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Light microscopic features of the rete testis, the vas efferens, the epididymis and the vas deferens in the adult rhesus monkey

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Abstract. The present study was carried out to determine the detailed histological and cytological features of the excurrent ducts of the male reproductive system in the rhesus monkey. The excurrent ducts show a regional difference in their histological features. The use of some of these features as histological markers and their possible functional significance are discussed.

The epithelial cells in the different components of the excurrent duct system possess cytological features which suggest their involvement in absorption and the secretion of different products into the lumen.

Keywords. Rhesus monkey; rete testis; vas efferens; opididymis; vas deferens.

Introduction

The rete testis, the vas efferens, the epididymis and the vas deferens constitute a part of the excurrent ducts of the male reproductive system. These ducts not only subserve a simple function of conducting spermatozoa from the testis but they also play a more sophisticated role in altering the milieu of the spermatozoa (Hamilton, 1975). There is reason to believe that as a result of this latter function of the excurrent ducts, spermatozoa undergo a process of maturation and acquire the ability to fertilise ova.

The microscopic structure of the excurrent ducts has been a subject of much interest and several studies have been carried out to identify the histological and cytological features associated with their diverse functions (Benoit, 1926; Reid and Cleland, 1957; Nicander, 1957a, b; Glover and Nicander, 1971; Hamilton, 1975; Hoffer, 1976; Kormano, 1977). Studies carried out so far have shown that although the general histological features of the excurrent ducts are essentially similar between species, there are subtle differences in their detailed cytological features.

The rhesus monkey is one of the species of non-human primates widely used in experimental studies aimed at understanding reproductive processes occurring in

Abbreviations : periodic acid Schiffs reagent, PAS.

man. All the same, little is known about the histological features of the excurrent ducts of the male reproductive system in this species, although, some histochemical (Arora–Dinakar *et al.*,1977a, b, c) and ultrastructural (Dym, 1974; Ramos and Dym, 1977) studies have been carried out. The present investigation was undertaken to fill this gap and to form a basis for further structural studies on excurrent ducts in the rhesus monkey.

Materials and methods

Six adult, healthy male rhesus monkeys were used in the present study. After anaesthetising the animals with an intramuscular dose of Ketlar (5 mg/kg body wt., Parke-Davis), the testis along with the associated excurrent ducts were dissected out from one side of the animal and fixed by immersion in Bouin's fluid. Nicks were made in the tissues with a scalpel blade to facilitate penetration of the fixative. After fixation, small pieces of tissue were dissected out from the following regions: the cranial part of the testis which included a part of the rete testis, the vas efferens, the epididymis and representative pieces from the scrotal and abdominal portions of the vas deferens. The fixed material was processed and embedded in paraffin; representative sections (6–10 μm thick) from different portions of the excurrent duct system were stained with haematoxylin eosin or Periodic Acid Schiff's reagent (PAS). The epididymis was serially sectioned at a thickness of 10 μm.

Regions corresponding to those tissues taken for paraffin sectioning were dissected out from the contralateral side of the animal, fixed in modified Karnovsky's fluid containing 4% paraformaldehyde and 1% glutaraldehyde, processed and embedded in araldite. The tissues were sectioned at 1–2 μm on a Reichert OMU_3 ultramicrotome and stained with 1% toluidine blue.

Several photomicrographs of the excurrent ducts fixed in Bouin's fluid were prepared at a constant final magnification and the height of the epithelial cells and sterocilia and the diameter of the lumen were determined from these photomicrographs (table 1).

Table 1. Dimensions of cell height, stereocilia height and lumen diameter in the vasa efferentia and different segments of the epididymis.

	Cell height μm	Stereocilia height μm	Lumen diameter μm
Vasa efferentia (22)	20·2 ± 8·23	.	101·43±31·79
Initial segment (15)	65·53±10·82	35·00±11·73	150·00±23·92
Middle segment (40)	53·63± 8·18	16·08± 8·43	141·00±29·81* 187·78±50·94**
Terminal segment (40)	31·31± 6·60	4·72± 2·64	269·10±57·51* 471·17±94·52**

* Proximal portion. ** Distal portion.
Figures in parenthesis indicate number of observations made.

Observations

General

The gross structural features of the testis and the associated excurrent ducts taken for the present study are shown in figure 1.

The rete testis constitutes a system of interconnected channels extending from the mediastinum to the tunica albuginea. Twelve to 17 efferent ducts emerge from the cranial pole of the testis and become highly convoluted before individual ducts open into the epididymal duct. The epididymal duct is also highly convoluted and it continues into the vas deferens. The initial part of the vas deferens is mildly convoluted.

The extratesticular portions of the excurrent ducts are surrounded by connective tissue comprising collagen, fibroblasts, macrophages, mast cells, nerve fibres and a network of blood and lymphatic vessels.

The epithelium lining the lumen of the excurrent ducts lies over a basement membrane which is surrounded by a muscularis. Intra-epithelial lymphocytes occur throughout the excurrent ducts. These are pleomorphic cells with a clear cytoplasm and an intensely heterochromatic nucleus (figure 13; figure 16). Blood vessels are seen to lie close to the basement membrane.

The luminal contents of the excurrent ducts show regional differences. The lumen in the rete testis, vas efferens and in the initial segment of the epididymis contains sparsely distributed spermatozoa and many immature germ cells (e.g., figure 4). Spermatozoal density increases progressively from the middle to the terminal segment where it is maximal. The proximal portion of the vas deferens also shows a high intraluminal concentration of spermatozoa. The number of immature germ cells is negligible; on the other hand, a large number of intraluminal macrophages are seen in areas of high spermatozoal density (figure 8).

Rete testis

The seminiferous tubules open into the rete testis through tubular extensions of the rete, the tubuli recti (figure 2). The junction between the seminiferous tubule and the tubulus rectus is distinguished by the presence of a knob-like protrusion composed of Sertoli cells (figure 2). It is not unusual to find spermatozoa associated with these Sertoli cells. The rete testis lying within the tunica albuginea opens into the proximal part of the efferent ducts (figure 3).

