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The Genital Tract of the Male Opossum, *Didelphis Marsupialis Virginiana*, and Other Marsupials.

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In this paper an attempt has been made to collect and summarize available information on the male genital tract of *D. marsupialis*. When literature was available, information on other members of the family Didelphidae and other marsupials has been compared and conclusions drawn concerning the similarities and differences in structure and function of the male genital tract within and between the two groups. Any unifying concept which will emerge may serve as a base to distinguish between marsupials and eutherian mammals, and for speculation.

The prostate gland of *D. marsupialis* is a carrot-shaped structure which originates from urethal epithelium, and in the adult surrounds the proximal urethra (Moore 1941). It varies in length and diameter according to the age of the animal (Chase, 1939; Oudemans, 1892). It is narrow and straight in young males and enlarged, curved and even looped in adults. Histologically, the opossum prostate gland is of tubular variety. The secretory glandular tubules encircle the urethra and are embedded in the urethral stroma but do not penetrate the overlying smooth muscle layers. The ducts of the tubular glands open individually into the urethra (Chase, 1939). This type of prostate gland, in which glandular components do not penetrate the muscle layers of the urethra, is called a diffuse or disseminate prostate gland (Fig. 1). This arrangement contrasts with that in which parenchymal cells penetrate muscle layers and form a discrete glandular body outside the urethra (Price and Williams-Ashman, 1961). The opossum prostate gland is subdivided into 3 parts, each of which in the adult male has a characteristic diameter and color (Figs. 1, 2, 3, 4).

Chase (1939) designated the short, most craniad region which is light brown in color as prostate I, the medial region which has the greatest diameter and is pink in color as prostate II, and the most caudad gray colored region with the smallest diameter as prostate III. Following prostate III the urethral diameter narrows abruptly into the short membranous urethra extending to Cowper's glands, from which it broadens and continues as a cavernous urethra (Fig. 1).

Price and Williams-Ashman (1961) suggested that the 3 differently colored regions of the opossum prostate gland probably differed in structure and function as do the different lobes of prostates in eutherian mammals. Martan & Allen (1965) and Hruban *et al.* (1965) referred to these regions as prostatic segments I, II, and III. Histological studies of the opossum prostate by Chase (1939) showed each colored region of the gland to have a characteristic epithelium. The epithelial cells were: cuboidal to low columnar in segment I; columnar with apical granular material in segment II; and cuboidal with clear cytoplasm in segment III. The secretory tubules in segment I had narrow lumina and were separated from each other by distinct layers of connective tissue, while these tubules in segments II and III had broader lumina and were separated by narrow streaks of connective tissue.

Martan and Allen (1965), using various staining techniques and histochemical reactions, found great diversity in the cytological and cytochemical characteristics of cells from individual segments (Figs. 2, 3, 4). The distinctive feature of segment I was the large cytoplasmic globules derived by apocrine secretion and present in the lumina of the secretory tubules. The globules contained granules and vesicles, some of which showed positive reactions for phospholipids, polysaccharides, acid phosphatase (Fig. 21), aliesterase, nucleoside diphosphatase and 5′-nucleotidase. Similar reactions occurred in the cytoplasm of epithelial cells. The finding of an unusually high content of iron in the luminal globules and epithelial cells prompted Hruban *et al.* (1965) to suggest that the presence of iron gives segment I its brown color (Fig. 18).

Two types of columnar cells designated A and B cells (Martan and Allen 1965) characterized the secretory epithelium of tubules in segment II (Fig. 7). The cytoplasm of the taller A cells was filled with round granules which showed positive histochemical reactions for mucopolysaccharides. The cytoplasm of the less elongated B cells contained large numbers of fusiform granules which were strongly reactive with various histochemical tests for protein (Fig. 8). The cytoplasmic network around granules in both cells gave positive reactions for aliesterase, leucine amino peptidase, 5′-nucleotidase and nucleoside diphosphatase but reactions of both types of granules were negative. Tests for RNA were positive with a netlike distribution similar to that for enzymes. Secretory material found in the lumina of the glands and ducts of segment II gave positive reactions similar to those for the granules of type A and B cells. The presence of cytoplasmic granules was also reported by Ladman and Soper (1963).

The cuboidal cells lining the secretory tubules of segment III contained large globules of glycogen (Figs. 9, 10). This high content of glycogen (2g per 100g of wet tissue, Martan and Allen, 1965), was the characteristic feature of segment III. The large size of the glycogen mass in the cytoplasm of the cuboidal cells resulted in a peripheral distribution of cytoplasmic RNA, Golgi complex, and mitochondria. Phospholipids were localized in the apical portion of the epithelial cells. The lumina of the glands and ducts were filled with glycogen globules forming islands in an amorphous mass of phospholipids. This mass also gave a positive reaction for acid phosphatase (Fig. 6). In the cytoplasm only randomly distributed large granules were positive for this enzyme. Hardin (1965) also reported the presence of glycogen in segment III.

Hruban *et al.*, (1965) used electron microscopy to study cytological diversity of the opossum prostate gland. The granules in A and B cells of segment II, were both limited by a single membrane. The elongated granules in B cells had a fila-

mentous dense center, which in cross-sections appeared hexagonal. This center was surrounded by a less dense matrix. The granules from A cells were moderately electron dense and sometimes contained a small dense disc. The authors described another type of cell found in the outermost zone of segment II, which they called C cells (Fig. 5). The cytoplasmic granules of these cells were heterogeneous in nature and many had highly complex cylindrical structures inside the granules. The luminal surfaces of epithelial cells of all three segments, were covered by microvilli. Glandular lumina of segment III contained electron transparent globules of glycogen surrounded by masses of smooth membranes. Most membranes were aligned in parallel stacks. Electron microscopic studies showed ferritin-like particles in the apical parts of epithelial cells and the lumina of segment I.

In their cytochemical studies, Hruban *et al.*, (1965) reported positive reactions for thiamine pyrophosphatase, adenosine diphosphatase, inosine diphosphatase, and adenosine triphosphatase in the luminal content of all three segments. The secretory material in the lumina of segment III was also strongly positive for peroxidase. The results of their biochemical studies are summarized in Table 1.

Table 1. Biochemical studies on opossum prostate

Segment		I	II	III
Zinc	Micrograms per gram of wet tissue	15;63;13	18;40;17	19;57;38
Copper		6;12	2; 6	2; 8
Calcium		37;10	12; 6	13; 7
Magnesium		16;35	15;15	16;18
Iodine		6.1;1.6	0.8	2.0
Glycogen	mg/g	1.6	1.3	31.8
Glycogen synthetase without glucose-6-P	units/g	—	5.5	2.7
Glycogen synthetase with glucose-6-P	units/g	—	14	32
Catalase	units/g/sec	39;55	7; 7;14	615;1290
Uricase	units/g/min	0	trace	0
Lactate dehydrogenase	units/g/min	349	424	296

Values for metals and catalase are given on individual animals; each value represents the mean of two determinations.

(From Z. Hruban *et al.*, J. Exper. Zool., 160, 81-105, 1965, Alan R. Liss Inc.)

Chase (1939) studied the influence of castration on the opossum prostate gland by removing the entire scrota with their contents from adults. Generally, prostates of castrated males were lighter than those of controls, however, histological obser-

vations showed different responses by individual segments to castration. In all segments, after 90 days, the tubular glands had narrower diameters and their cells were cuboidal. The lumina of the glands were also narrower with little or no secretion. In segments I and II the lumen of the prostatic urethra was wider than in control animals, and segments II and III showed a noticeable increase in connective tissue in castrates. Chase stated that the greatest changes were in segment I where in some castrates the tubular glands were either disorganized or their glandular character lost. In the same study, of three adult male hemicastrates injected twice a day with gonadotrophins for 20 days, only the two receiving the higher doses showed increased weight of prostate glands when compared to normal controls.

A comparative study of cells in prostate gland of members of 3 genera of the family Didelphidae was done by Martan *et al.*, 1967a. (Unpub. Ms.). Seven four-eyed opossums (*Philander opossum*, Brisson), six murine opossums (*Marmosa* sp., Gray), and four woolly opossums (*Caluromys derbianus* Allen), all males, were obtained commercially. The reproductive maturity of the opossums was judged by the presence of spermatogenesis in the testes, curvature of the prostatic urethra, and distinct colors of individual prostatic segments.

The gross features of the prostate glands of these opossums were strikingly similar to those described in *Didelphis marsupialis virginiana*. Similarity was also observed on the microscopic level but to a different degree in various segments. The epithelial cells of secretory tubules in segment I were columnar with a distinct brush border. A strong positive reaction for glycogen was observed in the epithelial cells and lumina of segment I in all opossums. In addition to glycogen, the lumina also contained large cytoplasmic globules formed probably by a process of apocrine secretion. The glycogen masses in all opossums were often tinged with Alcian blue, suggesting the presence of mucopolysaccharides.

Histochemical tests for iron were positive only in the epithelial cells and lumina of the four-eyed opossums but to a lesser extent than in North American opossum. Granules reacting positively for iron were also PAS positive and diastase resistant. Hruban *et al.* (1965) suggested that the light brown color of segment I of the North American opossum was probably due to its high content of iron. In other species, where the reaction for iron was weak or negative, the color of the first segment was pale gray (Table 2).

