Appearance of Peculiar Multivesicular Bodies in the Principal Cells of the Epididymal Duct after Efferent Duct Cutting in the Mouse

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Summary. Ligation of the efferent duct in the mouse results in appearance of PAS-positive inclusions in principal cells in the duct of the epididymal body, and the inclusions are considered to be formed by absorption of the luminal PAS-positive material produced in the epididymal head (Abe et al., 1982a). For better understanding of the significance of the inclusions, this study examined morphologic changes in the principal cells after efferent duct cutting, by light and electron microscopy.

Electron microscopy revealed that the principal cells containing PAS-positive inclusions are characterized by a nipple-like protrusion—micropapilla—on the luminal surface and by peculiar multivesicular bodies in the supranuclear cytoplasm. The micropapilla, 1-2 µm in height, contains ductules of 0.2-0.5 µm thickness which extend from its tip to the multivesicular bodies. The multivesicular bodies, measuring up to 10 µm in diameter, are larger toward the nucleus. They contain numerous vesicles, 40-100 nm in diameter, and an amorphous material that is similar to the luminal material of the epididymal duct. In addition, larger multivesicular bodies are heterogenous in structure and possess bundles of tubules 40 nm thick. There are dense bodies in the basal cytoplasm under the nucleus which appear to be transformed from the multivesicular bodies.

In summary, occurrence of the micropapilla and the giant multivesicular bodies are considered to represent an activated breakdown of the material ingested from the duct lumen.

The epididymal duct which is conspicuously convoluted and shows segmental differences in structure is thought to participate in functional maturation and the storage of spermatozoa (see reviews by Bedford, 1975; Hamilton, 1975). It has recently been demonstrated that the principal cells of the epididymal duct in the head of the epididymis secrete a specific glycoprotein which is bound to spermatozoa, providing them with the ability to fertilize ova (Lea et al., 1978; Olson and Hamilton, 1979; Faye et al., 1980; Moore, 1980). The spermatozoa bound with such an epididymal substance are stored in the duct of the body and tail of the epididymis (Lea et al., 1978). It has been also suggested that excessive substances not bound to spermatozoa may be absorbed in the distal regions of the epididymal duct (Lea et al., 1978; Faye et al., 1980).

The mouse epididymal duct is divided into 5 segments (I–V): Segments I, II, and III constitute the head of the epididymis; Segment IV, the body; and Segment V, the tail (Takano, 1980; Abe et al., 1982a, 1983a) (Fig. 1). Previously, we reported that PAS-positive inclusions appeared in the principal cells of Segment IV after efferent...
duct ligation which excluded the testicular fluid and spermatozoa from the epididymal duct, and we presumed that such inclusions were formed due to absorption of the luminal PAS-positive material, glycoprotein, secreted in Segment II (Abe et al., 1982a).

In this study, the detailed morphologic changes in the principal cells in Segment IV after efferent duct cutting were examined by light and electron microscopy. In particular, electron microscopy revealed that the PAS-positive inclusions appear as peculiar, very large multivesicular bodies associated with absorption of the luminal substance.

MATERIALS AND METHODS

Forty-nine male dd-mice were used in this study. The animals were anesthetized with pentobarbital sodium (Nembutal) injected intraperitoneally, the epididymis and testis were exposed through a median incision of the lower abdomen, and the efferent duct was cut on one side. The unoperated duct on the other side served as control. The animals were killed 1 to 4 weeks after operating, and the epididymis was examined.

For light microscopy, the epididymis was fixed in Bouin's fluid for 3 hrs, or 10% formalin-0.1M phosphate buffer (pH 7.4) for 20 hrs, then dehydrated, and embedded in paraffin or methacrylate (Bennett et al., 1976), respectively. Longitudinal sections of the epididymis were cut serially at 5 μm and stained with periodic acid-Schiff (PAS) reagent and hematoxylin.

