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AN ELECTRON MICROSCOPICAL STUDY OF ABSORBING CELLS IN THE POSTERIOR CAPUT EPIDIDYMIDIS OF RABBITS

By

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With 17 Figures in the Text

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Summary. The columnar cells in regions 3 and 4 of the ductus epididymidis in rabbits display ultrastructural features characteristic of *absorbing cells.* The stereocilia show basal anastomoses and often a fibrillar core continuous with a fibrillar web in the apical cytoplasm. Numerous invaginations of the slightly "downy" apical cell membrane and many thick-walled apical vesicles and vacuoles contain an opaque substance similar to that seen in the lumen. The vacuoles often contain small vesicles or bodies, probably formed from the vacuolar wall by budding. Numerous bodies or vacuoles with moderately dense contents are seen in the Golgi area and in the supranuclear and infranuclear cytoplasm in region 3. In region 4 they are denser and mainly seen above the nucleus. A high acid phosphatase activity was demonstrated in most dense and some light bodies. India ink introduced by way of the rete testis was taken up from the lumen into apical invaginations, vesicles and vacuoles and slowly transferred to denser bodies below the Golgi apparatus.

These observations are interpreted as evidence for a resorption of substances from the lumen by a pinocytotic process, and for their storage and perhaps digestion in the dense bodies, which appear to have a lysosomal character. The Golgi apparatus is large with many vesicles of two types and empty cisternae but few typical Golgi vacuoles. The partly granular endoplasmic reticulum is very well developed and has opaque contents. Microtubules run from the terminal bar region into the Golgi area. Thick-walled vesicles occur throughout the cytoplasm, sometimes in continuity with the cell membrane. The basal parts of the cell borders often interdigitate.

The cytoplasmic granules and vacuoles in the epididymal duct epithelium are generally considered to indicate a *secretory function.* On the other hand, many studies have shown that this epithelium has an *absorbing capacity*, especially in the caput epididymidis.

The spermatozoa are carried into the epididymis by a current of fluid from the seminiferous tubules. Most of this fluid is resorbed in the efferent ductules and the proximal part of the epididymal duct (CRABO and GUSTAFSSON 1964). Vital dyes (Trypan blue and India ink) are also absorbed from the luminal contents of these regions (VON MÖLLENDORFF 1920, WAGENSEIL 1928, YOUNG 1933, MASON and SHAVER 1952, SHAVER 1954).

Elaborate *regional variations* regarding the structure and cytochemistry of the duct epithelium have been demonstrated in the epididymides of dogs (HAMMAR 1898), rabbits (NICANDER 1957), rats (REID and CLELAND 1957), stallions, bulls, and rams (NICANDER 1958), and mice (ALLEN and SLATER 1961). The initial segment (region 1) of the rabbit epididymal duct has a high epithelium and few spermatozoa in the lumen. Regions 2 and 3 — at the caput flexure — contain increasing amounts of spermatozoa and an amorphous, eosinophilic substance stainable

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with periodic acid and Schiff's reagent. In region 4 the lumen is invariably crowded with spermatozoa but contains only small amounts of eosinophilic substance. The columnar cell cytoplasm of region 3 appears highly vacuolated, whereas region 4 has a lower epithelium with fewer vacuoles and numerous granules. Therefore, NICANDER (1957) suggested that the many cytoplasmic vacuoles of region 3 might contain material absorbed from the luminal contents instead of secretory precursors. The non-ciliated cells of the efferent ductules of guinea pigs show ultrastructural features suggesting absorption of fluid from the lumen (LADMAN and YOUNG 1958). In rabbits, the corresponding cells take up electron-dense particles from the luminal contents into apical vesicles and later into dense bodies at deeper levels of the cell (NICANDER 1960). BURGOS (1964) has recently shown that the columnar cells of the initial segment of the epididymal duct in hamsters perform a similar function. The fine structure of the human epididymis was studied in detail by HORSTMANN (1962), who found signs of pinocytosis and different types of dense bodies in the cytoplasm.

The aim of the present work was to study the fine structure of regions 3 and 4 of the rabbit epididymis with special reference to features suggesting pinocytosis and phagocytosis, including the uptake of colloidal particles from the luminar contents. Colloidal particles were found to be taken up by the columnar cells and transferred to dense cytoplasmic bodies. The possible lysosomal character of these dense bodies was also investigated by cytochemical methods for acid phosphatase. A preliminary report of the results has been published (NICANDER 1962).

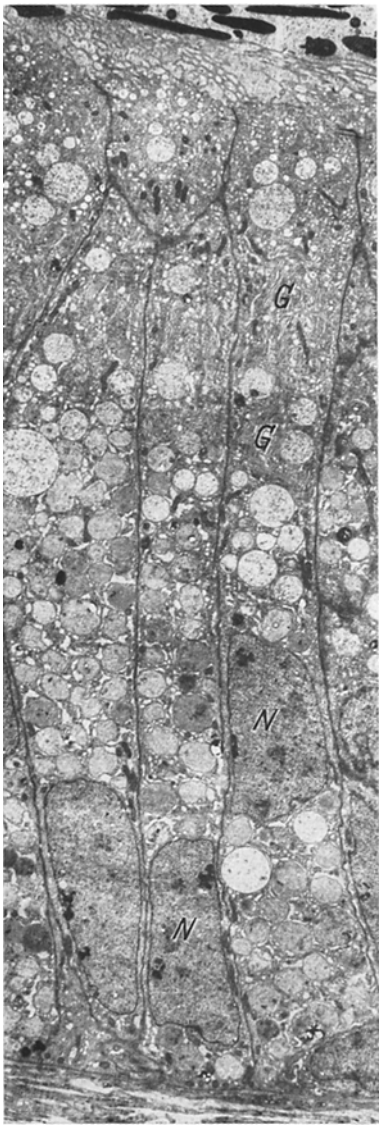
Material and Methods

Ten young, sexually mature rabbits were used for the study of normal fine structure. Small pieces rapidly cut out from regions 3 and 4 of the epididymal head were fixed for one hour in a buffered 2% OsO_4 solution with the addition of sucrose (CAULFIELD 1957). After rapid dehydration in ethyl alcohol the pieces were embedded in Epon (LUFT 1961), or in a mixture of methyl and buthyl methacrylate (1:9). Thin sections were cut with an LKB Ultrotome and picked up on formvar coated copper grids in the case of methacrylate embedded material. Epon sections were placed on uncoated grids and "stained" with uranyl acetate (WATSON 1958) or lead acetate (DALTON and ZEIGEL 1960). Electron microscopy was performed with a Siemens Elmiskop I at original magnifications from 1,000 to 12,000 diameters. Seven rabbits were used for phagocytosis experiments. Laparotomy was performed under ether anesthesia and about 0.1 ml of a commercial solution of India ink (Günther Wagner), diluted with Ringer's solution (1:20), was injected into the rete testis on one side. Then the testis was replaced into the scrotum. Fixation was performed after 1, 2, 3 or 4 days, respectively, as stated above, and the material was usually embedded in methacrylate.