The epithelial cells lining the rete show regional differences. Cells lining the tubuli recti are short columnar and possess an ovoid, indented nucleus. The cytoplasm of these cells is mostly homogenous but in the apical regions it has a vacuolated appearance (figure 11). The epithelium lining the rete in the mediastinum and in the tunica albuginea comprises mostly squamous cells and a few large cuboidal cells which are distinguished by their hyaline cytoplasm and rounded nucleus (figure 12). In the region close to the junction between the rete and the vas efferens the epithelium is composed of short columnar cells whose apical regions contain prominent cytoplasmic vacuoles (figure 13).

Vas efferens

The lumen of the vas efferens is lined by a pseudo-stratified columnar epithelium comprising ciliated and non-ciliated cells (figure 14). The height of the epithelial cells and the diameter of the lumen is indicated in table 1.

The non-ciliated cell constitutes the predominant cell-type and it is distinguished by a basally situated, indented, euchromatic nucleus. Its cytoplasm contains a large number of granular inclusions which stain with PAS. The apical surface of this cell type shows features characteristic of a brush-border (figure 14).

The ciliated cell has a pale cytoplasm containing a large number of mitochondria. The nucleus is round, centrally situated and contains euchromatin. The apical surface is lined by kinocilia associated with basal bodies which stain more deeply than the surrounding cytoplasm (figure 14).

Epididymis

The epididymis can be divided into an intial, a middle and a terminal segment on the basis of histological criteria defined by Glover and Nicander (1971). The dimensions of the epithelial cells, stereocilia and the lumen diameter in different segments of the epididymis are shown in table 1.

The initial segment is characterised by a high epithelium with long stereocilia. The lumen is generally devoid of spermatozoa but, when present, they are very sparsely distributed (figure 5).

In the middle segment the stereocilia are usually bent and spermatozoa are present in varying densities within the lumen (figure 6).

In the terminal segment the epithelium is lower than in other regions and the stereocilia are also shorter. The lumen is packed with spermatozoa which exhibit a distinctly spiral arrangement. The peritubular mucularis shows considerable thickening (figure 8).

Besides these histological differences, the lumen diameter of the epididymal duct increases progressively from the initial to the terminal segment (table 1). The epithelium in the distal part of the middle segment and in the proximal part of the terminal segment shows evaginations (figure 7) which in some sections appear as crypts.

Three distinct cell types can be discerned in the epithelium lining the epididymal duct. These are: (1) the principal cell, which constitutes the predominant cell type, (2) the pale cell and (3) the basal cell.

The principal cell extends from the basal lamina to the lumen. In the initial segment the principal cell is tall columnar and has an ovoid nucleus containing euchromatin (figure 5). The nuclear envelope has a regular outline which is a distinguishing feature of this segment. Clear vesicles and vacuoles fill the apical cytoplasm (figure 15). In the middle and terminal segments the principal cells become progressively shorter (figure 6 and figures 7 and 8) and the nuclear membrane displays indentations of increasing complexity (figure 16). Apical vesicles and vacuoles are few. The supranuclear region of principal cells shows negative images of the Golgi. Thecytoplasm cont ains well defined granules which stain deeply with toluidine blue. The granules are mostly distributed in the supranuclear region. Stereocilia extend into the lumen from the apical surface of principal cells (figures 15 and 16).

The pale cell is distinguished by an apically situated nucleus which is round and contains much heterochromatin. The basal part of the cell is slender and elongated whilst the apical part is broad. The perinuclear cytoplasm in most cells contains thread-like inclusions which are presumably mitochondria. In some cells the supranuclear cytoplasm contains either vacuoles or large granules which stain intensely with PAS. The apical surface lacks stereocilia (figure 16). Pale cells are seen most frequently in the middle segment and least so in the terminal segment.

The basal cell lies on the basement membrane and shows no connection with the lumen. The euchromatic nucleus is usually round and indented (figures 15 and 16). The cytoplasm of some basal cells contains granular material which stains metachromatically with toluidine blue.

Vas deferens

The pseudostratified epithelium is wavy and much taller in the scrotal (figure 9) than in the abdominal part of the vas deferens. In the abdominal portion of the vas deferens the epithelium forms deep projections into the lumen which show evidence of branching (figure 10).

The epithelial cells of the vas deferens can be distinguished into Type I and Type II cells based on thei r cytological characteristics.

The Type I cell is the predominant cell type. Cytological differences in this cell type are noticed between cells located in the proximal convoluted portion and in the distal straight portion of the vas deferens. The Type I cell in the former region (figure 17) exhibits extensions of the apical cytoplasm into the lumen. The nucleus is round or ovoid and the nuclear membrane is regular or mildly indented. In the distal straight segment of the vas deferens (figure 18), the Type I cell lacks apical extensions and the nucleus is ovoid and highly indented. The Type I cell in all regions of the vas deferens contains a vacuolated area above the nucleus which is distinct from the Golgi area.

The Type II cell (figures 17 and 18) resembles the pale cell in the epididymal epithelium in that it possesses a round nucleus which is usually heterochromatic but may contain euchromatin, depending on the functional state of the cell. Moreover, the cytoplasm contains a perinuclear population of mitochondria. Unlike the epididymal pale cell, however, the Type II cell does not contain vacuoles and granules which stain with PAS.

Basal cells resemble those present in the epididymal epithelium. However, basal cells containing metachromatic granules were not observed as frequently as in the epididymis.

Discussion

The present study has shown that the epithelium in the rete testis is of the simple type while in the vas efferens, epididymis and vas deferens the epithelium is pseudo-stratified. Besides a stable population of cells, the epithelium also contains a wandering population of intraepithelial lymphocytes which are presumed to be involved in preventing sperm antigens from reaching the blood stream and eliciting an putoimmune response (Dym and Romrell, 1975).

The possible functional significance of the histological and cytological features of the excurrent ducts can be better interpreted on the basis of what is already known of their physiology. Thus, the rete testis is not only an avenue through which spermatozoa traverse but a fairly substantial volume of fluid also courses through it (Tuck *et al.,* 1970). It is therefore not surprising that only a few, sparsely distributed spermatozoa can be observed in histological sections of the rete testis.