The tall columnar epithelial cells of segment II in all opossums were filled with secretory granules. The granules differed in shape and size from species to species. However, their staining properties were similar. With the exception of the woolly opossum, A and B granules were localized in different types of epithelial cells. The B granules gave a strong positive reaction for protein: the staining reactions of A granules with PAS and Luxon Fast blue MBS techniques indicated the presence of polysaccharides and phospholipids. The epithelial cells in segment II of the woolly opposum were of one type with two types of oval granules demonstrable within the cytoplasm of most cells. Some granules had staining reactions characteristic of A cell granules of other opossums, while other granules gave reactions typical of B cell granules. The shorter cells, lining the ducts of segment II in all opossums, also contained granules typical for this segment. The granules were smaller, their staining reactions weaker and they were fewer in number. In the woolly opossum some of the duct cells contained glycogen. In all opossums the same granules as described in cipthelial cells were found also in the lumina of secretory tubules and ducts.

The third segment of all investigated species was rich in glycogen. Glycogen was found in the columnar epithelial cells lining the secretory tubules and in the cuboidal cells lining the ducts. The only exception was middle zone of the four-eyed opossum where the complex secretory products gave staining reactions for polysaccharides, proteins, and phospholipids.

The characteristic feature of all lumina in segment III, in all opossums, was the presence of amorphous masses in which granules and globules of glycogen were embedded. The masses gave a weak histochemical reaction for mucopolysaccharides, and in the murine and woolly opossums, for phospholipids as well. Biochemical studies (Table 3) demonstrated that the third segment in all investigated species, had catalase activity several times higher than that observed in the livers of these animals. In addition, segment I of the four-eyed opossum contained a high degree of catalase (Table 3). The prostatic catalase may be localized in the cells in the region of microvilli, which, in the North American opossum, showed high peroxidase activity (Hruban *et al.*, 1965). The differences in the relative sizes of comparable prostatic segments (Table 2) will probably be reflected in ejaculates and related to the metabolic requirements of ejaculated spermotozoa.

The genital tract of male *Marmosa robinsoni*, another species of the family Didelphidae, was described by Barnes (1977). The author stated that the prostate gland was unusually large (3.59% of the male body weight) in sexually mature males, had the same three distinctive segments as reported for other opossums, and there were two pairs of Cowper's glands.

Three North American male opossums *(Didelphis Marsupialis virginiana)* were reared in captivity and killed at the age of six months (Martan *et al.*, 1967a.). Their prostatic urethrae were not curved, indicating reproductive immaturity (Chase 1939). The prostrates were, however, already differentiated into three segments with distinct colors. Cytological staining reactions of all segments were similar to those of adult males (Hruban *et al.*, 1965; Martan and Allen, 1965). The cellular secretory activity in the young animals, however, was weak and unequally distributed. The lumina contained fewer secretory products. The secretory activity in all three segments appeared earlier on the ventral side of the prostate gland than on the dorsal side. In all segments, the cells close to the periphery of the gland matured later than cells in more central locations. Mitotic figures were absent in segment I, many were observed in segment II, and the greatest number occurred in segment III. The mitotic activity in the last two segments was greater at the periphery of the gland than in the middle. These observations suggest that segment I differentiated first and the third segment last. Due to the morphological similarity of prostates in all investigated opossums, this differential maturation of segments of prostatic glands in subadult males may be indicative of all Didelphidae.

Development of the prostate gland in pouch opossum males was studied by Chase (1939) and Moore (1941). Chase reported that the first appearance of prostate gland buds was noticed at 13 days, the first branching of these primordia at 28-30 days and the tree like solid cords, were characteristic of the prostate gland at 70 days. For similar stages, Moore listed 15, 40 and 100 days of development. Moore believed that in young male opossums, the fully developed functional prostate was achieved at approximately one year of age.

Prostate gland development of the pouch opossum was greatly stimulated and accelerated by androgen injections (Moore 1941), especially in the older males. In

Didelphidae, the female embryos do not have prostatic primordia at any stage of their genital tract development, yet the androgen injections induced prostate gland buds on day 16 in opossum females injected from the third day in the pouch (Moore 1941). Continued treatment of pouch females resulted in greater prostatic growth than that in the untreated males but lesser than that of similarly treated males of the same age.

Burns (1949) stated that introduction of androgen into pouch opossum females not only induced the prostate gland development but their urogenital sinus was changed into the male bilobed form. After androgen treatment was stopped there was no immediate regression of induced prostate glands in the females. Both authors agreed that estrogen injections were highly toxic to pouch opossum males. In the surviving young males there was no development of prostate glands. In older males where prostatic development already had started, the glands were modified by estrogen (Moore 1941). The male form of the urogenital sinus in estrogen treated males, was changed into that of the 5-lobed female form (Burns 1949). The failure of gonadotrophins to elicit acceleration of prostatic development in pouch opossum males younger than 70 days, caused Moore and Morgan (1943) to doubt that the testes at this age were secreting any androgens.

The most unusual feature of the prostate glands in all investigated opossums was the amount of glycogen found in all prostatic segments, especially in segment III. Mann (1964) reported that the reducing sugar in the semen of many mammals, including the opossum *Didelphis marsupialis*, is fructose. The cells of accessory sex glands convert blood glucose into cell glycogen which by a system of enzymes is converted into fructose of seminal plasma. Because in segment III glycogen was found not only in the epithelial cells but also in the lumina of the tubular glands (Figs. 9, 10), the conversion to fructose should also happen there. The high acid phosphotase reactivity in the lumina of segment III supports this suggestion (Fig. 6). Other enzymes necessary for such conversion may be present in the secretory products of the other two prostatic segments. If this speculation is correct, the amount of seminal sugar in opossums does not depend on the immediate level of glucose in the circulating blood.

Phospholipids, which may be utilized in sperm metabolism were found in segments I and III of all investigated opossums but their histochemical reactivity was strong only in *Didelphis marsupialis*.

The third segment in all species had higher catalase activity than that observed in the livers of these animals (Table 3). Prostatic catalase, if present in the ejaculate, will prevent injury to spermatozoa by hydrogen peroxide. Biochemically the prostate gland of the opossum is just as complex as that of eutherian mammals (Table 1); however, the physiological roles of prostatic secretions in opossums are not known. Howarth (1950), using electrostimulation, obtained about 30cc of semen from the Australian marsupial *Trichosurus vulpecula* (brush possum). After the prostate was cut off, the same stimulation produced only a small quantity of fluid from the Cowper's glands. Howarth concluded that the prostate gland supplied the fluid for spermatozoa during ejaculation.

The ejaculate, at least in some Didelphidae, coagulates and forms copulatory plugs (vaginal plugs) located in the lateral vaginal canals of the female — (Hartman 1924, Biggers 1966, Barnes 1977). Hartman observed that though these canals, during the female's receptive period, were greatly distended with fluid, they were completely filled by the copulatory plugs following coitus. The author

was so amazed by the mass of the two plugs, which in his opinion was greater than the combined size of all male accessory sex glands, that he concluded that the copulatory plugs in *Didelphis marsupialis* were formed by the fluid content of the lateral vaginal canals of the female, coagulated by the secretions from the seminal plasma of the male. Hruban *et al.* (1965) failed to obtain coagulation by mixing together secretions from different prostatic segments of *Didelphis marsupialis*. Barnes (1977) reported that freshly deposited copulatory plugs in *Marmosa robinsoni* females can be localized by palpation and was successful in getting coagulation by mixing the secretion from the larger pair of bulbourethral glands with luminal contents of prostatic segment I. Both, Hartman and Barnes noted that the spermatozoa-rich fluid part of the ejaculate was located above the plugs, and Hartman speculated that just as in rodents, plug formation prevented the outflow of spermatozoa from the female genital tract. Martan and Shepherd (1976) through experimentation with copulatory plugs demonstrated that in the guinea pig, the copulatory plug ensures the sole paternity of the first male to mate, presumably the dominant male, and suggested that this may be the role of the plug in other mammals.

There are 3 pairs of Cowper's glands (bulbourethral gl.) in *Didelphis marsupialis* (Fig. 1). The morphology of these glands in adult males was described by Chase (1939), and Rubin (1944). The latter author also studied the development of these glands in the pouch males. The greatest lobes (Cowper's 1,) were enclosed by a layer of striated muscle surrounding the connective tissue capsule from which septa extended between the glandular tubules. The middle lobes (Cowper's 2,) and the smallest lobes (Cowper's 3,) had a common striated muscle enclosure but their glandular portions were separated by individual connective tissue capsules. All three pairs of Cowper's glands were of the compound tubular variety and their ducts ran parallel to the lumen of the urethra before joining into common duct emptying into the cavernous urethra. The authors described the secretion as eosinophilic and homogenous in all three glands. The individual ducts were lined with epithelial cells similar to those lining the secretory tubules and were filled with secretion.

After castration all lobes declined in weight, their secretory tubules became smaller and the inter-tubular connective tissue between those tubules wider (Chase 1939). These changes became visible in the largest lobes at 20 days, in the intermediate lobes at 65 days, but in the smallest lobes there was no change, even at 90 days after the operation. Rubin (1944) observed primordia of Cowper's glands 5 days after birth as paired bilateral thickenings of the solid urethral plate. At 32 days these primordia had already separated into 3 individual pairs of prospective adult glands. Between 75-84 days the glands were surrounded by a muscle layer and their epithelial cords had acquired lumina, except in the smallest lobes. However, Cowper's 3 glands subsequently showed a rapid developmental spurt and at 125 days all lobes exhibited characteristics of compound tubular glands, including secretory activity.