Acid phosphatase activity was studied in six rabbits. Three of these were used for light microscopy after freeze-drying and paraffin embedding *in vacuo*, or after fixation in cold, buffered formol and frozen sectioning. Gomori's (1952) procedure was followed. Material from three animals was prepared for electron microscopy, mainly according to the method of HOLT and HICKS (1962 a, b). Frozen sections cut at 40—50 μ were incubated for 10 minutes at pH 5. Control sections were incubated for the same time in substrate with 4% formol. All sections were then processed through osmium "fixation" and embedded in Epon.

Observations

A. Fine Structure. The normal fine structure of the epithelium is fundamentally similar within the two regions. The extremes regarding epithelial height and vacuolization are illustrated in Fig. 1 (from the middle part of region 3) and Fig. 2 (from the middle part of region 4). The following description is largely



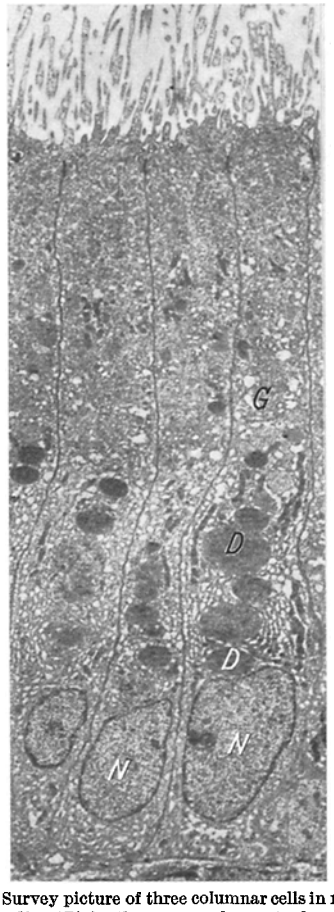


Fig. 2. Survey picture of three columnar cells in region 4, with dense bodies (*D*) in the supranuclear cytoplasm. In this extreme case no vacuoles are seen above the Golgi apparatus (*G*). *N* nuclei. Methacrylate. 3,600 ×

Fig. 1. Survey picture of three columnar cells in region 3. The cytoplasm below the Golgi apparatus (*G*) is occupied by vacuoles with opaque contents. The Golgi and apical zones show fewer vacuoles with lighter, more granulated material. *N* nuclei. Epon. 3,000 ×

based on an intermediate cell type from the proximal part of region 4. Some features characteristic of region 3 are also depicted. For descriptive purpose the columnar cells will be subdivided into apical zone, including the stereocilia, Golgi zone, supranuclear zone, nuclear zone, and infranuclear zone.

Apical zone. The *stereocilia* are long, slender protrusions of the apical cytoplasm, frequently interconnected by anastomoses. The tufts formed by this arrangement are generally bent, apparently in the direction of the intraluminar current (Fig. 3). One very coarse protrusion, containing various cytoplasmic structures, sometimes originates from the centre of the cell apex. Swollen stereocilia with very light contents have also been observed. They seem to represent

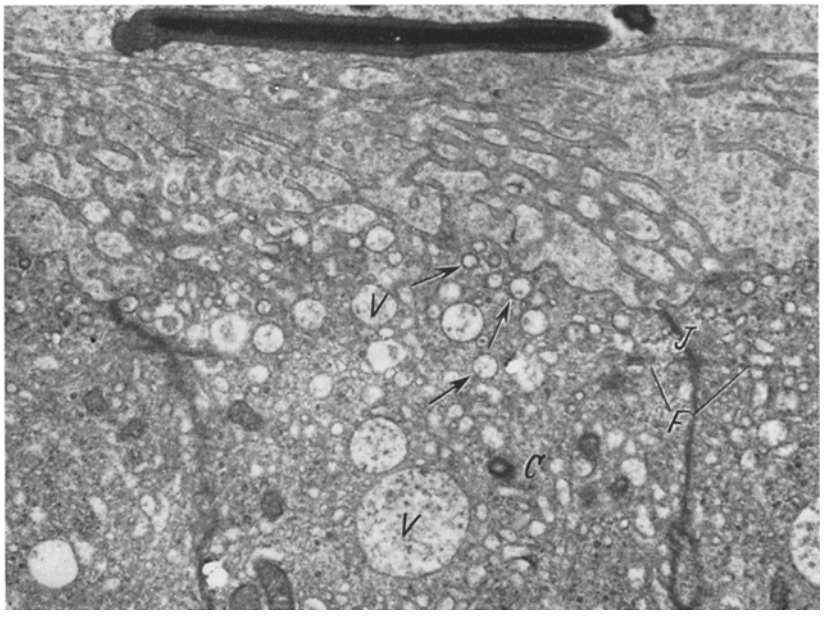


Fig. 3. Enlarged apical part of the intermediate cell in Fig. 1. An opaque, flocculent substance is seen between the anastomosing stereocilia and in the apical vesicles (arrows) and vacuoles (*V*). *F* fibrillar streak from junctional complex (*J*), *C* centriole. Epon. 12,000 ×

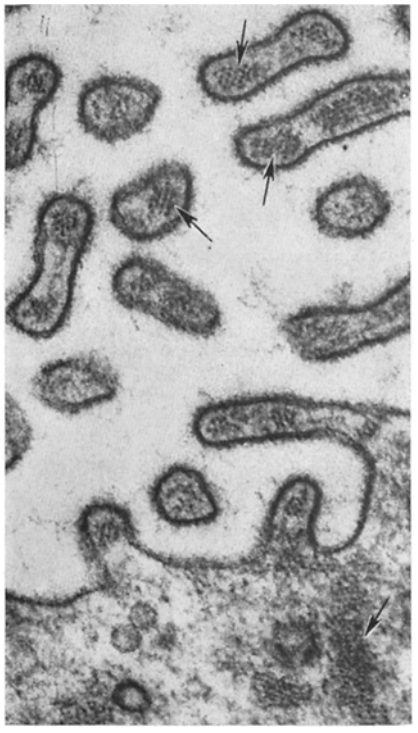


Fig. 4. Region 4. Apical cytoplasma and stereocilia with bundles of fine fibrils (arrows). The outer surface of the cell membrane is downy. Epon. 65,000 ×

artefacts. Most stereocilia show a core of material which is more opaque than the apical cytoplasm, and a longitudinal striation is often visible in this material. In favourable cross sections groups of dense dots less then 50 Å in diameter are seen (Fig. 4). The dots obviously represent cross sections of fine, dense fibrils arranged in bundles. Many of these bundles run down in the apical cytoplasm where they reach and mingle with the dense, fibrillar substance present along the junctional complexes (terminal bars).