The rete testis fluid (RTF) is believed to be a mixture of fluids secreted by the seminiferous tubules and by the rete itself (Tuck *et al.,* 1970). The cellular origin of the substances secreted by the rete has not yet been identified. The cytological features of the epithelial cells in the rete testis suggest that the cells having a hyaline cytoplasm and those having cytoplasmic vacuoles possibly contribute to the rete testis secretion; cells with such features occur in the tubuli recti, amidst the squamous cells in the mediastinum and intratunical portions of the rete testis and at the junction between the rete testis and th e vas efferens. Since the secretory cells in these regions of the rete are cytologically different from one another, it is likely that each of these 3 different cell types contribute different substances to the RTF.

The vas efferens contains non-ciliated and ciliated cells. The non-ciliated cells constitute the predominant cell-type and they contain PAS positive granules as seen in other exocrine cells. The ciliated cell lacks such cytoplasmic inclusions but contains mitochondria which presumably constitute an energy source for ciliary movement. These cytological features are in consonance with a view that the non-ciliated cells have a secretory role while ciliated cells subserve a function of transporting the constituents of the vas efferens by ciliary movement.

The physiology of the epididymis has been more widely investigated than any other component of the excurrent ducts of the male reproductive system (Hamilton, 1975). Spermatozoa in the epididymis are known to undergo a process of maturation (Bedford, 1975). The epididymis is also known to function as a storehouse for spermatozoa (Glover and icander, 1971). Glover and Nicander (1971) suggested a nomenclature of initial and middle segments to such of the regions of the epididymis concerned with the maturation of spermatozoa and terminal segment to denote the region where spermatozoa are stored. The advantage of using this nomenclature against the classical terminology of caput, corpus and cauda to define the different regions of the epididymis has been discussed in detail by these authors. The present study has shown that it is also possible to distinguish an initial, a middle and a terminal segment in the epididymis of the rhesus monkey on the basis of histological criteria described by Glover and Nicander (1971).

Apart from the presence of intraepithelial lymphocytes, the epididymal epithe lium of the rhesus monkey also contains 3 distinct cell types as has been found in the rat (Reid and Cleland, 1957). The cytological features of the principal cell in the monkey epididymis are similar to those of the principal cell in the rate epididymis. The principal cell in the rat has been implicated with a function of fluid absorption (Hamilton, 1975) and it is likely that in the monkey also these cells have a similar role.

The second cell type in the epididymal epithelium of the rat is the clear cell (Reid and Cleland, 1957) which is present only in the middle and terminal segments. These cells have been implicated with a secretory function and ultrastructural studies on the epididymis of the rat have shown that the clear cell in the middle segment is different from that found in the terminal segment (Anand Kumar *et al.,* in press).

In the rhesus monkey there is no cell type which is comparable to the clear cell of the rat epididymis. However, the second cell type in the epithelium of the monkey epididymis is the pale cell which is present in all the 3 segments. A similar cell type has been described in the human vas deferens (Hoffer, 1976). The cytological features of the pale cell show that they either contain an abundance of mitochondria, or PAS positive granules, or cytoplasmic vacuoles. These features suggest that pale cells may have a secretory function.

The third cell type in the epididymal epithelium, the basal cell, is essentially similar in structure in the rhesus monkey and in the rat. The function of this cell type is not known. However, the presence of cytoplasmic droplets which stain metachromatically with toluidine blue in some cells suggests that these droplets may be lysosomes in which case it is likely that basal cells play a role in sequestering and disposing of the products absorbed by the epithelial cells. Further studies are needed to substantiate this view.

The epithelium of the vas deferens comprises Type I and Type II cells and basal cells. The Type I cell in the proximal, convoluted portion of the vas deferens has apical extensions and a round to ovoid nucleus whose membrane may be smooth or mildly indented. However, in the more distal portions of the vas deferens, the Type I cell lacks apical extensions and possesses a highly indented, ovoid nucleus. The Type II cell resembles the pale cell in the epididymal epithelium. These cytological features of the epithelial cells of the vas deferens clearly suggest that the vas deferens in the rhesus monkey is not merely a simple conduit for the passage of spermatozoa, but it may also secrete substances which are possibly related to the survival of spermatozoa.

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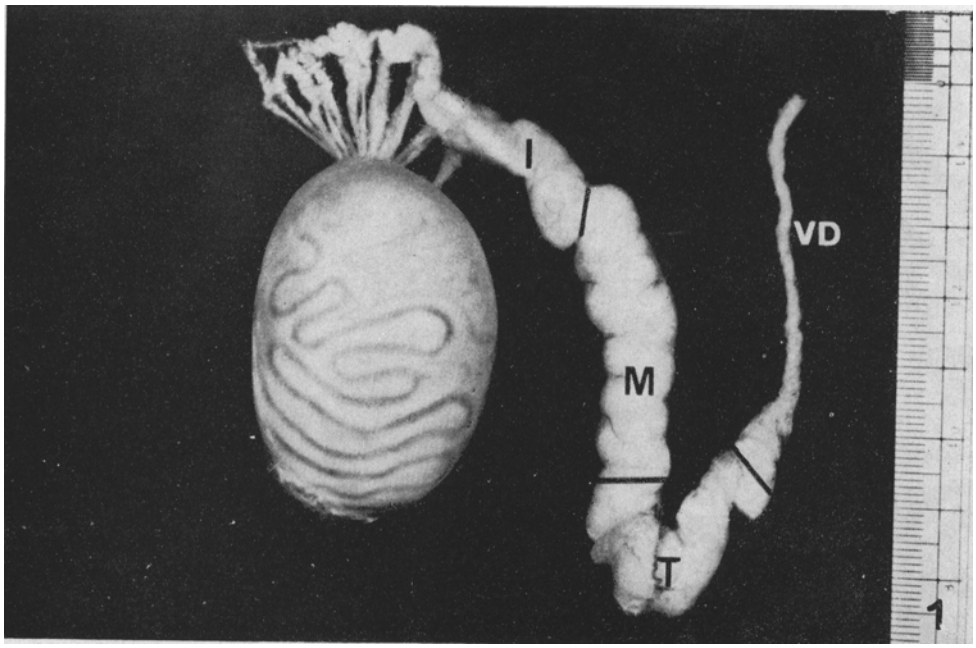
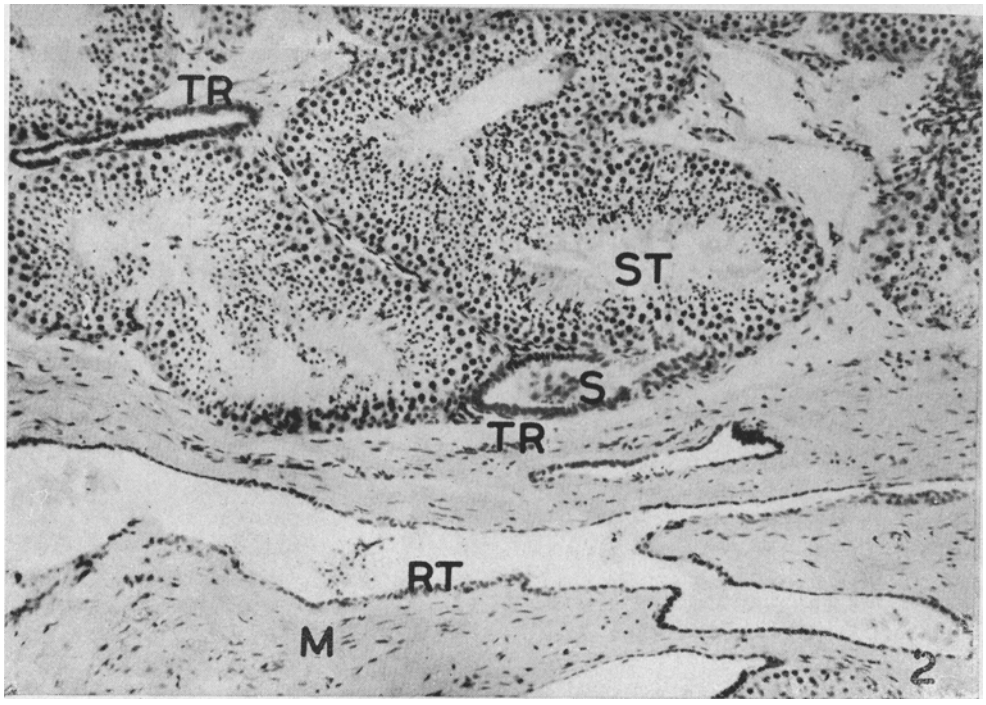
Figure 1. Gross morphology of the excurrent ducts of the male reproductive system in the rhesus monkey.