Treatment of younger pouch males (5-39 days) with androgens caused a precocious gain in the size of Cowper's glands, and advanced differentiation of secretory tubules and canalization of the ducts. If treatment was prolonged, there was an inhibition of morphological development (Rubin 1944). In males older than 70 days, androgens stimulated normal development and resulted in glands comparable to those of much older untreated males. Gonadotrophins stimulated

Cowper's gland differentiation in young males of all ages while castration had no effect on normal development of the glands up to 100 days. Treatment with estrogens produced hyperplasia and abnormal development of Cowper's glands in young males. According to Rubin (1944), bulbovestibular glands (Bartholin gl.) of the opossum female are equivalent to the smallest pair of lobes (Cowper's 3,) in the males.

Simple techniques have been used to examine the histochemistry of the Cowper's glands secretions (Martan, unpublished observations). Secretory material in all lobes showed strong reactions for neutral and acid mucopolysaccharides and weak reactions for general protein. Secretions of the two smaller pairs of lobes were strongly positive for sulfated acid mucopolysaccharides. In the smallest lobes, the homogenous secretory material was mixed with unstained vacuoles. In the middle lobes, similar homogenous material contained globular bodies of various sizes. In the largest lobes, the secretion was homogenous and without either globules or vacuoles. In lumina with little secretion, the material in all lobes was granular rather than homogenous. Glycogen was not detected in any of the lobes by the techniques used.

In *Didelphis marsupialis* males the only accessory sex glands present were those originating from the embryonic urogenital sinus, namely the prostate gland and Cowper's glands. Even the urogenital sinuses of female embryos were competent to differentiate into these glands either under natural conditions (bulbovestibular gl., Rubin 1944) or under hormonal stimulation (prostate gl. Moore 1941). It is of interest that in didelphids and other marsupials (Tyndale-Biscoe 1973), vasa deferentia do not cross the ureters to open into the urethra (Fig. 1). Further, the accessory sex glands which in eutherian mammals originate from the embryonic Wolffian duct (seminal vesicles and ampullary gl.) are absent.

Rodger and Hughes (1973) in a detailed study of the accessory sex glands of the Australian marsupials separated species into two groups; those with a carrot-shaped prostate and those with a heart-shaped prostate. They reported that all prostates were of the diffuse type and segmented, and at least in some, these segments differed in the coloration. The authors named the segments anterior, central and posterior in the carrot shaped group, and dorsal and ventral in the heart shaped group. Histologically all segments consisted of simple tubular glands, lined with simple columnar epithelium. The height of epithelial cells was characteristic for segments and species. The secretion, at least in some segments, included round globules and spheres, some of which according to the authors originated from apocrine type of secretory activity of the epithelial cells. The histochemical analyses of all species investigated indicated that the chief secretory materials of the prostate glands were mucosubstances; though in some species, lipids were present. The authors noticed in several species similar segmentation of the prostate gland as reported for *D. marsupialis* (Chase 1939) and suggested that this type of prostate gland may resemble the ancestral marsupial pattern.

Barbour (1981) noticed apocrine secretion in his study of the prostate gland in the hairy-nosed wombat. Histochemical analysis of prostatic cells and their secretions revealed some proteins, and abundant iron and glycogen. The author was so impressed with the amount of glycogen secreted (especially in the posterior segment) that he considered glycogen to be the main product of this gland.

In the genital tract of the Australian marsupial males, Cowper's glands were always present and the number reported was from one to three pairs (Rodger and

Hughes 1973). The histology of Cowper's glands was more uniform among Australian species than that of the prostate glands, and similar to the microanatomy observed in *D. marsupialis* (Chase 1939, and Rubin, 1944). Histochemical studies revealed glycogen and lipids in the cells of the Cowper's glands of some species and the authors suggested that the glands generally secreted mucous material.

While the morphology of the prostate glands in the American and Australian marsupials appeared similar, biochemical studies of the seminal plasma, which is mainly the product of prostate gland (Howarth 1950), showed differences in composition of seminal sugars (Rodger and White 1973). In several species of Australian marsupials Rodger and White (1974, 1975a, 1976) reported N-acetylglucosamine and glucose to be the principal seminal sugars while fructose (the main sugar in the semen of eutherian mammals) was practically absent. In *Didelphis marsupialis*, Mann (1964) reported fructose, secreted by the prostate gland, to be the principal sugar in the seminal plasma. If fructose will be confirmed as the principal sugar in the seminal fluid of other members in the family Didelphidae, it will represent a significant difference in sperm metabolic requirements between Australian and American marsupials, and establish the first uniform difference between Australian marsupials and the eutherian mammals. According to Rodger and White (1975a), in Australian marsupials with the carrot shaped, three segmented type of prostate, the main site of glucose was the posterior segment, while a high level of N-acetylglucosamine was found in the central segment. Interestingly, the similar prostatic segments in *Didelphis marsupialis* (Martan and Allen 1965), showed a high glycogen content and multitudes of cytoplasmic granules. (Figs. 4, 7, 8, 9, and 10)

Biochemical studies of the prostate of the brush-tailed possum *Trichosurus vulpecula* (Cook et al. 1978) revealed high concentrations of zinc in the central segment, and a high activity of testosterone 5α reductase in the posterior segment of the gland. The authors suggested the possibility of testicular and epididymal androgen transport by blood vessels of the prostatic urethra and the prostate gland.

Little is known about the biochemistry of the secretory material produced by the Cowper's glands. The simple histochemical test done by Rodger and Hughes (1973) on the Australian marsupials and the present author on *D. marsupialis*, imply the existence of mucoproteins. Hearn (1975) reported great reduction in weights of prostate and Cowper's glands in 60 days castrated and hypophysectomized wallabies (*Macropus eugenii*), and Carrodus and Bolliger (1939) observed that injections of estrogen caused a decline in the size and weight of the prostate gland, especially in the posterior segment.

Setchell (1977) stated that in marsupials, coagulating glands were missing. However, copulatory plugs in the lateral vaginal canals in the females of some Australian marsupials (Kean 1959, Tyndale-Biscoe 1973) and some American marsupials (Hartman 1924, Biggers 1966, and Barnes 1977) were found. Copulatory plug formation occurs also in some eutherian mammals in which the presence of coagulating glands was not established (Price and Williams-Ashman 1961). The latter authors discussed at length the confusion which exists in the nomenclature of different prostatic lobes in the eutherian mammals and regarded coagulating glands as a part of the prostate gland, comparable to the anterior lobes. Earlier, Engle (1926) expressed a similar opinion and looked upon the coagulating glands in the rat and guinea pig as proximal lobes of the prostate glands and not as a distinct organ as reported by Walker (1910).

The sequential segments of the disseminate prostate gland in the marsupials are regarded by some authors (Rodger and Hughes 1973) as an adaptation for the production of different secretions in the absence of the other accessory sex glands (seminal vesicles and ampullary gl.). Other workers (Price and Williams-Ashman 1961) considered the difference between these segments as comparable to those observed in the prostatic lobes of eutherian mammals. Much more information must be gathered before any comparison in prostatic secretions between eutherian mammals and marsupials can be made. However, if one wishes to speculate, the recent report by Barnes (1977) that secretory products of the prostatic segment I in *Marmosa robinsoni* coagulated the secretion of the larger pair of Cowper's glands would point to this segment as functionally comparable to the anterior lobes of the prostate (coagulating gland) of the eutherian mammals. In addition to the above-mentioned copulatory plug formation, secretion of the prostate gland and Cowper's glands provides a vehicle to carry spermatozoa during ejaculation (Howarth 1950) and a favorable medium for their survival and function in the female genital tract (Price and Williams-Ashman 1961).

The ductuli efferentes in *Didelphis marsupialis* form a wedge-shaped body above the vascular plexus of the hilus of the testis (Fig. 11). In the adult males, this segment of the genital tract, is of yellowish-green color and is separated from the epididymis by a shallow groove (Martan *et al.*, 1967b). Chase (1939) reported that the testis was connected to the epididymis by a single efferent ductule while Ladman (1967) identified up to three ductuli efferentes in *D. marsupialis*. The simple columnar epithelium of these ducts, resting on the prominent basement membrane, was composed of ciliated cells and cells with short microvilli according to the latter author. This disagreement among experts may be explained by the observation that a single efferent duct divides into three to four ductuli in *Didelphis azarae* (Noqueira *et al.*, 1977).

Martan *et al.*, (1967b.) found that the highly convoluted ductuli efferentes, surrounded by abundant stroma, had wider lumina in their middle portion than at either end (Fig. 12) and reported low columnar and basal cells in their epithelia. Apical parts of the columnar cells showed a strong alkaline phosphatase reaction (Fig. 16), and contained granules of different sizes in some of which histochemical reactions denoted materials containing mucoprotein (Fig. 17) and possibly phospholipids. Ferric ferricyanide and dihydroxy dinaphthyl disulphide (DDD) methods indicated the presence of sulfhydryl groups in some of the granules (Figs. 14, 15). Some of the columnar cells had only microvilli, but others had both cilia and microvilli. The authors proposed that the epithelial cells of ductuli efferentes in *D. marsupialis* had absorptive and probably secretory functions; supported by Ladman (1967).