The apical *plasma membrane* appears coarser and more heavily stained than the lateral cell membrane, and a thin layer of a downy material adheres to its luminar surface (Fig. 4). The membrane is often invaginated into the apical cytoplasm and appears still coarser in such areas (Fig. 5). The lateral cell borders show a few desmosomes at various levels of the epithelium, and a conspicuous *junctional complex* is seen near the luminar surface at the level of the terminal bar of

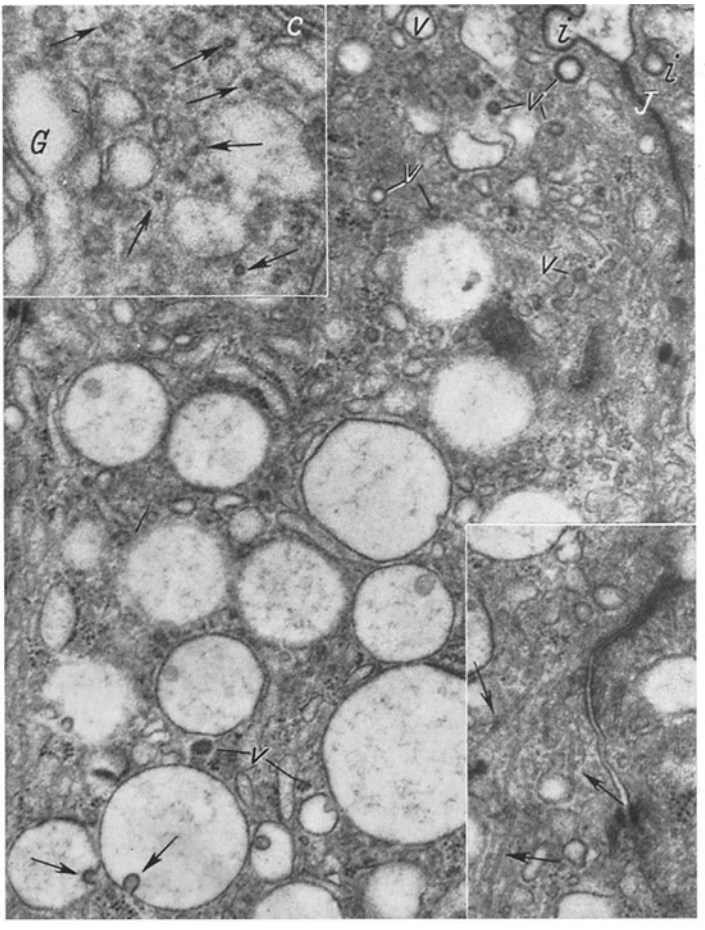


Fig. 6b

Fig. 5

Fig. 6a

Fig. 5. Apical cytoplasm from a cell in region 4, with thick-walled invaginations (*i*) and vesicles (*v*), junctional complex (*J*), vacuoles, and numerous elements of the mostly smooth endoplasmic reticulum. Note the invaginations of the vacuolar membrane into the vacuole (arrows). Epon. 22,000 ×

Fig. 6. a (bottom right). Junctional complex and microtubules (arrows). Epon. 27,000 ×. b (top left). Section through part of the Golgi zone to show cross sectioned microtubules (arrows) between the Golgi complex (*G*) and the cell border (*c*). Epon. 50,000 ×

light microscopy (Fig. 5). It is similar to the junctional complexes described by FARQUHAR and PALADE (1963) in various epithelia. Its juxtaluminar "tight junction" (zonula occludens) is very long, 0.5—1 μ, and some electron dense material is condensed in the cytoplasm along it. Larger amounts of dense material are seen at the level of the "intermediate junction" (zonula adhaerens) from where it radiates into the adjacent cytoplasm as a narrow, slightly fibrillar streak, perpendicular to the lateral cell border. In sections cut near the periphery of a cell this streak bridges the cell apex. Some long, straight or slightly curved *microtubules* with a diameter about 250 Å appear to originate from this peripheral fibrillar material near the junctional complex (Fig. 6a). They are very rarely seen in the apical cytoplasm above the level of this streak. The microtubules seem to run down into the Golgi zone, where they are seen both in the centre and in the

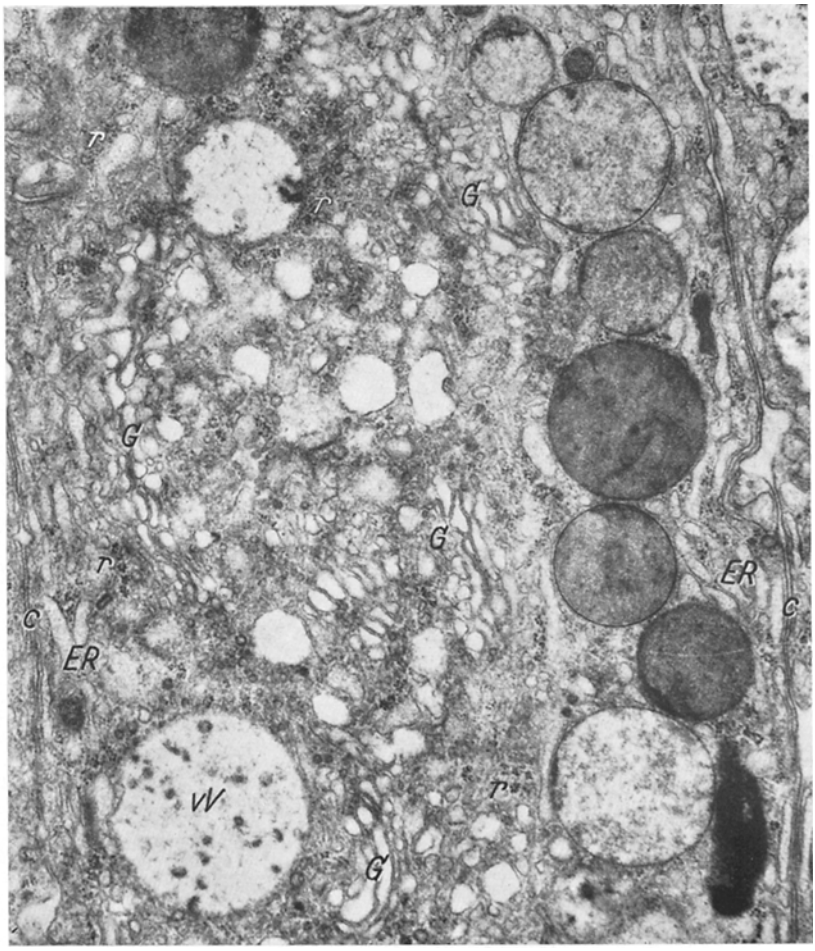


Fig. 7. Longitudinal section of Golgi zone, region 4, with Golgi apparatus (*G*), vacuoles, one with vesicles (*vV*), dense bodies, endoplasmic reticulum with opaque contents (*ER*), and free ribosomes (*r*). *c* cell border. Epon. 18.000 ×

periphery (Fig. 6 b) of the cell. They could never be demonstrated at deeper levels of the cell.

