangement of the microvilli is apparent.
The arrows denote respectively:
1. vesicular dilation of the intercellular space
2. ribosomal granules arranged in a "rosette"
3. sub-surface vesicle apparently opening to the
lumen of the deferent duct between the bases
of the microvilli
4. mass of microvilli fused above the basal level.

Osmium tetroxide fixation; araldite embedding; uranium
acetate stain.

X 20,000

ABBREVIATIONS

cm . . . . cell membrane
m . . . . mitochondrion
mv . . . . microvilli
r . . . . ribosomes
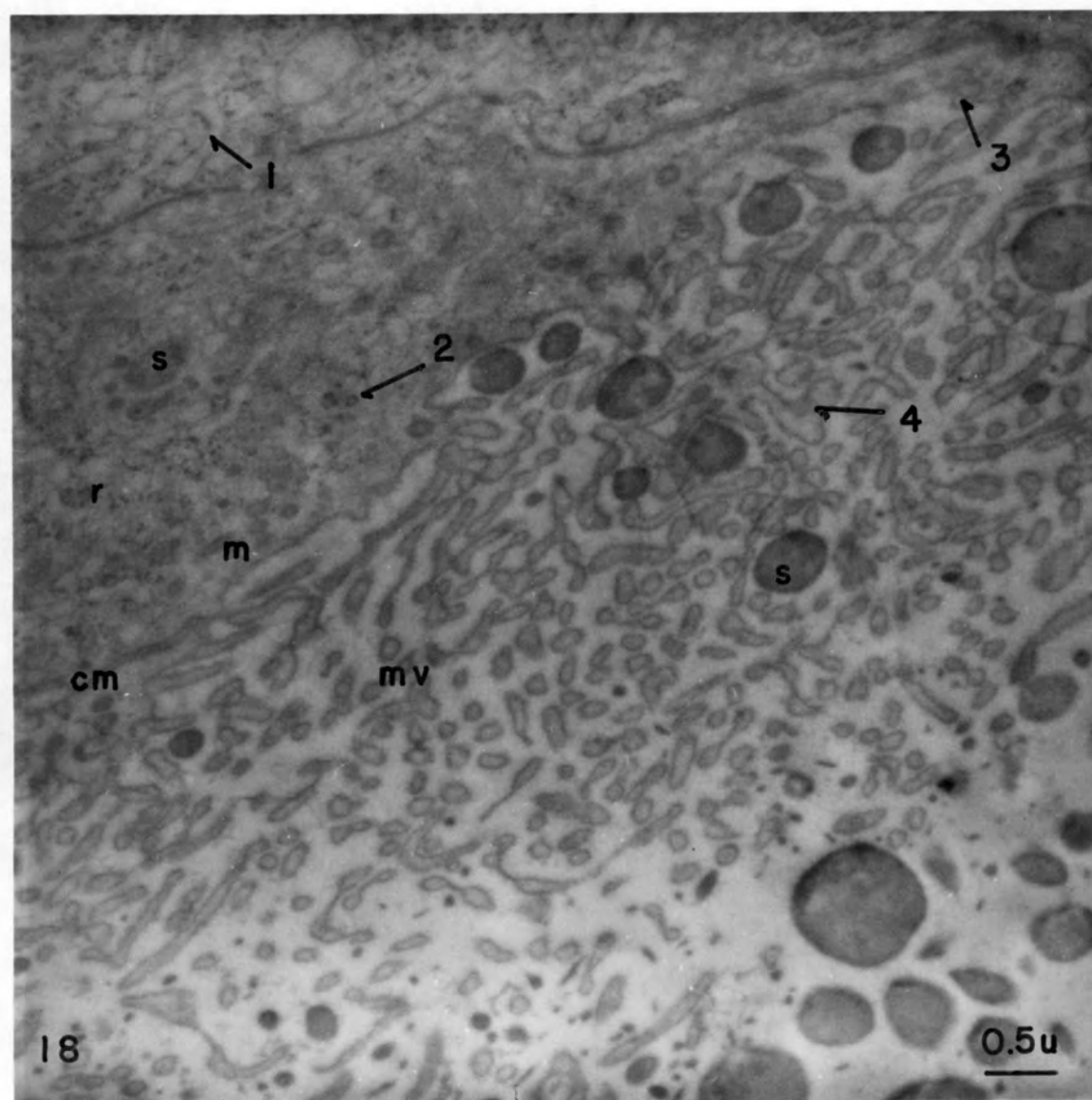s . . . . secretion droplet

PLATE XV

PLATE XVI

Figure 19. Boundary region between a giant and a prismatic cell of the anterior segment. Widely dilated cisternae of the endoplasmic reticulum occupy most of the cytoplasm of the giant cell appearing in the lower half of this micrograph. Less dilated tubular and cisternal elements appear in the prismatic cell (upper half of the micrograph).

Potassium permanganate fixation; araldite embedding.

X 20,000

ABBREVIATIONS

cm . . . . cell membranes
cp . . . . cytoplasmic matrix
d . . . . dictyosome
er . . . . endoplasmic reticulum
m . . . . mitochondrion
mvb . . . . multivesicular body
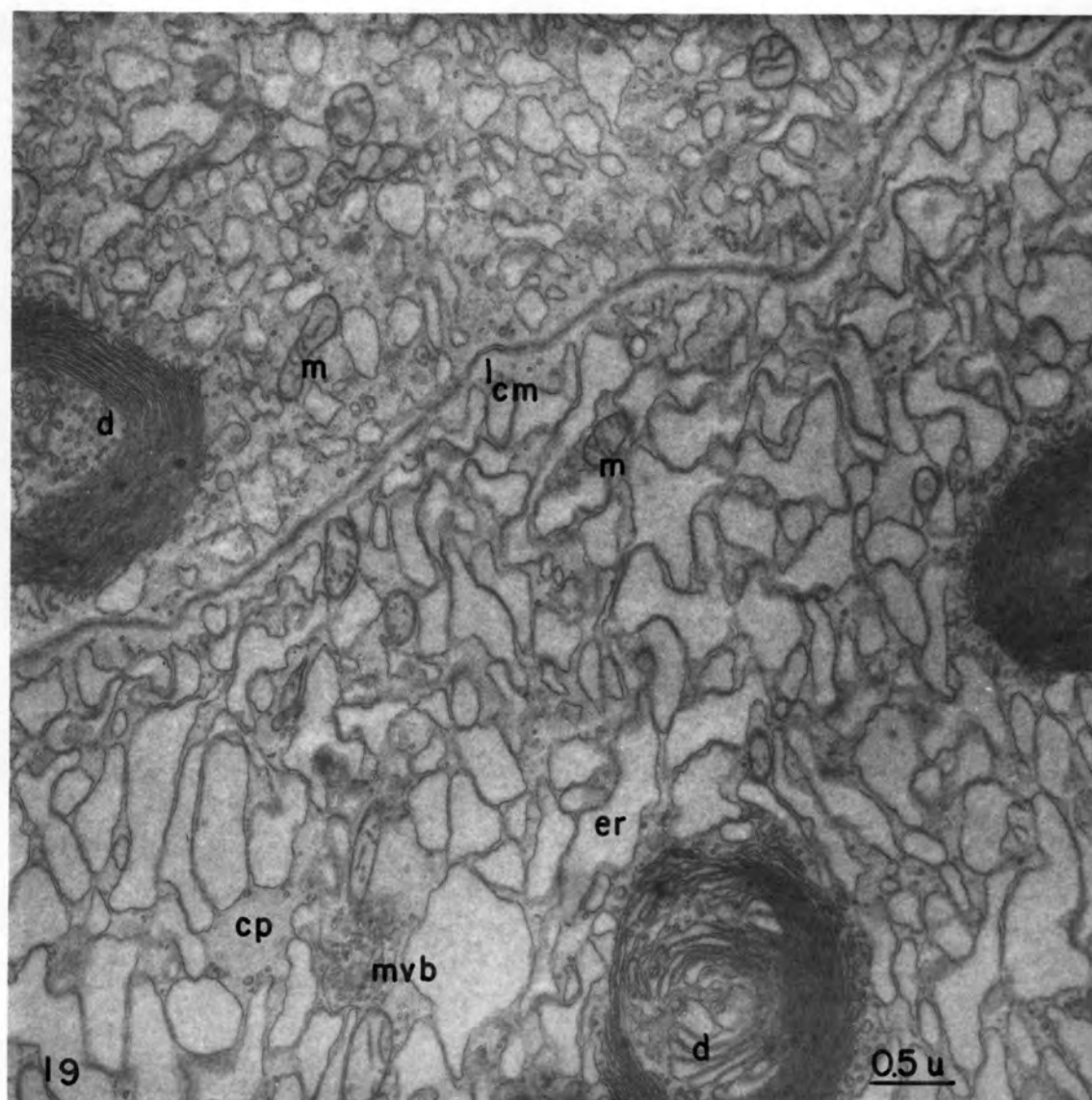
PLATE XVI

19   0.5 u

PLATE XVII

Figure 20. An area of giant cell cytoplasm illustrating the widely
dilated cisternae of the endoplasmic reticulum and a
highly elaborated Golgi structure. Mitochondria appear
dispersed among the cisternae. The arrows identify
regions where particularly intimate contact exists
between the mitochondrial limiting membrane and
the adjacent walls of cisternae.

Potassium permanganate fixation; araldite embedding.

