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Uptake of Colloidal Particles by Cells of the Ductuli Efferentes of the Hamster*

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Summary. Ductuli efferentes epithelium of the hamster consists of a single layer of cells resting upon a typical basement membrane. Two cell types, ciliated and non-ciliated, which are held together by a junctional complex, are distinguished in this epithelium.

The ciliated cells present numerous cilia having prominent basal bodies. These lie in the apical cytoplasm surrounded by a feltwork of filaments. Throughout the cell dense particles of the glycogen type are abundant.

The non-ciliated cells are interspersed among the others without regular sequence. They are consistently more numerous toward the ductus epididymidis. The luminal surface shows a variable number of microvilli, canaliculi and vesicles. Colloidal mercuric sulfide (SHg) was injected into the rete testis as particulate tracer material, in order to identify the cellular type specializing in absorption and to study the mechanism of transport of these particles. Particles of the tracer were selectively incorporated into non-ciliated cells (apical vesicles, canaliculi and vacuoles). The functional significance of these morphological and experimental findings is discussed.

Introduction

The ductuli efferentes of hamsters constitute a complex system of tubules through which spermatozoa pass from the testis towards the epididymis. These ductules in the rat, were studied by von Möllendorff (1920) who found trypan blue within the epithelium after subcutaneous injection. von Möllendorff postulated the similarity of this epithelium and the cells of the proximal convoluted tubules of the kidney, as to their capacity of reabsorption. Since then, in spite of numerous indirect observations of reabsorption at the level of the efferent ducts (van Wagenen, 1925; Wagenseil, 1928; Young, 1933; Mason and Shavers, 1952; Holstein, 1964) a direct demonstration was given as late as 1959 by Burgos et al., when they found incorporation of Fe 59 that had been injected two hours before into rete testis. The non-ciliated cell type seemed to be in charge of Fe 59 absorption. The similarity in the structure of this cell type and that of the proximal convoluted tubules of the kidney was pointed out by Burgos (1957), Young and Ladman (1957), Ladman and Young (1958). In this paper we offer direct proof by demonstrating the incorporation of previously injected colloidal particles by non-ciliated cells. At the same time we describe the ultrastructural characteristics of both cellular types of the epithelium and advance an interpretation on the mechanism of transport of the colloidal particles.

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Materials and Methods

Twenty five male hamsters (*Cricetus auratus*) 6 months old and weighing 120 grams, were used. They were anesthetized with nembutal administered intraperitoneally. On exposing the vascular pole of the testis, the rete testis was injected with a 0.1 ml solution of colloidal mercuric sulfide, 8% (Hille Laboratories, Chicago) in distilled water containing 2 mg of mercuric sulfide (BURGOS, 1964). Five minutes later the efferent tracts were fixed "in situ" in cold 1% OsO_4, phosphate buffered to pH 7.3 (MILLONIG, 1961), administered by drops placed upon the tissue simultaneosly with its injection into the rete testis. After 10 min fixation, ductuli efferentes were removed and placed in fresh fixative to complete 2 hours of fixation. The material was dehydrated in ethyl alcohol and embedded in epon (LUFT, 1961). Ultrathin sections were obtained with a Huxley ultramicrotome and were stained with lead citrate and uranyl acetate (REYNOLDS, 1962); they were covered with a thin layer of carbon and examined in a Siemens Elmiskop I electron microscope operated at 60 KV and low amperage acceleration, to avoid sublimation, of mercury through the beam's action.

Results

Histology

The 5 to 12 efferent ductuli of the hamster originate near the vascular pole of the testis by partitioning of the extratesticular cone of the rete testis (MONTORZI and STATI, 1964). In the rat, ductuli efferentes originate from many extratesticular cones of the rete testis (ROOSEN-RUNGE, 1961).

Ductuli efferentes are lined by a simple columnar epithelium consisting of two cell types; ciliated and non-ciliated. Both rest on a well-defined basement membrane (Fig. 1b). In the region proximal to the testicle there is a corresponding ciliated cell for each non-ciliated cell (1/1), while in the distal region the proportion of the non-ciliated cells increase up to double (1/2). Close to the rete testis the diameter of the lumen of each ductule is about 300 microns; in the vicinity of the caput epidydymis the lumen diameter decreases to 150 microns. The height of the epithelium remains almost constant.

Outside the epithelium some plain muscular fibers are arranged in a single layer surrounding each ductule. This layer becomes more evident in the distal third (Figs. 1, 1b, 9).

Cytology

Ciliated and non-ciliated columnar cells have the following ultrastructural characteristics: *Non-ciliated cells:* Their nuclei tend to lie in the basal region and are often irregular in outline (Fig. 1). Nuclear pores are infrequent. The apical border of the cells shows thin microvilli that are frequently fused in pairs. These microvilli vary in number and pattern. In some cells (Fig. 5) they are numerous, long and regular, while in others they appear short, irregular and less numerous (Fig. 4).