For electron microscopy, the epididymis was fixed with 3% paraformaldehyde-1% glutaraldehyde-0.1 M cacodylate buffer (pH 7.4) for 2 hrs, post-fixed with 2% OsO₄ for 2 hrs, block-stained with 0.5% uranyl acetate over night, dehydrated, and embedded in Epon. Ultrathin sections stained with lead citrate were examined in a Hitachi HS-9 electron microscope.

RESULTS

Normal epididymis

The epididymal duct was lined with a single layer of columnar epithelial cells consisting mainly of the principal cells. The principal cells had long microvilli and stereocilia; they varied in structural details in the different segments, as reported previously (Abe et al., 1983a).

This study dealt mainly with the principal cells in Segment IV. They were 12-15 μm in height and 8-10 μm in width; the nucleus was basally located (Fig. 2a). The two adjacent cells were connected with a junctional complex on the lateral cell boundary just below the free surface (Fig. 3).

The apical cytoplasm contained round coated vesicles of about 50 nm in diameter,
Giant Multivesicular Bodies in the Epididymal Principal Cells

123

and non-coated vesicles of about 100–400 nm in diameter. In the supranuclear cytoplasm, multivesicular bodies, measuring 0.5–1.0 μm in diameter, were sporadically seen. They contained many vesicles of 40–100 nm in diameter and an amorphous material of low or moderate density. The principal cells usually contained 4–5 multivesicular bodies in a sectional profile (Fig. 3). Above the nucleus there were well-developed Golgi complexes, forming a Golgi area as large as the nucleus.

Cisterns of rough endoplasmic reticulum and dense bodies were distributed in the infranuclear cytoplasm. The latter, 0.2–0.6 μm in diameter, had heterogenous contents, including an amorphous or finely granulated material, granules, membranous or myelin figures, and lipid droplets. In addition, peculiar rod-shaped inclusions, 0.3–0.5 μm in width and 1–5 μm in length, were found among the dense bodies. The inclusions contained a bundle of fine, straight cylindrical tubules, 40 nm in diameter, as detailed in our previous paper (Abe et al., 1983b).

The lumen in Segment IV contained abundant spermatozoa and a moderately PAS-positive material which appeared as an opaque flocculent material by electron microscopy.

Fig. 2. Light microscopy of the epididymal duct in Segment IV. PAS-hematoxylin. ×500. a. Normal. The lumen contains spermatozoa and PAS-positive material. The epithelial cells have no PAS-positive inclusions. b. One week after efferent duct cutting. The lumen contains strongly PAS-positive material but no spermatozoa. PAS-positive hyaline granules (HG) are seen in the lumen. The epithelial cells contain accumulated PAS-positive granules (arrows). c. One week after efferent duct cutting. Methacrylate section. The PAS-positive hyaline granules (HG) in the lumen are clearly demonstrated. d. Four weeks after efferent duct cutting. The epithelial cells contain PAS-positive inclusions (arrows).
The epididymis one week after efferent duct cutting

Light microscopy showed the lumen in Segment IV, which contains few spermatozoa, to be filled with a strongly PAS-positive material (Fig. 2b). In addition, PAS-positive hyaline granules or globules, 2-30 μm in diameter, and clumps of varying forms were seen in the lumen (Fig. 2b). They were clearly demonstrated in the methacrylate sections (Fig. 2c).

In the epithelium, some of the principal cells had PAS-positive inclusions, measuring up to about 8 μm in diameter, which were accumulated in the supranuclear region (Fig. 2b). The inclusions were generally larger near the nucleus. In Segment IV, the inclusion-containing cells were more numerous in the proximal portion than in the distal portion.

Electron microscopy revealed a lumen containing generally opaque and flocculent material. The PAS-positive substance occurred as coagulated masses which were rounded or irregular in contour (Fig. 4a). Small, irregularly-shaped fragmentary cell-debris were occasionally seen around the masses (Fig. 4a).

The principal cells containing PAS-positive inclusions were characterized by the following prominent features: 1) nipple-like protrusions on the luminal surface which will be designated as micropapilla in this study; 2) ductules and tubules continuous to the surface membrane; and 3) giant multivesicular bodies (Fig. 5).