The cytoplasm of the columnar cell is characterized by an opaque matrix and an abundance of vesicles, vacuoles, cisternae, and lamellae. Large *apical vesicles* near the luminar border have about the same size as the apical invaginations and show the same coarse limiting membrane (Fig. 5). They are particularly numerous in region 3 where they contain an opaque, flocculent material and a few denser particles with a diffuse outline. A material with identical structure occurs in the lumen, filling the space not occupied by stereocilia and spermatozoa (Fig. 3). In region 4 these vesicles contain smaller amounts of a more delicate precipitate (Fig. 5), similar to the luminal contents of this region. The most apical cytoplasm also contains numerous microvesicles less than 80 mμ in diameter. At somewhat deeper levels of the apical zone there are many vacuoles. In region 3 they generally have contents similar to the apical vesicles, but in region 4 most of them

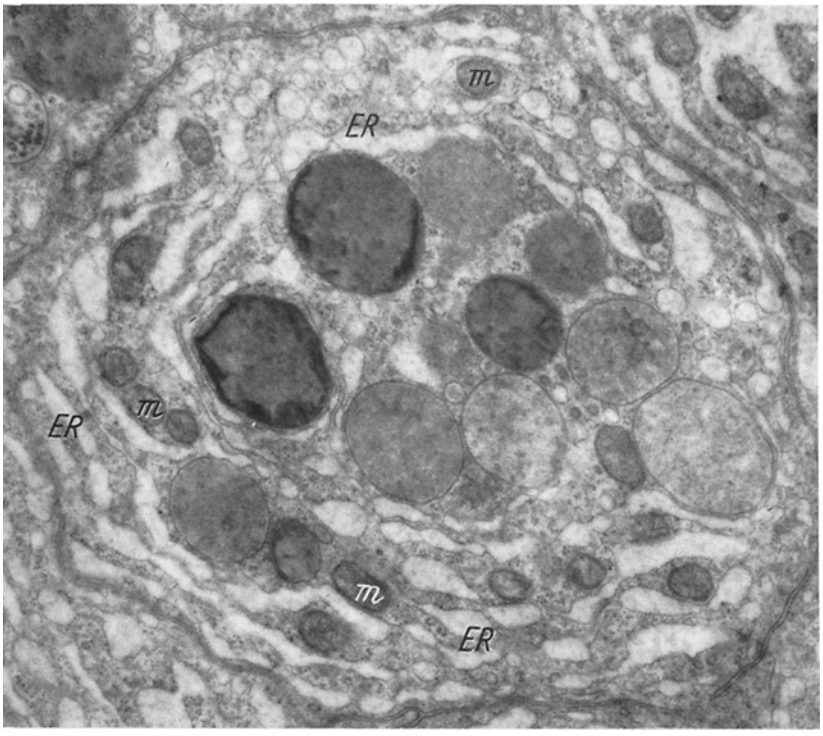


Fig. 8. Cross section of supranuclear zone, region 4. The cisternae of the endoplasmic reticulum (*ER*) show a concentric arrangement in the periphery, mingled with cross sectioned mitochondria (*m*). The centre contains dense bodies of various density and structure. Epon. 18,000 ×

also contain varying numbers of small (50—80 mμ) vesicles of somewhat irregular shape and apparently with opaque contents (Figs. 7, 9). Such vacuoles are reminiscent of large multivesiculated bodies. Cytoplasmic "buds" protruding into the vacuole are seen in many cells (Fig. 5). Numerous small, spherical vesicles are also present throughout the cytoplasm. They are similar to the Golgi vesicles, but some of them display a coarse membrane similar to that of the apical invaginations and large vesicles (Fig. 5). Some elements belonging to the endoplasmic reticulum are also present. They always have opaque slightly flocculent contents (Fig. 10). Ribosomes are also seen, but generally free in small clusters not immediately associated with membranes (Fig. 7). The few, rather small mitochondria have a dark matrix. They are mainly seen near the Golgi region. Centrioles are often observed near the luminar surface (Fig. 3).

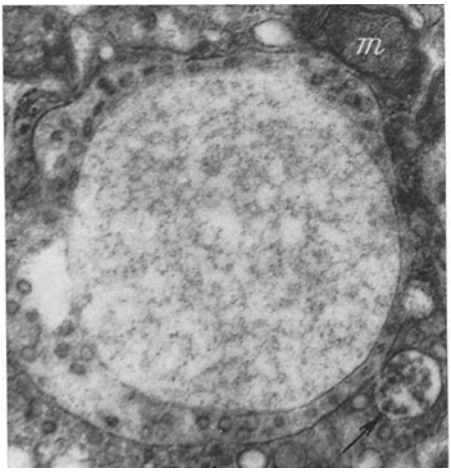


Fig. 9. Detail of the supranuclear cytoplasm, region 4, to show a multivesicular body apparently containing a large vacuole with flocculent contents. *m* mitochondrion. A small vacuole with vesicles is also seen (arrow). Epon. 26,000 ×

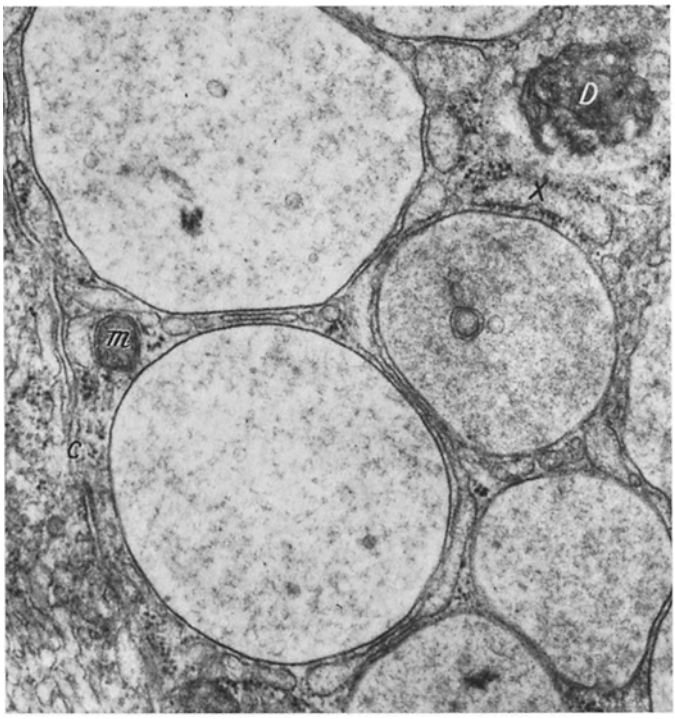


Fig. 10. Periphery of a cell from the supranuclear zone, region 3. Large vacuoles contain a somewhat flocculent material and a few vesicular structures. Narrow elements of the endoplasmic reticulum run between the vacuoles. One cisterna (x) seems to have ribosomes attached to its membrane. c cell border, m mitochondrion, D dense body. Epon. 30,000 ×

The *Golgi zone* is dominated by the large Golgi apparatus, which is mainly built up of piles of winding lamellae arranged in a way suggesting the classical three-dimensional net of light microscopy (Fig. 1). In cross sections the Golgi apparatus is generally shaped like a horseshoe, an S or the numeral 6. The Golgi membranes form pairs enclosing apparently empty spaces often dilated into elongated vacuoles (Fig. 7). Numerous small, spherical Golgi vesicles are also present, as well as some darker vesicles (Fig. 7). The cytoplasm surrounding the Golgi apparatus contains mitochondria, endoplasmic reticulum, ribosomes, and microtubules similar to those in the apical zone. "Multivesiculated bodies" — large vesicles filled with small, spherical vesicles of uniform size — are sometimes seen. A few vacuoles and dense bodies, similar to those in the supranuclear zone are frequently observed. They are often arranged in rows between the Golgi apparatus and the lateral cell membrane (Fig. 7). In one cell in region 4, a very large vacuole was seen, containing a degenerating sperm tail (Fig. 11).