X 20,000

ABBREVIATIONS

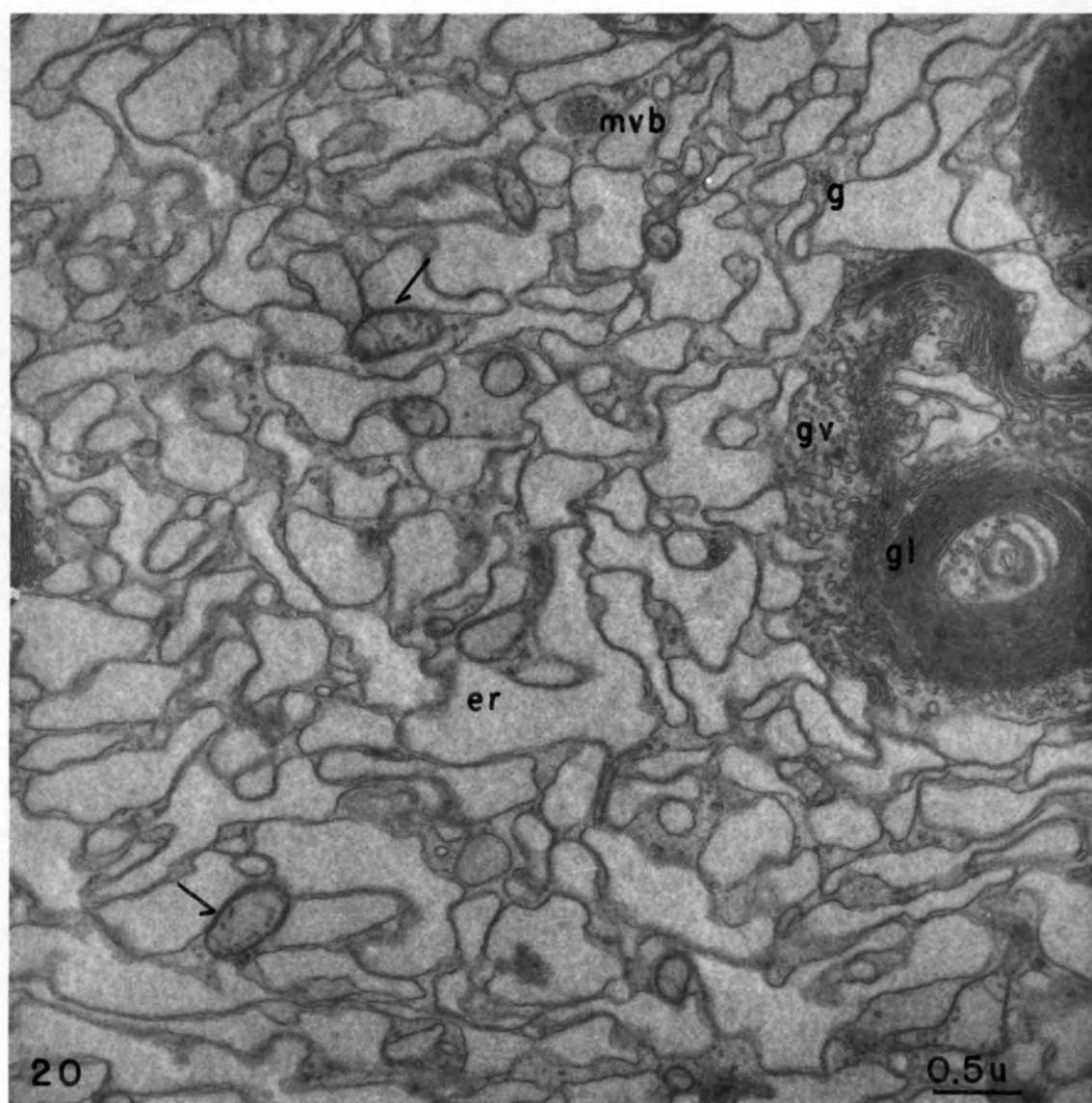|	
er	endoplasmic reticlum
g	dense granule
gl	Golgi lamellae
gv	Golgi vesicles
mvb	multivesicular body

PLATE XVII

PLATE XVIII

Figure 21. Mitochondrion from a prismatic cell of the anterior
segment showing the bilaminar limiting membrane and
the internal cristal membranes. At the arrow the
membranes of a crista are continuous with the inner
lamella of the limiting membrane.

Potassium permanganate fixation; araldite embedding.

X 80,000

Figure 22. Mitochondrion from a prismatic cell, again illustrating
the bilaminar limiting membrane and the internal
cristae. At the arrow the membranes of a crista are
continuous with the mitochondrial limiting membrane.

Potassium permanganate fixation; araldite embedding.

X 110,000

ABBREVIATIONS

er . . . . endoplasmic reticulum
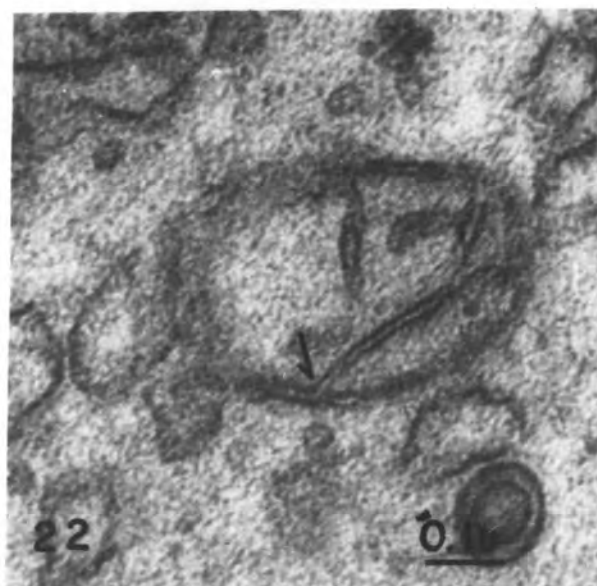g . . . . granules

PLATE XVIII

PLATE XIX

Figure 23. A single Golgi structure illustrating the form typical
of this organelle in the giant cells of the anterior
segment. The Golgi lamellae are arranged in whorls,
around which appear the Golgi vesicles. The interior
of the whorl is filled with dilated vacuolar elements.
The arrows denote respectively:
1. regions where the outermost membrane
doublets are apparently undergoing
fragmentation
2. points where the inner limiting membrane
of the mitochondrion is continuous with
the limiting membranes of cristae.
This Golgi body gives the impression of two centres
enclosed within a few whorls of Golgi lamellae.

Permanganate fixation; araldite embedding.

X 40,000

ABBREVIATIONS

er . . . . endoplasmic reticulum
g . . . . granules
gl . . . . Golgi lamellae
gv . . . . Golgi vesicles
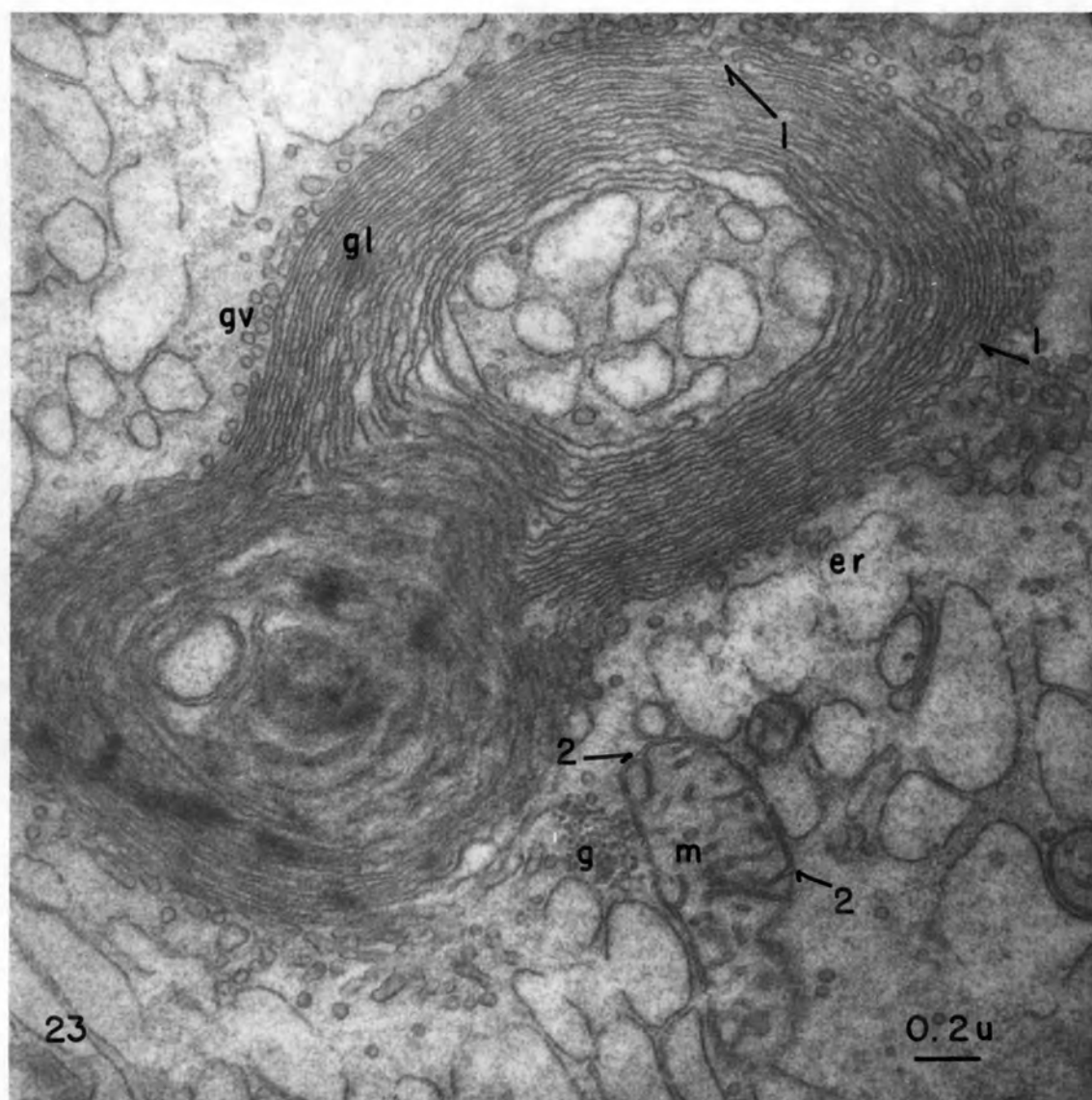m . . . . mitochondrion

PLATE XIX

PLATE XX

Figure 24. Golgi structure in a giant cell. The arrows emphasize
respectively:
1. intimate contact between the limiting
membrane of a mitochondrion and an
adjacent cisterna of the endoplasm
reticulum
2. intimate contact of the endoplasmic
reticulum and the outer lamella of
the Golgi apparatus.
The unidentified structure at lower right has been
observed only once in the cells of the sow bug
vas deferens.

Potassium permanganate fixation; araldite embedding.