Thirteen efferent ducts are seen to emerge from the cranial pole of the testis and join the epididymal duct in the initial segment of the epididymis (I). Middle segment (M); terminal segment (T); vas deferens (VD).

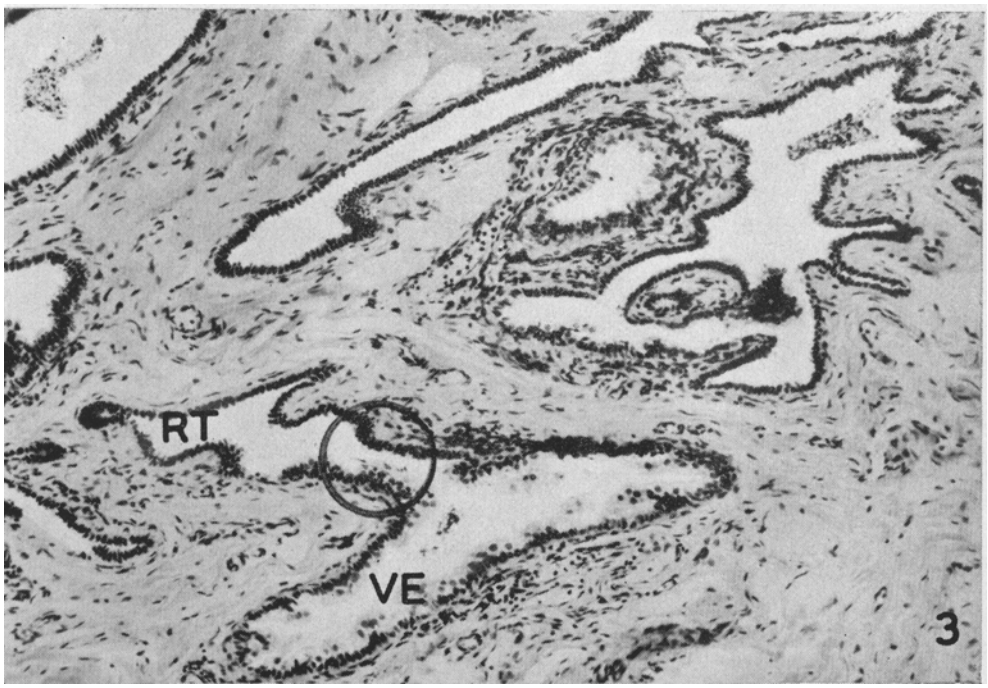
Figure 2. Photomicrograph of the rete testis (RT) surrounded by fibrous tissue of the mediastinum (M).

Tubular extensions of the rete, *viz.,* the tubuli recti (TR) connect the seminiferous tubules (ST) with the RT. Note the knob-like protrusion of Sertoli cells (S) at the junction between the ST and TR × 130.