The supranuclear zone is characterized by numerous dense bodies and a few vacuoles, which rarely contain small vesicles. The vacuoles have more flocculent and opaque contents than in the apical zone. The dense bodies generally have spherical or oval shape, but their internal structure is very varying. The most common type has a dark greyish, slightly mottled structure, often with a few very dark peripheral streaks or spots (Figs. 7, 8). In a few cells, a large "multi-

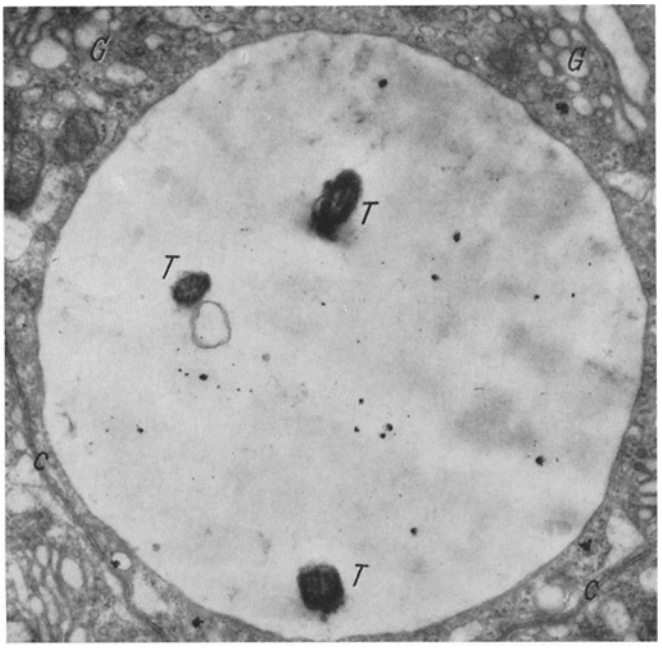


Fig. 11. Cross section of Golgi zone, region 4, to show large vacuole with degenerating sperm tail (*T*). *G* Golgi apparatus. *c* cell border. Epon. 18,000 ×

vesiculated body" with a more homogeneous, membrane bounded central sphere was seen (Fig. 9). In region 3, dense bodies are rare and the many vacuoles differ from the apical ones only through their more homogeneous contents (Figs. 7, 10). The cisternae of the endoplasmic reticulum are ofter "rough surfaced" within this area of the cell. In cross sections a concentric arrangement of the cisternae is often suggested (Fig. 8). Numerous free ribosomes are also present, as well as many slender mitochondria, elongated along the axis of the cell.

The *nuclear zone* is mainly occupied by the nucleus surrounded by a narrow rim of cytoplasm with some endoplasmic reticulum. The periphery of the nucleus often shows impressions caused by adjacent dense bodies or vacuoles. The perinuclear space appears to be continuous with the endoplasmic reticulum. Nuclear inclusions were not observed.

The *infranuclear zone* varies much in height. In region 3 it is quite similar in character to the supranuclear zone (Fig. 1). In region 4, on the other hand, it shows only a few dense bodies, or vacuoles with very dense particles. The endoplasmic reticulum is well developed and the cisternae are generally parallel to the long axis of the cell (Fig. 12). Many slender mitochondria occur, apparently oriented at random. They are often accumulated near the basal cell membrane, but never near the border to a basal cell. The basal cytoplasm often sends off branches interdigitating with those of adjacent columnar and basal cells, but no infoldings were seen along the basal surface facing the basement membrane. Some thick-walled vesicles are generally seen near the interdigitations, and sometimes such vesicles seem to arise from or coalesce with the lateral cell membrane (Figs. 12, 13).

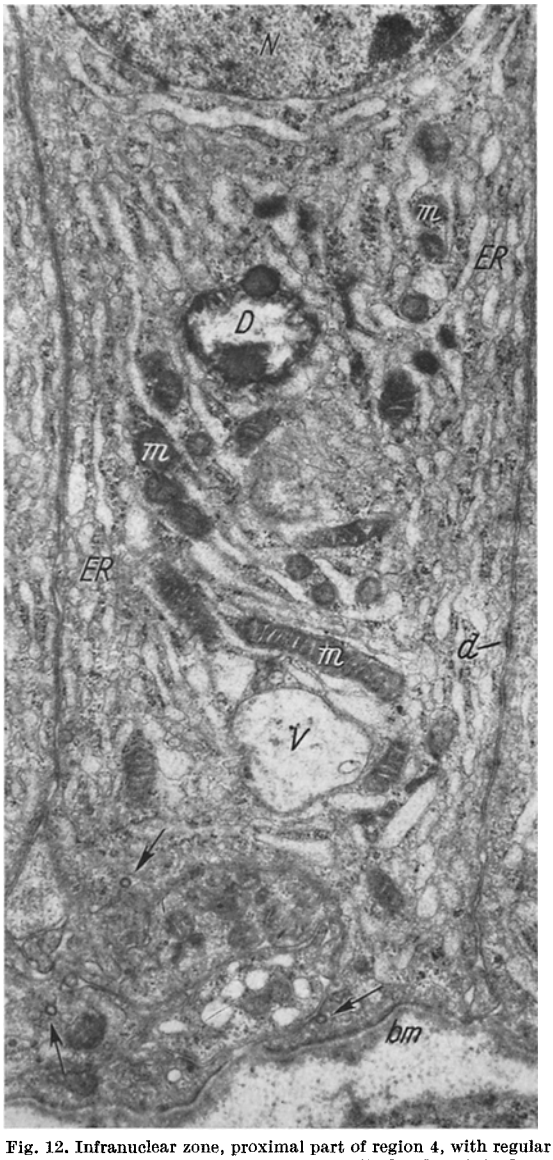


Fig. 12. Infranuclear zone, proximal part of region 4, with regular endoplasmic reticulum (*ER*), numerous mitochondria (*m*), dense body (*D*), and vacuole (*V*). Note the interlocking with adjacent, probably basal cells (lower left), and the thick-walled vesicles in this area (arrows). *N* nucleus, *d* desmosome, *bm* basement membrane. Epon. 14,000 ×

The *basal cells* (Fig. 13) are elongated along the basement membrane, with a rather scanty cytoplasm and many protrusions. The nuclear outline is less regular than in the columnar cells. A small Golgi apparatus and numerous mitochondria and small vesicles are present, as well as a sparse endoplasmic reticulum. A few thick-walled vesicles, small vacuoles or dense bodies are sometimes seen.

B. Absorption of India ink. A preliminary experiment with a concentrated suspension (1:5) caused a marked damage of the fine structure of the columnar cells, with swollen cell apices containing giant vacuoles. The more dilute (1:20) suspension used for the definitive series did not produce any visible damage to the ultrastructure of the resorbing cells.

Twenty-four hours after the ink was injected into the rete testis the ink mass had reached region 4. The electron micrographs showed scattered particles or most often typical conglomerates of carbon particles in the lumen of this region. In many areas, numerous particles were seen in the apical vacuoles of the columnar cells, always between the small vesicles normally present in these vacuoles. Similar particles were sometimes located within the invaginations of the apical cell membrane or in the adjacent apical vesicles (Fig. 14).

