

An Electron Microscopic Study of the Ductuli Efferentes and Rete Testis of the Guinea Pig*

By AARON J. LADMAN, Ph.D.†, AND WILLIAM C. YOUNG, Ph.D.§

WITH THE TECHNICAL ASSISTANCE OF ARTHUR J. MITCHELL

(From the Department of Anatomy, Harvard Medical School, Boston)

PLATES 109 TO 119

(Received for publication, September 3, 1957)

ABSTRACT

The ductuli efferentes and rete testis of the guinea pig were isolated by micro dissection, fixed in cold buffered osmium tetroxide, and sectioned for examination with the light and electron microscopes.

Proximal and distal segments of the ductuli efferentes were identified and their respective cytological organizations characterized. The cytological components of the rete testis are briefly described and figured.

Non-ciliated and ciliated cells are found in both segments of the ductuli efferentes. The non-ciliated cells have a microvillous border, mitochondria, a Golgi complex, an ubiquitous endoplasmic reticulum, and numerous cytoplasmic vacuoles. The ciliated cells contain more mitochondria, an endoplasmic reticulum with a relatively sparse distribution, and few, if any, cytoplasmic vacuoles. A regional difference exists in proximal and distal segments based on the distribution, size, number, and electron opacity of the cytoplasmic vacuoles. Attention was paid to the disposition of the endoplasmic reticulum and its relation to the system of cytoplasmic vacuoles. These findings are interpreted as suggesting that the continuity of the vacuolar system with elements of the endoplasmic reticulum represents a pathway for transfer of large quantities of fluid, an activity which has long been ascribed to the epithelium of the ductuli efferentes.

Periductular capillaries possess pore-like apertures in their endothelia similar to those in other tissues known to engage in fluid transfer.

The capacity for reabsorption by the epithelial cells of the ductuli efferentes was first postulated by von Möllendorff (1920, 1922). He found that these cells store subcutaneously injected vital dyes such

as trypan blue or pyrrhol blue very much like the cells of the proximal convoluted tubules of the kidney. Direct evidence that reabsorption from the lumen occurs in the ductuli efferentes has never been provided, but indirect evidence is abundant and varied (van Wagenen, 1925; Wagenseil, 1928; Young, 1933; Mason and Shaver, 1952). With electron microscopy, a more detailed comparison of the ductuli efferentes with the nephron became possible, and it was anticipated that, even though only a degree of homology in function exists, some similarity in structure would be seen. That this expectation was partially realized will be evident from what follows, as it was from the data summarized in our preliminary abstract (Young and Ladman, 1957) and from a concurrent study on the hamster reported by Burgos (1957).

* This investigation was supported in part by research grant M-504(C4) from the National Institute of Mental Health of the National Institutes of Health, United States Public Health Service, and in part by research grant B903(C) from the National Institute of Neurological Diseases and Blindness of the National Institutes of Health, United States Public Health Service.

† United States Public Health Service Special Research Fellow, 1955-57.

§ Professor of Anatomy, University of Kansas School of Medicine. Population Council Fellow and Visiting Professor of Anatomy, Harvard Medical School, 1956-57.

Materials and Methods

Ten adult guinea pigs were used. Under light nembu-tal anesthesia the testes were removed from the scrotum through a midline abdominal incision. With the aid of a dissecting microscope the ductuli efferentes were located, the surrounding fat dissected away, proximal and distal segments identified, and excised separately with a sharp razor. Rete tubules were removed from one animal. The tissues were fixed immediately in cold (0–5°C.) buffered 1 per cent osmium tetroxide (Palade, 1956 a), containing 0.4 M sucrose. After 30 to 45 minutes, they were washed in distilled water for a few minutes, dehydrated within 2 hours in ascending concentrations of methanol, and then infiltrated with three changes of *n*-butyl methacrylate, the last change containing 2 per cent luperco CDB.¹ The specimens were embedded in partially polymerized methacrylate at 60°C., and polymerization was completed overnight at that temperature. Sections 25 to 50 μ in thickness were cut on a Porter-Blum, mechanically advanced, thin-sectioning microtome, picked up on carbon-coated and celloidin-covered copper grids, and examined in an RCA electron microscope EMU 2E without removing the plastic. The microscope was equipped with a 15 or 25 mil externally centerable condenser aperture and a 50 μ externally centerable objective aperture. The objective was compensated electrostatically. Original micrographs were taken at magnifications of 1,275 to 10,800 diameters, on Kodak contrast plates, and developed in Kodak D-19 or D-11 to enhance contrast.

Thicker sections (1 to 2 μ) from some of the blocks of ductuli efferentes were picked up on coverslips, dried, and stained by the periodic acid Schiff technique (PAS), and examined for the reaction of these tissues to this procedure.

In four animals the fat was dissected away and ligatures were placed around the proximal ends of the ductuli efferentes on one side, as a means of stopping the passage of fluid and spermatozoa from the seminiferous tubules into the ductuli efferentes. Three and 6 days later (two animals each), when the tissues were removed, the testes from the two sides were weighed; and parts of the ductuli efferentes distal to the ligature were prepared for electron microscopic study.

OBSERVATIONS

General Orientation:

The cytological features of the ductuli efferentes have been well described and beautifully illustrated in a variety of mammals, including the guinea pig (Benoit, 1926). In histological section

the ductuli efferentes are seen to possess a tall columnar epithelium consisting of ciliated and non-ciliated cells. These cells rest upon a distinct basement membrane beneath which scanty amounts of connective tissue, fibroblasts, some smooth muscle cells, and periductular capillaries are found. *In situ* these components are surrounded by an abundance of adipose tissue. Confirmatory evidence for this basic structure is provided by our work, but, as would be expected, the use of newer methods has contributed additional information with respect to the character of these cells.

An observation, which we feel is of considerable physiological importance, is the regional difference in structure between proximal and distal segments of the ductuli efferentes. The non-ciliated columnar cells of the proximal segment (Fig. 1) have many small supranuclear vacuoles (*v*) that are stained moderately or heavily by the PAS technique, and a prominently stained apical border. In the distal segment (Fig. 2), a greater number of cells contain vacuoles that are larger and relatively poorly stained or unstained. They are distributed more uniformly in the cytoplasm and are found both above and below the nucleus. In addition, an occasional cell is encountered that possesses many heavily staining globules in the supranuclear and apical regions and bears considerable resemblance to a goblet cell (Fig. 2,*).

Low magnification electron micrographs reveal clearly the features observable with the light microscope. In both segments, non-ciliated and ciliated cells are present (Figs. 3 to 5). The former possess a microvillous or brush border, numerous cytoplasmic vacuoles (*v*) of varying density, mitochondria (*m*), and a prominent basement membrane (*bm*). The ciliated cells often contain microvilli of irregular form (Fig. 9), few if any vacuoles, and a greater number of mitochondria.

A principal difference between the cells of the proximal and distal segments lies in the number, size, distribution, and electron opacity of the vacuolar component. In the former (Fig. 3), many vacuoles (*v*) of varying density are found in the supranuclear cytoplasm; in the latter, a large number of clear vacuoles is present both above and below the nucleus (Fig. 4). An extreme condition, encountered in cells of the distal segment, is illustrated in Fig. 5, where the vacuoles closest to the nuclei appear to have coalesced into large areas or lakes which are not bounded by sharp membranes. In the living state these electron transparent lakes

¹ Luperco CDB is a 1:1 mixture of 2,4-dichlorobenzoyl peroxide in dibutyl phthalate, manufactured by Novadel-Agene Corporation, Buffalo, New York.

are presumed to have a high fluid content that leaves little residue after osmium fixation. One may ask if the extreme form of the vacuolar structure in Fig. 5 represents the appearance *in situ* or is an artifact of preparative procedures. The argument in favor of artifact finds little support, however, for the plasma membranes of the cells and the mitochondrial cristae are intact, and the mitochondria are not vacuolated or exploded as they are when cytological preservation is poor.

Another form of non-ciliated cell, which is encountered only rarely in the distal segment, is depicted in Fig. 2 and is thought to resemble a goblet cell. The apical portion of one of these cells is shown in Fig. 15, in which a number of membrane-bounded globules containing moderate to intensely electron opaque materials is located in the lumen of a ductulus efferens in the vicinity of two spermatozoa. From the few observations of cells having this form, it may be premature to attempt to classify non-ciliated cells on the basis of their different appearances without first establishing that these differences are transitions or modifications of a single cell type in a different phase of its metabolic cycle. Tentatively, however, the possibility that there is more than one non-ciliated cell type must be considered.

Fine Structure:

1. *General.*—The apical regions of non-ciliated and ciliated cells are illustrated in Fig. 6. The principal cytoplasmic features of these two cell types are: in the former, a prominent striated, brush or microvillous border (*mv*, also Figs. 7 and 8) approximately 1 micron in width; a large, widely distributed apical population of tubular and vesicular elements of the endoplasmic reticulum (*er*) in its various forms², round or oval profiles of mitochondria (*m*); and many large, round or oval vacuoles (*v*) filled with light to moderately electron opaque material. In the ciliated cell, sections of very fine tubules representing profiles of elements

² In this presentation the dilated canaliculi opening between the microvilli are presumed to be an element of the endoplasmic reticulum and have been treated as such here. We are aware, however, that these structures may actually represent smooth surfaced tubular invaginations of the cell surface, a possibility which merits consideration and further investigation. Similar appearing apical canaliculi of the proximal convoluted tubule of the kidney have not been termed as elements of the endoplasmic reticulum.

of endoplasmic reticulum (also see Fig. 9) lie beneath the basal bodies. The exceedingly sparse endoplasmic reticulum as well as the small size of its elements imparts a more homogeneous appearance to this region of the cytoplasm than is found in the apical end of the non-ciliated cell (*cf.* Figs. 8 and 9). Mitochondria (*m*) similar in appearance to those in non-ciliated cells are more numerous in ciliated cells. Both cell types have conspicuous Golgi complexes (*gc*, also see Fig. 10). Terminal bars (*tb*), represented by increased densities of portions of the apically adjacent plasma membranes, are usually found.