FIGS. 1–2



FIGS. 3–4

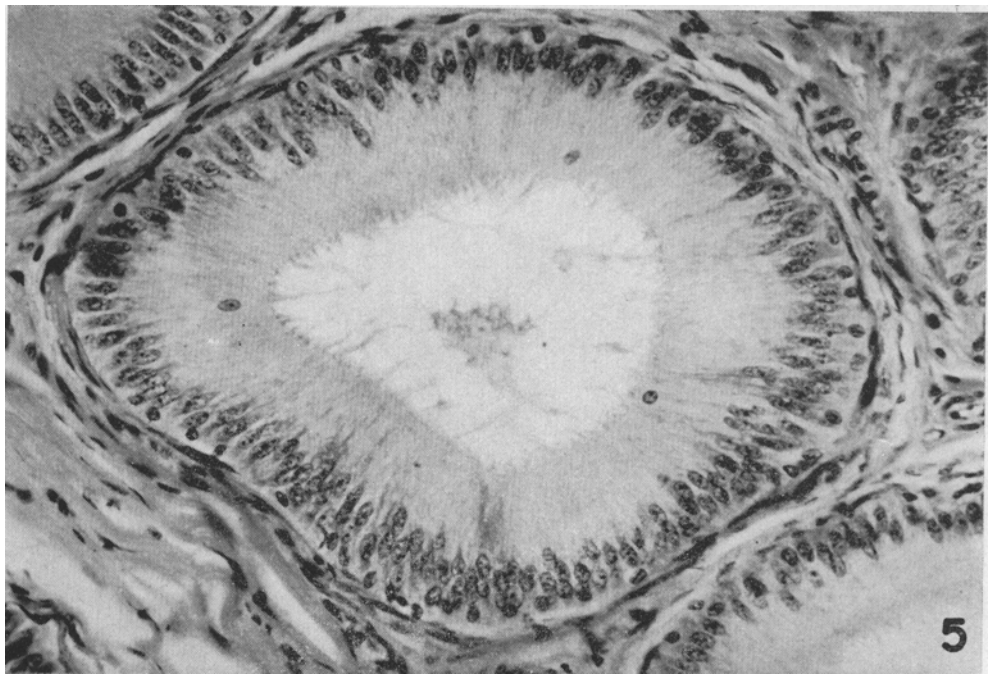
Figure 3. Photomicrograph showing the junction between the intratunical portion of the rete testis (RT) and the vasa efferentia (VE) which is characterised by a wavy epithelium.
Cytological features of circled area are shown in figure **13.** × 130.

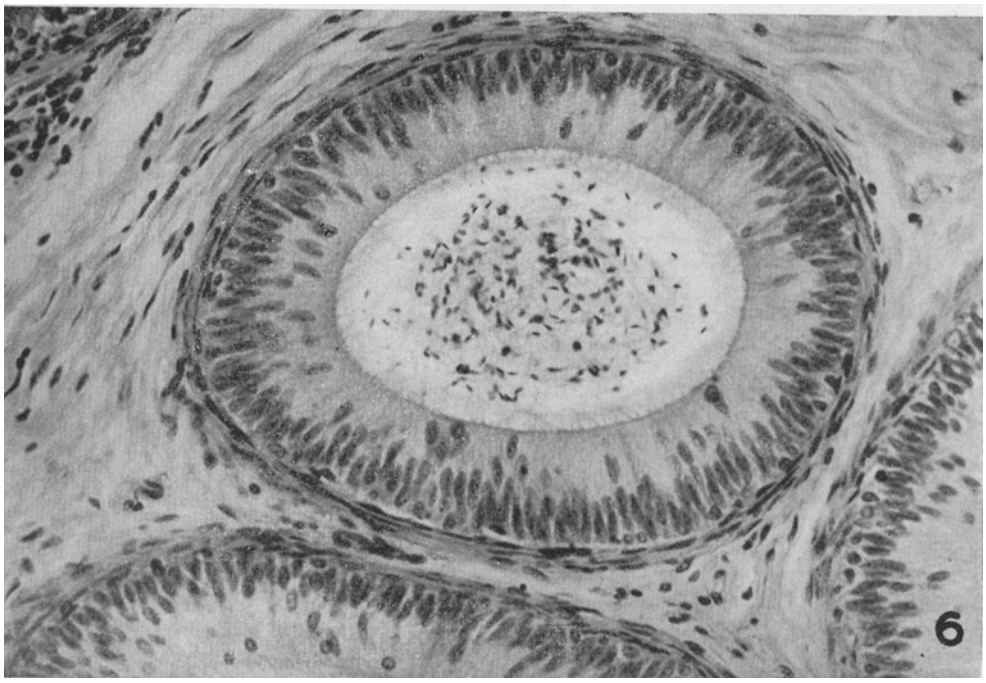
Figure 4. Photomicrograph of efferent ducts in cross-section. The epithelium comprises non-ciliated cells in which the nucleus is located basally and ciliated cells with a centrally located nucleus. The non-ciliated cells constitute the predominant cell type. The lumen contains spermatozoa and immature germ cells. × 320.

Figure 5. Photomicrograph of the epididymal duct in the initial segment. The epithelial cells are tall and have long, straight stereocilia. The lumen is devoid of spermatozoa but contains some coagulum. The peritubular muscularis is only a few layers thick. × 300.

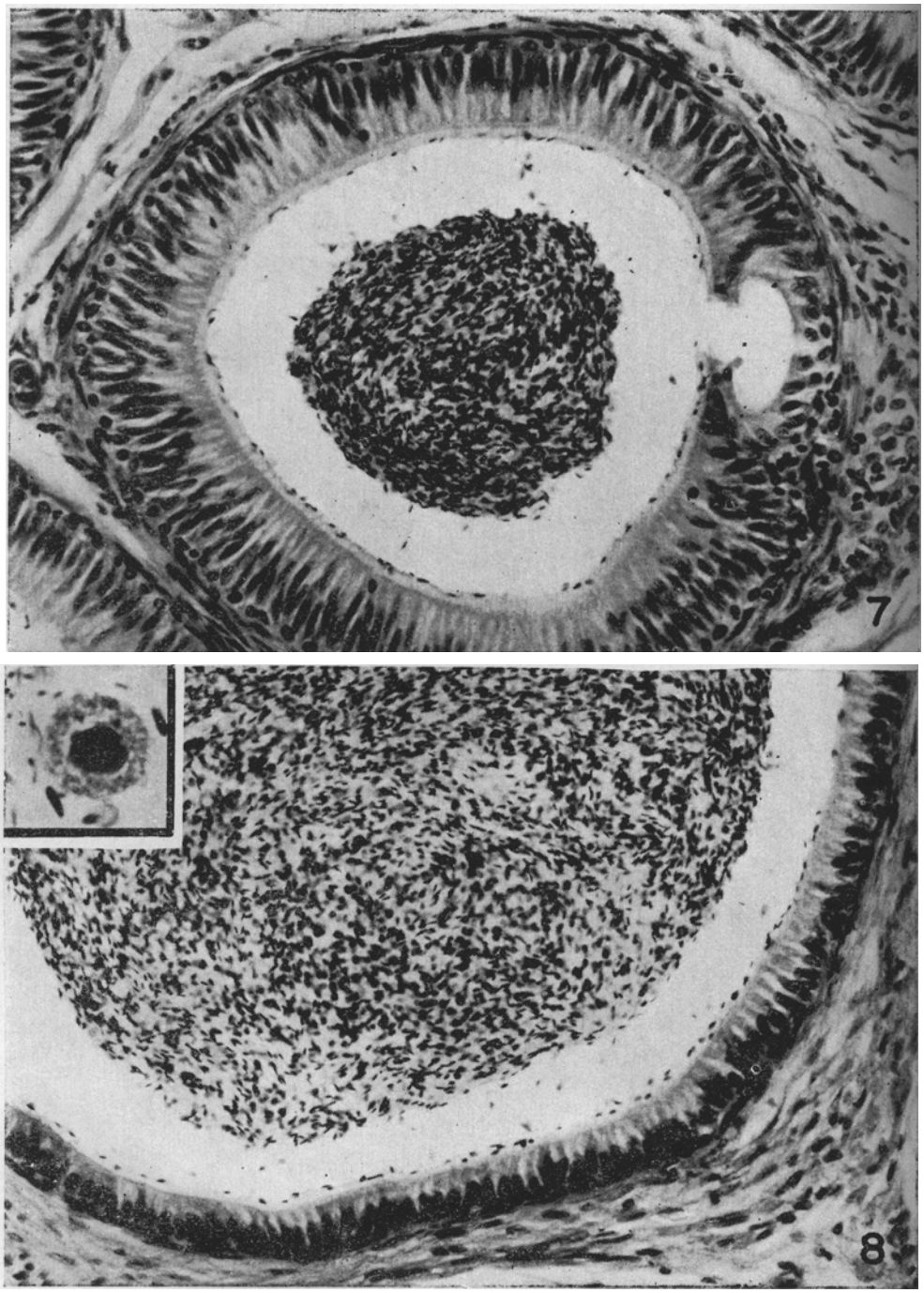
Figure 6. Photomicrograph of the epididymal duct in the middle segment of the epididymis.