The wedge-shaped segment containing the ductuli efferentes from six months old opossum males did not have the yellowish green color of the adults (Martan *et al.*, 1967b). This lack of specific coloration and the absence of apical granules giving positive reactions with DDD and ferric ferricyanide in the epithelial cells, prompted the researchers to suggest that both, the coloration and the granules, were under testicular hormone control and associated with maturity.

The anatomical and histological structures of the ductuli efferentes of the four-eyed (*Philander opossum*, Brisson), murine (Marmosa sp., Gray), and woolly (*Caluromys derbianus*, Allen) opossums were similar to those described for adul *Didelphis marsupialis* (Martan *et al.*, 1967b.). However, their coloration was

grayish pink and the apical granules, which give a positive reaction for sulfhydryl groups, were missing. In the columnar cells of the ductuli efferentes in the four-eyed opossum, another type of specific structure was observed in their apical cytoplasm (Figs. 19, 20). This single basophilic body, not limited by a membrane, was 1.5μm in diameter and contained RNA and protein. Thus the only observed differences in ductuli efferentes among the representatives of four genera of the family Didelphidae were on the cytological and cytochemical level (Figs. 13, 14, 17, 19). These differences probably represent the modified requirements of spermatozoa in different species. The lack of studies on the ductuli efferentes in the Australian marsupials prevents comparison between the two groups.

The epididymal duct in *Didelphis marsupialis*, with its anatomical divisions into caput, corpus, and cauda, resembled the epididymides of eutherian mammals (Snydle 1975). Using histological criteria, Snydle divided the epididymis of the opossum into eight segments. He observed that in the middle of the corpus (segment 5), single spermatozoa suddenly associated into pairs. In each pair, the two spermatozoa were attached to one another by the fusion of limited areas of cell membranes overlying their acrosomes. This pairing of spermatozoa was accompanied by strong histochemical reactions for alkaline phosphatase, polysaccharides, and acid mucopolysaccharides. The reactions occurred in both, the cytoplasm of the epididymal cells lining segment five and in the lumen of the duct. Electron microscopic studies have shown changes in the spermatozoan plasma membranes occurring in the same segment. Barnes (1977) reported that in *Marmosa robinsoni*, pairing of the spermatozoa occurred in the cauda epididymis. Pairing of spermatozoa is a common feature in American opossums and unknown in Australian marsupials. The functional aspects of the pairing of sperm are only speculative. However, in the guinea pigs, formation of another type of spermatozoan association, called rouleaux, was reported to be dependent on testicular androgens and not necessary for the sperm fertility (McGlinn *et al.*, 1979). On the other hand, spermatozoa in rouleaux were protected against phagocytosis by leukocytes in the female uterus while single spermatozoa were not protected (Martan and Shepherd 1973). High levels of serotonin were found in the epididymides and ductuli efferentes of *Didelphis marsupilais*, accompanied with even higher levels of an unknown, probably indole-like compound (Anderson *et al.*, 1979). Positive histochemical reactions were observed in the interductular connective tissue, mast cells, apical parts of epididymal epithelial cells, and subepithelial bands (Figs. 22, 23, 24, 25, 26). The cells forming these subepithelial bands had a high RNA content (Fig. 28). The authors proposed that the bands represented a myoepithelial layer under the epididymal epithelium (Fig. 27). If a myoepithelial layer will be observed to be characteristic of the epididymides of other marsupials, it will represent a distinct difference between the genital tract of marsupials and that of eutherian mammals. Chase (1939) described the ciliated columnar epithelium in the epididymis and the vas deferens of *Didelphis marsupialis*. The cilia in the epididymal duct were not confirmed by any other researcher and there is no detailed report on the vas deferens of any marsupial.

The epididymides of the Australian marsupials had anatomical divisions similar to those of eutherian mammals (Cummins 1980), and the passage of spermatozoa through the epididymal duct was estimated to last between eleven and thirteen days (Setchel and Carrick 1973). In *Trichosurus vulpecula* the epididymis had higher testosterone 5 α-reductase activity than the testis (Cook *et al.*, 1978),

and spermatozoa acquired their progressive motility in the distal corpus epididymis (Temple-Smith and Bedford 1976).

The elimination of unused spermatozoa by spermatorrhea, first reported in Australian marsupials (Carrodus and Bolliger 1939, Bolliger 1942, Woolley 1964) and later found in some American species, (Biggers 1966, Barness 1977) appears to be another similarity between the two marsupial groups and in contrast to the majority of eutherian mammals. The occurrence of both spermatorrhea and coagulation of the ejaculate in marsupials led Rodger and White (1975b) to speculate that the mechanism of ejaculation in this group is different from that of eutherian mammals.

In conclusion, the marsupial male genital tract is structurally more stereotyped than that of his eutherian counterpart. The accessory sex glands originating from the embryonic urogenital sinus are present, while accessory sex glands originating from the embryonic Wolffian ducts are missing. All prostate glands so far reported were of the diffuse (disseminate) type. Comparative studies when done, have confirmed the morphological uniformity among the members of the groups studied. Differences when reported were on the subcellular and cytochemical levels only. The products of marsupial accessory sex glands coagulate when ejaculated, forming vaginal plugs. Plugs have been observed in both the American and Australian marsupials. Similarly, there have been several reports of spermatorrhea in both groups, which suggest functional similarities of marsupial sex glands and ducts.

Additional information on the genital ducts of marsupials is needed before any comparison within this group and with eutherian mammals can be made. Pairing of spermatozoa in the epididymal duct of American marsupials provides a distinct example of differences between them and the Australian group. Reports that the principal sugars of seminal plasma in Australian marsupials were glucose and N-acetylglucosamine rather than fructose, established a significant difference between this group and the eutherian mammals. On the other hand, if fructose will be confirmed as the main sugar in the seminal fluid of American marsupials, an additional difference between the two marsupial groups will become evident. However, apocrine type of secretion and an unusually high amount of glycogen reported in prostatic secretions in both the American and Australian marsupials point to their functional similarity.

The morphological and physiological patterns of the marsupial male genital tract seem to be emerging. Many more species need to be studied before more definite conclusions are drawn.

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The author gratefully acknowledges Dr. Benjamin A. Shepherd for his assistance in reading and proofing of the manuscript.

Table 2.

Oppossums	Body weight in grams	Prostate weight in grams	Prostate weight as % of body weights*	Colors and weights of the individual segments expressed as % of total prostate weight*					
				Segment I		Segment II		Segment III	
North American***	1160-5280	7.4-13.0	0.26 ± 0.03 (13)	11.3 ± 1 (8)	light brown	53.6 ± 1.8 (8)	pink	35.1 ± 2.1 (8)	dark grey
Four-eyed	345-1100	0.67-2.0	0.19 ± 0.01 (7)	9.8 ± 1.1 (3)	pale grey	57.0 ± 1.3 (3)	pink	33.2 ± 0.5 (3)	dark grey
Woolly	333-362	1.67-2.19	0.56 ± 0.02 (4)	40.2 ± 5.4 (4)	pale grey**	47.9 ± 5.6 (4)	pink	11.9 ± 1.8 (4)	dark grey
Murine	45-80	1.1-2.15	2.71 ± 0.44 (6)	17.1 ± 1.3 (5)	pale grey	26.5 ± 1.6 (5)	pink	56.5 ± 1.5 (5)	dark grey

*Numbers in brackets denote number of animals studied.
The ± figure is the estimated standard deviation of the mean.
**Segment I of woolly opossum had gelatinous consistency.
***After Hruban *et al.*, 1965 and (unpublished data.
From J. Martan *et al.*, 1967a., unpublished manuscript)

Table 3. Catalase and Uricase Activity of Livers and Prostates

Opossums	Catalase*				Uricase*			
	Liver	Segment			Liver	Segment		
		I	II	III		I	II	III
North American**	2.58 ± 0.43 (9)	1.37 ± 0.47 (5)	0.41 ± 0.08 (3)	35.36 ± 4.28 (6)	0.91 ± 0.06 (9)	0-trace (6)	0-trace (3)	0-trace (6)
Four-eyed	4.38 ± 0.13 (4)	10.23 ± 5.72 (3)	0.67 ± 0.11 (3)	166.2 ± 22.5 (4)	0.55 ± 0.01 (4)	0.42 ± 0.09 (3)	0.49 ± 0.12 (3)	0-trace (4)
Woolly	1.48 ± 0.41 (4)	4.07 ± 3.04 (3)	0.32 ± 0.08 (2)	16.62 ± 2.32 (2)	0.20 ± 0.03 (2)	trace (3)	0-trace (3)	0-trace (3)
Murine	5.10 ± 1.07 (4)	—	1.66 (1)	26.23 ± 7.88 (3)	0.95 ± 0.18 (4)	—	0 (1)	0-trace (3)

*Catalase and uricase activities are expressed in units per gram of nitrogen.
Numbers in brackets denote number of specimens from different animals.
The ± figure is the estimated standard deviation of the mean.

**After Hruban *et al.*, 1965 and (unpublished data.
From J. Martan *et al.*, 1967a., unpublished manuscript)

EXPLANATION OF FIGURES

Fig. 1. Male opossum, *Didelphis marsupialis virginiana* genital tract. (Redrawn and modified from C.R. Moore, Physiol. Zool. 14:1-45, 1941.)

Fig. 2 to 4. Longitudinal sections through the opossum prostate showing separation of the segments (I on the left, III on the right). The first segment shows a pale inner zone. The subdivision of the second and third segment into outer zone, middle zone and inner (secretory ducts) zone is evident in some stains. A thin superficial muscular capsule is recognizable in some areas. x 3.