The fine structural organization of the cilia conforms to that described by Fawcett and Porter (1954) for cilia of human and mouse oviducts. In favorable sections, lateral extensions or spurs protrude from the ciliary basal body (Fig. 9). We have also observed a tubular extension between the central pair of ciliary filaments and two lateral pairs of filaments (Fig. 16, upper arrow). The lower arrow in Fig. 16 shows a group of three smaller circular profiles at the site of a lateral pair of filaments. The plane of transverse section of this cilium was slightly closer to the basal body than the sectioned shaft of the cilium above, yet not below the level where a central pair of filaments is absent.

2. *Endoplasmic Reticulum.*—The well developed endoplasmic reticulum, in addition to the vacuolar system, is a principal characteristic of the non-ciliated cells. As indicated above, the endoplasmic reticulum is abundant in the apical cytoplasm. A portion of its total organization is viewed to better advantage in sections parallel to the cell's long axis (Fig. 7), where the elements of the endoplasmic reticulum are principally tubular in form and show much interjoining and considerable branching. The material within the confines of the membranes forming the walls of the tubules usually has a slightly greater electron density than the background cell cytoplasm; this material must be considered an integral part of the endoplasmic reticulum system as recently emphasized (Porter and Palade, 1957). The conspicuous intermingling of these tubular components is also a feature in the non-ciliated cells of the ductuli efferentes in hamsters (Burgos, 1957). Other extensive ramifications and interrelations of the endoplasmic reticulum are shown in Fig. 8. Many tubular elements (*er*) are easily identified. At numeral 1, several large crypt-like dilatations or cisternae open onto

the luminal surface at the bases of the microvilli. The membranes lining similar dilatations are also in continuity with the membranes limiting the fine tubular elements of the endoplasmic reticulum (numeral 2). The latter are usually smooth surfaced in this region of the cytoplasm. However, some tubules (numerals 3 and 4) have adherent to their membranes dense particles approximately 150 A in diameter, which have been described often and recently identified biochemically as RNA-rich particles by Palade and Siekevitz (1956) in their study of liver microsomes. The major portion of these particles is found free in the cytoplasm and is not associated with membranes. The latter statement also applies to the disposition of these particles in the apical cytoplasm of ciliated cells (Fig. 9).

At the supranuclear level, the elements of the endoplasmic reticulum are both vesicular and tubular (Figs. 10 and 11), and they are in intimate relation with the numerous vacuolar elements (*v*) in the cytoplasm, particularly in the cells of the distal segment (Figs. 10, 11, and 14). The relationship is considered more extensively below.

At the basal ends of the cells, the disposition of the endoplasmic reticulum is dependent on the segment of the ductulus efferens from which the section was taken. In the proximal segment (Fig. 12), elements of the endoplasmic reticulum appear vesicular or tubular with one or two flattened cisternae having dense RNA-rich particles adherent to their membranes. Many other dense particles are free in the basal cytoplasm. In a cell of the distal segment (Fig. 13), a tubular element (*er*) surrounds the vacuole (*v*). The apparent union of a tubular profile of the endoplasmic reticulum with the plasma membrane is at the left in Fig. 13.

3. *Mitochondria*.—The form and disposition of these organelles has been briefly noted. Their structure resembles that of the mitochondria in the cells of kidney tubules (Pease, 1955 *b, c*), although rarely are they as long and filamentous. Their long cristae are well defined and, in many micrographs, they traverse the width of the organelles (Figs. 10, 12, 14). In addition there are very small, extremely dense particulate bodies within the mitochondrial matrix resembling the dark bodies in mitochondria of the kidney and some other tissues (Figs. 10 and 12).

4. *The Relationship of the Endoplasmic Reticulum to the Vacuolar System*.—The possibility that the

endoplasmic reticulum establishes intimate relations with the system of large vacuoles in the cytoplasm of the non-ciliated cells has been mentioned. The nature of this relationship and the further association of the endoplasmic reticulum with the nuclear envelope and plasma membranes remain to be described.

In Fig. 14 a variety of forms and dispositions of the endoplasmic reticulum is represented. Profiles of elements of the reticulum can be seen between many vacuoles (upper left, *er*). In a particularly favorable area (upper vacuole, *v*) fine tubules (*er*) surround the right margin, and the membrane bounding the vacuole is continuous in some places with the walls of these tubules. This structural relationship, we feel, demonstrates the continuity of the vacuolar contents with the cavities enclosed by the membranes of the endoplasmic reticulum. At *er'*, two cisternal elements of the endoplasmic reticulum are in almost longitudinal section between vacuoles. Inasmuch as the vacuolar system is apparently connected to the tubular elements of the endoplasmic reticulum, we tend to regard the vacuoles as localized expansions of the reticulum, providing a greater volume for the accommodation of fluid materials.

The endoplasmic reticulum (*er''*), in proximity with the plasma membranes, shows union of the walls of the tubular elements with the plasma membranes and the confluence of their bounded spaces. A similar junction of endoplasmic reticulum and plasma membrane is visible in Fig. 13. Palade (1956 *b*) has illustrated the continuity of infolded plasma membranes with elements of the endoplasmic reticulum in macrophages. Similar relationships were found in striated muscle (Porter and Palade, 1957) and in cells of the mammalian choroid plexus (Wislocki and Ladman, 1958).

In the micrograph of a cell of a distal segment (Fig. 11) a portion of the nuclear envelope or perinuclear cisterna (*) is everted to form a small bleb, the contents of which resemble the material filling the many vacuoles near it. Watson (1955) has shown the direct continuity of the membranes forming the nuclear envelope with elements of the endoplasmic reticulum. On the basis of Watson's observations and the similarity of the material within the vacuoles and nuclear envelope (Fig. 11), the suggestion is made that the vacuoles have continuity with the nuclear envelope as well as with the other parts of the endoplasmic reticulum.

It appears evident that the vacuolar system of

the non-ciliated cells is one part of the ubiquitous internal membranous network comprising the endoplasmic reticulum. The cavities enclosed by the bounding membranes of this system have been observed to communicate with the intercellular space as well as with the lumen and to provide a means of facilitating the exchange of materials between the lumen, the cells, and the interstitial fluid.

5. *Connective Tissue*.—Relatively few elements of connective tissue enclose the epithelium of the ductuli efferentes. Fibroblasts, prominent reticular fibers, and an occasional smooth muscle cell are found beneath the moderately electron opaque component of the basement membrane (Figs. 1 to 4, 12, and 13). Arterioles are observed in the connective tissue, and a portion of one is shown in Fig. 17. Parts of three endothelial cells are separated from a smooth muscle cell (*smc*) by a basement membrane (*bm*). Another band of electron opaque material is continuous around the smooth muscle cell. Elements of endoplasmic reticulum (*er*) are more abundant in the endothelial cells than in the smooth muscle cell. In the endothelium of capillaries, fenestrations approximately 250 to 450 Å in diameter are found (Fig. 18).

In sections of the basal regions of cells, we have repeatedly looked for profiles which could be identified as sections of peripheral nerve. We were unable to convince ourselves, however, that any of the structures we saw were sections of nerve, and not portions of other cellular elements.

6. *Effects of Ligation*.—The fine structure of the ligated ductuli efferentes was so traumatized that our efforts to compare the number and appearance of the vacuoles on the operated and control sides were fruitless. Reparative processes may well require more than the 3 or 6 days which elapsed in our experiment.

TABLE I
*Effect of Ligation of Ductuli Efferentes on
Weight of Testis*

Animal	Interval after ligation	Ligated side	Control on opposite side	Differences between ligated and control testis
	<i>days</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
1	3	3.34	2.31	+1.03
2	3	2.34	1.66	+0.68
3	6	2.80	2.53	+0.27
4	6	2.60	2.37	+0.23

The effect on the testes, on the other hand, was clear, probably because there was no interference with the afferent and efferent vessels of this organ. Without exception, the testes on the operated side were more taut and larger than those on the unoperated side (Table I). Indications are, as van Wagenen (1925) found in the rat, that the peak in size is reached relatively soon after the operation, and by day 6, the decrease in size associated with atrophy has begun.

Rete Testis:

Examples of the epithelium of the rete are shown (Figs. 19 to 21). It is composed of rather low cuboidal cells with prominent round or oval nuclei and scant amounts of cytoplasm. Sometimes the cells are flattened (Fig. 20). The epithelium rests on a definitive basement membrane, with very few connective tissue cells or fibers subjacent to it. Lipid droplets are prominent in some of the cells (Fig. 20, *lip*). In Fig. 21 small round or elongate mitochondria possessing long prominent cristae are in the cytoplasm. A well developed endoplasmic reticulum (*er*) with tubular and cisternal elements is observed; many of these have dense RNA-rich particles attached to them. Elsewhere, an intimate relation or continuity with the plasma membrane is seen. Also figured here is an outline of a Golgi complex (*gc*). The over-all impression gained from the appearance of the cells is one of great cellular activity.

DISCUSSION

The essential structural features of the ductuli efferentes as revealed by electron microscopy are not unlike those described by Benoit (1926). The epithelium is composed of ciliated cells and cells possessing a brush border. The principal additions to our knowledge of the tissue, apart from those expected from the use of the higher resolutions, are explained by the changes in thought with respect to the function of these tubules since Benoit's careful study was made. At that time the non-ciliated cells were assumed to be secretory; the experiments suggesting that they might also be reabsorptive were not performed until later (Wagenseil, 1928; Young, 1933; Mason and Shaver, 1952). Interpretation of what we have seen is influenced by this newer development.

It is our opinion that the clear vacuoles, which are a dominating feature of the non-ciliated cells in the electron micrographs, correspond to the

large vacuoles given so much emphasis by Benoit. He describes them as located in the apical end of the cells, formed from granules of secretion which have been enlarged and dissolved, and destined to pass through the brush border into the lumen. Our interpretation is that these vacuoles are not the products of a secretory process. Instead, they are thought to contain fluid which has entered the cells from the lumen and is destined to traverse the fine tubular or canalicular system visible in electron micrographs and to leave the cells through the lateral and basal surfaces.