X 24,000

ABBREVIATIONS

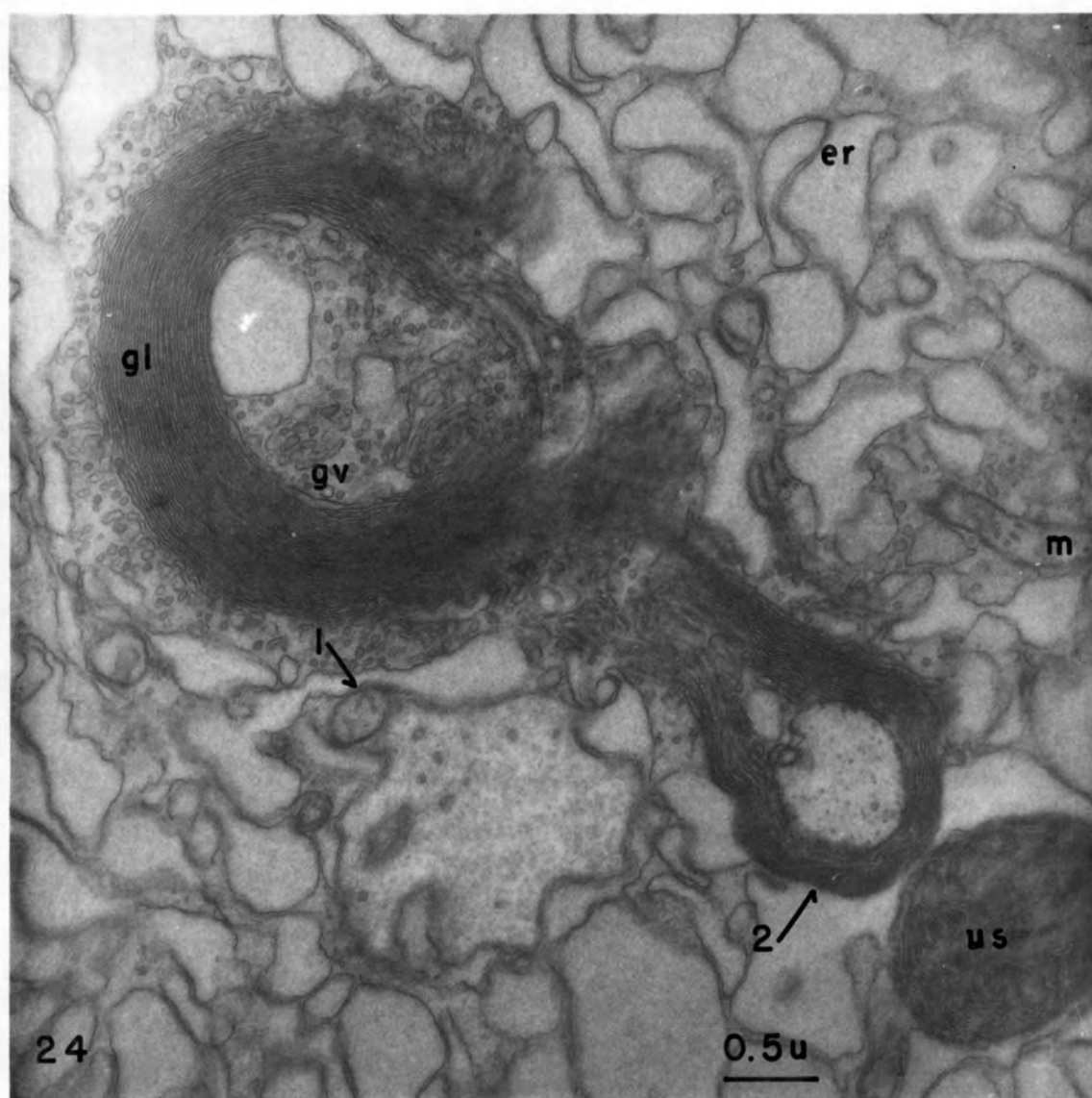|	
er	endoplasmic reticulum
gl	Golgi lamellae
gv	Golgi vesicles
m	mitochondrion
us	unidentified structure

PLATE XX

PLATE XXI

Figure 25. Golgi structures in a giant cell. The characteristic lamellar and vesicular components of the organelle are well illustrated. A few small dense granules appear within the central region of the lower Golgi body. Arrows draw attention to:

1. terminal dilation of the space within a membrane doublet
2. close association of membranes of one Golgi structure with an adjacent cisternal wall
3. continuity of the membranes of a crista with the inner limiting membrane of a mitochondrion.

Potassium permanganate fixation; araldite embedding.

X 40,000

ABBREVIATIONS

er . . . . endoplasmic reticulum
g . . . . dense granules
gl . . . . Golgi lamellae
gv . . . . Golgi vesicles
mvb . . . . multivesicular body
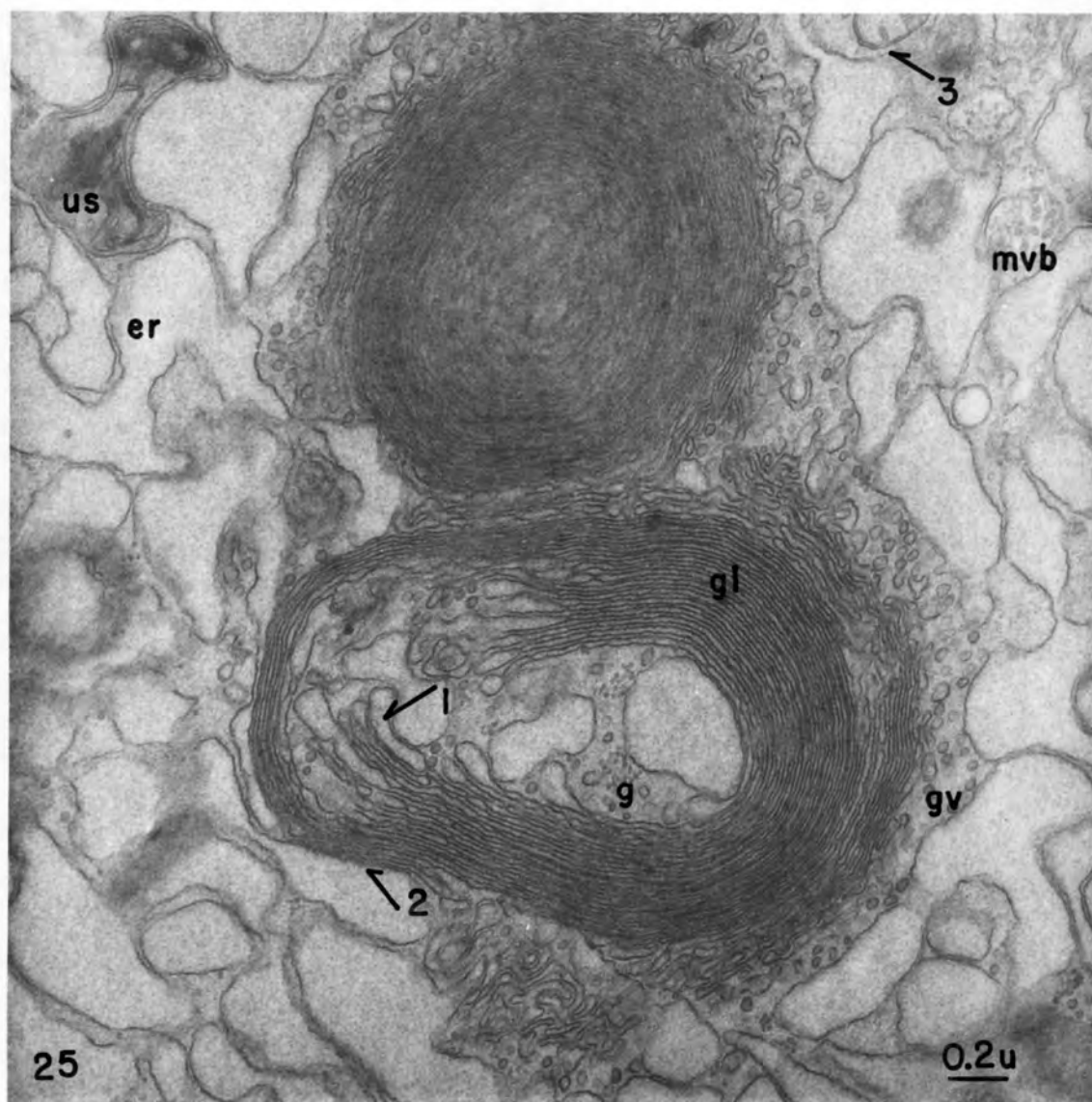us . . . . unidentified structure

PLATE XXI

PLATE XXII

Figure 26. A single giant cell dictyosome. This organelle shows
vacuoles formed by dilation of the space within a
membrane doublets.

Potassium permanganate fixation; araldite embedding.