The micropapilla was a cytoplasmic protrusion, measuring 1-3 μm wide and 1-2 μm high, and lacking stereocilia on its surface (Fig. 6, 7). At its base, bundles of microfilaments were distributed between the cell organelles. The micropapilla was always associated with opaque coagulated material in the lumen (Fig. 4a, 6a).
Fig. 4. Segment IV of the epididymal duct after efferent duct cutting. a. One week after efferent duct cutting. The lumen contains coagulated masses (*) and flocculent material but no spermatozoa. Arrows indicate cell debris. A small micropapilla (MP) is seen on the luminal surface of a principal cell. ×5,000. b. One week after efferent duct cutting. Numerous multivesicular bodies are accumulated in the supranuclear cytoplasm of a principal cell. ×7,500. c. One week after efferent duct cutting. The principal cell contains many multivesicular bodies of varying size. A very large multivesicular body (MVB) is shown at the center. ×6,000. d. Four weeks after efferent duct cutting. Most of the cell is occupied by a large multivesicular body (MBV). D dense bodies. A micropapilla (MP) with no ductules is seen. ×6,000.
At the tip of the micropapilla, 2-50 ductules opened to the lumen. They were 0.2-0.5 μm in diameter, ran almost parallel, and occasionally branched into fine tubules, 50-100 nm thick (Fig. 6, 7). The ductules contained an opaque amorphous substance that was continuous to the luminal coagulated material.

There were branching tubules, 50-100 nm in diameter, which were continuous to the luminal surface membrane around the micropapilla (Fig. 6b, c, 7b). As a whole, they formed a labyrinthine conglomeration lying 1-4 μm below the luminal surface (Fig. 6c, 7c, 8c).

Many coated and non-coated vesicles were distributed in the apical cytoplasm (Fig. 6a, 7, 8a). The coated vesicles, about 50 nm in diameter, appeared to bud off from the tubules and the luminal surface membrane. The non-coated vesicles were 100-400 nm in diameter; some of them contained an amorphous or flocculent material, and others appeared clear without visible contents (Fig. 8). The non-coated vesicles were also continuous with the tubules (Fig. 8a). The vesicles and tubules were packed below the micropapilla (Fig. 7a, 8a, c).

Multivesicular bodies, varying in diameter, were accumulated in the supranuclear cytoplasm (Fig. 4b, c, 6a, 7, 8). They sometimes occupied almost all the supranuclear area. The multivesicular bodies were generally larger in the deeper area near the nucleus. They were frequently very large to form giant multivesicular bodies, measuring up to 10 μm in diameter (Fig. 4c). The multivesicular bodies were often connected by tubules or sometimes fused directly to each other (Fig. 7, 8). The ductules and tubules occasionally opened to the bodies. Thus, the multivesicular bodies directly or indirectly continued to the lumen by the ductules and tubules (Fig. 7).

The contents of the multivesicular bodies consisted mainly of the following three
Fig. 6. One week after efferent duct cutting.  

a. The micropapilla (MP) in a perpendicular section has ductules (D) opening to the lumen. The micropapilla is associated with coagulated material (⁎) in the lumen. MVB multivesicular bodies contain various numbers of vesicles. ×14,000.  

b. Micropapilla in cross section reveals many ductules (D). Tubules (T) opening to the luminal surface around the micropapilla are shown. ×18,000.  

c. Apical cytoplasm sectioned obliquely to the surface at the base of the micropapilla. A group of ductules (D) extending from the tip of the micropapilla to the apical cytoplasm is shown. T tubules opening to the luminal surface around the micropapilla. L labyrinthine conglomerate of tubules. ×18,000
components; an amorphous material, vesicles, and heterogenous figures (Fig. 8, 9). The smaller multivesicular bodies near the cell surface usually contained an amorphous material and small numbers of vesicles without any heterogenous figure (Fig. 9a). The larger bodies near the nucleus generally contained numerous vesicles and heterogenous figures (Fig. 9a).