Definitive evidence for such a process should be provided by a reduced number of vacuoles in the cells from ductuli efferentes that had been separated from the testis. As we have noted, however, the trauma following this operation is severe, and we cannot be sure that the reduction in the number of vacuoles on the operated side is not partly the consequence of surgical injury *per se*. In our work better evidence for the hypothesis of reabsorption comes from the distention and increase in size of the testis following ligation of the ductuli efferentes. This operation does not interfere with the afferent and efferent vessels of the testis (Harrison, 1949), and the change in size is due to the accumulation of fluid within the blocked-off seminiferous tubules. In addition, when the head of the epididymis was ligated, the testis did not become turgid with fluid as it did when the ligature was placed around the proximal part of the ductuli efferentes (Young, 1933).

Information obtained from the microscopic study should be analyzed for evidence it may provide for reabsorptive activity. If the ductuli efferentes, derived from the embryonic mesonephros, are the site at which fluid leaving the seminiferous tubules reenters the blood, as do water and water-soluble substances in the nephron, some degree of structural similarity should be seen. As nearly as we can tell from a comparison of our micrographs with the published descriptions of the nephron, the resemblance is not great. Most striking is the fact that microvilli are present on the free surface of the non-ciliated cells as they are on the cells of the proximal convoluted tubule (Sjöstrand and Rhodin, 1953; Pease, 1955, *b, c*). In the kidney, Sjöstrand and Rhodin described manifold basal infoldings in the proximal tubule. Pease (1955 *b, c*) confirmed this observation and extended it to include the distal and collecting tubules. These workers believe that the highly

developed basal intussusceptions or interdigitations are important in the exchange of fluid. Recent analysis of serial sections by Ruska, Moore, and Weinstock (1957) add further details supporting this interpretation. Elaborate infoldings of the type found in the nephron have not been seen in the ductuli efferentes; lateral and basal interdigitations are numerous, but they are relatively inconsequential compared with those in the kidney. Many vacuoles in the cells of the ductuli efferentes resemble the clear vacuoles in the apical cytoplasm of the cells of the proximal convoluted tubules (Pease, 1955 *b, c*; Clark, 1957). It may also be significant that both types of vacuoles have openings onto the luminal surface. On the other hand, these electron transparent vacuoles in the ductuli efferentes are much larger and occupy much more of the cells. In many cells at the distal end of the tubules, supranuclear and infranuclear vacuoles are so numerous that the cell is almost filled with them.

As single items, the morphological evidence of a similarity in function is not convincing, but taken in the aggregate, the case is somewhat stronger. The ductuli efferentes as we see them in the adult mammal are far removed from the mesonephros of the adult amphibian (Fawcett, 1954), to say nothing of the tubular elements in the metanephros. The intracellular organization in the cells of the distal segment of the ductuli efferentes may represent a highly specialized stage in the disposition of fluid and solutes which bears only a vestigial resemblance to that in the kidney.

We would recall that the vacuolar system outlined above is continuous with a variety of elements of the endoplasmic reticulum. The continuity of the walls of small tubules of the endoplasmic reticulum with the bounding membranes of the vacuoles, as well as with the plasma membrane, has been pointed out. In one micrograph (Fig. 11), the junction of the vacuolar system with a dilatation of the nuclear envelope or perinuclear cisterna may be adduced. In view of these findings, we feel that the vacuolar system is a part of the endoplasmic reticulum which appears as local expansions or dilatations within the network. If this interpretation is correct, the endoplasmic reticulum in this cell type has taken a form which makes possible the mobilization of large amounts of fluid materials in the smallest cytoplasmic volume.

This accumulation of fluid and the extent of its occurrence in the distal ends of the tubules is

puzzling. An explanation of what is taking place should be sought, and the conditions under which this fluid collects in greater or lesser amounts than we have seen should be ascertained. In addition, the possibility of a connection between posterior pituitary antidiuretic substances and the water exchange suggested by the presence of the clear vacuoles and by the other data should be explored.

The regional differences in the structure of the proximal and distal segments of the ductuli efferentes are of interest. Those described in the present study are assumed to be related to the regional differences in the deposition of trypan blue (Young, 1933) and, like the latter, to be indicative of regional differences in function. Whether they are qualitative or merely differences in degree cannot now be decided. The fact that regional differences are found in the ductuli efferentes as well as in the renal tubules may be more than mere coincidence.

The perforations noted as occurring in the endothelium of the periductular capillaries are similar to the fenestrations in glomerular and in peritubular capillaries of the kidney (Pease, 1955 *a*, *c*; Yamada, 1955) and in the capillaries of the chorioid plexus (Maxwell and Pease, 1956; Wislocki and Ladman, 1958). Observations by Luft and Hechter (1957) point to the possibility that such pore-like interruptions may perhaps be related to tissue anoxia. They add, however, that judgment should be withheld until more data are available. To us it is interesting that these endothelial discontinuities are found in tissues related to the transport of large volumes of fluid.

BIBLIOGRAPHY

- Benoit, M. J., Recherches anatomiques, cytologiques et histophysiologiques sur les voies excrétrices du testicule, chez les mammifères, *Arch. Anat. Histol. et Embryol.*, 1926, **5**, 173.
- Burgos, M. H., Fine structure of the efferent ducts of the hamster testis, *Anat. Rec.*, 1957, **127**, 401.
- Clark, S. L., Jr., Cellular differentiation in the kidneys of newborn mice studied with the electron microscope, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 349.
- Fawcett, D. W., The submicroscopic structure of the kidney in *Rana pipiens*, *Anat. Rec.*, 1954, **120**, 733.
- Fawcett, D. W., and Porter, K. R., A study of the fine structure of ciliated epithelia, *J. Morphol.*, 1954, **94**, 221.
- Harrison, R. G., The comparative anatomy of the blood supply to the mammalian testis, *Proc. Zool. Soc. London*, 1949, **119**, 325.
- Luft, J., and Hechter, O., An electron microscopic correlation of structure with function in the isolated perfused cow adrenal, preliminary observations, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 615.
- Mason, K. E., and Shaver, S. L., Some functions of the caput epididymis, *Ann. New York Acad. Sc.*, 1952, **55**, 585.
- Maxwell, D. S., and Pease, D. C., The electron microscopy of the choroid plexus, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 467.
- von Möllendorff, W., Vitale Färbungen an tierischen Zellen, *Ergebn. Physiol.*, 1920, **18**, 141.
- von Möllendorff, W., Philipp Stöhr's Lehrbuch der Histologie und der mikroskopischen Anatomie des Menschen, Mit Einschluss der mikroskopischen Technik, **19**, Jena, Gustav Fischer, 1922.
- Palade, G. E., Intracisternal granules in the exocrine cells of the pancreas, *J. Biophysic. and Biochem. Cytol.*, 1956 *a*, **2**, 417.
- Palade, G. E., Studies on the endoplasmic reticulum. II. Simple dispositions in cells *in situ*, *J. Biophysic and Biochem. Cytol.*, 1956 *b*, **2**, 547.
- Palade, G. E., and Siekevitz, P., Liver microsomes. An integrated morphological and biochemical study, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 171.
- Pease, D. C., Electron microscopy of the vascular bed of the kidney cortex, *Anat. Rec.*, 1955 *a*, **121**, 701.
- Pease, D. C., Electron microscopy of the tubular cells of the kidney cortex, *Anat. Rec.*, 1955 *b*, **121**, 723.
- Pease, D. C., Fine structures of the kidney seen by electron microscopy, *J. Histochem. and Cytochem.*, 1955 *c*, **3**, 295.
- Porter, K. R., and Palade, G. E., Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 269.
- Ruska, H., Moore, D. H., and Weinstock, J., The base of the proximal convoluted tubule cells of rat kidney, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 249.
- Sjöstrand, F. S., and Rhodin, J., The ultrastructure of the proximal convoluted tubules of the mouse kidney as revealed by high resolution electron microscopy, *Exp. Cell Research*, 1953, **4**, 426.
- van Wagenen, G., Changes in the testis of the rat following ligation of the ductuli efferentia, *Anat. Rec.*, 1925, **29**, 399.
- Wagenseil, F., Experimentale untersuchungen am Nebenhoden der Maus, *Z. Zellforsch.*, 1928, **7**, 141.
- Watson, M. L., The nuclear envelope. Its structure and relation to cytoplasmic membranes, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 257.
- Wislocki, G. B., and Ladman, A. J., The fine structure of the mammalian chorioid plexus, *in* The Production, Circulation and Absorption of the Cerebro-

- spinal Fluid, a Ciba Foundation Symposium, (G. E. W. Wolstenholme and C. M. O'Connor, editors), Boston, Little Brown, 1958, in press.
- Yamada, E., The fine structure of the renal glomerulus of the mouse, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 551.
- Young, W. C., Die Resorption in der Ductuli Efferentes der Maus und ihre Bedeutung für das Problem der Unterbindung im Hoden-Nebenhodensystem, *Z. Zellforsch.*, 1933, **17**, 729.
- Young, W. C., and Ladman, A. J., The structural organization of the guinea pig's ductuli efferentes as revealed by electron microscopy, *Anat. Rec.*, 1957, **127**, 490.