2. Mallory hematoxylin stain.

3. Mitochondrial stain after Kull.

4. Periodic acid-Schiff reaction after diastase digestion and Alcian blue reaction.

Fig. 5. Transition between the outer (top and middle (bottom)) zone of the second segment. The pale cells in the outer zone probably represent C-cells. The dark cells in the middle zone are A-cells. Both zones contain moderately dense B-cells. Periodic acid-Schiff reaction. x 300.

Fig. 6. Acid phosphatase reaction on the third segment shows localization of the enzyme in villi and in lumina. x 300.

(Figs. 2, 3, 4, 5 and 6 from Hruban *et al.*, J. Exper. Zool. 160:81-105, 1965 Alan R. Liss, Inc.)

Fig. 7. A and B cells in the epithelium of segment II of the prostate gland. Type A cells are characterized by the presence of rounded secretory granules and a greater height than the type B cells which are typified by the presence of elongate secretory granules. Ludford osmium tetroxide method, paraffin embedded, 3 μm. x 1,750.

Fig. 8. Differential staining of A and B cells following fixation in 4.0% formaldehyde containing 1.0% cadmium chloride and refixation (after sectioning and mounting) for three hours in 5% mercuric chloride at 50°C. The rounded granules in the "A" cells stain red after treatment with the periodic acid-Schiff procedure while the elongate granules of the B cells stain purple after treatment with Mallory's phosphotungstic acid hematoxylin. Paraffin embedded, 2 μm. x 1,750.

Fig. 9. Distribution of glycogen in segment III of the prostate gland. Dense accumulation of this material are seen in the epithelium and large dense mass is present in the lumen. Periodic acid-Schiff reaction hematoxylin counterstain. Bouin fixation, 8 μm frozen section. x 700.

Fig. 10. Identical to figure 9 but with omission of hematoxylin counterstain. Large numbers of periodic acid-Schiff reactive globules are present in the glandular lumen. x 700.

(Figs 7, 8, 9 and 10 from Martan and Allen, J. Exper. Zool. 159:209-229, 1965 Alan R. Liss, Inc.)

Fig. 11. Gross appearance of the testis and epididymis of adult North American opossum. Cauda (C) of the epididymis is detached from the testis. Ductuli efferentes (arrow), epididymis (E), testis (T), vascular hilum (V). x 3.5.

Fig. 12. Section through the ductuli efferentes and adjacent epididymis (E). The ductuli efferentes near testis (arrow) have narrow lumina. Wide lumina are seen in the major portion of the ductuli efferentes (D). North American opossum, hematoxylin-eosin, 6 μm section x 30.

Fig. 13. Cross section of a ductulus efferens of the woolly opossum, showing distribution of large, periodic acid-Schiff reactive, diastase resistant, granules in epithelial cells. Lumen (L). Periodic acid-Schiff-Alcian blue reaction, 6 μm section x 2,000. (Compare with Fig. 17)

Fig. 14. Cross section of a ductulus efferens of the North American opossum. Ferric ferricyanide reaction demonstrating localization of granules with sulfhydryl groups. Lumen (L). 5 μm section x 530.

(Figs 11, 12, 13 and 14 from Martan *et al.*, 1967b. J. Morph. 121:81-101. Alan R. Liss, Inc.)

Fig. 15. Same as in Fig. 14. Sulfhydryl groups in granules demonstrated by DDD method. Connective tissue (CT). x 1,300.

Fig. 16. Distribution of alkaline phosphatase in cross-section of ductulus efferens of North American opossum. The reaction product is restricted to the apical region of epithelial cells. Lumen (L) 15 μm freezing microtome section. x 600.

Fig. 17. Distribution of periodic acid-Schiff positive, diastase resistant granules in the epithelial cells of ductuli efferentes of North American opossum. Nuclei (N). Periodic acid-Schiff-Alcian blue reaction, 6 μm section x 2,000. (Compare with Fig. 13)

Fig. 18. Localization of iron in the apices of cells and in cytoplasmic globules in lumina in segment I of the prostatic complex. Turnbull blue reaction x 300.

Fig. 19. Ribose nucleic acid revealed in basophilic bodies (arrows) stained with azure B reaction at pH 4.0. One body in lumen (L). Nuclei (N). 7 μm section x 2,000.

Fig. 20. Basophilic bodies (arrows) are positive with Luxol Fast Blue MBS, suggesting the presence of phospholipid material. Nuclei (N) 7 μm section x 1,500.

Fig. 21. Distribution of acid phosphatase in segment I of the prostatic complex. The reaction is restricted to apical portion of intact epithelial cells and cytoplasmic vesicles released into tubular lumina. Frozen section, Gomori method, x 565.

(Figs. 15, 16, 17, 19 and 20 from Martan *et al.*, 1967. J. Morph., 121:81-101. Alan R. Liss, Inc.)

(Fig. 18 from Hruban *et al.*, 1965, J. Exp. Zool. 160:81-105. Alan R. Liss, Inc.)

Fig. 22. Section through the cauda epididymis. Note the subepithelial band (arrow), epithelial granules (g) and interductular connective tissue x 528.

Fig. 23. Excitatory light quenching of induced yellow fluorescence. Compare with Fig. 22., x 528.

(Figs. 22 and 23, from Anderson *et al.*, 1979, J. Anat. 129:141-149 Cambridge U. Press)

Fig. 24. Section through the cauda epididymis of the opossum. Note the blood vessel (arrowhead) and mast cells arrow; x 300.

Fig. 25. Section treated with $NaBH_4$. Compare with Fig. 24. x 300.

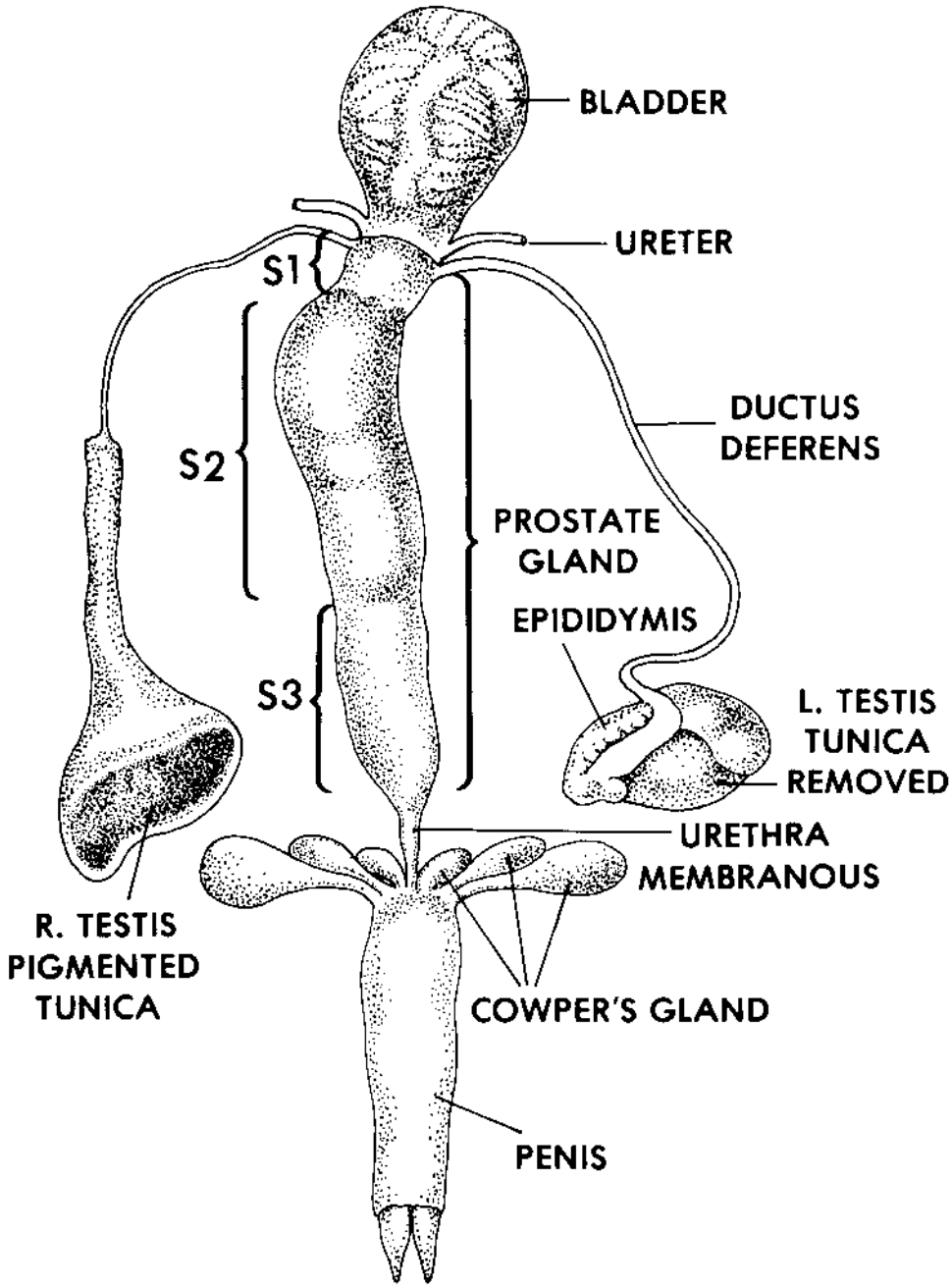
Fig. 26. Cuada epididymis following reinduction of fluorescence with gaseous formaldehyde. Compare with Figs. 24 and 25. x 300.