The amorphous material appeared similar in density to the coagulated material in the lumen (Fig. 8, 9a). The material was particularly accumulated at the opening of the ductules or tubules (Fig. 8). Minute crystalline structures of about 15 nm periodicity were occasionally found in the amorphous material (Fig. 10c). They were also seen in the ductules of the micropapilla and in the lumen of the epididymal duct (Fig. 10b). In addition, similar crystalline structures were also found in the membrane bound fragmentary debris in the lumen of the duct (Fig. 10a).

The multivesicular bodies that were continuous to the lumen of the epididymal duct through the ductules contained a few vesicles (Fig. 7). The ductules contained few or no vesicles. Similar but smaller vesicles, about 40 nm in diameter, were seen, though not frequently, in the duct lumen.

The heterogenous figures, such as granular masses of varying density, foamy figures, irregular myeline figures, or fine granules, were also seen in the multivesicular bodies (Fig. 9). They were generally contained in large quantities in larger bodies,
Fig. 8. One week after efferent duct cutting.  

**a.** Multivesicular bodies often continue to ductules and tubules (arrows). In the multivesicular bodies, amorphous material is massive (★), especially at the opening of the ductule, and continuous to that in the ductule. Non-coated vesicles (V) containing an amorphous material which is similar to that in the lumen (upper right) and multivesicular bodies occasionally connect with tubules (T) from the luminal surface. ×20,000.  

**b.** In the multivesicular body at the center, amorphous material (★) occupies the pole toward the opening of a tubule, and vesicles are packed toward the opposite pole. ×24,000.  

**c.** Amorphous material forms a mass (★) at the opening of a ductule. L labyrinthine of tubules. ×20,000
Fig. 9. Legend on the opposite page.
but they were not found in smaller ones near the luminal surface. In very large bodies, they tended to be aggregated toward the basal side.

The fine tubules, 40 nm in diameter, were straight and formed bundles which were composed of 10–50 tubules arranged in a hexagonal pattern (Fig. 9). In slightly oblique cross sections, as reported previously (Abe et al., 1983b), the wall of the tubules appeared membranous on one side, whereas it consisted of regularly arranged spikes on the other (Fig. 9ab). Large multivesicular bodies contained as many as 20 bundles of the tubules.

In addition, dense bodies were present in the basal cytoplasm (Fig. 9c). They were

Fig. 9. Principal cells one week after efferent duct cutting. a. Multivesicular bodies, which are accumulated in the supranuclear cytoplasm, are smaller toward the luminal surface (upper left) and larger in the deeper area (lower right). Larger multivesicular bodies have dense heterogenous figures (H). Arrows indicate bundles of fine, cylindrical tubules. D dense bodies. ×12,000. b. Heterogenous figures and vesicles in the multivesicular body. Longitudinal (1), cross (2), and slightly oblique (3) sections of the bundles of cylindrical tubules are shown. A slightly oblique section reveals regularly arranged spikes on one side of the walls. ×70,000. c. Basal cytoplasm showing dense bodies (D). The larger dense body (upper) contains bundles of cylindrical tubules as seen in the multivesicular body. ×17,000

Fig. 10. Crystalline structures. ×60,000. a. Membrane bound fragmentary debris in the duct lumen occasionally includes crystalline structures (arrows). b. Opening of a ductule at the tip of a micropapilla is represented (lower). Crystalline structures are seen in the epididymal duct lumen (arrows) and in the ductule (double arrow) of the micropapilla. c. Various structures in the multivesicular body are shown. Crystalline structures (arrow) are found in the amorphous material.
larger and more numerous than in normal cases. They were usually irregular in shape, measuring up to about 3 μm in diameter. Intermediate forms were recognized between the multivesicular bodies and dense bodies, as some of the dense bodies contained bundles of the fine tubules seen in the multivesicular bodies (Fig. 9c).

The principal cells with no multivesicular bodies appeared almost normal in their fine structure.

**The epididymis four weeks after efferent duct cutting**

At this stage, the PAS-positive inclusions were more frequent and larger than before. Some of the principal cells were distended with a large, round inclusion measuring 10 to 15 μm in diameter (Fig. 2d).