EXPLANATION OF PLATES

Abbreviations

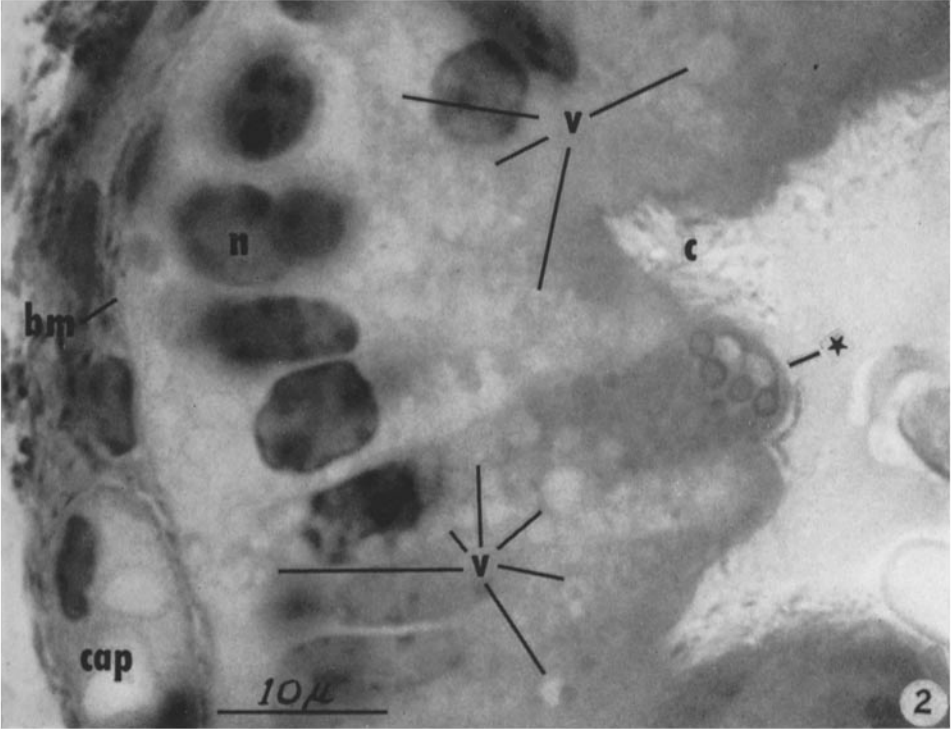
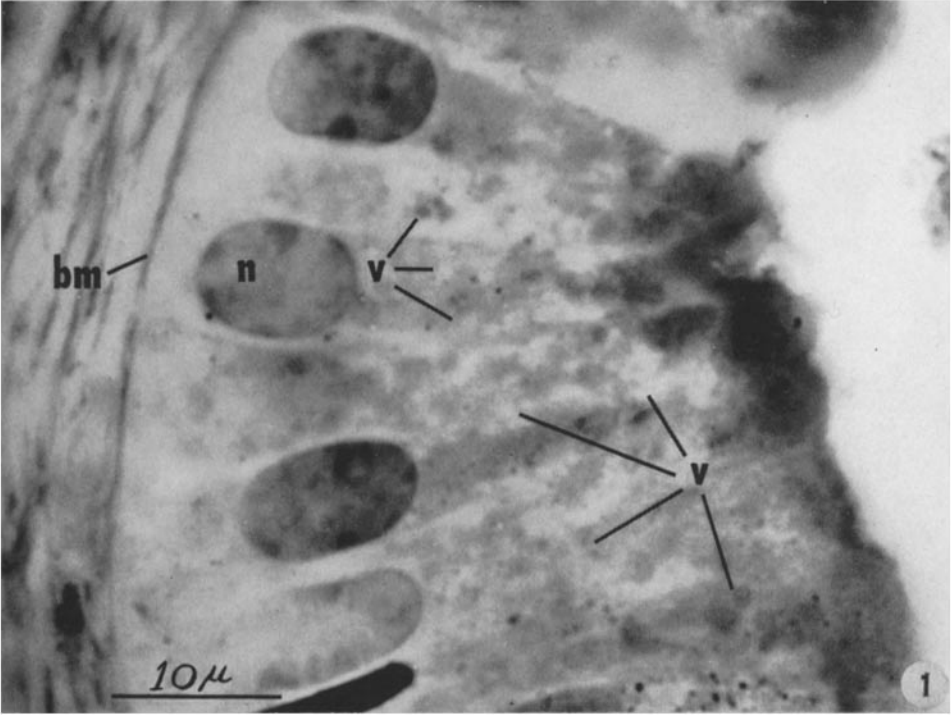
<i>bm</i> , basement membrane.	<i>mv</i> , microvillus.
<i>c</i> , cilia.	<i>n</i> , nucleus.
<i>cap</i> , capillary.	<i>ncl</i> , nucleolus.
<i>er</i> , endoplasmic reticulum.	<i>np</i> , nuclear pore.
<i>gc</i> , Golgi complex.	<i>pm</i> , plasma membrane.
<i>lip</i> , lipide droplet.	<i>smc</i> , smooth muscle cell.
<i>lu</i> , lumen.	<i>sp</i> , ciliary spur.
<i>m</i> , mitochondria.	

The solid line in each figure represents one micron unless otherwise indicated.

PLATE 109

FIG. 1. A light micrograph of a thick (1 to 2 μ) section of the proximal part of a ductulus efferens fixed in buffered osmium tetroxide and stained with periodic acid Schiff (PAS) and hematoxylin, showing numerous vacuoles (*v*), some of which are stained by the PAS method (compare with Fig. 3). The apical surfaces of the cells at the right are stained by PAS, as is the basement membrane (*bm*). *n*, nucleus. $\times 2000$.

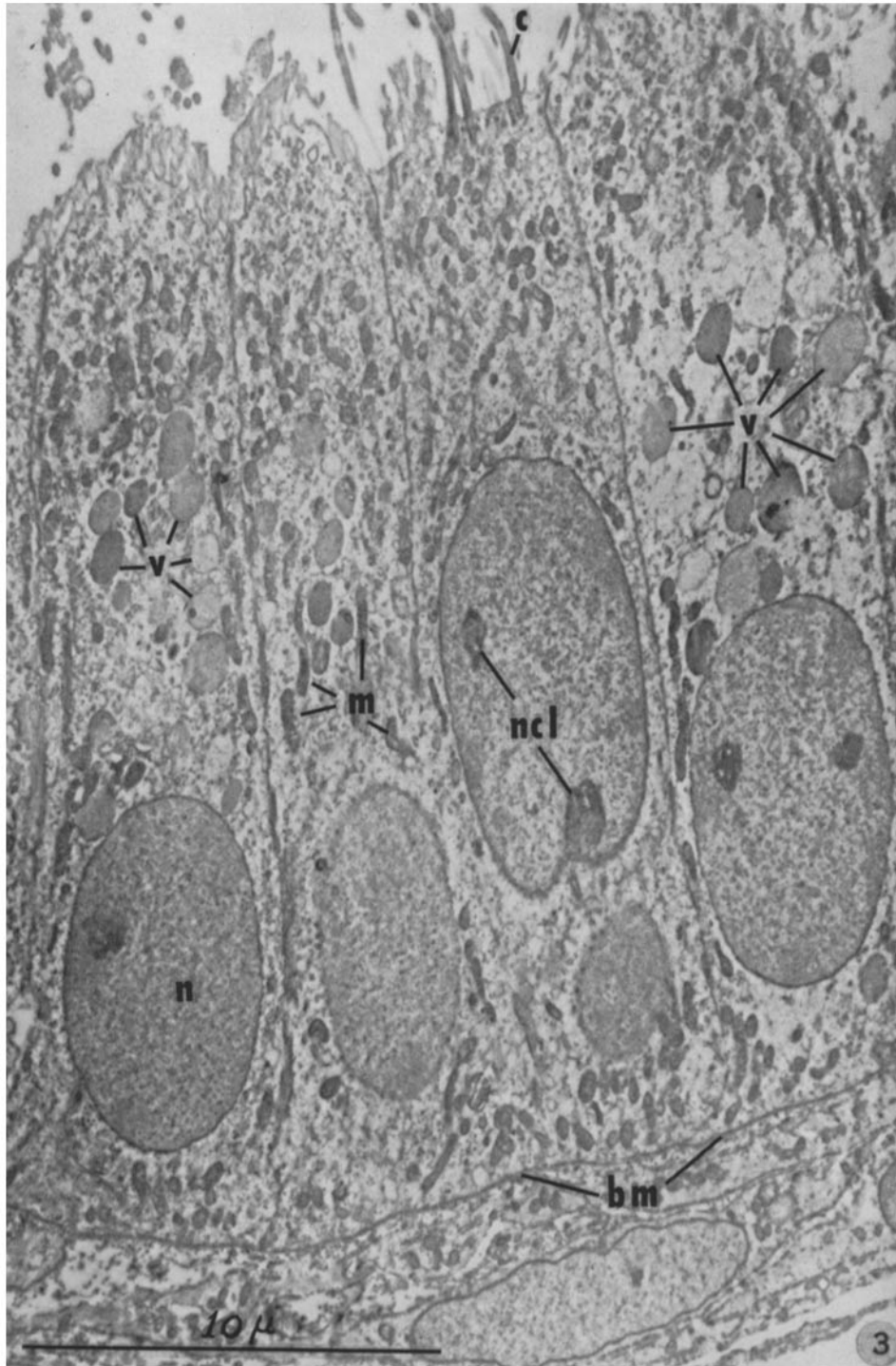
FIG. 2. A light micrograph of a section similar to Fig. 1, taken from the distal part of a ductulus efferens. Note the greater number of vacuoles (*v*), their larger size, and the tendency to a more uniform distribution within the cytoplasm, *i.e.* below as well as above the nucleus (compare with Figs. 4 and 5). In an occasional cell (*) the apical portion is filled with many vacuoles which display a prominent PAS reaction. $\times 2000$.



(Ladman and Young: Ductuli efferentes and rete testis)

PLATE 110

FIG. 3. An electron micrograph of the proximal part of a ductulus efferens, showing the distribution of cytoplasmic constituents in the ciliated and non-ciliated cells. The tall columnar cells rest on a well defined basement membrane (*bm*). The nuclei (*n*) are situated in the lower third of the cells and possess one or two prominent nucleoli (*ncl*). Mitochondria (*m*) are distributed throughout the cytoplasm. Numerous vacuoles (*v*) containing material which varies in the degree of its electron opacity are usually located in a supranuclear position in the non-ciliated cells. These cells have a prominent striated or microvillous border. The ciliated cells possess a greater number of mitochondria (see Fig. 6) and few, if any, vacuoles. $\times 6000$.