Two days after the injection, the ink mass had passed into region 5 and the electron microscope did not reveal any particles in the luminar contents of region 4, or in the apical invaginations and vesicles. Many cells showed particles in vacuoles and dense bodies immediately above the Golgi apparatus.

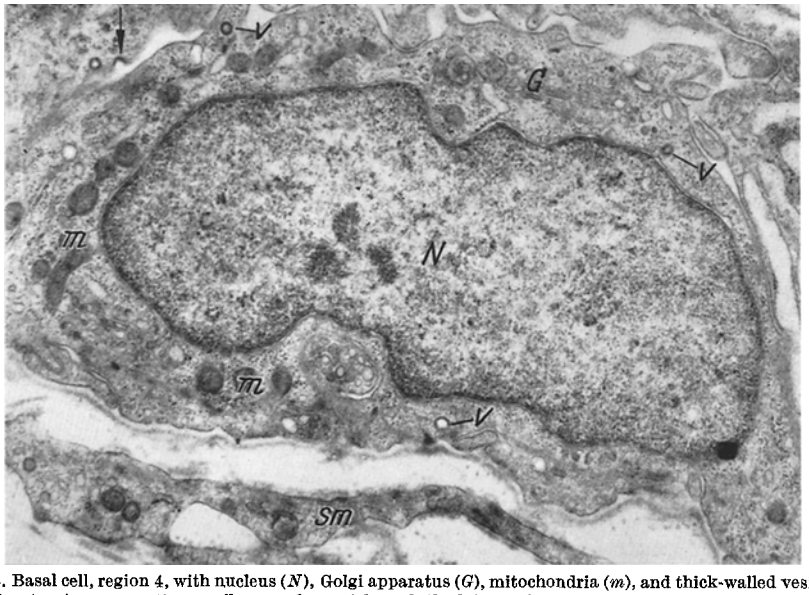


Fig. 13. Basal cell, region 4, with nucleus (*N*), Golgi apparatus (*G*), mitochondria (*m*), and thick-walled vesicles (*v*) Note the structure suggesting confluence of a vesicle with the lateral plasma membrane in an adjacent columnar cell (arrow), *sm* smooth muscle cell. Epon. 14,000 ×

After *three* and *four days*, no carbon particles were seen in the vacuoles, but many dense bodies in the supranuclear and Golgi zones were loaded with particles (Fig. 15). No intracellular carbon particles were ever observed outside the vacuoles and dense bodies, except for those few ones seen in apical invaginations and vesicles after 24 hours.

C. Acid phosphatase activity. In sections of freeze-dried material, incubated for 10 minutes, well preserved cells show a heavy precipitate in cytoplasmic granules. In region 3 these granules fill the cytoplasm except the apical zone (Fig. 16). In region 4 they are mainly present in the supranuclear zone. A weak, brownish stain is seen outside the granules. In areas with distinct freezing damage to the cells, the whole epithelium is diffusely and uniformly stained. Similar results are obtained after formol fixation, though a somewhat longer incubation time is required to produce a distinct reaction and the diffuse background colour is more pronounced. Repeated freezing and thawing of the tissue block before sectioning destroyed the localization to granules. No precipitate was seen in the control sections.

The electron microscopical study of acid phosphatase activity showed lead precipitates in most dense bodies and vacuoles in the supranuclear and Golgi zones (Fig. 17), and sometimes also in the infranuclear zone. In region 4, the particles were abundant in most dense bodies and generally few in the vacuoles. They were never concentrated near the periphery of a vacuole or dense body. No activity was demonstrated in the vacuoles of the apical zone. The preservation of fine structure was not very satisfactory, but in those cells where the Golgi apparatus could be discerned it seldom contained any precipitate, though the adjacent cytoplasm often showed some fine particles of lead phosphate mingled with the ribosomes.

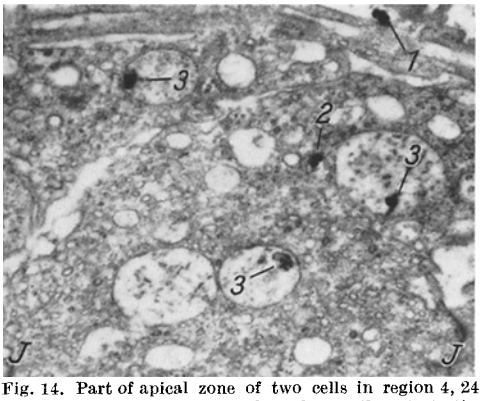


Fig. 14. Part of apical zone of two cells in region 4, 24 hours after the injection of India ink into the rete testis. Conglomerates of carbon particles are seen in the lumen (*1*), in an apical vesicle (*2*), and in vacuoles (*3*). *J* junctional complex. Methacrylate. 14,000 ×

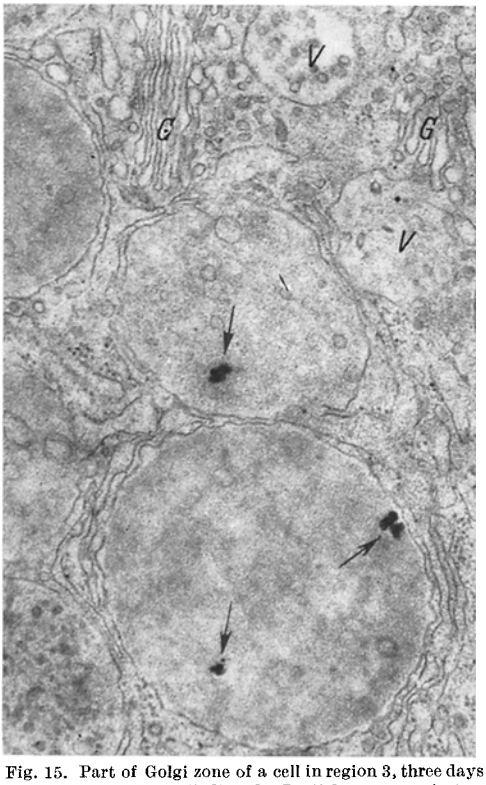


Fig. 15. Part of Golgi zone of a cell in region 3, three days after the injection of India ink. Particles are seen in two opaque bodies (arrows). *G* Golgi apparatus, *V* vacuoles. Epon. 26,000 ×

The control sections showed no precipitate in any structure.

Discussion

The observations made on the apical cytoplasm of the columnar cells in regions 3 and 4 are in good agreement with the concept that these cells have a resorbing function. The many apical vesicles are similar to those in cell types performing pinocytosis or phagocytosis. Particles of India ink are also taken up from the lumen into apical vesicles and vacuoles, apparently together with opaque material occurring in the lumen. The carbon particles are then slowly transferred to vacuoles or dense bodies in the Golgi and supranuclear regions, but they are never seen in other structures. All these features are reminiscent of observations made on macrophages phagocytizing India ink (KARRER 1960), and cells taking up Trypan blue (SCHMIDT 1960, TRUMP 1961) or hemoglobin (MILLER 1960, ERICSSON 1964) from the luminar contents of the proximal kidney tubules. BURGOS (1964) has also shown that mercury sulphide injected into the rete testis is rapidly taken up in a similar way by cells in the most proximal part of the epididymal duct of hamsters.