X 60,000

ABBREVIATIONS

er . . . . endoplasmic reticulum
gl . . . . Golgi lamellae
gv . . . . Golgi vesicles
vac . . . . vacuole
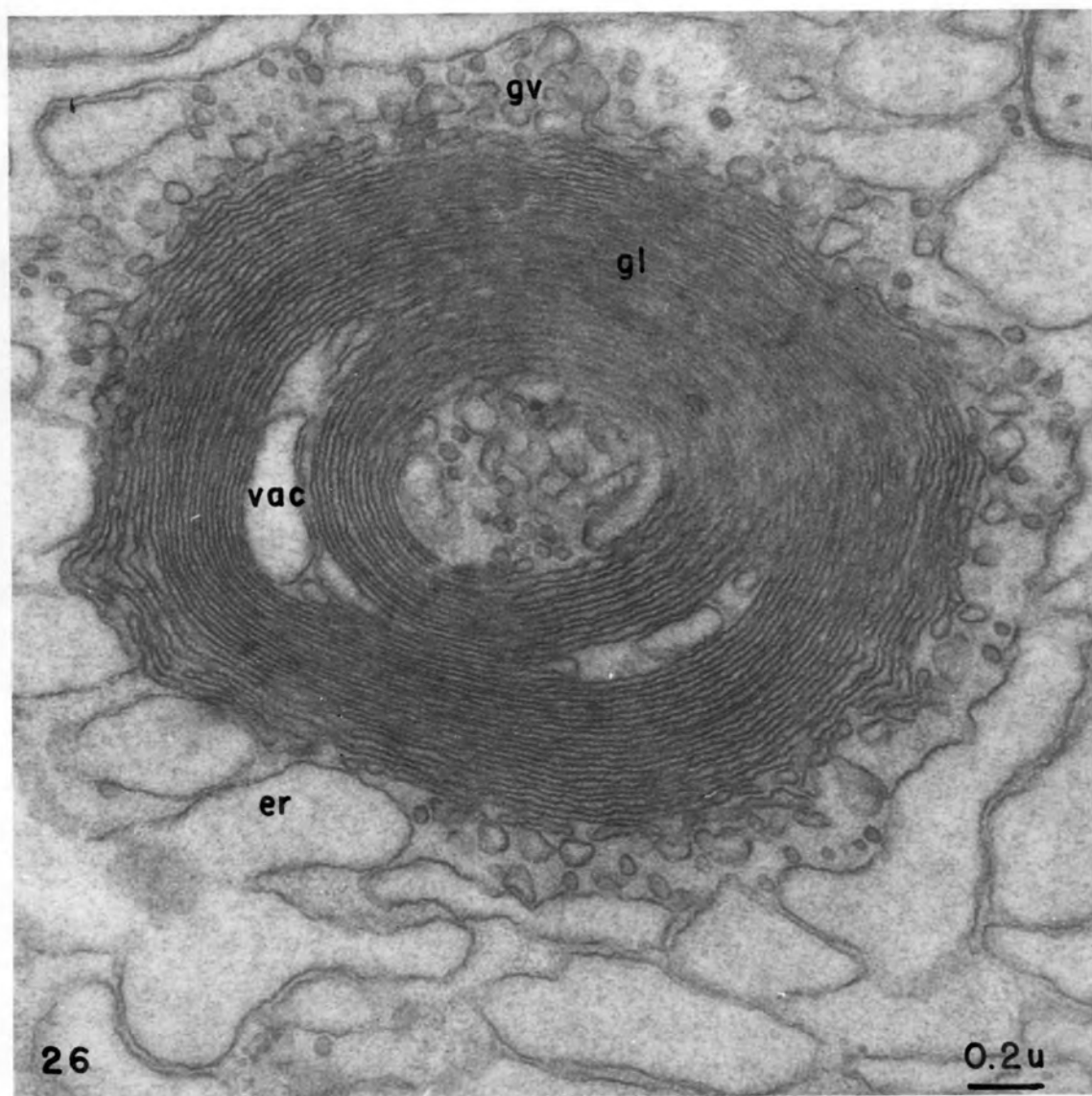
PLATE XXII

26 0.2u

PLATE XXIII

Figure 27. An elongate Golgi structure in a prismatic cell of the anterior segment. This dictyosome is comprised of only six membrane doublets, compared to twenty in the giant cell dictyosome appearing in figure 26. At the arrow appears a group of vesicles apparently "budded" from the exposed ends of the lamellae. The membranes of the endoplasmic reticulum are liberally sprinkled with ribosomal granules adhered to their outer surfaces.

Osmium tetroxide fixation; araldite embedding; uranium acetate stain.

X 60,000

ABBREVIATIONS

dg . . . . dense granule
er . . . . endoplasmic reticulum
gl . . . . Golgi lamellae
gv . . . . Golgi vesicles
m . . . . mitochondrion

PLATE XXIII

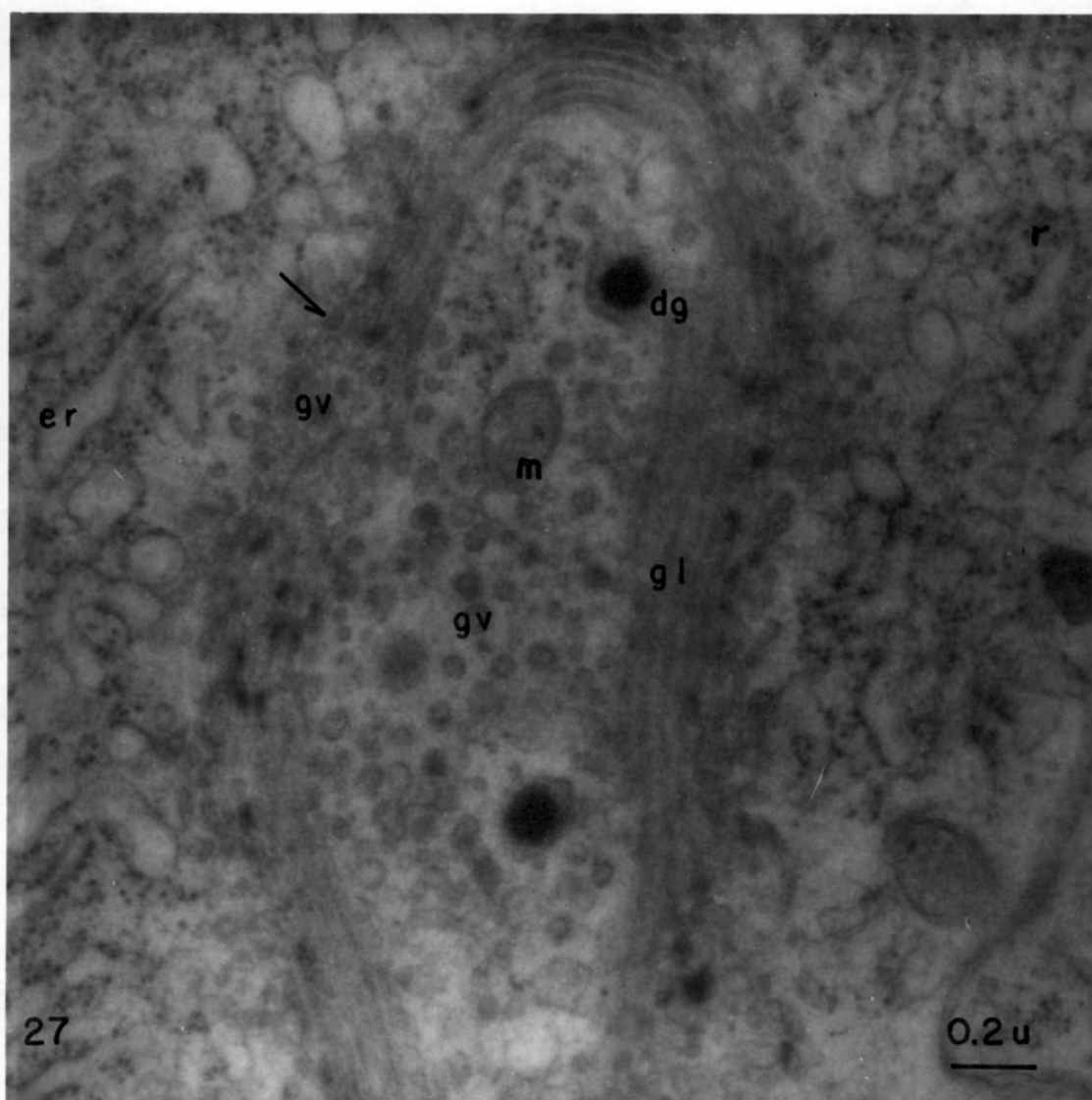27   0.2 u

PLATE XXIV

Figure 28. Elongate Golgi element in a structural cell of the
posterior segment. At the arrow, vesicles were
apparently being formed by "budding" from the ends
of the Golgi lamellae.

Osmium tetroxide fixation; methacrylate embedding.

X 45,000

ABBREVIATIONS

dg . . . . dense granule
er . . . . endoplasmic reticulum
gl . . . . Golgi lamellae
gv . . . . Golgi vesicles
m . . . . mitochondrion
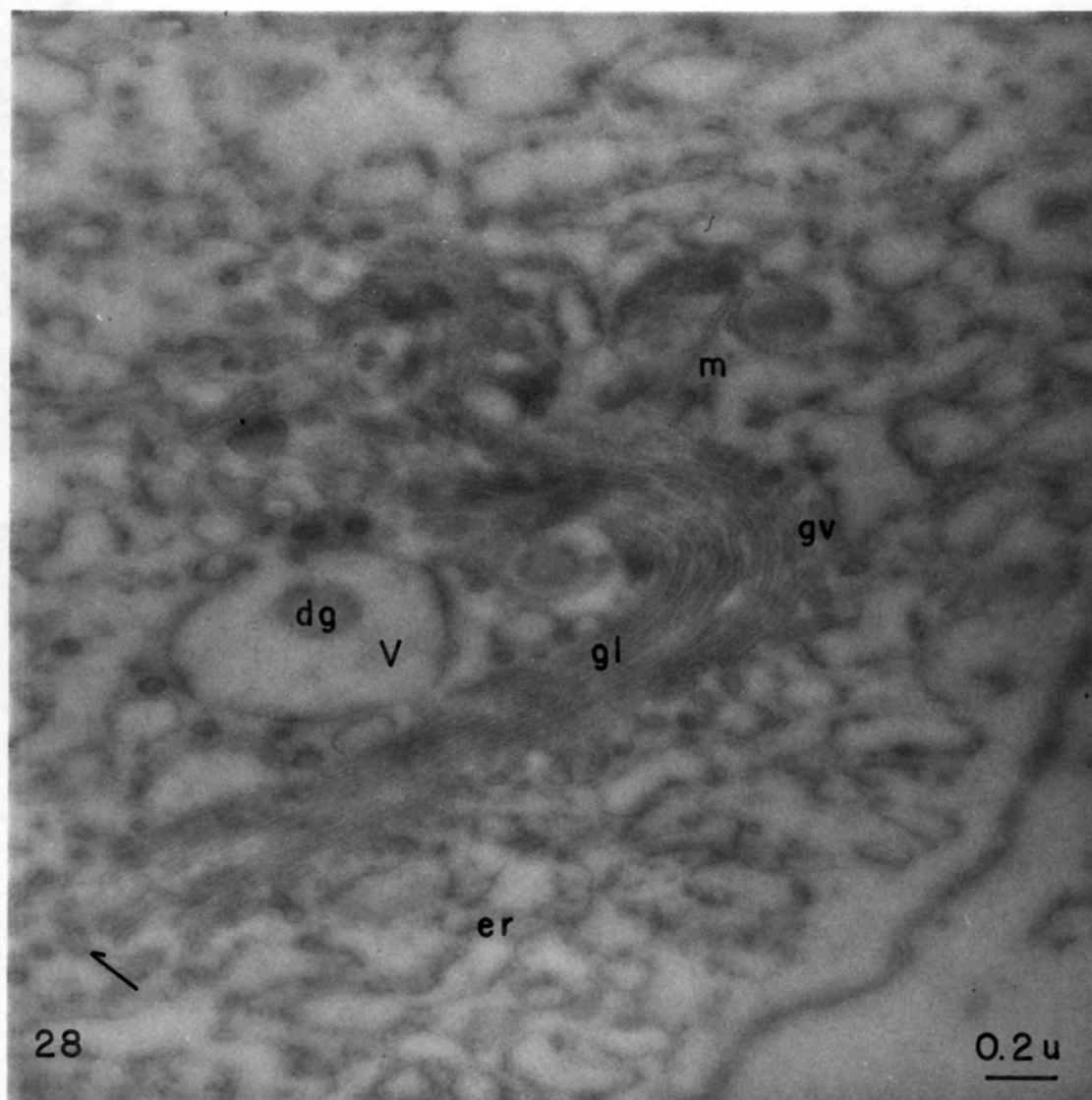V . . . . vacuole

PLATE XXIV

PLATE XXV

Figure 29.   Elongate Golgi structure in a structural cell of the
anterior segment (prismatic cell). The Golgi lamellae
are without granules, whereas the adjacent membranes of
the endoplasmic reticulum are liberally covered with
dense ribosomal granules.