(Ladman and Young: Ductuli efferentes and rete testis)

PLATE 111

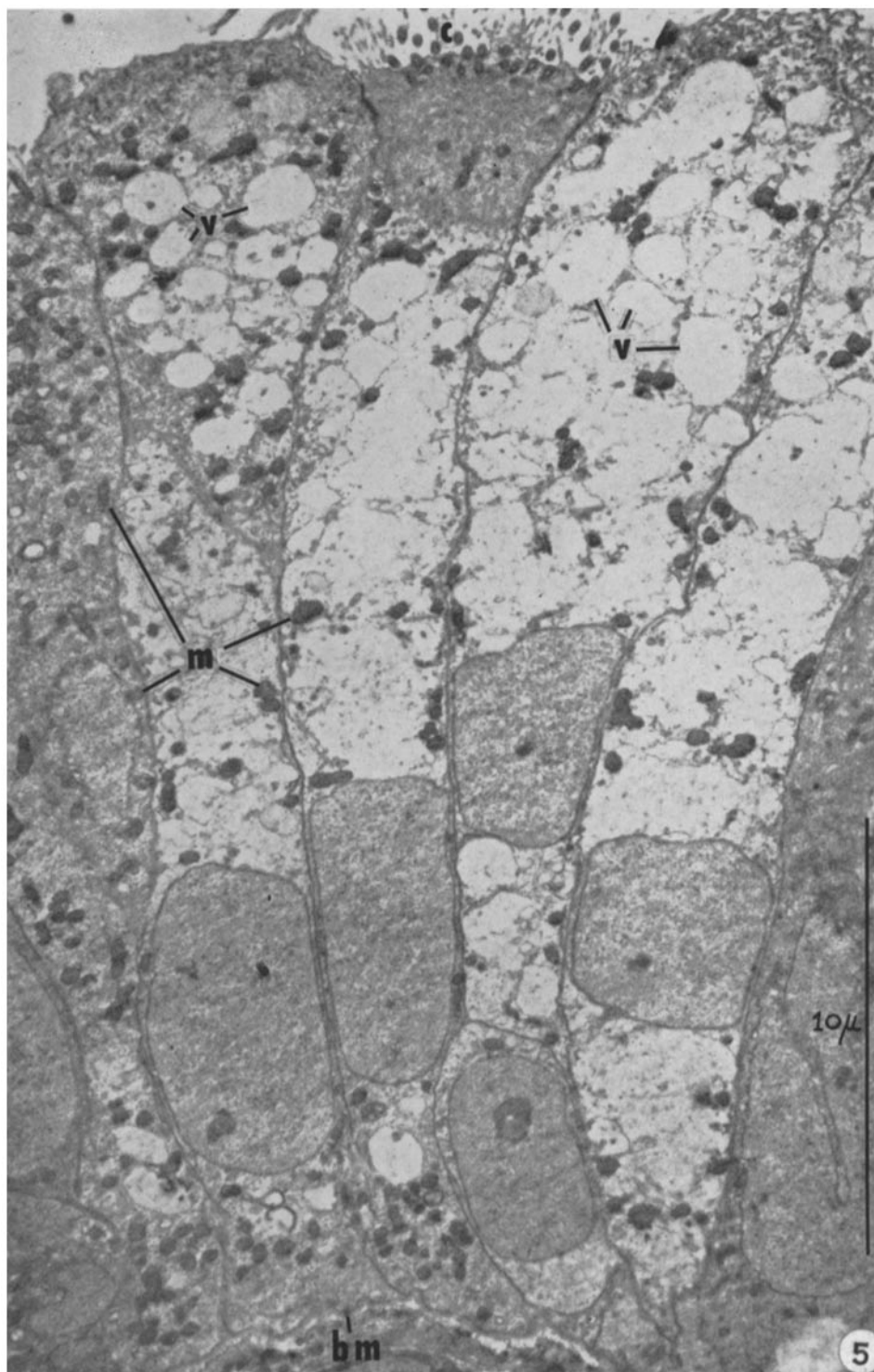
FIG. 4. An electron micrograph of the distal part of a ductulus efferens, showing two types of cells in a slightly oblique section. The apical end of the non-ciliated cell containing large numbers of relatively clear vacuoles (*v*) is not shown, although a portion of another non-ciliated cell with numerous tubular components of the endoplasmic reticulum (*er*) can be identified. The vacuoles in the former cell have a basal distribution in addition to their supranuclear location. The vacuoles (*v*) in the non-ciliated cell at the right have a moderate electron opacity. Apical portions of two ciliated cells bordering the non-ciliated cell show large numbers of mitochondria, as well as ciliary basal bodies. $\times 7,000$.



(Ladman and Young: Ductuli efferentes and rete testis)

PLATE 112

FIG. 5. Another view of the distal part of a ductulus efferens, showing the apparent loss of continuity of some of the limiting membranes of the vacuoles (*v*) in the areas closest to the nucleus. $\times 6000$.

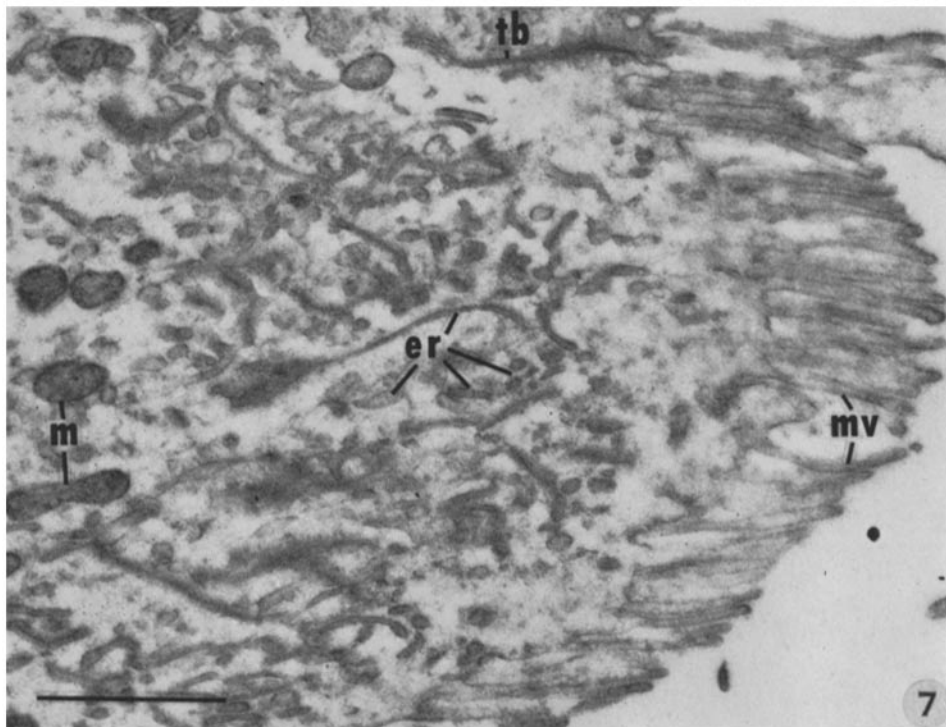
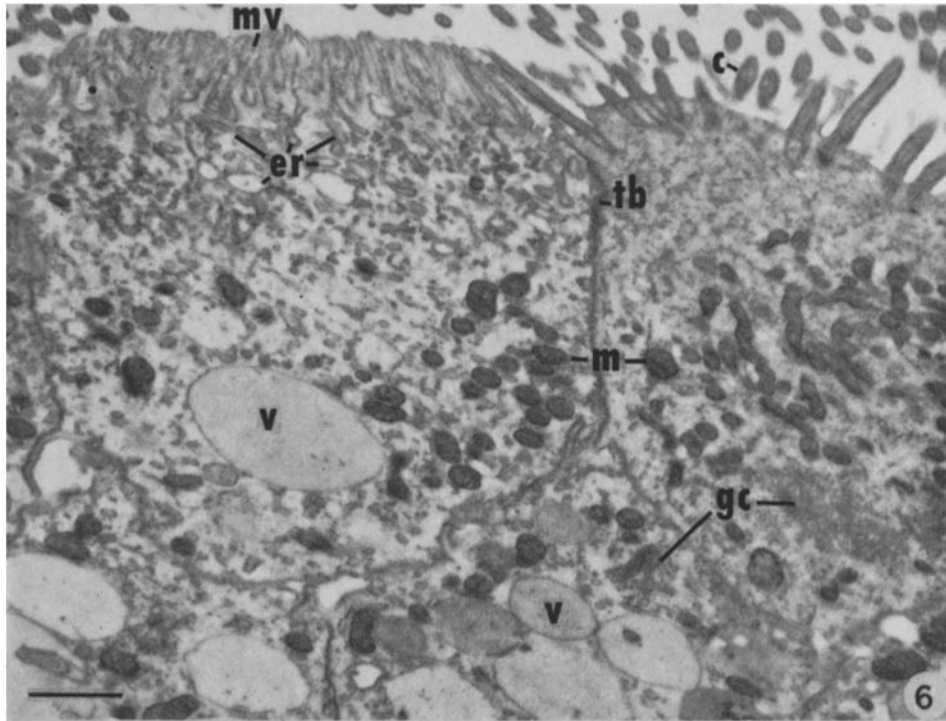


(Ladman and Young: Ductuli efferentes and rete testis)

PLATE 113

FIG. 6. Structural organization of the apical portions of a ciliated and a non-ciliated cell. The non-ciliated cell has a distinct microvillous border (*mv*), immediately beneath which are numerous dilated cisternal, as well as tubular elements of the endoplasmic reticulum (*er*), mitochondria (*m*), and some vacuoles (*v*). In the ciliated cell, sections of very fine tubules representing the endoplasmic reticulum are seen beneath the basal bodies of the cilia; mitochondria are more plentiful. Parts of two Golgi complexes are indicated (*gc*), as is a terminal bar (*tb*) between the cells. $\times 13,000$.

FIG. 7. A more highly magnified portion of a non-ciliated cell, showing the microvillous border, long intertwining and anastomosing tubules of the endoplasmic reticulum (*er*), some mitochondria (*m*), and a terminal bar (*tb*). $\times 25,000$.