The lateral plasma membrane has few sinuosities; there are junctional complexes with neighbouring cells. These junctions are of three types: Zonula occludens, desmosome and zonula adhaerens as described for other epithelia (BRIGHTMAN and PALAY, 1963; FARQUHAR and PALADE, 1963; ROIG DE VARGAS-LINARES and BURGOS, 1965). The 100 Å intercellular space widens occasionsally forming small dilatations with which some pinocytotic vesicles anastomose (Fig. 8). In the basal region the cytoplasmic membrane shows a few folds and scanty interdigitations (Figs. 1, 10).

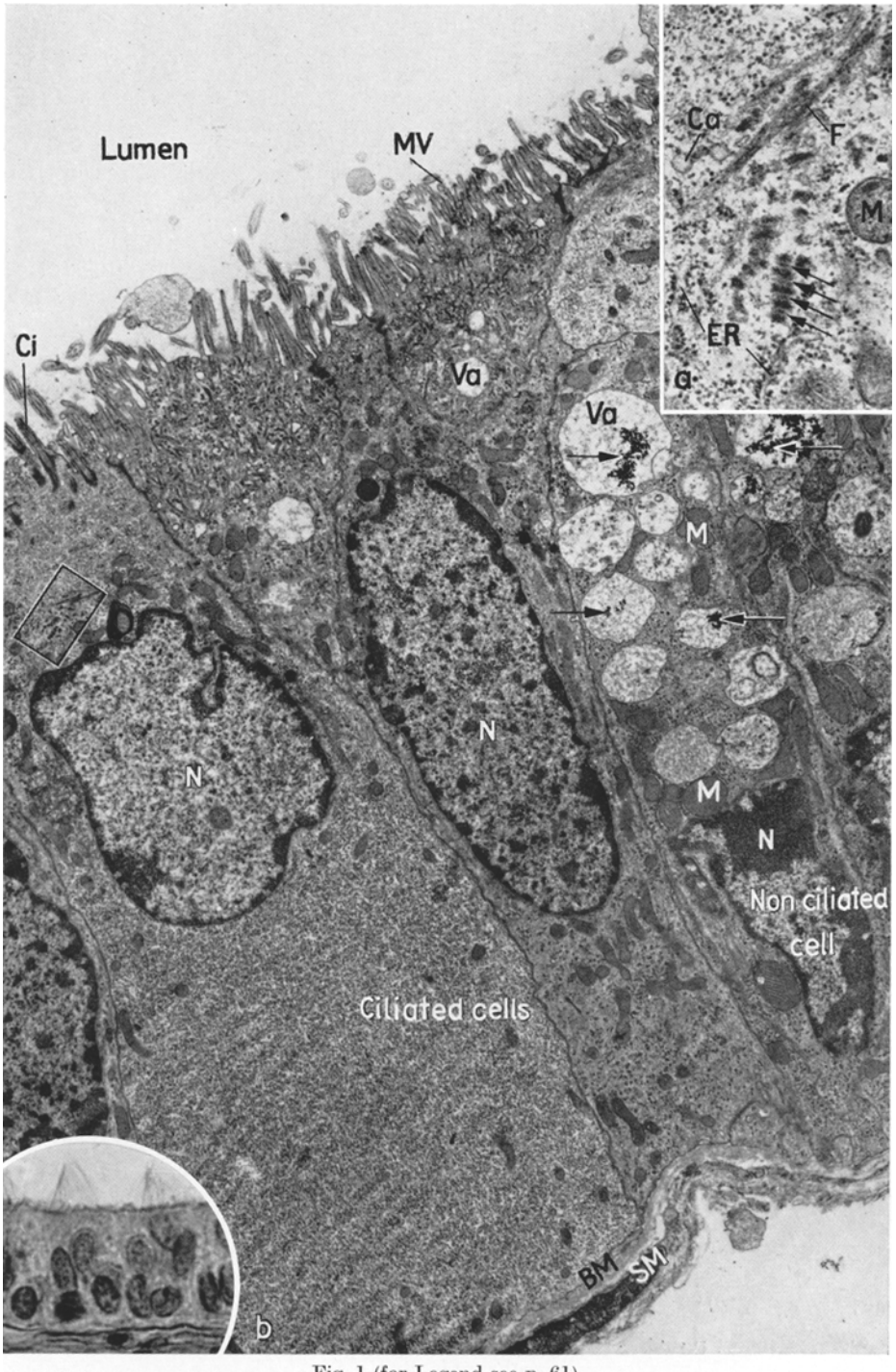


Fig. 1 (for Legend see p. 61)