Osmium tetroxide fixation; araldite embedding; uranium
acetate stain.

X 60,000

ABBREVIATIONS

cm . . . . cell membrane
dg . . . . dense granule
er . . . . endoplasmic reticulum
gl . . . . Golgi lamellae
gv . . . . Golgi vesicles
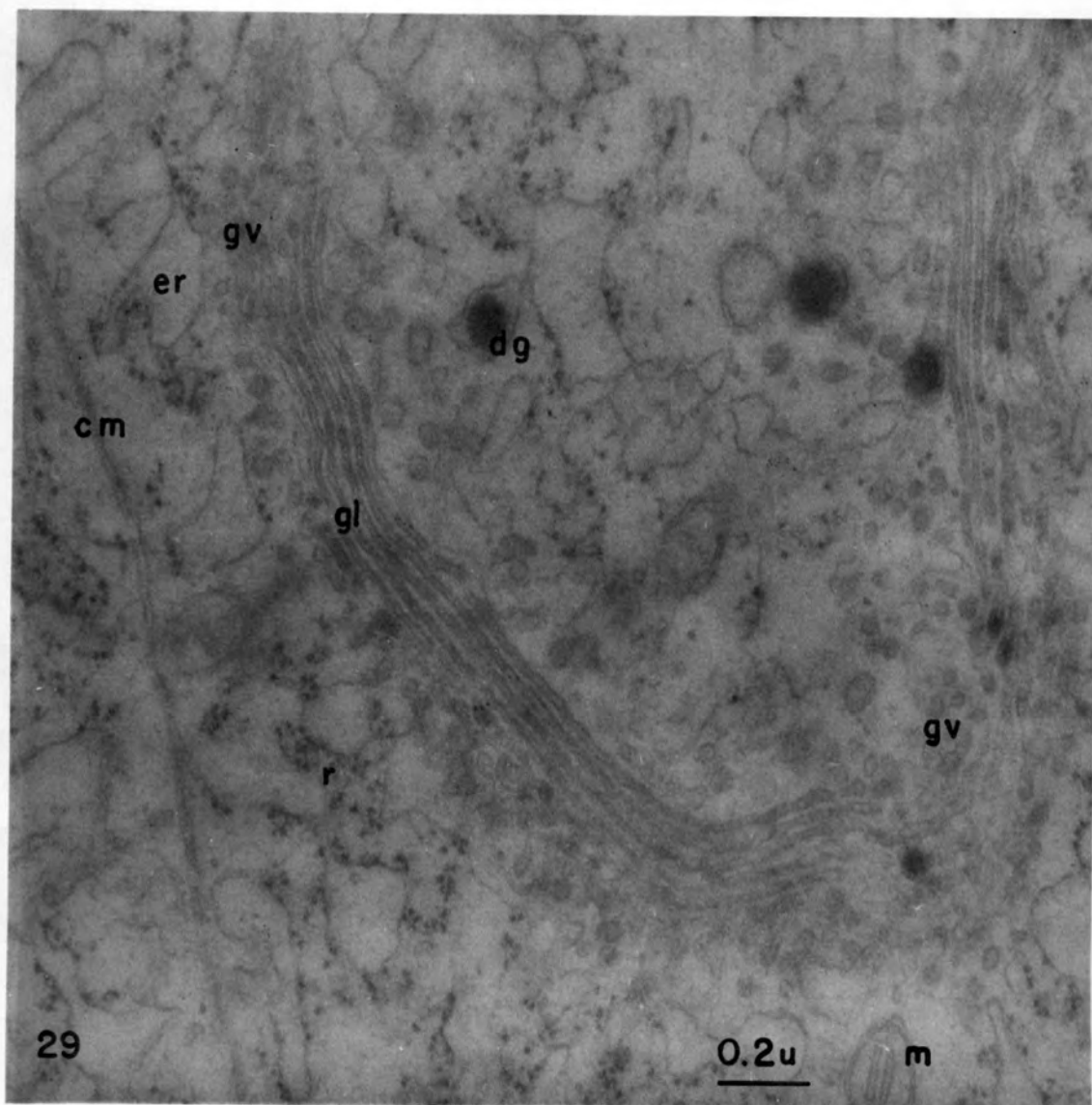m . . . . mitochondrion

PLATE XXV

PLATE XXVI

Figure 30. A cluster of vesicles representing an extreme
tangential section of the Golgi apparatus in a
giant cell. The lamellar system of the structure
has been entirely excluded from the section owing
to the obliquity of the section.

Potassium permanganate fixation; araldite embedding.

X 20,000

ABBREVIATIONS

er . . . . endoplasmic reticulum
gv . . . . Golgi vesicles
m . . . . mitochondrion
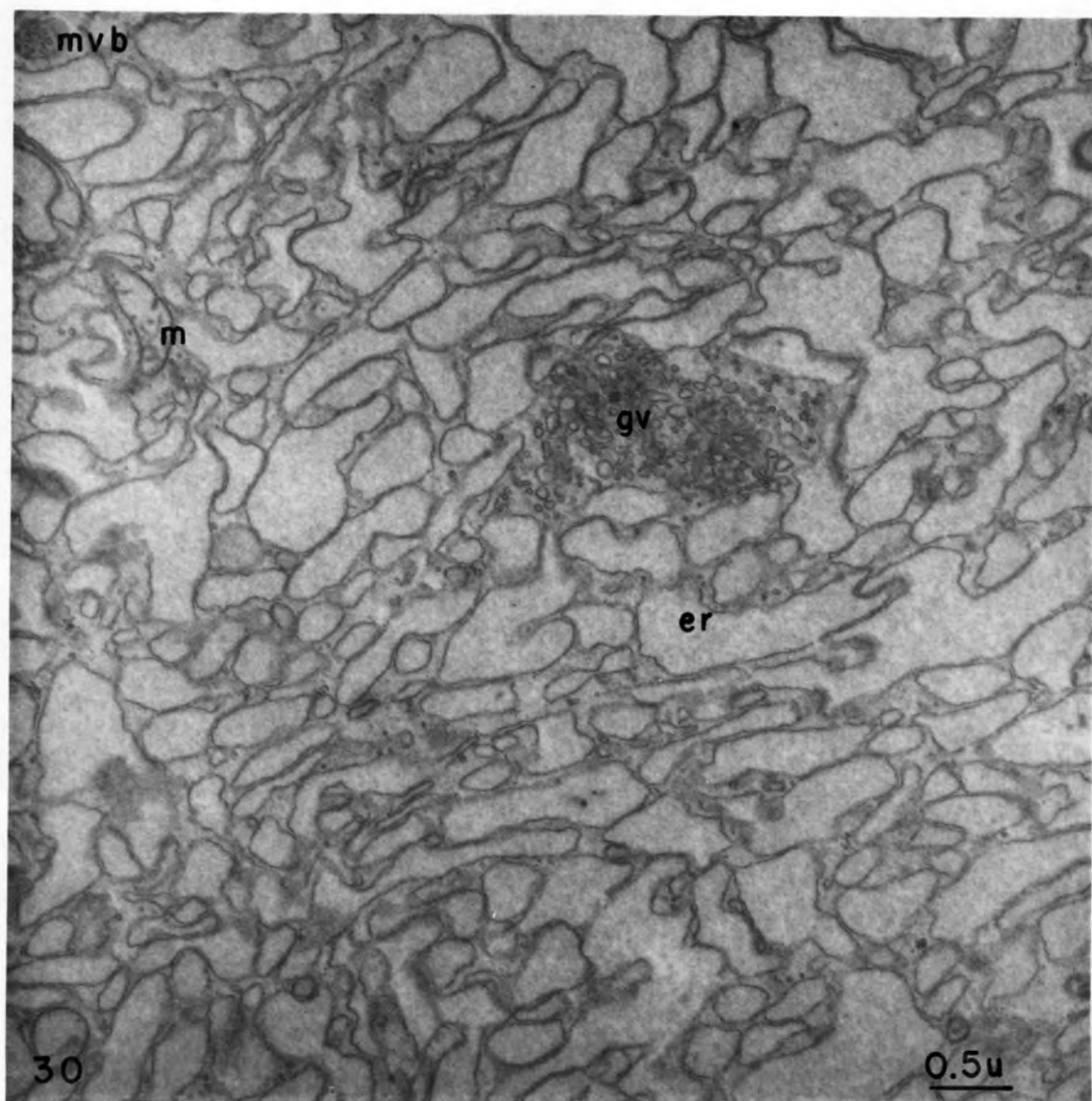mvb . . . . multivesicular body

PLATE XXVI

PLATE XXVII

Figure 31. Low power electron micrograph illustrating the
distribution of Golgi structures amongst the
cisternae of the endoplasmic reticulum in a
giant cell. At the upper right in the micrograph
a portion of an adjacent prismatic cell can be seen;
the relatively small tubules and cisternae of the
endoplasmic reticulum in this cell provide a marked
contrast to the extremely dilated cisternal elements
which entirely fill the cytoplasm of the giant cell.
At the lower right the surface of the giant cell is
overlain by a thin brush bordered extension of the
prismatic cell cytoplasm.

Potassium permanganate fixation; araldite embedding.