(Figs. 24, 25 and 26, from Anderson *et al.*, 1979, J. Anat. 129:141-149 Cambridge U. Press)

Fig. 27. Nuclei (arrow) in the subepithelial band of the opossum cauda epididymis, Feulgen, x 320.

Fig. 28. Cross-section of cauda epididymis of the opossum exhibiting RNA positive subepithelial band, methyl green-pyronin Y, x 400.

(Figs. 27 and 28, from Anderson *et al.*, 1979, J. Anat. 129:141-149 Cambridge U. Press)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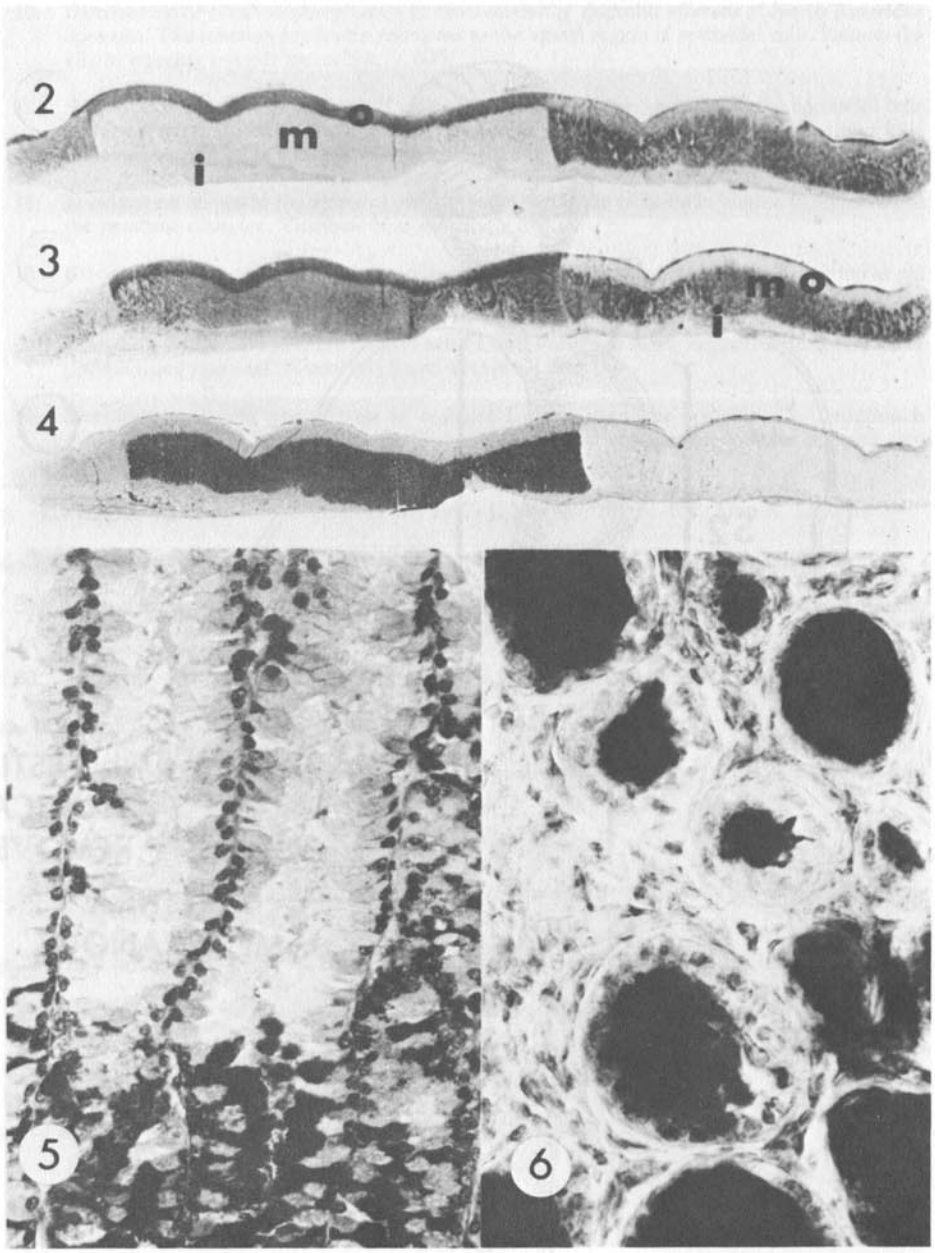
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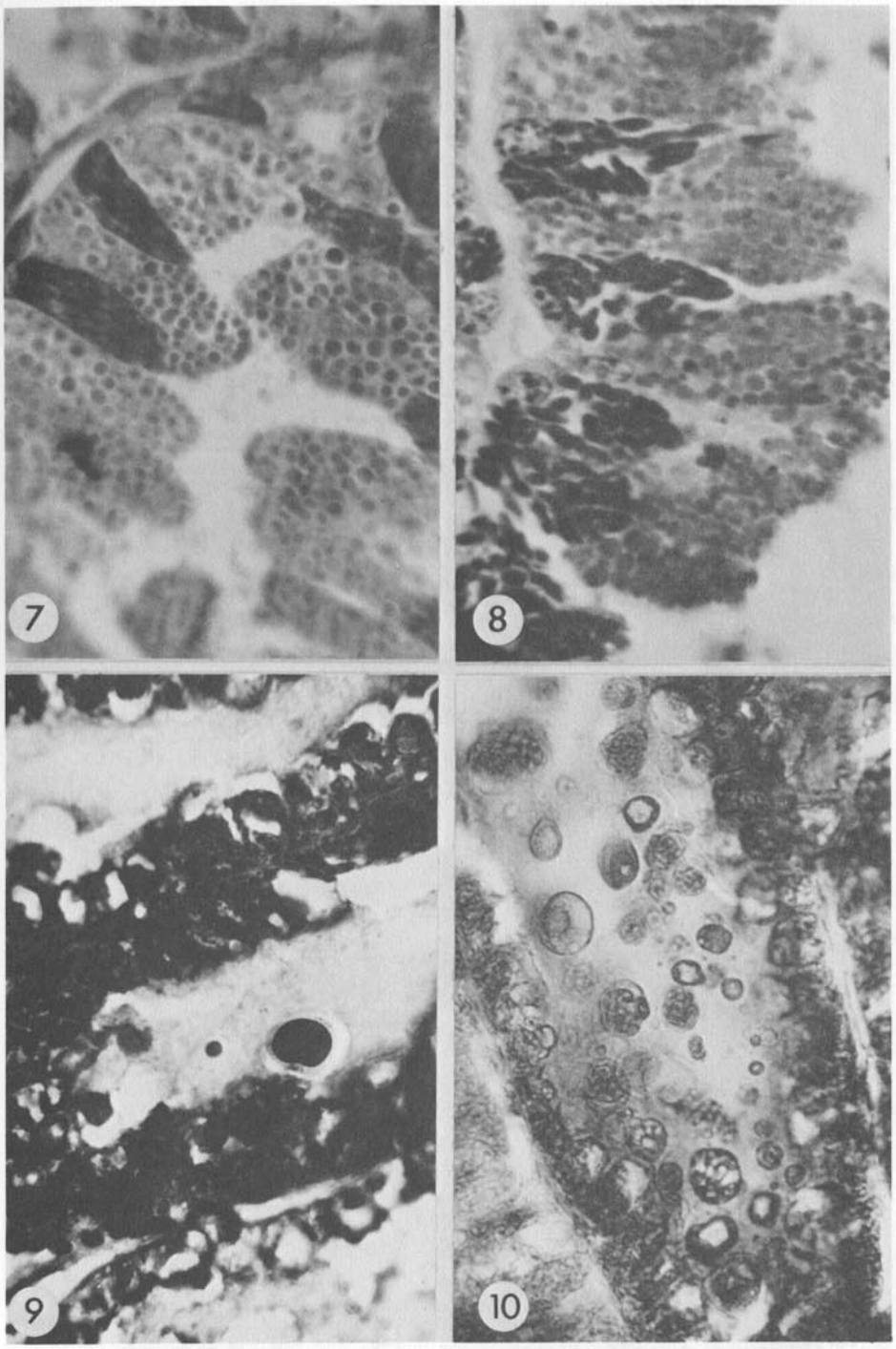
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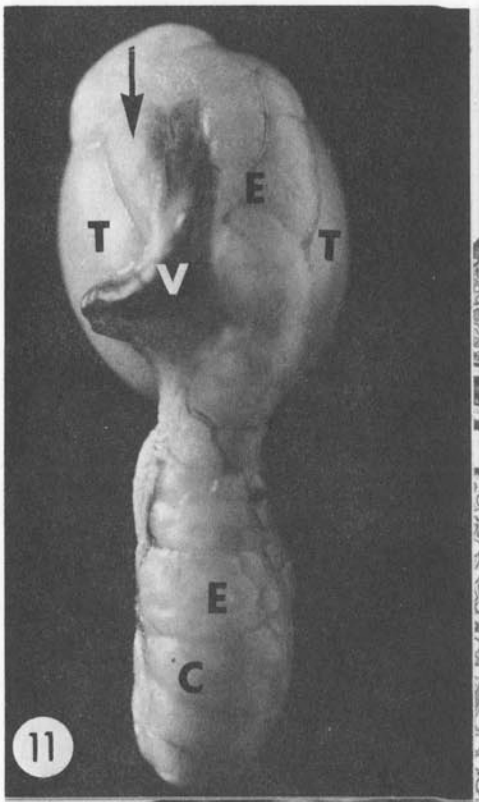