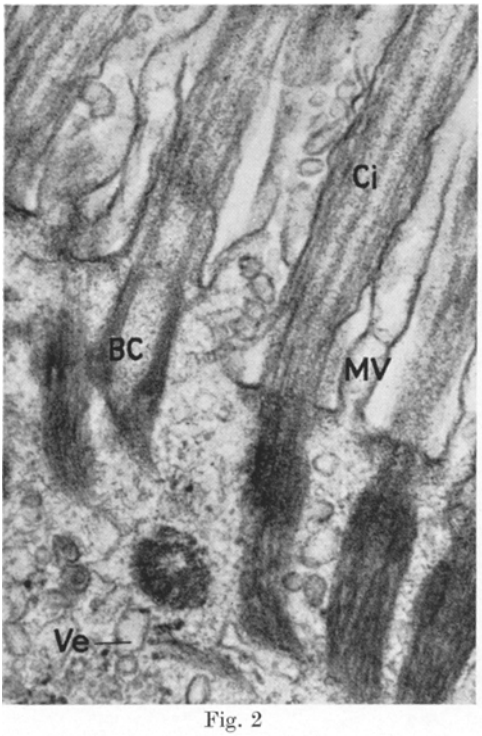


Fig. 2

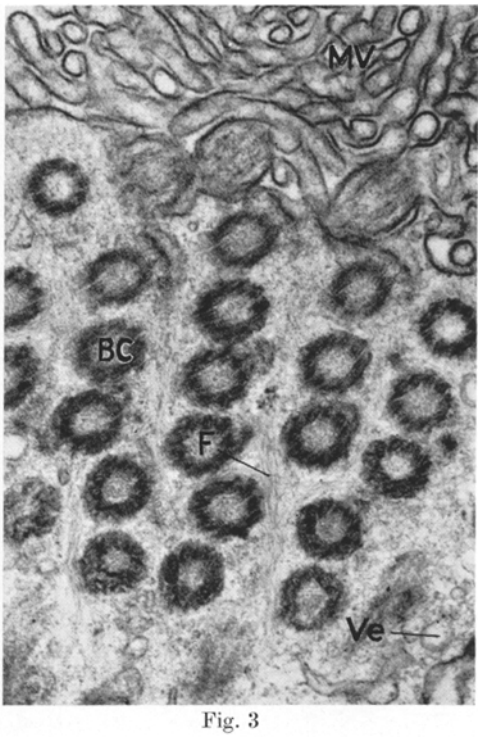


Fig. 3

Fig. 2. Sagittal section of a ciliated cell (apical cytoplasm) *Ci* cilia; *MV* microvilli; *Bc* Basal body; *Ve* vesicles. 40,000×

Fig. 3. Cross section of a ciliated cell (apical cytoplasm). The basal bodies (*BC*) are surrounded by a feltwork of filaments (*F*). *MV* microvilli. 33,000×

At the apical region, the plasma membrane, approximately 100 Å in thickness, carries an irregular extraneous coat made of delicate filaments which contribute to increase the total thickness of the cell wall to about 200 Å (Figs. 4, 6, 7). The plasma membrane and its cover show invaginations which are continuous with canaliculi and pinocytotic vesicles between the bases of adjacent microvilli. These canaliculi and vesicles show an even thicker wall due to the addition of a cytoplasmic granular material lining the inner aspect of their membrane (Figs. 4, 6, 7). The canaliculi are frequently connected to the vesicles (Fig. 4). Canaliculi and vesicles are much more abundant than those in non-ciliated cells which have irregular and scanty microvilli (compare Figs. 4 and 5).

Fig. 1. Low power electronmicrograph of the epithelium of the ductuli efferentes showing ciliated and non-ciliated cells. Non-ciliated cells contain a number of vacuoles some of which enclose SHg particles (arrow). *BM* basement membrane; *Ci* cilia; *M* mitochondrion; *MV* microvilli; *N* nucleus; *Va* vacuole; *SM* smooth muscle. 6,000×. At higher magnification a portion of the ciliated cell cytoplasm shows bundles of filaments (*F*); ciliary rootlets with periodic structure (arrows); endoplasmic reticulum (*ER*) and mitochondria (*M*) (inset 1a) 32,000×. A photomicrograph of ductuli efferentes shows the nuclear arrangement of the columnar cells (1b). 1,700×