X 12,000

ABBREVIATIONS

d . . . . dictyosome
er . . . . endoplasmic reticulum
G . . . . giant cell
m . . . . mitochondrion
mv . . . . microvilli of brush
border
P . . . . prismatic cell
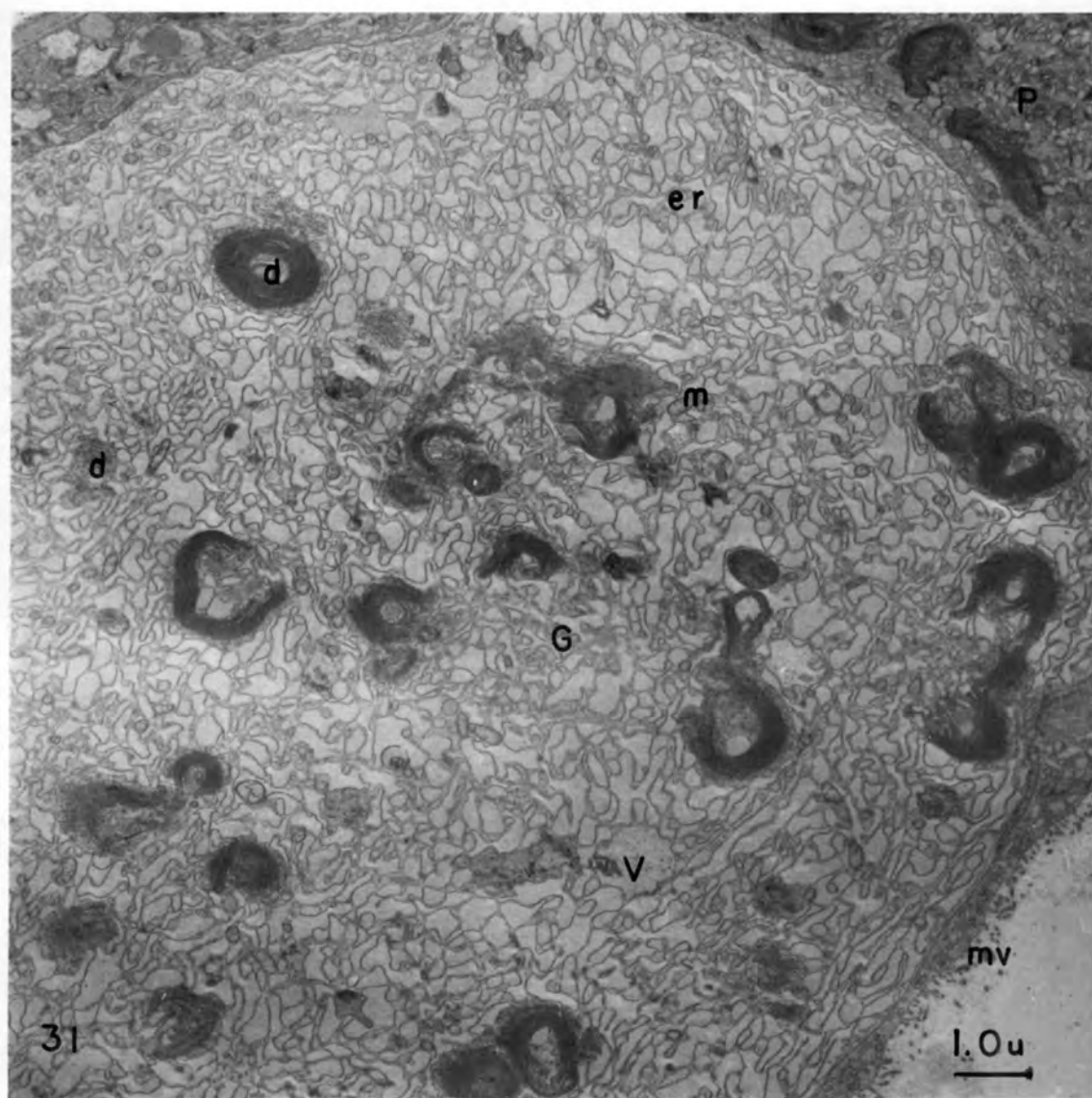
PLATE XXVII

31

PLATE XXVIII

Figure 32. A cup-shaped dictyosome in a giant cell. Cisternae of
the endoplasmic reticulum extend into the interior of
the cup at its open side. The arrows indicate
respectively:
1. terminal dilation of the space within a
    membrane doublet
2. close apposition of Golgi lamellae and the
    wall of an adjacent cisterna
3. site of fragmentation of peripheral lamellae
    of the dictyosome
4. vesicles apparently "budded" from the ends
    of the lamellae.

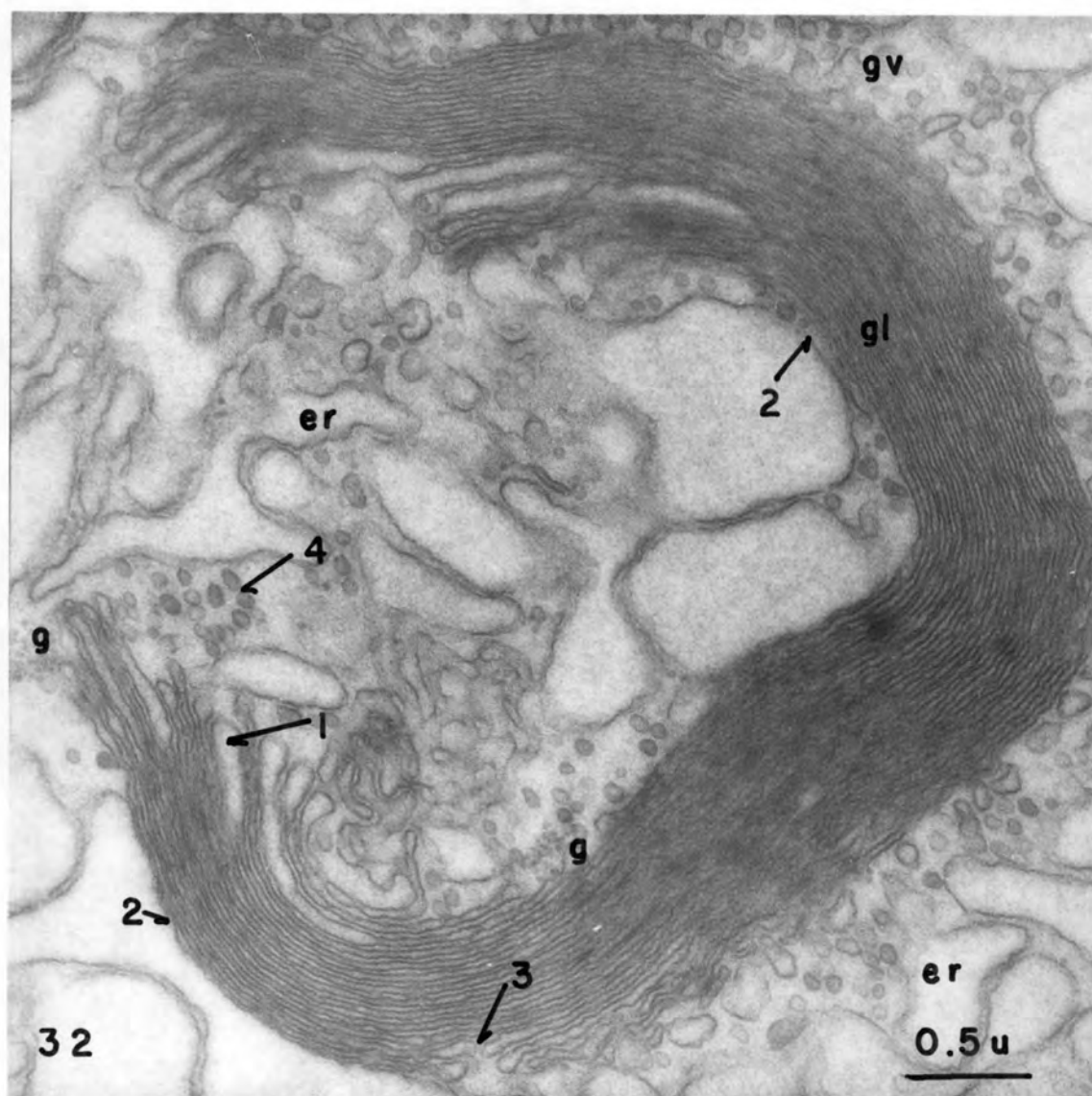Potassium permanganate fixation; araldite embedding.

X 35,000

ABBREVIATIONS

er . . . . endoplasmic reticulum
g . . . . dense granules
gl . . . . Golgi lamellae
gv . . . . Golgi vesicles

PLATE XXVIII

PLATE XXIX

Figure 33. Highly vacuolated region of the cytoplasm of a prismatic cell in the vicinity of a dictyosome. The vacuolar walls are distinguished from the membranes of the endoplasmic reticulum by the absence of ribosomal particles from the surfaces of the former. At the right, a small portion of the cell nucleus is included in the section. The characteristic double nuclear membrane is evident. The arrows denote:

    1. pores in the membrane which are apparently sealed by diaphragms
    2. a cluster of ribosomal particles arranged in a rosette.

Osmium tetroxide fixation; araldite embedding; uranium acetate stain.