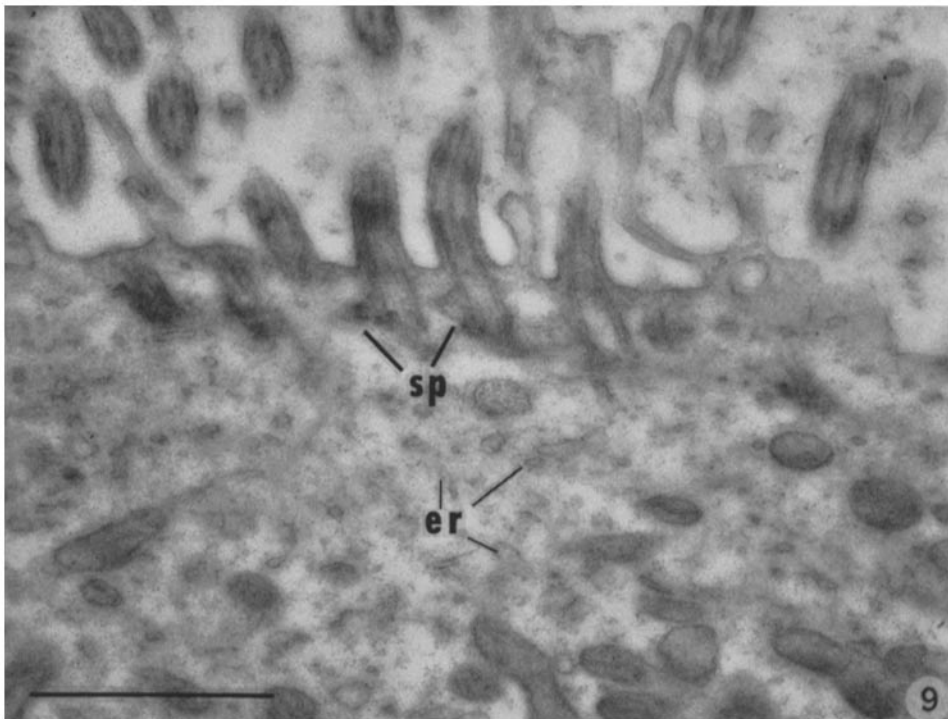
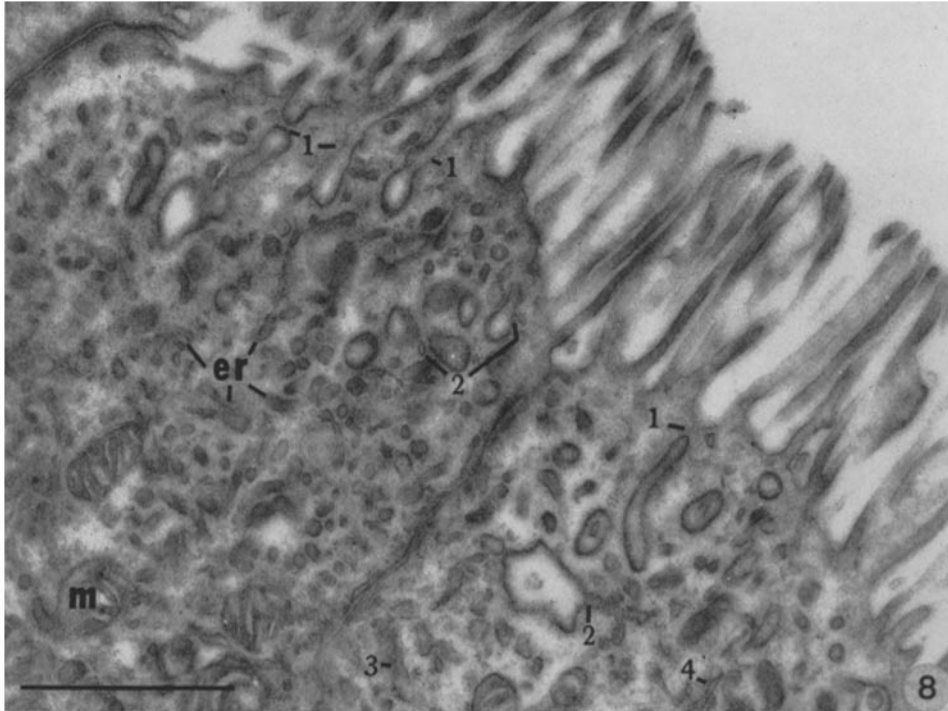


(Ladman and Young: Ductuli efferentes and rete testis)

PLATE 114

FIG. 8. Apical ends of non-ciliated cells. The microvilli are extensions of the cytoplasm bounded by plasma membranes. Between the microvilli, the walls of large cisternae of the endoplasmic reticulum are continuous with the plasma membrane (1). Deeper in the cytoplasm these large cisternae communicate with the small tubular elements of the endoplasmic reticulum (2). Some of these tubular elements, which are usually smooth surfaced, bear dense particles on their membranes (3), whereas others are wholly covered with particles (4). $\times 28,000$.

FIG. 9. Apical end of a ciliated cell, showing the basal bodies of the cilia with their lateral spurs, the paucity of elements of the endoplasmic reticulum (*er*), and occasional microvilli. $\times 32,000$.

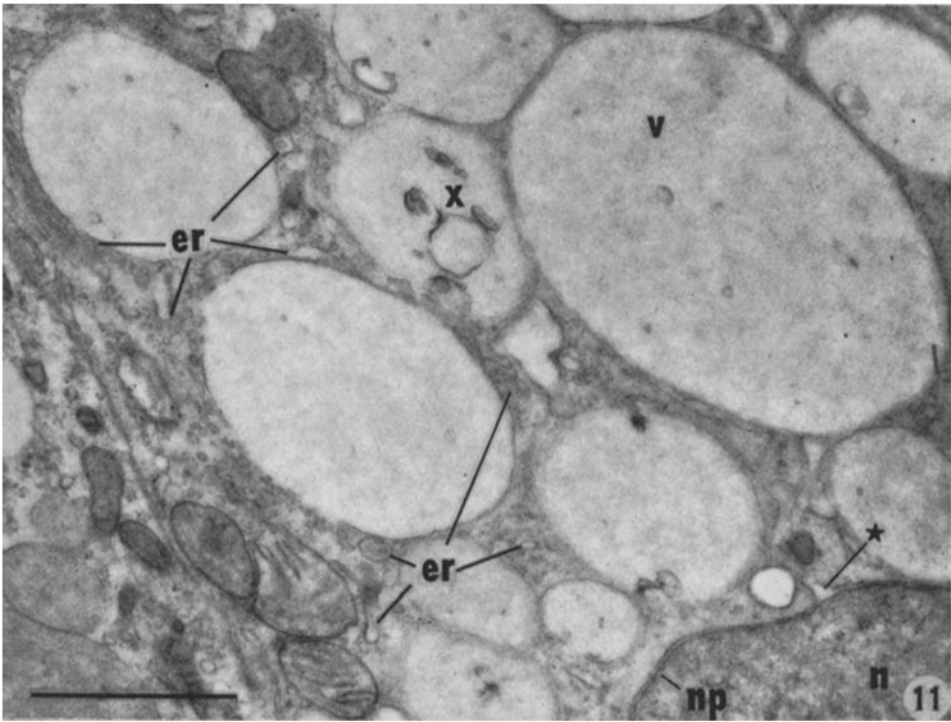
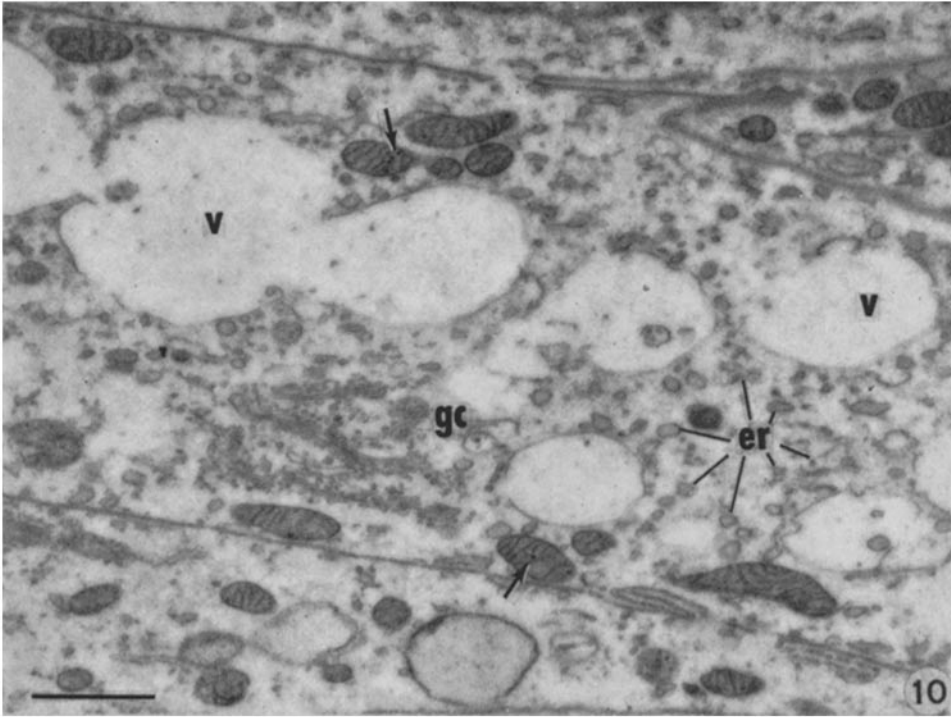


(Ladman and Young: Ductuli efferentes and rete testis)

PLATE 115

FIG. 10. Supranuclear portion of a non-ciliated cell, showing a well formed Golgi complex (*gc*), profiles of tubules of endoplasmic reticulum (*er*), confluent vacuoles and interdigitations of adjacent plasma membranes (lower right). The arrows point to dense bodies within the mitochondrial matrix. $\times 16,000$.

FIG. 11. Another supranuclear portion of a non-ciliated cell, showing a nuclear pore (*np*), numerous vacuoles (*v*) surrounded by profiles of endoplasmic reticulum (*er*), and occasional membranes found in the vacuoles (*x*). At the star a portion of the nuclear envelope is everted, and the enclosed substance shows electron opacity similar to that of the surrounding vacuoles. $\times 27,000$.