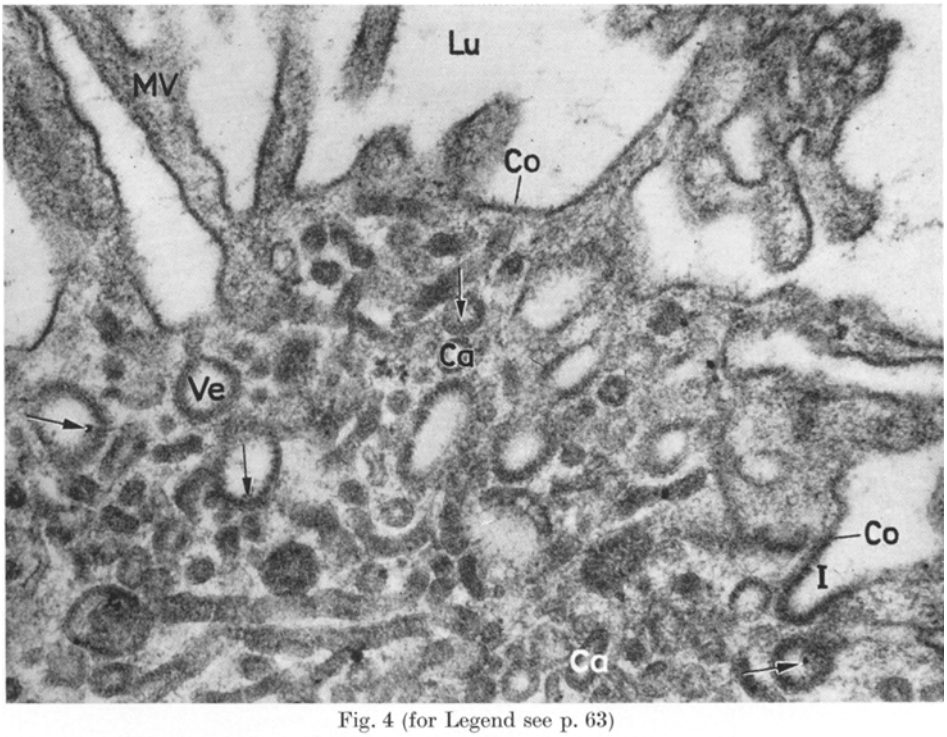
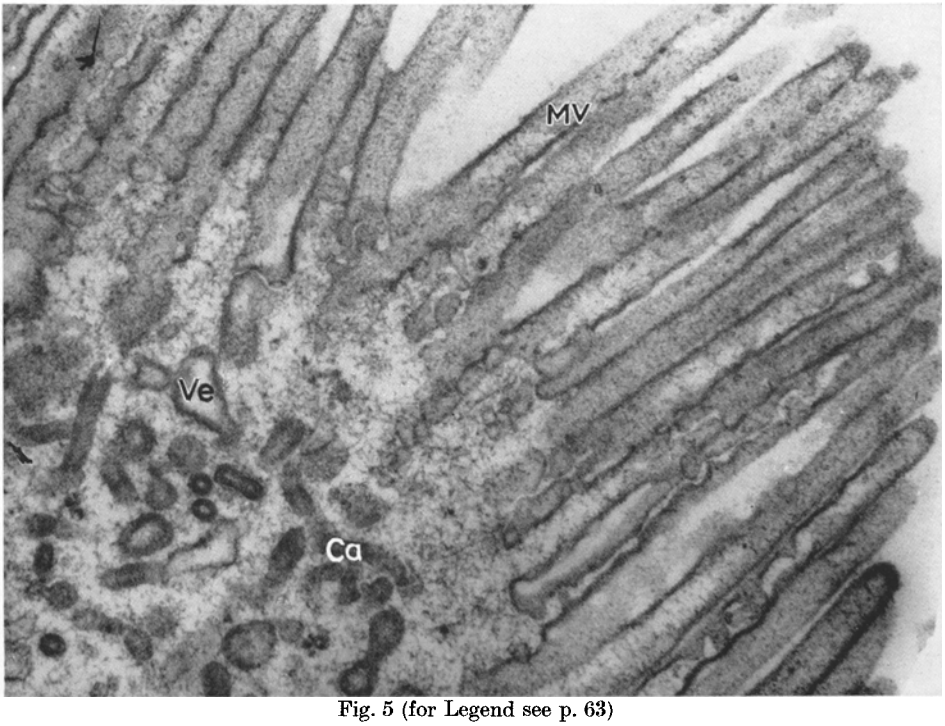


Fig. 4 (for Legend see p. 63)