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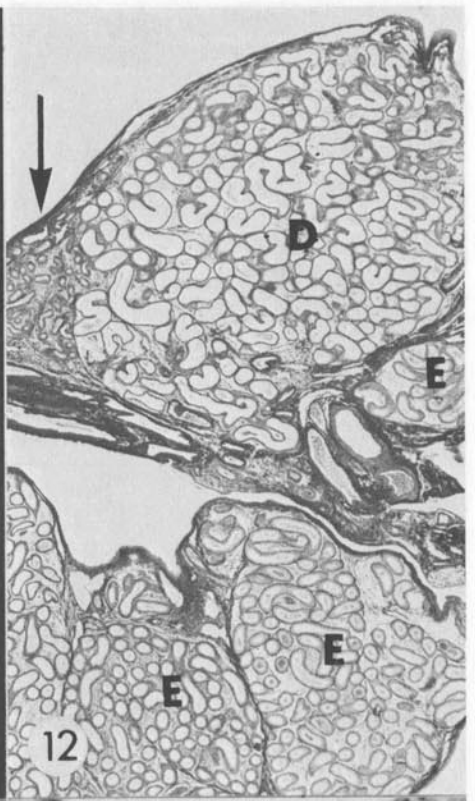

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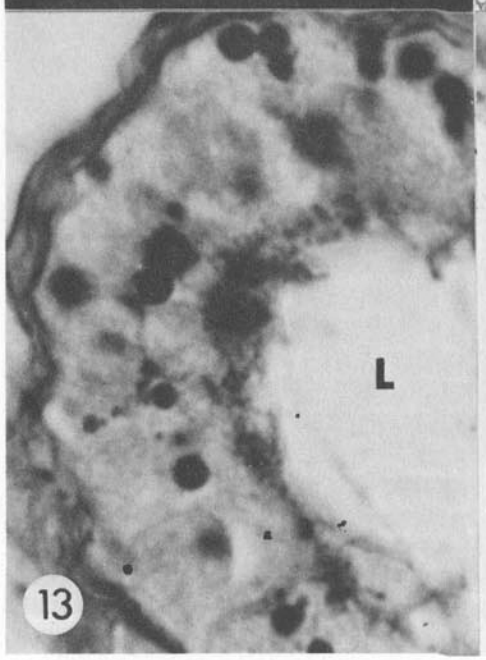

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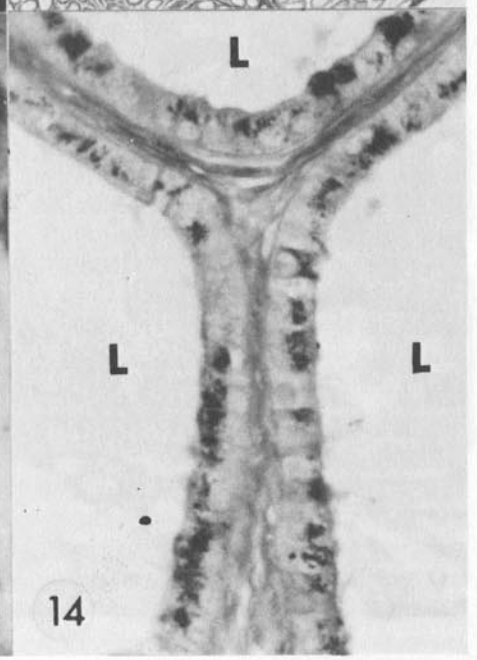