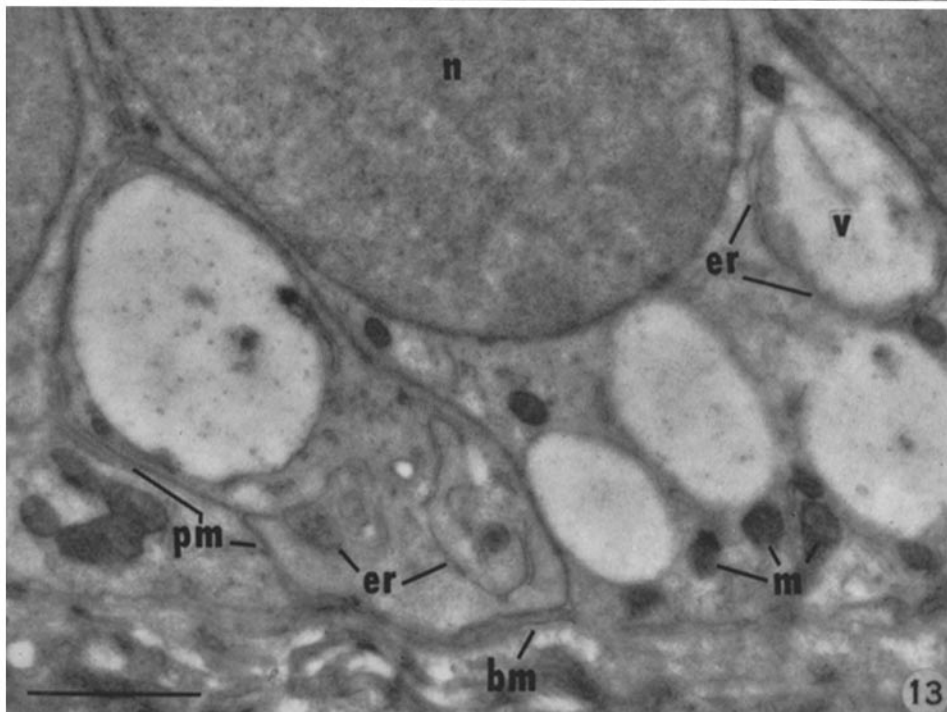
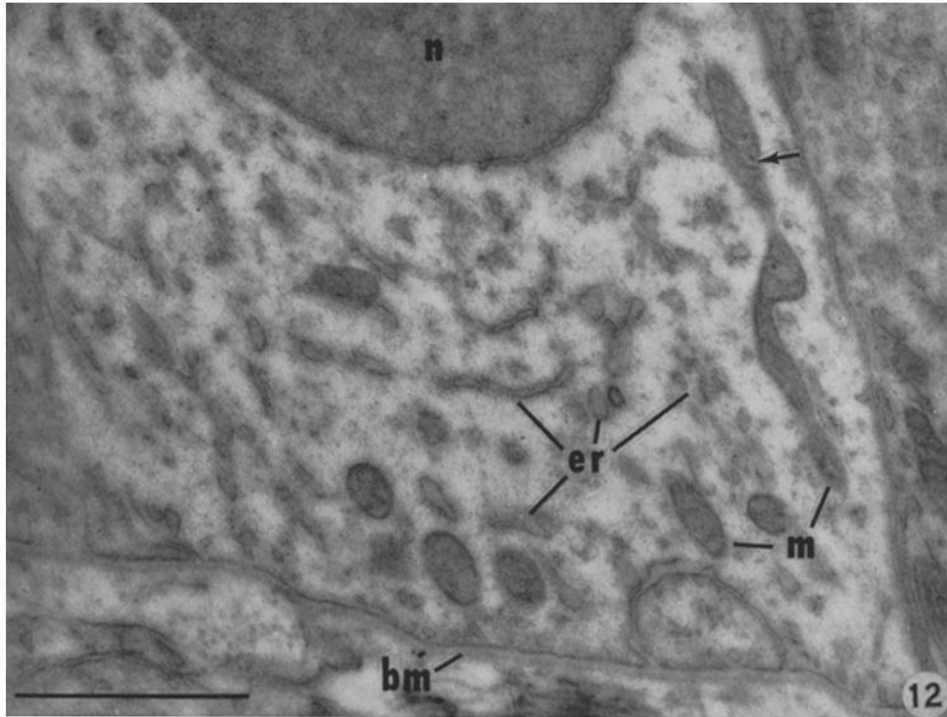


(Ladman and Young: Ductuli efferentes and rete testis)

PLATE 116

FIG. 12. Basal end of a cell from the proximal part of a ductulus efferens, showing profiles of the tubular components of the endoplasmic reticulum (*er*), mitochondria (*m*), and the prominent basement membrane. Some of the membranes of the endoplasmic reticulum possess the dense RNA-rich particles. Many of these are also found free in the cytoplasm. A portion of the interdigitation with an adjacent cell is shown at the lower right. The arrow points to a dense body within the mitochondrial matrix. $\times 31,000$.

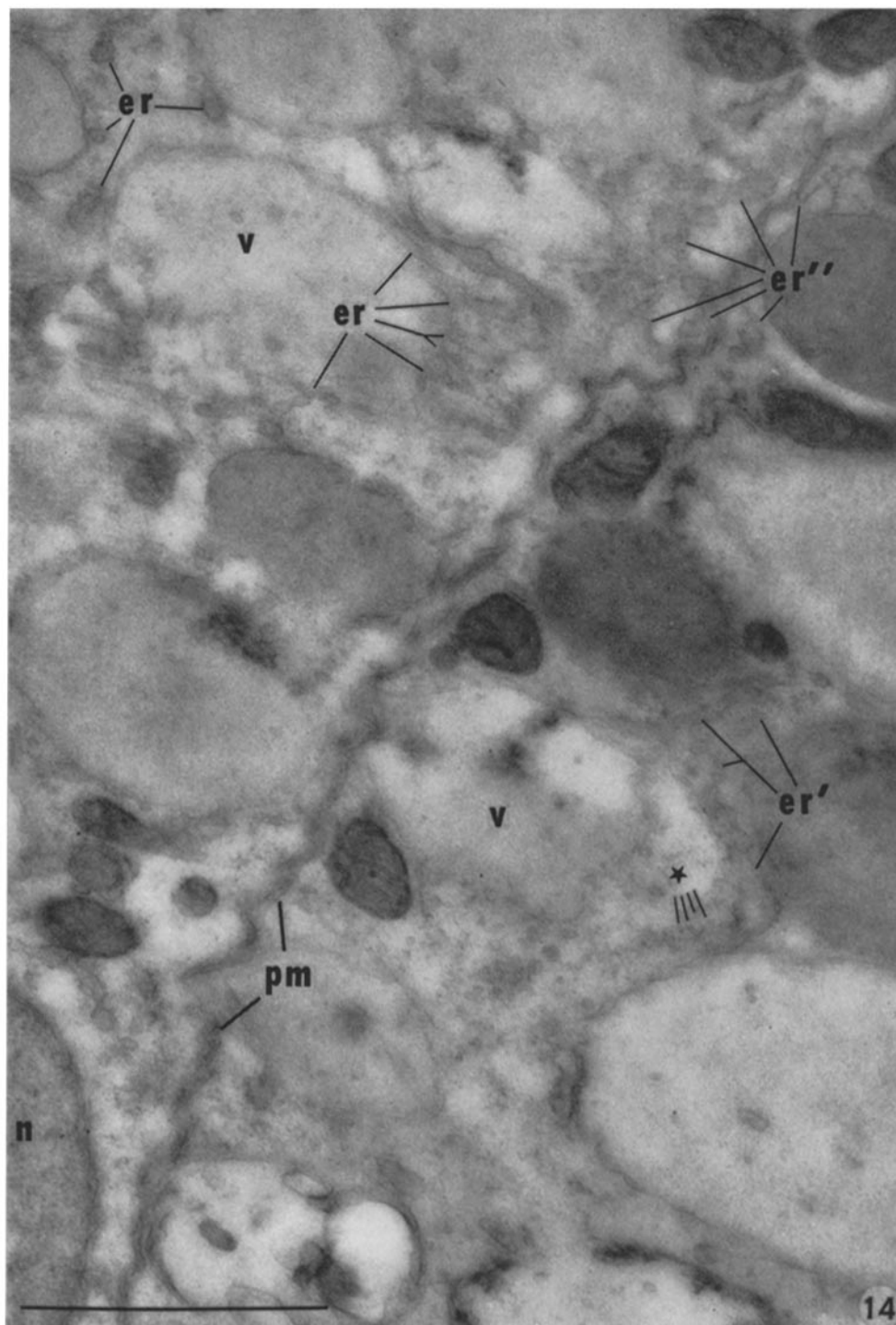
FIG. 13. Basal end of a cell from the distal part of a ductulus efferens, showing large vacuoles and the distribution of elements of endoplasmic reticulum about the vacuoles. The membranes of the endoplasmic reticulum (*er*) appear to be continuous with the infolding of the plasma membrane (*pm*) shown at the lower left. $\times 23,000$.



(Ladman and Young: Ductuli efferentes and rete testis)

PLATE 117

FIG. 14. Supranuclear portions of two cells in the distal end of a ductulus efferens, showing the relations of various parts of the endoplasmic reticulum to the vacuoles and plasma membrane. At the upper left, profiles of the tubular components of the endoplasmic reticulum (*er*) are shown between these vacuoles. At the right margin of the upper vacuole labelled *v*, parts of the tubular network of the endoplasmic reticulum (*er*) appear to open into the substance of the vacuole. At *er'*, the course of the larger tubules or cisternae of endoplasmic reticulum form intercommunications between the vacuoles, and passing between the larger tubules to the vacuoles are smaller canaliculi, approximately 90 to 110 Å in diameter (*). In favorable places along the plasma membrane, the walls of many tubules as well as fine canaliculi of endoplasmic reticulum (*er''*) are in continuity with the plasma membrane. The substance (*s*) enclosed by the membranes of the endoplasmic reticulum, and thus presumably the vacuolar contents, is thereby rendered easily exchangeable with the material of the intercellular space. $\times 44,000$.