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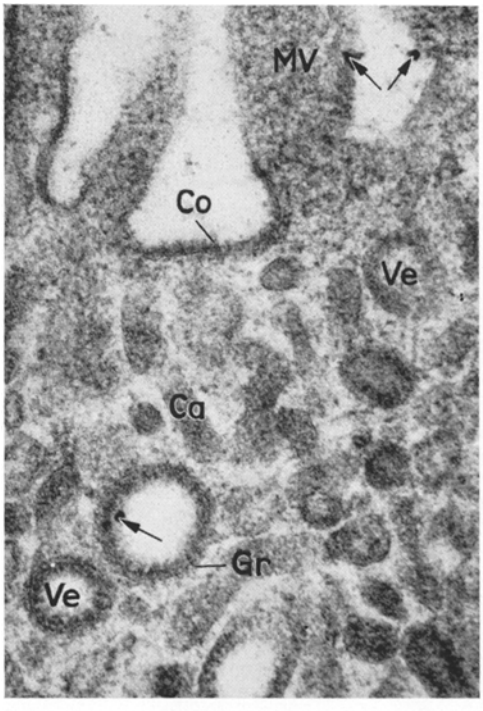

Fig. 6

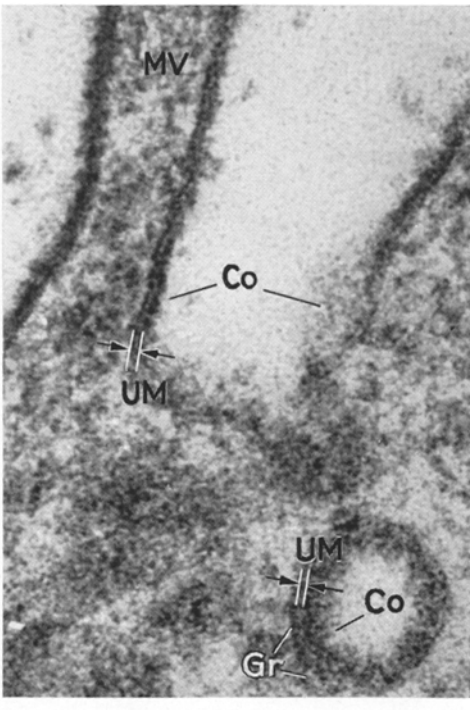

Fig. 7

Fig. 6. Non-ciliated cell (apical cytoplasm) *Ca* canaliculus; *Co* filamentous covering with adherent SHg particles (arrows). *MV* microvilli; *Ve* vesicles; *Gr* Cytoplasmatic coating. 80,000×

Fig. 7. High power electron micrograph of a non-ciliated cell (apical cytoplasm) *Co* filamentous coating; *Gr* cytoplasmic coating; *MV* microvilli; *UM* unit membrane. 464,000×

Vacuoles of different sizes whose walls are formed of an 80 Å membrane and which contain delicate filaments and granules present an increasing density and smaller size as they approach the nuclear region (Figs. 1, 8). Around the vacuoles one finds lengthened or ovoid mitochondria with a dense and granular matrix and transversal or irregular cristae (Fig. 8). Next to the vacuoles and mitochondria, cisternae of rough endoplasmic reticulum can be observed which occasionally show an apparent continuity with the lumen of the vacuoles (Fig. 8). Besides, there are Golgi elements and dense bodies, bounded by a single membrane, tentatively identified as lysosomes.

Fig. 4. Non-ciliated cells (apical surface), showing irregular microvilli (*MV*), canaliculi (*Ca*) and vesicles (*Ve*) which contain SHg particles (arrows). A thin filamentous coating is apparent on the outer aspect of the apical plasmalemma (*Co*). *I* vesicular invagination; *Lu* lumen. 40,000×

Fig. 5. A second type of non-ciliated cell (apical surface). The slender microvilli (*MV*) are closely packed. The canaliculi (*Ca*) and vesicles (*Ve*) present smaller diameter than the ones shown in Fig. 4. 40,000×