The height of the epithelial cells is considerably less than that in the initial segment. Stereocilia are bent and shorter than those present in the initial segment. Several spermatozoa are evenly dispersed in the lumen. The peritubular muscularis is a few layers thick. × 300.





FIGS 5–6



FIGS. 7–8

Figure 7. Photomicrograph of the epididymal duct in the proximal portion of the terminal segment of the epididymis.

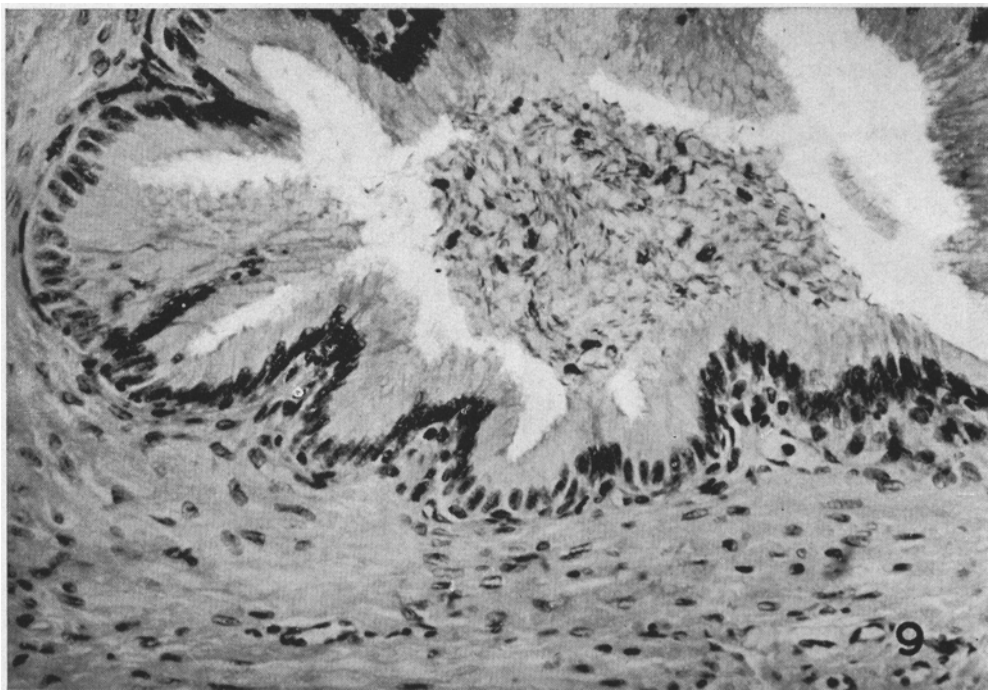
A ductular evagination is also shown in this picture. The spermatozoa appear to be more concentrated than in the middle segment. × 300.

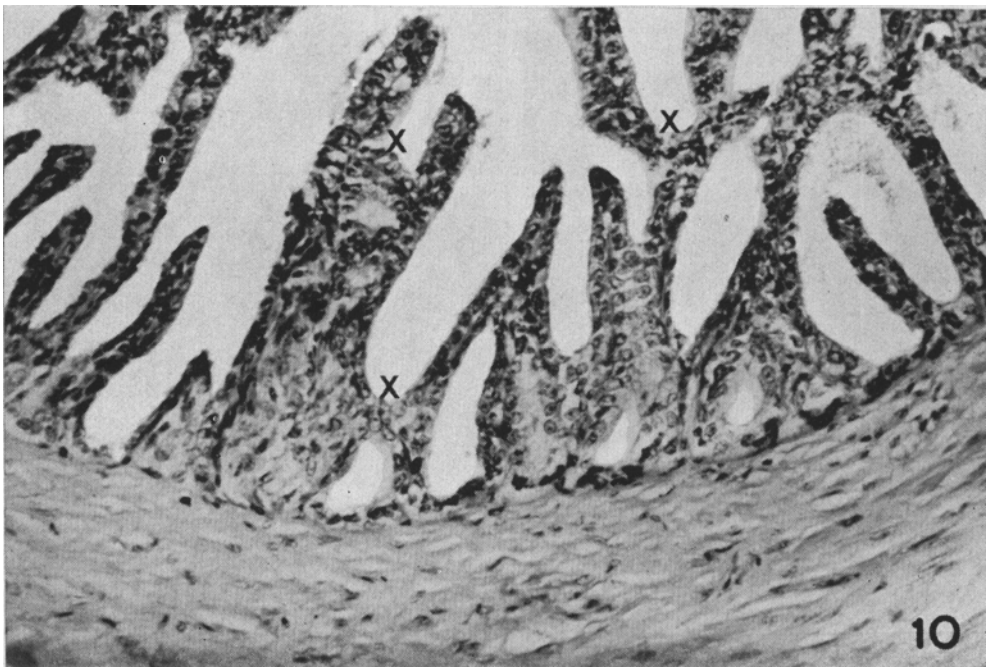
Figure 8. Photomicrograph of the epididymal duct in the terminal segment of the epididymis.