(Ladman and Young: Ductuli efferentes and rete testis)

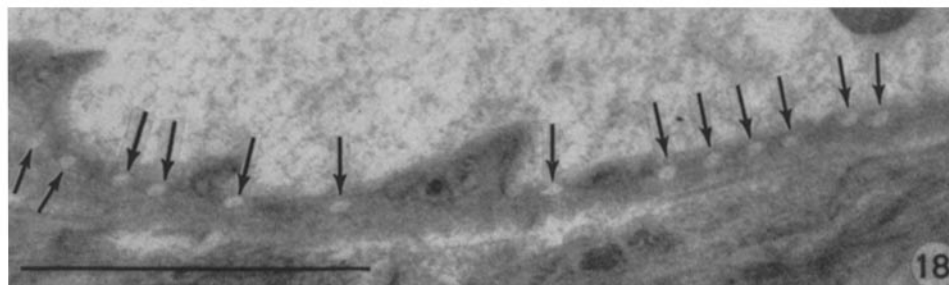
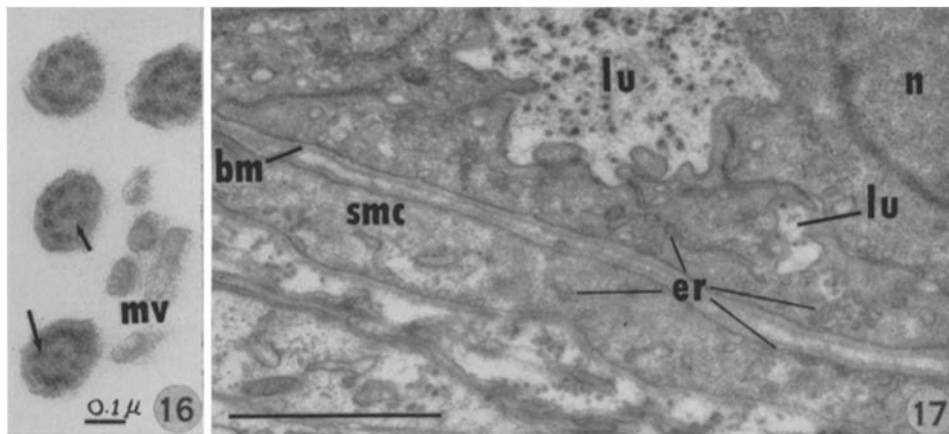
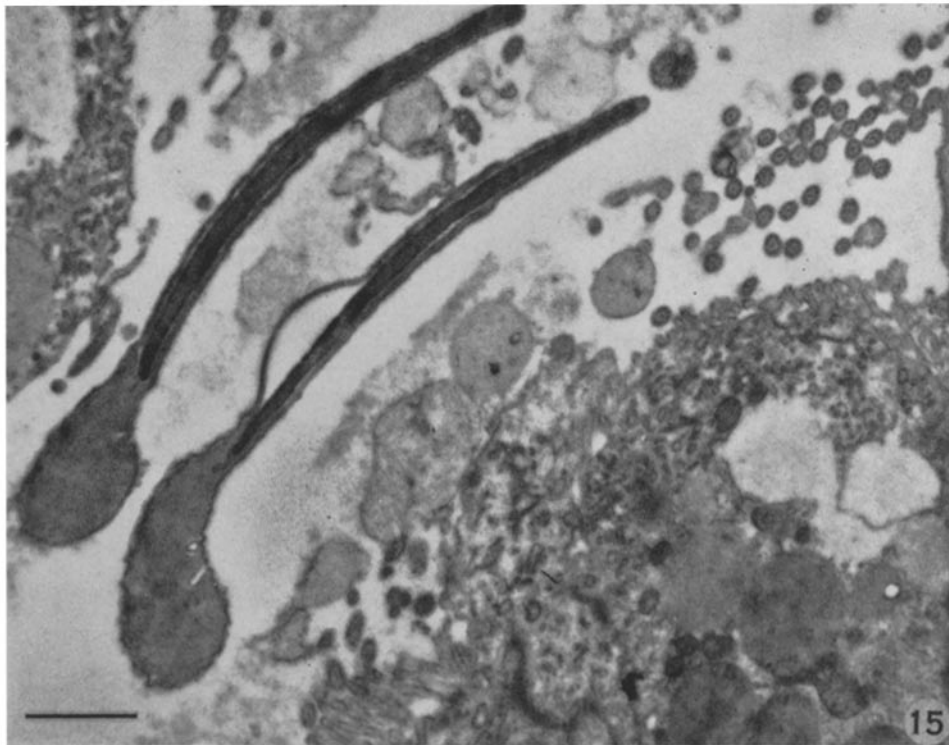
PLATE 118

FIG. 15. Section through a ductulus efferens, containing two spermatozoa in the lumen, and showing transverse sections of some cilia (upper right) and membrane-bounded, moderately dense globules which may be a secretory product of the cells. $\times 15,000$.

FIG. 16. Higher magnification of cilia and microvilli, showing the well known pairs of peripheral fibrils, an association of the central pair of fibrils with two groups of peripheral pairs (upper arrow), and, at a plane of section nearer the base of the cilium, the separation of fibrils into more numerous smaller tubular components (lower arrow). $\times 52,000$.

FIG. 17. Small arteriole, showing portions of three endothelial cells, an underlying smooth muscle cell (*smc*), and surrounding basement membranes (*bm*). Endoplasmic reticulum (*er*) is more extensive in the endothelium than in the smooth muscle cell. $\times 28,000$.

FIG. 18. An enlarged portion of the wall of a peritubular capillary, showing the fenestrated appearance of the endothelium (arrows). These pore-like structures measure approximately 250 to 450 Å in diameter. $\times 47,000$.



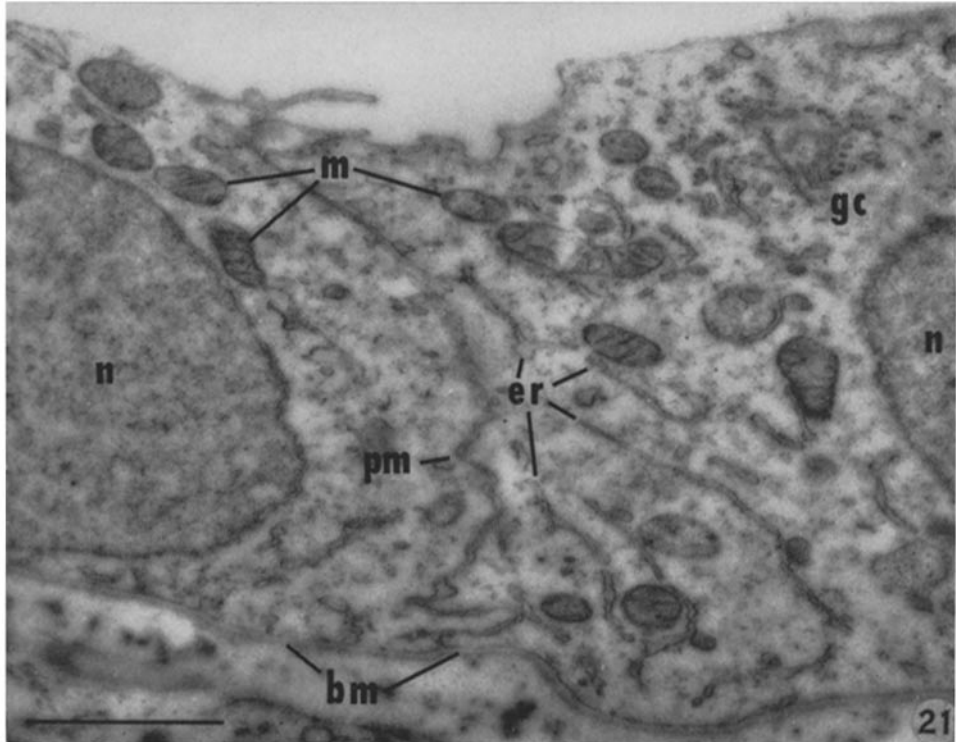
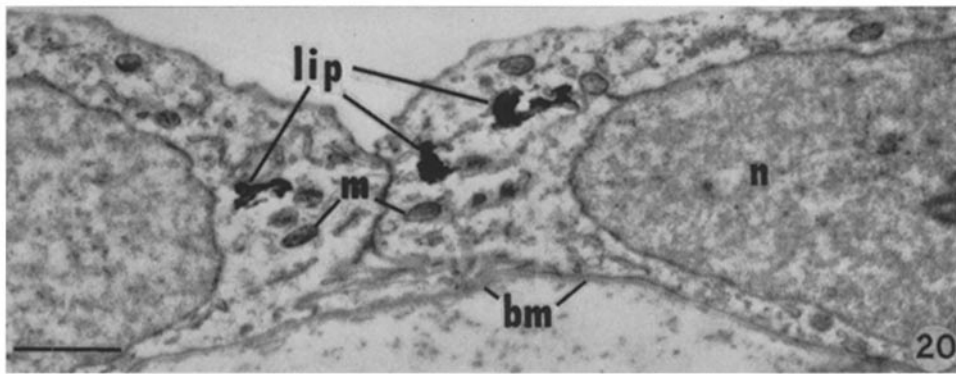
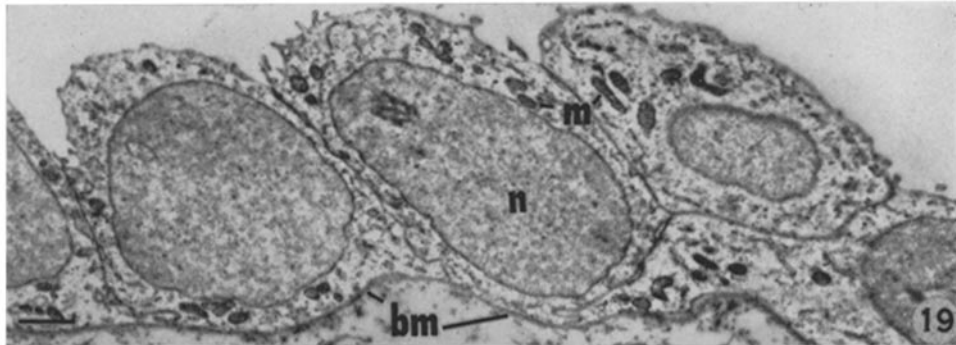
(Ladman and Young: Ductuli efferentes and rete testis)

PLATE 119

FIG. 19. Lower magnification of the rete testis showing the low cuboidal epithelium resting on a basement membrane. $\times 7000$.

FIG. 20. Higher magnification showing lipide droplets (*lip*) in some of the cells. $\times 14,000$.

FIG. 21. Two adjacent cells from the rete testis revealing mitochondria (*m*), a portion of a Golgi complex (*gc*), and a rather extensive endoplasmic reticulum (*er*). The latter in many places possesses dense RNA-rich particles. Portions of the endoplasmic reticulum appear to be continuous with the plasma membrane (*pm*). $\times 27,000$.



(Ladman and Young: Ductuli efferentes and rete testis)