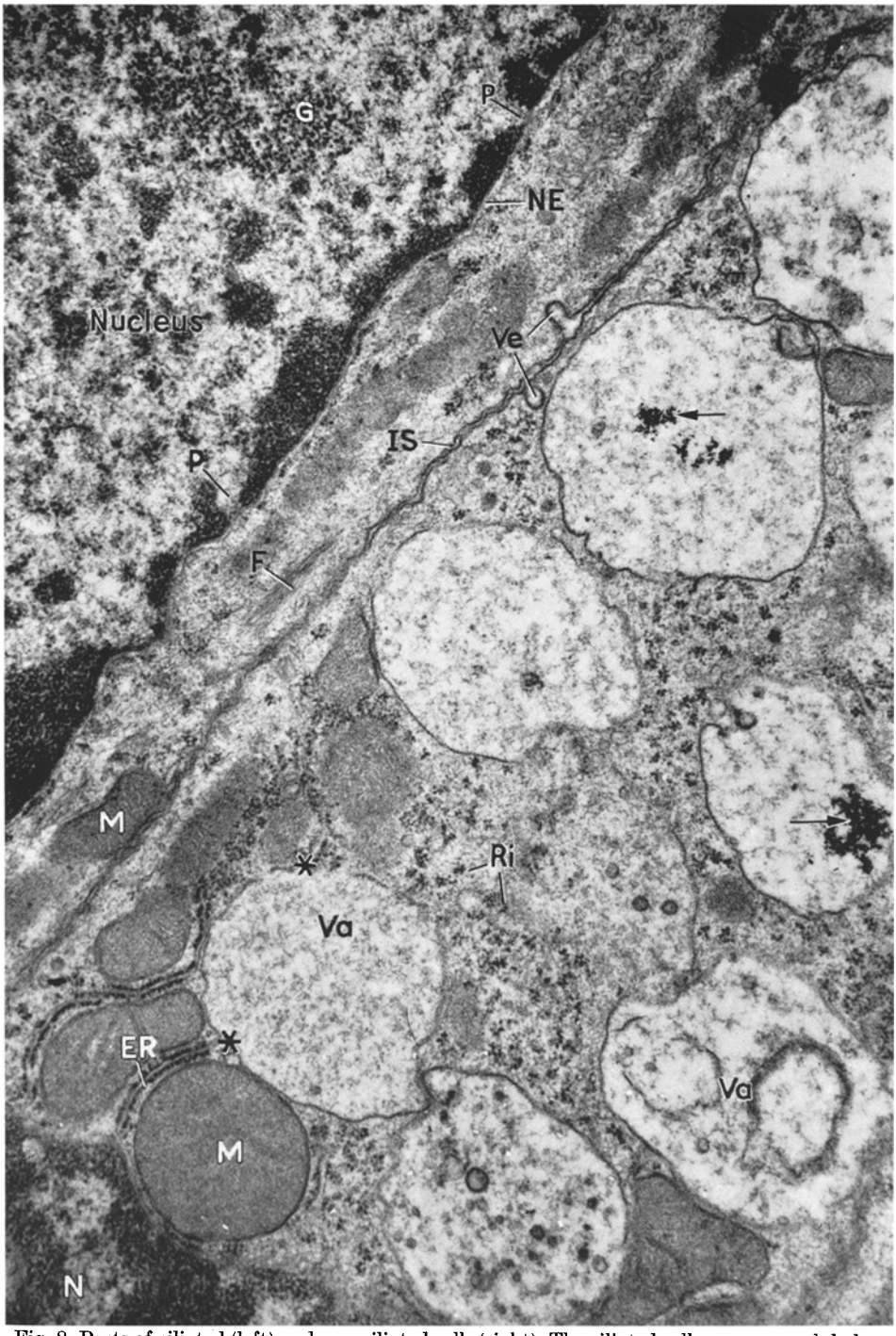

Fig. 8. Parts of ciliated (left) and non-ciliated cells (right). The ciliated cell possesses a glubolar nucleus containing dense granules (*G*). Pores (*P*) are abundant at the nuclear envelope (*NE*). The nonciliated cell encloses large supranuclear vacuoles (*Va*) intermingled with mitochondria (*M*). The latter are surrounded by cisternae of the endoplasmic reticulum (*ER*) which may be in apparent continuity with vacuoles (*). *R* ribosomes; arrows: SHg particles; *Ve* vesicles; *F* filaments; *IS* intercellular cleft; *N* nucleus. 28,000×