Cell height and stereocilia height are much reduced as compared with the initial and middle segments. Intraluminal macrophages, distinguished by their large, round nuclei, are numerous (details are seen in enlargement in inset; section stained with toluidine blue; × 1300). The peritubular muscularis is multi-layered. × 300.

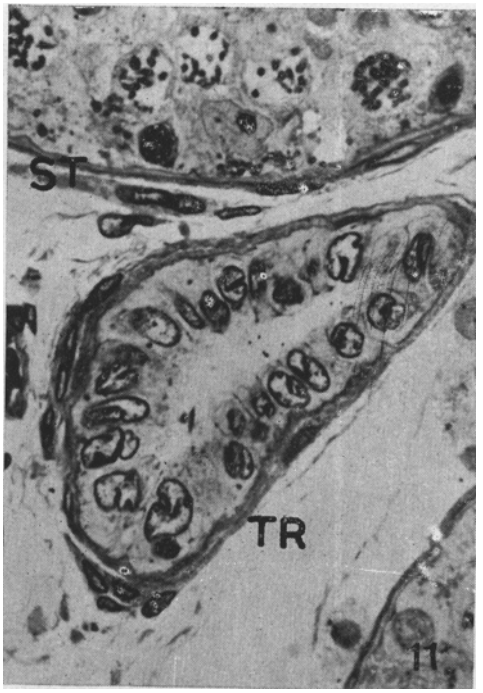
Figure 9. Photomicrograph of the scrotal portion of the vas deferens. The epithelium is wavy. The lumen contains spermatozoa and the muscular investment consists of many layers of smooth muscle cells. × 300.

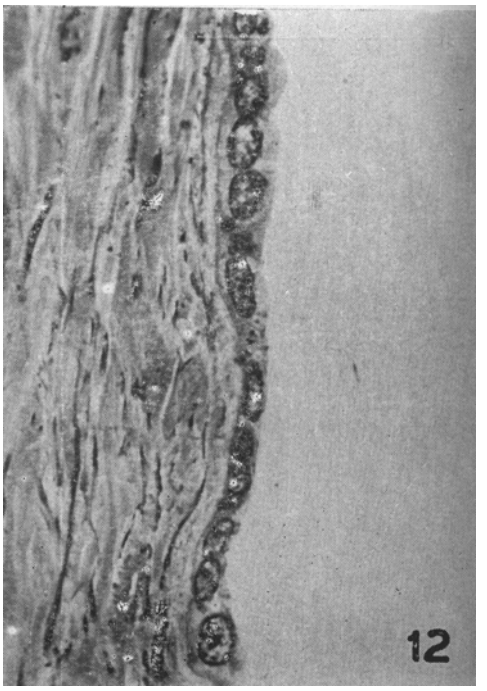
Figure 10. Photomicrograph of the abdominal portion of the vas deferens. The epithelium forms deep projections into the lumen. Branching of the epithelium occurs at sites marked with an X.

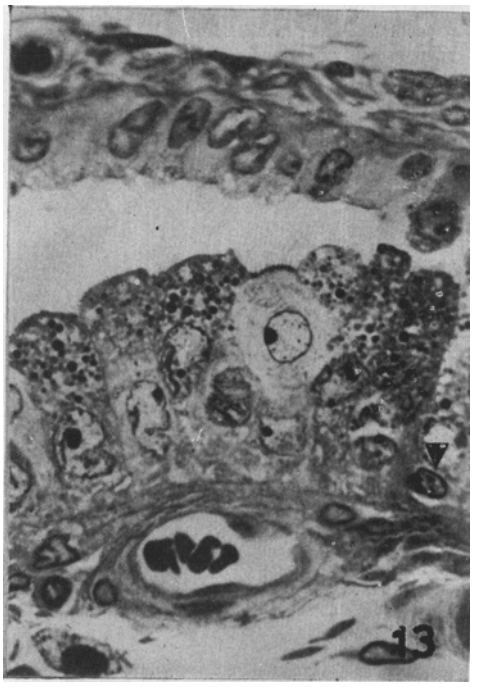


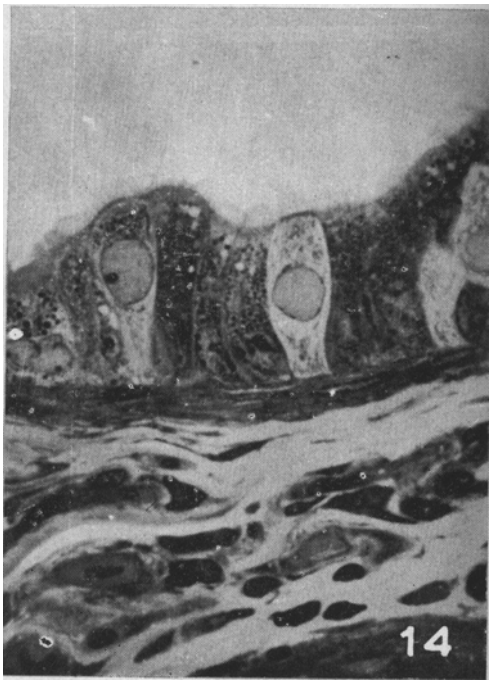


FIGS. 9–10









FIGS. 11–14

Figure 11. Photomicrograph illustrating a cross-section of a tubulus rectus (TR) lying close to a seminiferous tubule (ST).

The epithelium consists of short columnar cells with an indented, ovoid nucleus and a hyaline apical cytoplasm. × 900.

Figure 12. Photomicrograph illustrating squamous cells and a few large cells with round nuclei.

These cell types are representative of the epithelium lining the rete testis lying in the mediastinum and in the tunica albuginea. × 900.

Figure 13. Photomicrograph illustrating the junctional area between the rete testis and the vas efferens (area encircled in figure 3).