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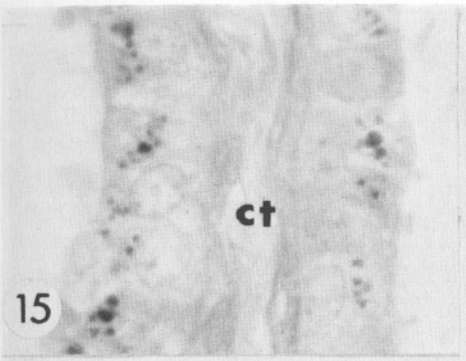
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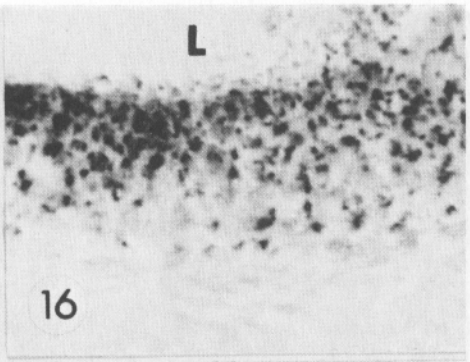
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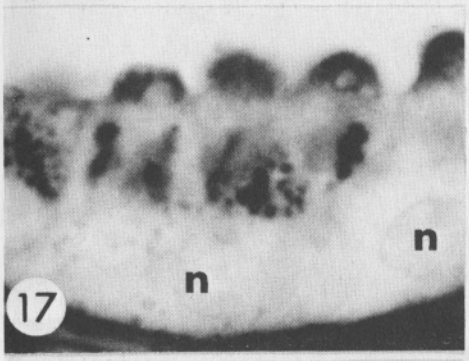
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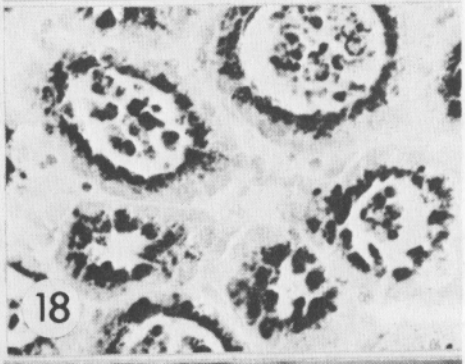
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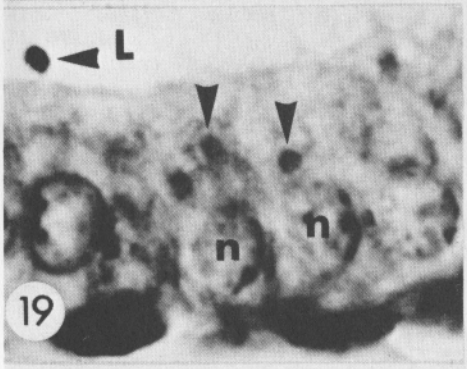
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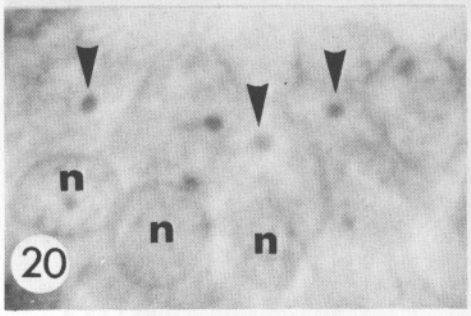
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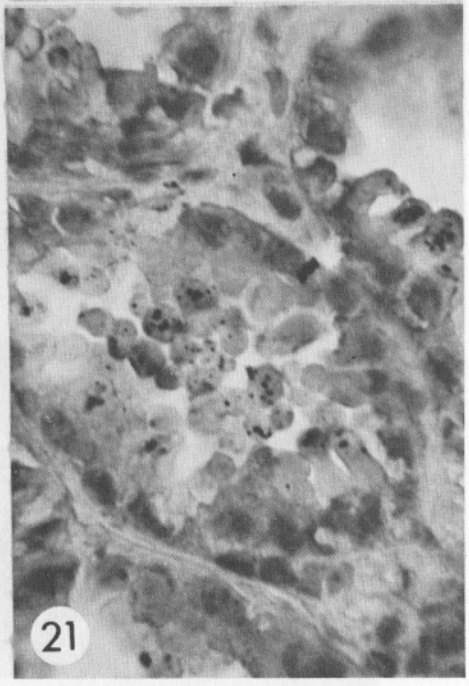
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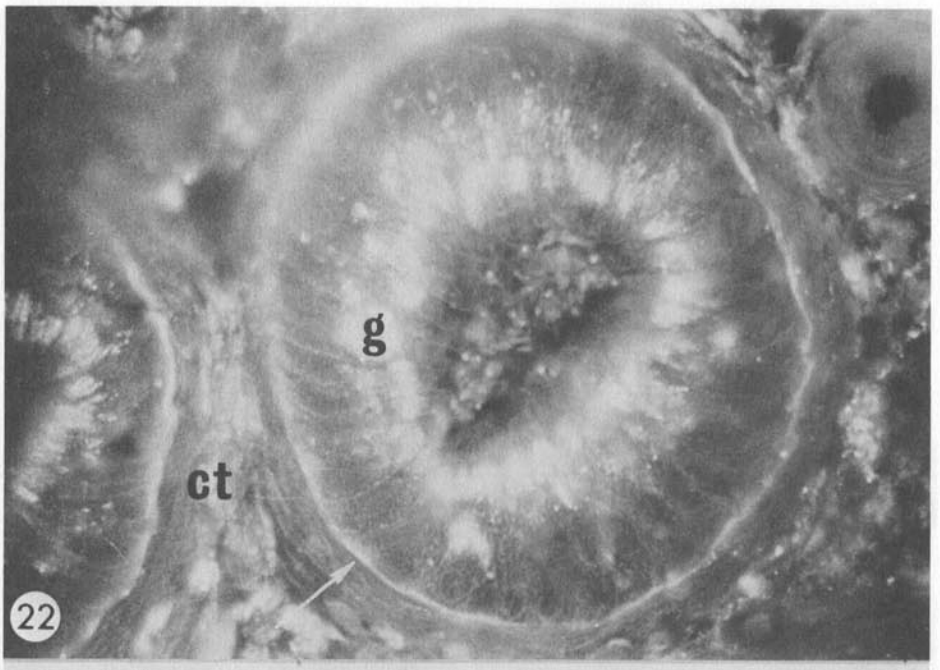
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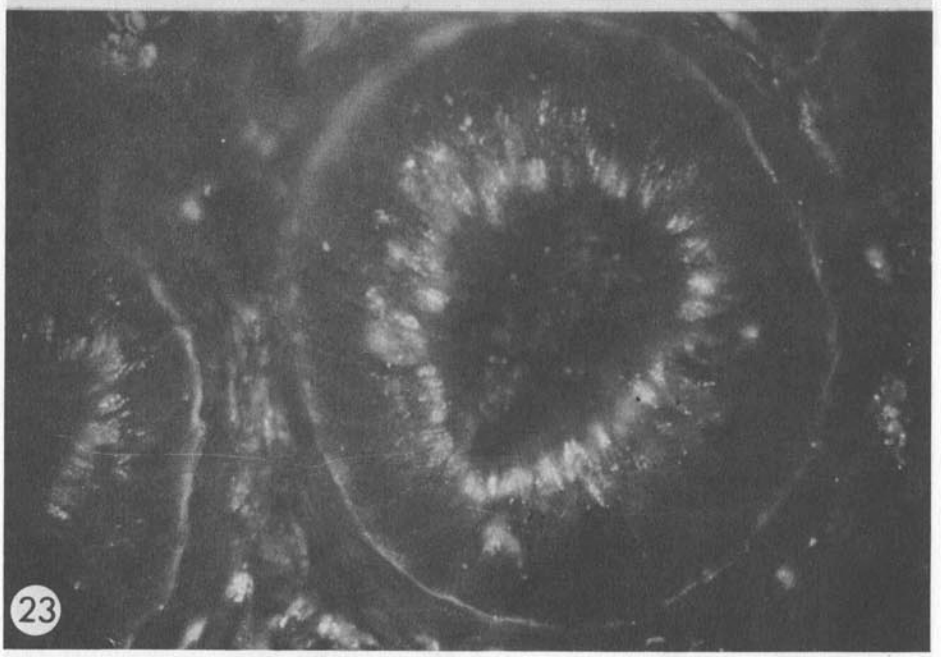
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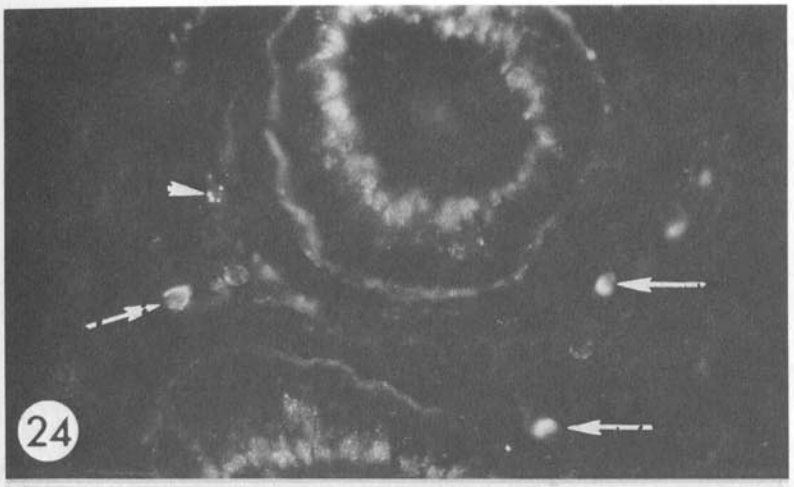
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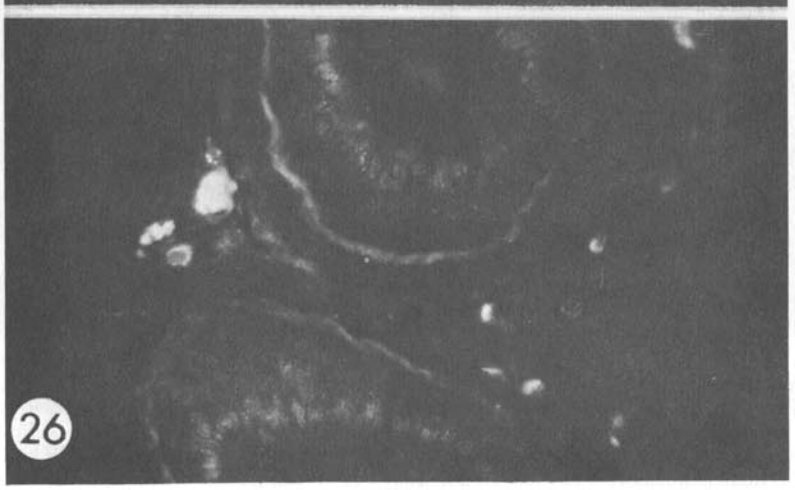
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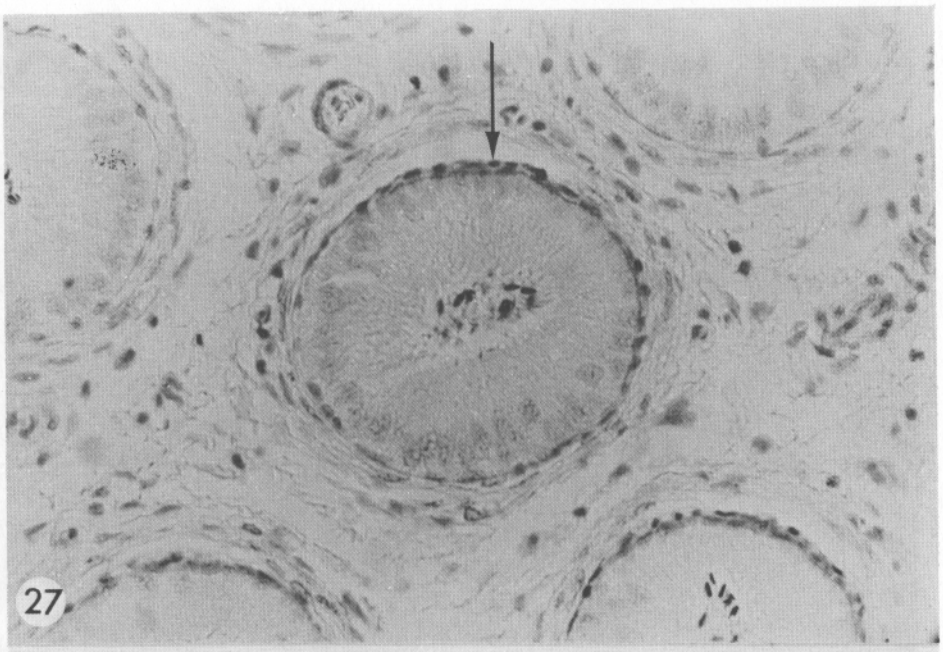

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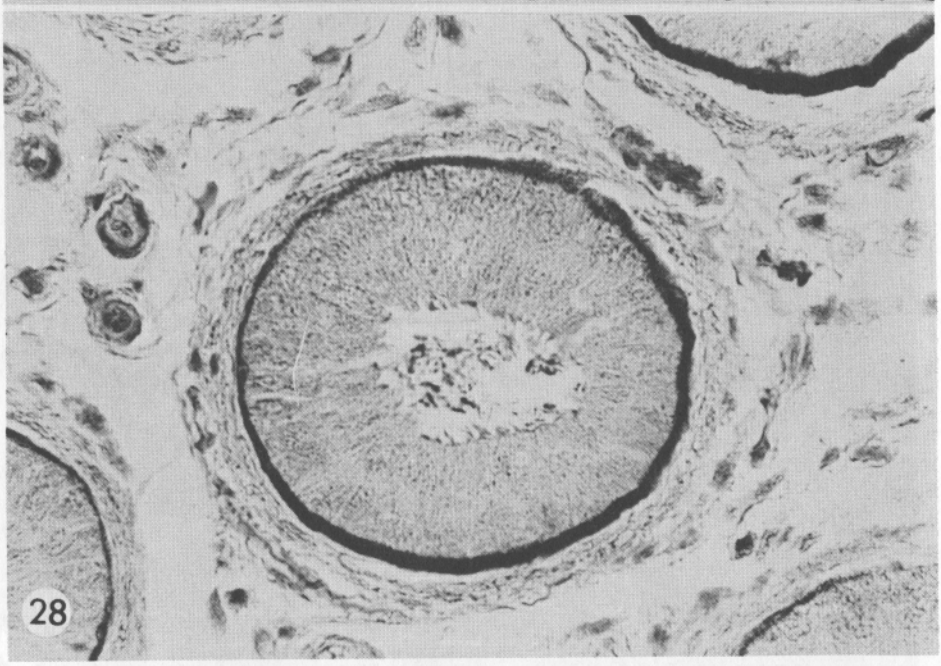

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