X 30,000

ABBREVIATIONS

chr . . . . heterochromatic mass
d . . . . dictyosome
m . . . . mitochondrion
nm . . . . nuclear membrane
r . . . . ribosomes
V . . . . vacuole

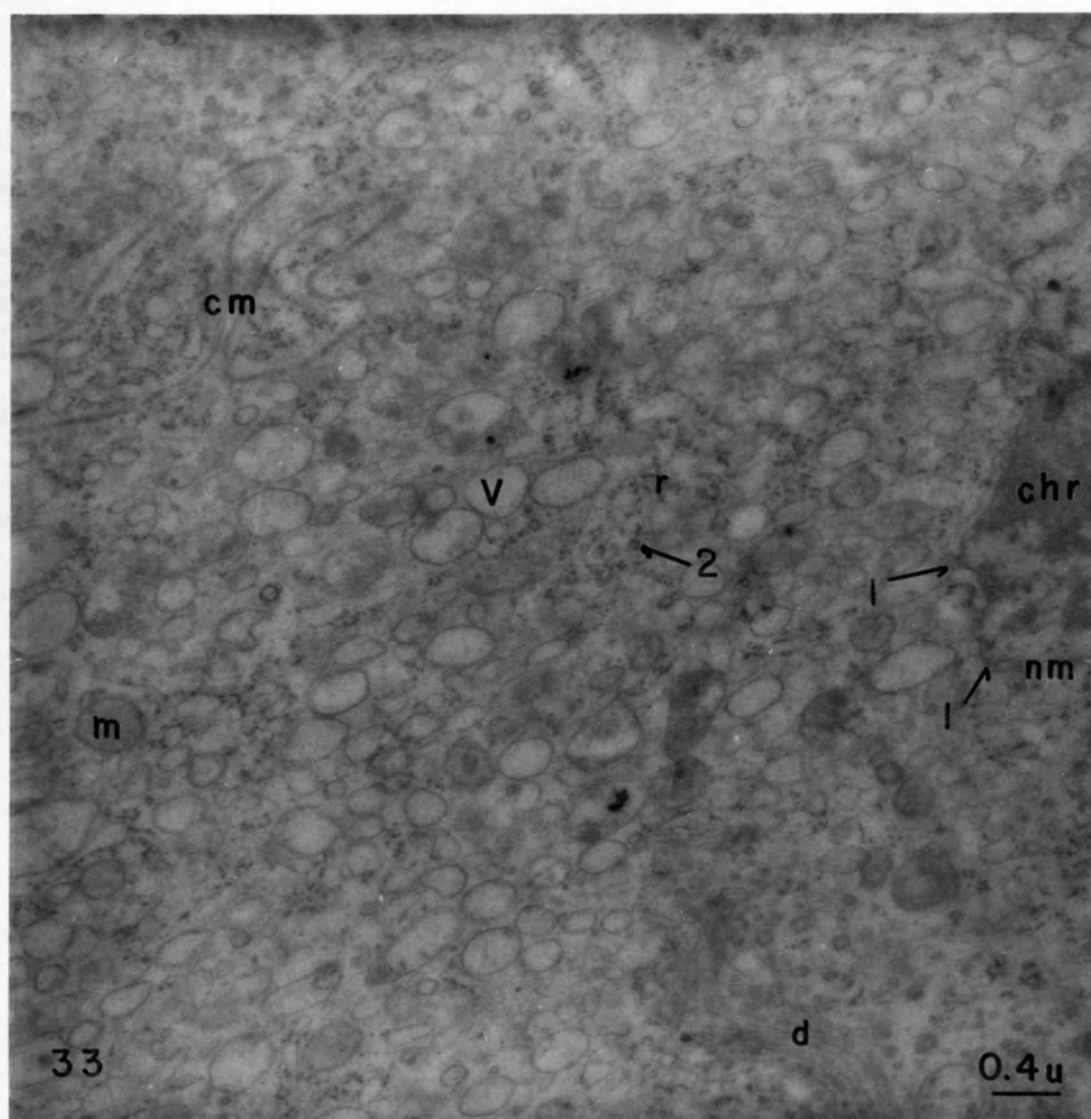PLATE XXIX

104

PLATE XXX

Figure 34.   A region of a prismatic cell similar to that shown in
             figure 33.   Vacuolar walls are identified by the absence
             of ribosomes from their surfaces.

             Osmium tetroxide fixation; araldite embedding; uranium
             acetate stain.

                              X 30,000

                           ABBREVIATIONS

             d . . . . dictyosome
             er . . . . endoplasmic reticulum
             m . . . . mitochondrion
             r . . . . ribosome
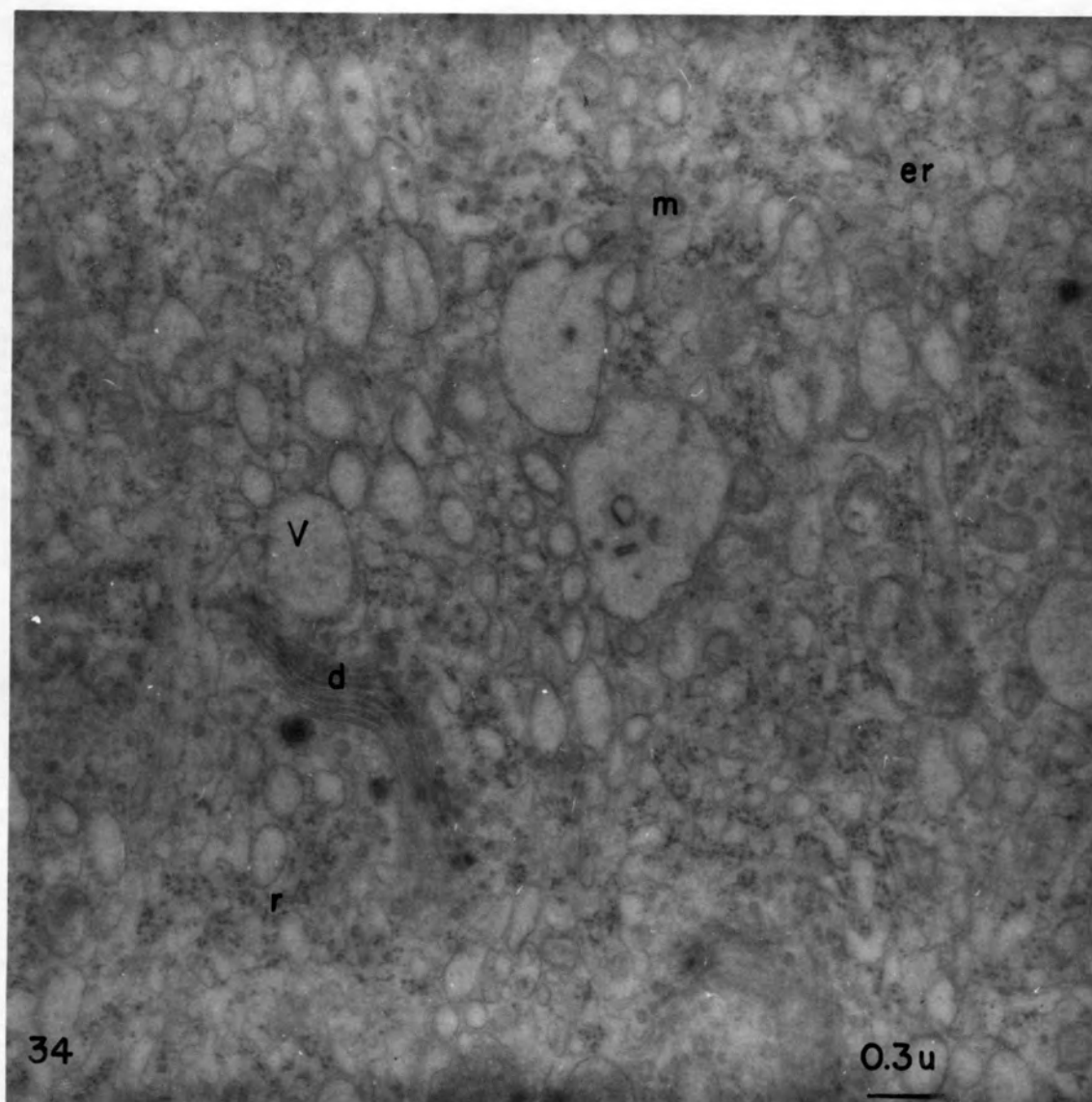   V . . . . vacuole

PLATE XXX

PLATE XXXI

Figure 35. Portions of two prismatic cells and a giant cell
(lower third of micrograph) to illustrate interlocking
of the surfaces of adjacent cells. The difference in
the degree of distention of the reticulum cisternae
in the two cell types is very apparent. The arrows
indicate small expanded regions of the intercellular
space.

Potassium permanganate fixation; araldite embedding.

X 21,000

ABBREVIATIONS

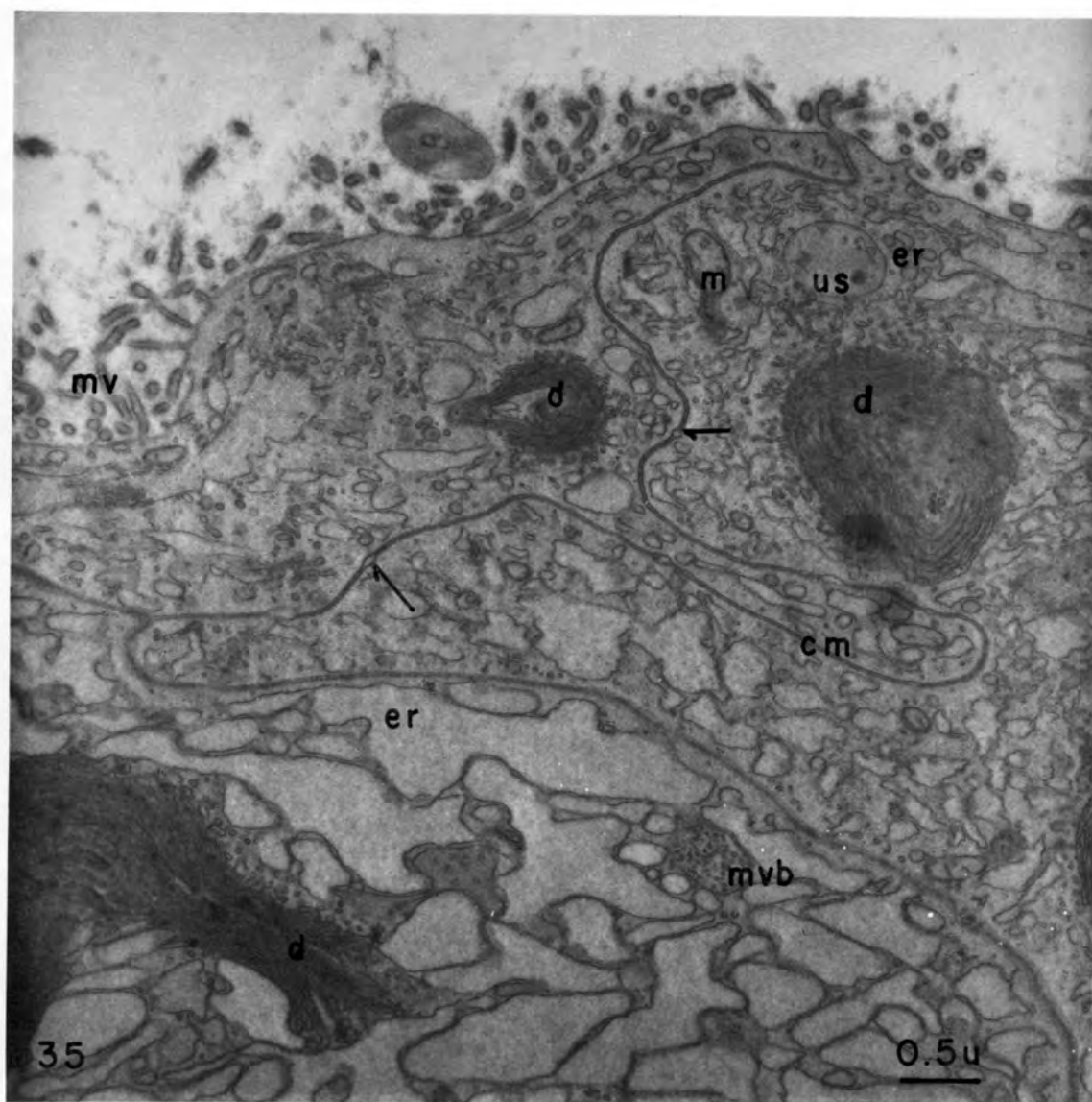|	
cm	cell membranes
d	dictyosome
er	endoplasmic reticulum
m	mitochondrion
mv	microvilli
mvb	multivesicular body
us	unidentified structure