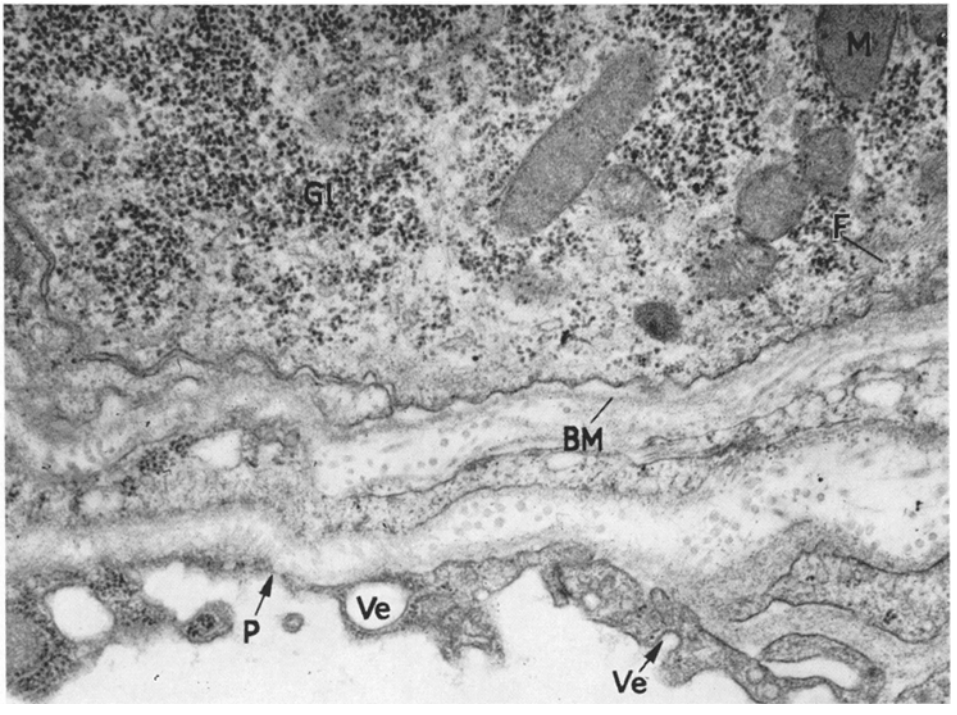

Fig. 9. Ciliated cell (basal part). Glycogen particles (*Gl*) conglomerate toward this zone of the cytoplasm. *M* mitochondrion; *F* filaments. The lower side of the micrograph comprises a capillary surrounded by basement membrane (*BM*). The endothelium encompasses pynocitosis vesicles (*Ve*) and is interrupted by a typical pore (*P*) 24,000×

The cytoplasm also contains numerous polyribosomes (Figs. 8, 10), and cytoplasmic filaments in contact with the desmosomes. No secretory granules were observed.

Ciliated cells. These have a large nucleus, in the central or apical region. In general, nuclei are similar to those of the non-ciliated cells. They usually show a higher number of dense granules and pores (Figs. 1, 8).

At the apical surface of these cells, long cilia with well defined basal bodies and rootlets are observed (Figs. 1,2,3). Among the cilia, variable in number, short and irregular microvilli are found (Figs. 1,2,3), and the apical cytoplasm contains some vesicles and canaliculi, whose walls do not show the covering found in non-ciliated cells.

The cytoplasm has numerous elongate mitochondria with transverse cristae and a dense matrix. These are located in the apical region in close topographical relation to dense bodies. The cytoplasm contains filaments, 50 Å thick, which are apparently related to desmosomes. They form an apical feltwork surrounding the basal bodies of the cilia (Fig. 3), or bundles within the cytoplasm (Fig. 8). Very abundant 200 to 300 Å particles, deeply stained with lead, having the characteristics of particulated glycogen, may be seen distributed throughout the cytoplasm (Figs. 1, 9).

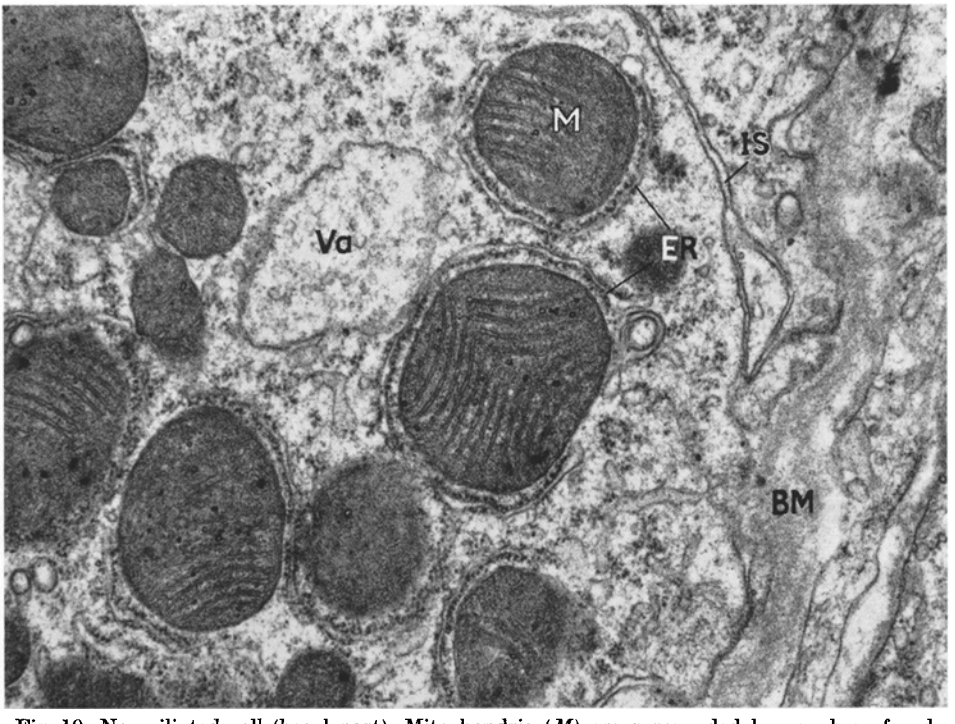

Fig. 10. Non-ciliated cell (basal part). Mitochondria (*M*) are surrounded by rough surfaced cisternae (*ER*). *Va* vacuole; *BM* basement membrane, *IS* intercellular cleft. 29,000×

The lateral borders of these cells are slightly sinuous and exhibit the three types of junctional complexes as described for non-ciliated cells. The basal part is rather smooth, occasionally with invaginations or interdigitations (Fig. 9).