The short columnar cells of the rete at the junction possess an ovoid, indented nucleus and apically situated cytoplasmic vacuoles. The epithelium of the vas efferens has non-ciliated and ciliated cells. The rete epithelium lies towards the upper end of the photomicrograph. The epithelium of the vas efferens lies towards the lower end of the photomicrograph.

Figure 14. Photomicrograph showing cytological features of the non-ciliated and ciliated cells.

The non-ciliated cell contains a basally located, ovoid, indented, euchromatic nucleus and deeply staining cytoplasmic granules. The ciliated cell possesses a centrally placed, round, euchromatic nucleus. The cytoplasm contains numerous thread-like mitochondria. Dense granules are occasionally seen as in the ciliated cell marked with an arrow. × 900.

Figure 15. Photomicrograph of cell types in the initial segment of the epididymis. The principal cell (P) is tall with long stereocilia. The basally situated nucleus is ovoid and has a smooth nuclear membrane. Negative images of the Golgi apparatus are seen above the nucleus. Clear vesicles and vacuoles are prominent in the apical cytoplasm. Pale cells of two types, one containing an abundance of mitochondria (arrow-head) and the other containing vacuoles (arrow) and a basal cell (B) can be seen × 900.

Figure 16. Photomicrograph illustrating cell types in the terminal segment of the epididymis.

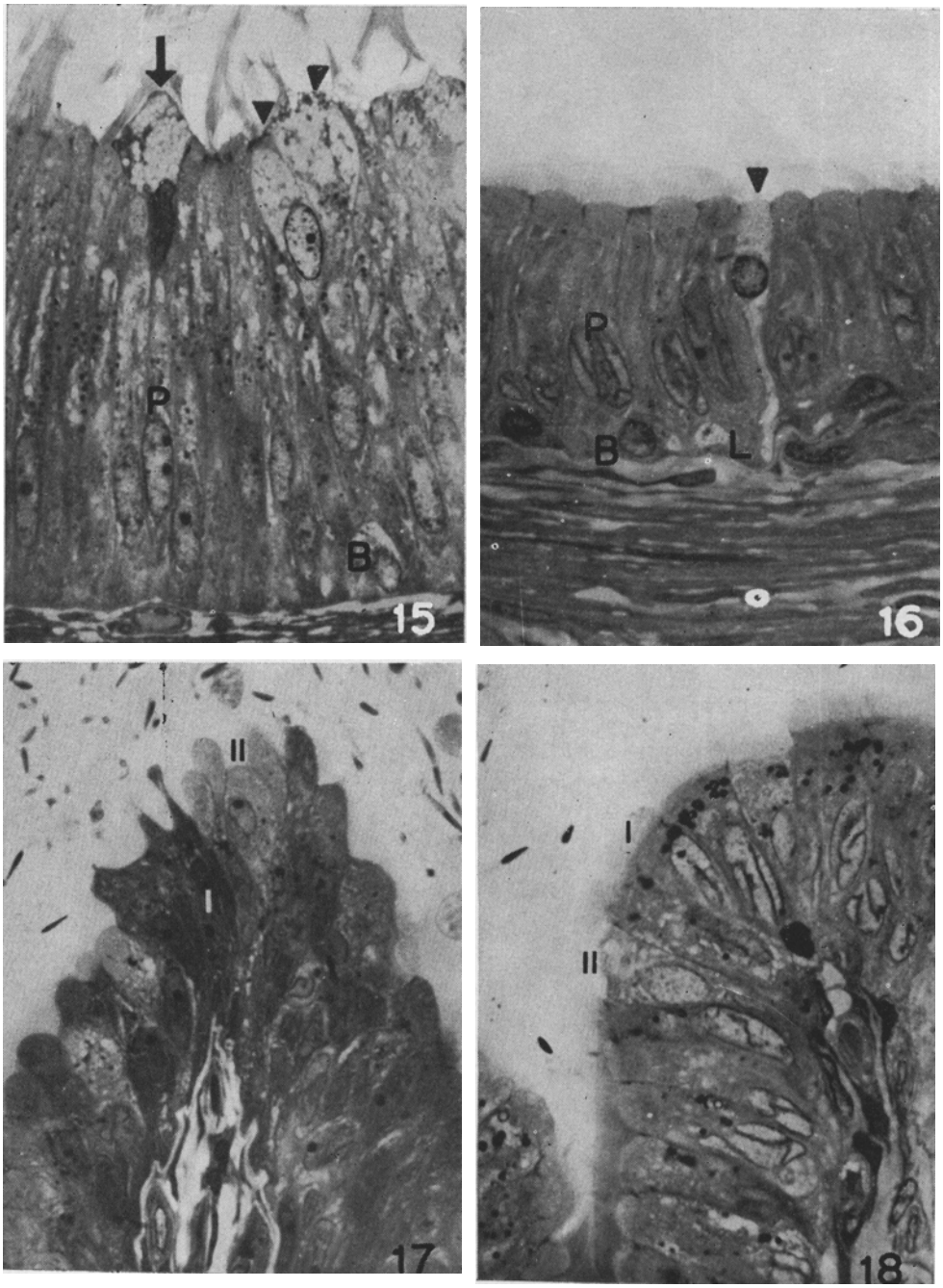
Principal cells (P) possess a highly indented nucleus. Clear vesicles and vacuoles are not evident. The pale cell (arrow head) is seen to extend from the base of the epithelium to the lumen. A round heterochromatic nucleus lies in the apical part of the cell. An intraepithelial lymphocyte (L) is seen. Basal cell (B). × 900.

Figure 17. Photomicrograph illustrating cell types in the proximal convoluted portion of the vas deferens.

The predominant Type I cell (I) in this region usually possesses slender extensions of the apical cytoplasm which show terminal expansions. The nucleus is devoid with few indentations. The Type II cell (II) resembles the pale cell containing mitochondria of the epididymal epithelium. × 900.

Figure 18. Photomicrograph of cell types in the distal straight portion of the vas deferens.

The Type I cell (I) in this region lacks apical extensions; instead the luminal surface is lined by microvilli. The nucleus is highly indented. The cell possesses a vacuolated cytoplasm. Type II Cell (II). × 900.



FIGS. 15–18