The observations made are interpreted as strong evidence for the shift of apical vacuoles to deeper levels of the cell and their transformation to dense bodies, probably by concentration of their contents, and perhaps also by their confluence with preexisting dense bodies. Since the introduction of the small amounts of India ink used did not produce any noticeable alterations of the fine structure of the epithelium, it seems reasonable to conclude that the observed process is a normal function of the columnar cells of regions 3 and 4, which take

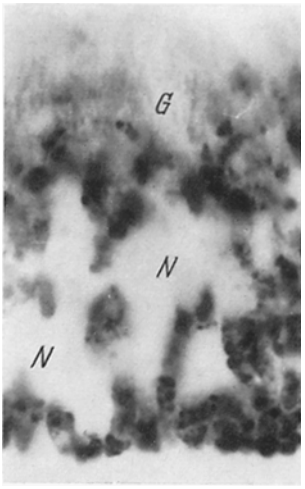


Fig. 16. Epithelium in region 3, to show localization of acid phosphatase activity to granules in the supranuclear and infranuclear zones *N* nuclear zone, *G* Golgi zone. Freeze-dried. Photomicrograph. 1.400 ×

Fig. 17. Part of supranuclear zone, region 3. Precipitate of lead phosphate in all bodies, but more in the dark (*D*) than in the light (*L*) ones. Gomori's technique for acid phosphatase, as modified by Holt and Hicks. Epon. 20.000 ×

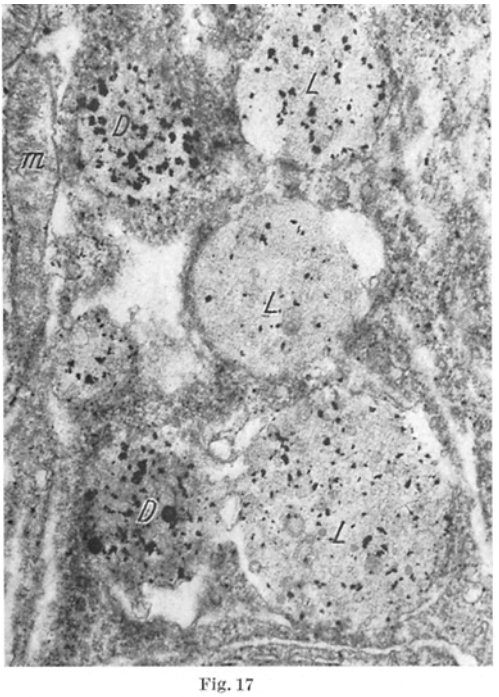


Fig. 17

up material from the luminar contents and transfer it to the many supranuclear granules as suggested in a previous paper (NICANDER 1957). Moreover, a high acid phosphatase activity is seen in many dense bodies and vacuoles. Freezing damage to the cells spoils the localization of the lead precipitate to the granules. It is therefore possible that these vacuoles and granules are similar to de Duve's lysosomes (DE DUVE 1959, 1963, NOVIKOFF 1961, 1963). Cytochemical and biochemical studies have shown a high activity of other "lysosomal enzymes", like amino peptidase (ARVY 1962) and some glucosidases (CONCHIE, FINDLAY and LEVVY 1959, HAYASHI 1964) in the epididymis of some mammals. The function of such "lysosomal" bodies is probably the digestion of material taken up from the luminar contents, like hemoglobin taken up by the cells of the proximal kidney tubules (MILLER 1960, ERICSSON 1964), though this process requires further investigation.

The ultimate fate of the India ink taken up was not revealed. Light microscopical studies of the phagocytosis of India ink by cells in the proximal part of the rat epididymal duct (SHAVER 1954) and the most distal part of the rabbit epididymis (RICHARD 1930) showed passage of the particles from the epithelium into macrophages in the connective tissue. In the present study, very few particles were seen to pass into the infranuclear cytoplasm during four days. This discrepancy may depend upon regional or species variations, or the much smaller amounts of marker substances used for the present study.

The stereocilia have the character of long, somewhat irregular microvilli, frequently with basal anastomoses. Similar bridges were observed in dog and human epididymides by HORSTMANN (1961, 1962), who also noted vesicles in

some stereocilia. Such vesicles were also observed in the hamster by BURGOS (1964). Preliminary studies revealed similar vesicles in other regions of the rabbit epididymis, though they were not seen in region 3 or 4. A longitudinal fibrillar structure was observed by ZETTERQVIST (1956) in the striated border of absorbing cells in the mouse jejunum, and HANSEN and HERMAN (1962) produced evidence for the presence of a similar structure in the brush border of the proximal convoluted tubules in mouse kidneys. In epididymal and absorbing intestinal cells the fibrillar structure apparently joins the fibrillar apical cytoplasm to form the terminal web recently restudied in many cell types by LEBLOND, PUCHTLER and CLERMONT (1960). The continuity of the fibrillar streak with the junctional complex gives support to this interpretation. Moreover, the microtubules running from this apical "web" into deeper layers of the cell are reminiscent of the fibres described by the same authors to run from the terminal web toward the cell base in some columnar cell types of rats. They could also explain BENOIT's (1926) statement that stereocilia have rootlets in the Golgi apparatus.

Microtubules have been revealed in many cell types (cf. SLAUTTERBACK 1963), especially since glutar aldehyde was introduced as a fixative for electron microscopy (BEHNKE 1964, SANDBORN, KOEN, MCNABB and MOORE 1964). They are sometimes considered to take part in the transport of fluids (cf. SLAUTTERBACK 1963 and SANDBORN, KOEN, MCNABB and MOORE 1964), though this concept seems less well founded than that of their role as a cytoskeleton in highly asymmetrical cells (PORTER, LEDBETTER and BADENHOUSEN 1964).

No evidence was found for the participation of the stereocilia in the secretory process of classical epididymal histology. This is in agreement with some earlier light and electron microscopical studies (BENOIT 1926, NICANDER 1957, HORSTMANN 1961, 1962). The stereocilia appear to be related to the microvilli of other resorbing cell types, but the coarse ones are also reminiscent of the "ruffles" of alveolar macrophages (KARRER 1960). They do not seem to take part in the pinocytotic process, but the recent demonstration of a selective absorption of sodium ions by the epithelium of the caput epididymidis in bulls (CRABO and GUSTAFSSON 1964) indicates the presence in the epididymis of other resorbing mechanisms than the pinocytotic uptake of macromolecules.

A thicker membrane in the invaginations of the apical cell membrane of proximal convoluted tubule cells of mouse kidneys was noticed by RHODIN (1954). Similar observations have been made repeatedly in this and other cell types performing pinocytosis (TRUMP 1961, ERICSSON 1964). Moreover, an external coat or an internal bristle coat often occur at the site of such invaginations (cf FAWCETT 1964, ROTH and PORTER 1964), which suggests different methods of uptake. The electron micrographs obtained during the present study do not clearly show whether the thickened membrane of the apical and basal invaginations has such coats, but the downy material often seen all over the external surface of the apical cell membrane may represent an extraneous coat similar to that in amebae (BRANDT and PAPPAS 1962) and some resorbing cell types in mammals (cf BURGOS 1964). The many thick-walled vesicles may have a pinocytotic origin, but they could also, together with the "dark vesicles" in the Golgi area, correspond to the "coated vesicles" recently observed in rat liver cells by NOVIKOFF and SHIN (1964), who consider them to be derived from the Golgi lamellae or the

endoplasmic reticulum and to perform intracellular transport. This problem will be the subject of further studies.