Basement Membrane. Both cellular types rest on a well-defined basement membrane that has an amorphous component of moderate electron density and borders on fibrils of the subjacent connective tissue (Figs. 9, 10). Quite nearby, isolated muscular fibers may be seen. Blood capillaries are near the epithelium, having a thin endothelium with pores and vesicles and its own basement membrane (Fig. 9).

Absorption of Colloidal Particles

The particles of colloidal mercuric sulfide, injected at the level of the rete testis five minutes before fixation, were recognized among the microvilli, canaliculi and apical vesicles and in cytoplasmic vacuoles of non-ciliated cells (Figs. 1, 4, 6, 8). These particles adhere to the extraneous coat of filaments which lines the plasma membrane at the cell surface and at the internal face of the canaliculi and apical vesicles (Figs. 4, 6). The colloidal particles adhere to the internal wall of the apical vesicles and canaliculi without forming conglomerates. Inside the large vacuoles, conglomerate of particles are formed in the lumen with no adherence to the internal wall of the vacuoles; they are intermingled with other substances of low electron density. Among these substances, electron lucent spaces are observed (Fig. 8).

We have observed no vacuoles with these particles to open up in the basal region, nor colloidal particles incorporated by ciliated cells. In no case were free particles found in the cytoplasm.

Discussion

This paper contributes the first direct demonstration, at the electron microscopic level, of absorption by specific epithelial cells of the efferent ducts of the testis. Previous studies had offered only indirect proof. BURGOS et al. (1959) had shown incorporation of Fe 59, but were unable to determine the cellular type in charge of the process.

The non-ciliated cells, now shown to specialize in this process of absorption, exhibit ultrastructural similarities to the cells of the proximal convoluted tubules of the kidney, i.e.: the presence of microvilli, apical canaliculi, vesicles and vacuoles with contents of variable density. This similarity was already pointed out by BURGOS (1957) and LADMAN and YOUNG (1958), and an important role in absorption processes was predicted for these structures (BURGOS, 1957; MILLER, 1960; MILLER and PALADE, 1964).

It is notewothy that the characteristics and distribution of microvilli should vary from one cell to another and that this variation is accompanied by changes in the apical vesicles and canaliculi. We found that fewer microvilli correspond to more apical vesicles and canaliculi, and vice versa. There is then a probable interrelation between membranes projecting into the cell and ones evaginating from it.

The mechanism of absorption must involve displacement of underlying cytoplasm, consumption of energy and a flux of membrane similar to those postulated by BENNETT (1956).

The surface of the microvilli presents a clear filamentous covering in some places similar to the covering described by BRANDT and PAPPAS (1960) in the cell membrane of the amoeba. This filamentous coat becomes more constantly visible between the implantation bases of the microvilli. Colloidal particles do not make direct contact with the cell membrane but appear to be incorporated in the filamentous extraneous coat in a way resembling the uptake of colloidal particles by cells of the epididymis (BURGOS, 1964).

In the large vacuoles, the filamentous material loses adherence to the vacuolar membrane and conglomerates freely in the lumen. We have not observed vacuoles as dilated and as numerous as those pointed out by LADMAN and YOUNG (1958) in cells of the efferent ducts of guninea pigs.

The ciliated cells possess characteristics somewhat similar in several respects to those described by other authors in different epithelia (FAWCETT and PORTER, 1956; RHODIN and DALHAMN, 1956; LADMAN and YOUNG, 1958; RHODIN, 1962; BRIGHTMAN and PALAY, 1963) but one's attention is called to the presence of abundant particulate glycogen in the cytoplasm. This abundance in the efferent ducts of the hamster is not observed in other ciliated epithelia. Its function escapes us, but one could think of an energy source for ciliary function.

The existence of a network of filaments among the basal bodies was pointed out by ALLEN (1965) in *Tetrahymena pyriformis* and interpreted as a sustaining apparatus for the basal bodies and a coordinator and propagator of ciliary movements.

The surface coat of the non-ciliated cells and its apparent role in the incorporation of colloidal particles suggests that it contains substances specialized for adherence of specific types of molecules. A similar conclusion was drawn by Brandt and Pappas (1960); Burgos (1960, 1964); Favard and Carasso (1964); and Fawcett (1965).

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