PLATE XXXI

PLATE XXXII

Figure 36. A specialized region (intercellular attachment area)
of the intercellular boundary between two prismatic
cells appears in the centre of this micrograph (A).
A similar accumulation of rounded and elongate profiles
occurs between the cell boundary and the basement
membrane at lower right.

Potassium permanganate fixation; araldite embedding.

X 50,000

ABBREVIATIONS

A . . . . intercellular attachment
area
b . . . . basement membrane
cm . . . . cell membrane
er . . . . endoplasmic reticulum
g . . . . dense granules
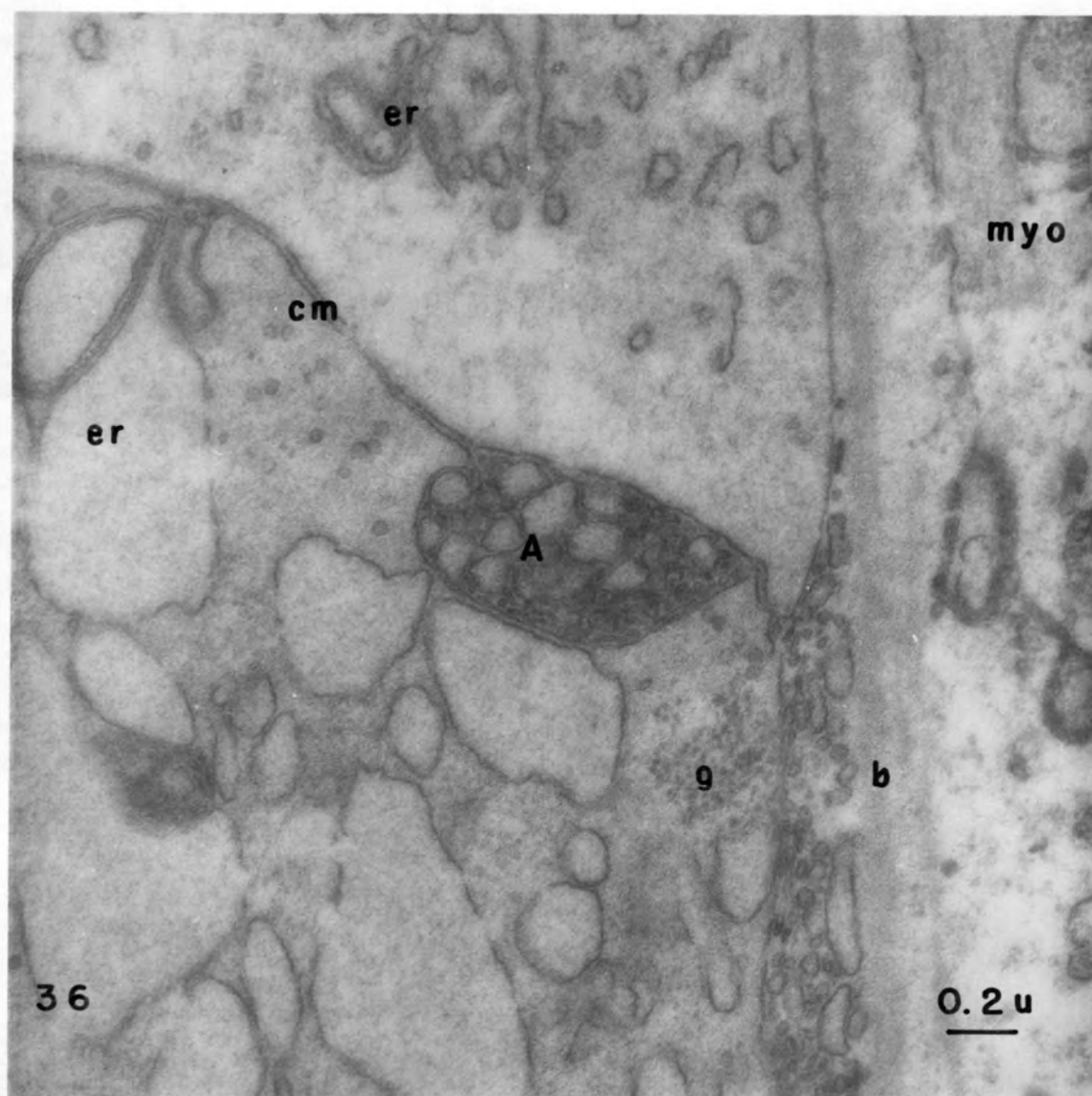myo . . . . myoepithelial cells

PLATE XXXII

PLATE XXXIII

Figure 37. A limited area of the cytoplasm of three prismatic cells
in the upper segment of the vas deferens. At the upper
left and lower right in the micrograph infolding of the
intercellular surfaces is apparent. The mitochondria
in this osmium fixed, methacrylate embedded specimen
exhibit a matrix density markedly higher than that of
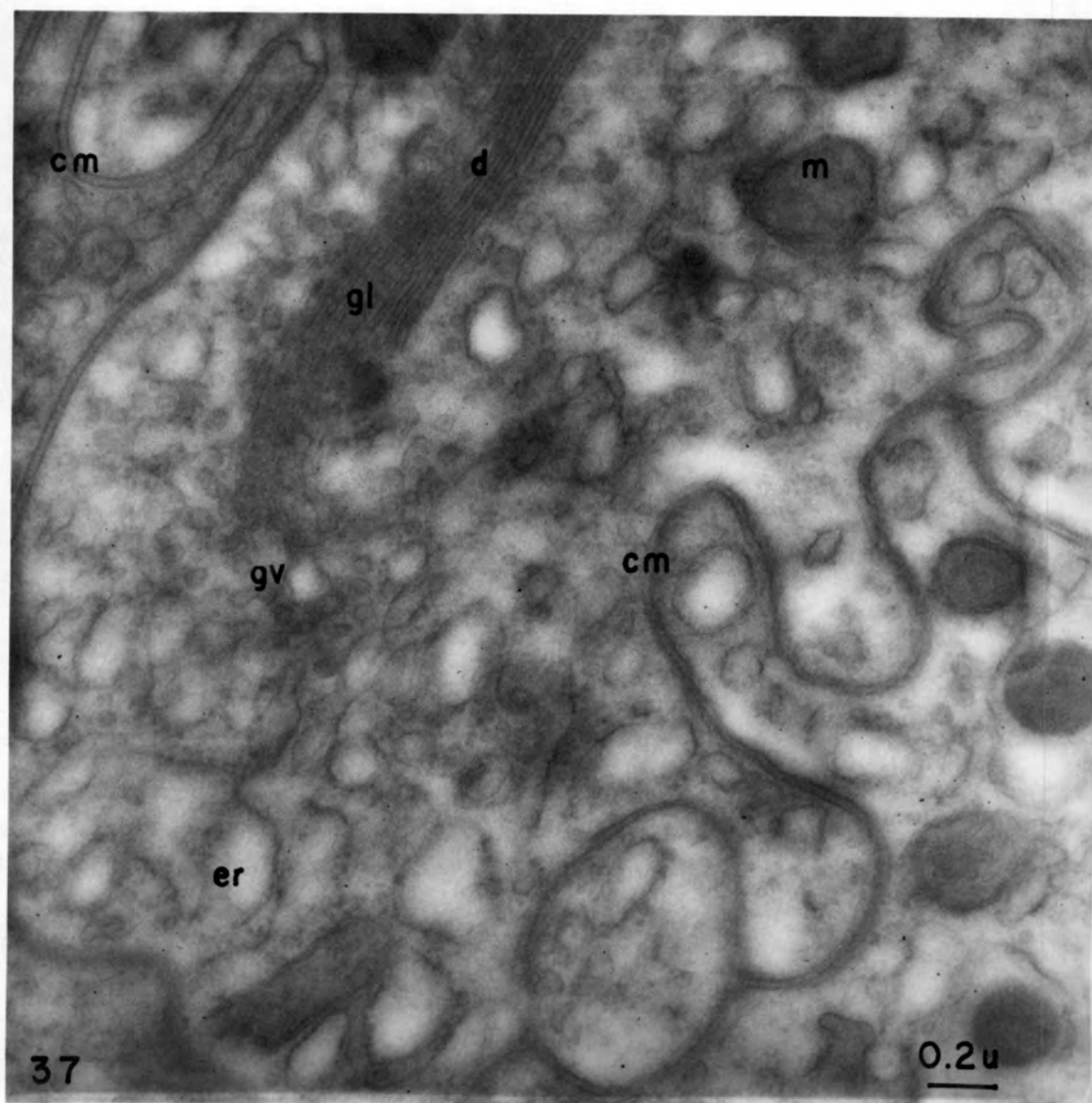the adjacent cytoplasm.

Osmium tetroxide fixation; methacrylate embedding.

X 50,000

ABBREVIATIONS

c . . . . cell membrane
d . . . . dictyosome
er . . . . endoplasmic reticulum
gl . . . . Golgi lamellae
gv . . . . Golgi vesicles
m . . . . mitochondrion

PLATE XXXIII

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