The seemingly multivesicular character of the larger vacuoles is reminiscent of observations of the incorporation of pinocytotic markers and some other absorbed substances into multivesicular bodies (FARQUHAR and PALADE 1962, ROBBINS, MARCUS and GONATAS 1964). The pictures suggesting formation of "internal vesicles" by budding from the vacuolar wall are reminiscent of some observations on pinocytotic vacuoles, food vacuoles, and cytolysomes in certain protozoa (BRANDT 1958, SCHNEIDER 1964, BRANDES and BERTINI 1964). Preliminary studies on other epididymal cell types have given further evidence for this mechanism which would mean that the "internal vesicles" are small, membrane bounded spheres of cytoplasm. On the other hand, acid phosphatase activity was present in some of these vacuoles in agreement with observations on multivesicular bodies in other cell types (NOVIKOFF 1961, 1963, ROBBINS, MARCUS, and GONATAS 1964). It has been suggested that the "internal vesicles" represent Golgi vesicles which have carried acid phosphatase into the multivesicular body (NOVIKOFF and SHIN 1964), though the mode of entrance of the vesicles into the vacuole has not been explained. The whole problem of the intracellular transport of "lysosomal enzymes" and their incorporation into different types of lysosomal structures is still poorly understood, and the mechanism may well vary with the cell type or even within one cell (cf MILLER 1962, DE DUVE 1963, NOVIKOFF 1963, STRAUS 1964, and ERICSSON 1964). The columnar cells of the rabbit epididymal head appear to be very favourable objects for studies of this problem.

A marked uncertainty also pertains to the process by which pinocytotic and digestive vacuoles are transformed to dense bodies, generally with a higher acid phosphatase activity. Confluence with preexisting dense bodies is one probable mechanism, which seems to be frequent in the proximal convoluted tubule cells of the rat kidney (STRAUS 1964, ERICSSON 1964). Pictures suggesting such a process were very rare in the present material. It seems probable that water is withdrawn from the vacuolar contents and that enzymatic processes change the contents until residual bodies containing lipofuscin are formed (cf NOVIKOFF 1961, DE DUVE 1963). Many cytoplasmic granules in the rabbit epididymal epithelium have been reported to show many characteristics of lipofuscin (NICANDER 1957).

The large Golgi apparatus shows the well-known fine structure first observed by DALTON and FELIX (1954) in mouse epididymal cells, though the vacuoles are less conspicuous in rabbits. The "dark vesicles" have apparently not been described before, though preliminary studies have shown them to occur frequently in the Golgi area of testicular and epididymal cells from many species. They may, however, be akin to the "coated vesicles" discussed above. The function of the Golgi apparatus is still poorly understood, but its participation in the formation of zymogen granules appears well established (cf CARO and PALADE 1964). ZEIGEL and DALTON (1962) suggest that the Golgi vacuoles of protein-secreting cells contain "fluid materials segregated from the secretory product." This would explain the fact that the ergastoplasmic cisternae, which manufacture proteins for the zymogen granules (CARO and PALADE 1964), have much more opaque contents than the Golgi cisternae and vacuoles. However, no pictures suggesting

the formation of granules were seen in the Golgi apparatus of the epididymal cells studied. Since their well developed endoplasmic reticulum is generally only sparsely granulated, it is even doubtful whether its contents are mainly proteinaceous substances. On the other hand, it seems probable that the synthesis of enzymes for the "lysosomal" structures takes place in the granulated regions of the endoplasmic reticulum. The lead precipitates often seen in such areas in material prepared for the demonstration of acid phosphatase activity might indicate true enzyme activity, in accordance with observations on neurons (NOVIKOFF 1963) and certain ciliated protozoa (CARASSO, FAVARD, and GOLDFISCHER 1964). No acid phosphatase acitvity could be established in the Golgi apparatus, though it is present there in some other regions of the rabbit epididymis (NICANDER, unpublished observations) as well as in the rat epididymis (KUFF and DALTON 1959, NOVIKOFF, GOLDFISCHER, ESSNER and IACIOFANO 1961). Further studies with better methods may well reveal similar conditions in regions 3 and 4.

The nature of the material taken up from the lumen is not known. NICANDER (1957) found that it is stained by periodic acid and Schiff's reagent. Some electron dense material is also seen in the more proximal regions of the duct, in the efferent ductules, and even in the seminiferous tubules (NICANDER, unpublished observations). It therefore seems probable that the material is carried with the fluid from the testis and somehwat concentrated when most of this fluid is resorbed in the efferent ductules and the initial segment of the duct. The uptake of débris from the testis by cells in the epididymal head was suggested by WAGENSEIL (1928), MASON and SHAVER (1952), and MASON (1954). The phagocyticed sperm tail once observed is reminiscent of similar observations in the efferent ductules of bulls (NICANDER 1963) and in other epididymal regions of rabbits and rats, especially under certain experimental conditions (unpublished observations).

HAMMAR (1897) observed a region with highly vacuolated columnar cells in the posterior caput epididymidis of dogs. This region obviously corresponds to region 3 in rabbits and some other species (NICANDER 1957, 1958). The many vacuoles were interpreted by HAMMAR as stages in a lively secretory process. Later studies of the epididymis have generally also considered the presence of many vacuoles and granules in epididymal columnar cells as evidence for secretion. The present study casts severe doubt on such generalizations based on purely static morphological evidence. Though the regional differences regarding epididymal structure, and probably also function, are greater than generally realized, it may be pointed out that preliminary electron microscopical studies on the general fine structure of the rabbit, rat and dog epididymides have revealed the same basic ultrastructure, with signs of pinocytosis, as well as the presence of many vacuoles and dense bodies with a high acid phosphatase activity. A similar fine structure has also been reported for the human epididymis (HORSTMANN 1962). It has recently been suggested (REID and CLELAND 1957, SCOTT, WALES, WALLACE and WHITE 1963) that the epididymis resembles the kidney tubules in its resorptive capacity and regional complexity. On the other hand, some recent biochemical studies (cf MANN 1964) have been interpreted as evidence for the addition of material to the epididymal contents by a secretory process. These studies have apparently not considered the possibility that the concentration of substances by a selective resorption of fluid might simulate the addition of these substances by

secretion, but the observation (SCOTT, WALES, WALLACE and WHITE 1963) that glyceryl-phosphoryl choline, present in high concentration in the epididymal contents (cf MANN 1964), is synthesized in the rabbit epididymis is strong evidence for a true secretion in this case. The mechanism of this process, if present in regions 3 and 4, was not revealed by the present study, but it seems improbable that the vacuoles and dense bodies are concerned. Obviously, substances of this kind are hardly demonstrable in electron micrographs. Studies of male accessory genital glands producing carbohydrates have also failed to demonstrate distinct secretion pictures like those seen in serous and mucous gland cells (NICANDER and SCHANTZ 1961, SCHANTZ 1964).

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