Electron microscopy revealed that the principal cells sometimes possessed a single huge multivesicular body which measured up to 15 μm in diameter and occupied most of the cytoplasm (Fig. 4d). The nucleus was dislocated basalwards. The multivesicular body usually consisted of an amorphous material, vesicles and heterogenous figures. The heterogenous figures were often located densely on the basal side. No typical micropapilla was seen, and the tubules and ductules were poorly developed. Large dense bodies were often found near the giant multivesicular body.

**DISCUSSION**

In the mouse, efferent duct cutting induced accumulation of PAS-positive inclusions in the principal cells in Segment IV. As reported in our previous paper (Abe et al., 1982a), similar inclusions appeared after ligation of the efferent duct or the epididymal duct proximal to Segment II, but they were not seen after ligation of the epididymal duct at Segment III or at the junction of Segments III and IV. Therefore, it is likely that, when Segment IV is free of spermatozoa and receives the luminal contents from Segment II, the PAS-positive inclusions accumulate in the principal cells in Segment IV.

The principal cells in Segment II are considered to secrete the PAS-positive material which is transported together with spermatozoa in the lumen (Takano, 1980; Abe et al., 1982a). In the rat, rabbit, and mouse, recent studies have demonstrated that the specific epididymal glycoprotein, most probably bound to spermatozoa in order to provide them with fertilizing ability, is produced in the proximal region of the duct (Lea et al., 1978; Olson and Hamilton, 1979; Faye et al., 1980; Moore, 1980). The glycoprotein is similar in localization to the PAS-positive material observed in the mouse epididymal duct (Abe et al., 1982a). It is therefore likely that the PAS-positive material in the epididymal duct lumen contains the specific glycoprotein. Our previous experiments suggest that the PAS-positive material, if not bound to spermatozoa, is absorbed by the principal cells in Segment IV (Abe et al., 1982a, b).

Electron microscopy revealed that the PAS-positive inclusions appear as large multivesicular bodies and that the multivesicular bodies are in communication with the lumen through the ductules or tubules. The possibility that the luminal coagulated material around the micropapilla is discharged from the ductules cannot be completely discounted, but it is more reasonable to consider that the luminal material is taken into the multivesicular bodies, in light of the following findings: 1) The amorphous material in the multivesicular bodies is massive closest to the ductules which are directly communicated with the bodies. This finding suggests that the massive material has been poured from the ductules into the bodies. 2) The crystalline structures found in
the multivesicular bodies are considered to be derived from the debris in the duct lumen. 3) It is unlikely that the contents of the multivesicular bodies are discharged into the duct lumen, because vesicles are less numerous in the multivesicular bodies near the luminal surface and not found in the ductules, and because they are rare in the luminal coagulated material around the micropapilla. 4) The principal cells contain many multivesicular bodies which become larger in the deeper region near the nucleus a week after operation. At 4 weeks the cells contain a single or a few giant multivesicular bodies. Thus, it is believed that the multivesicular bodies are formed due to absorption of the luminal material and that they become fused and enlarged in size as they are transported to the deeper region of the cells, and eventually form a giant multivesicular body.

The micropapilla is always associated with the dense, coagulated material which appears as PAS-positive hyaline globules or clumps in the duct lumen. The material seems to be related to formation of the PAS-positive inclusions or giant multivesicular bodies. Electron microscopy indicates that the dense PAS-positive material in the lumen appears as coagulated masses which may be heterogenous in origin. Thus the micropapilla with ductules may be a differentiated structure for absorbing the coagulated material. When the principal cells in Segment IV are in contact with the coagulated material, a micropapilla with ductules is presumably formed on the cell surface, and prominent PAS-positive inclusions or large multivesicular bodies appear in the cells. The contact with coagulated material occurs in small numbers for the cells lining the duct, because the inclusions are produced in only some of the principal cells.

Around the micropapilla are seen well-developed tubules, which appear to transport the luminal material into the multivesicular bodies. The development of similar tubules is known in the intestinal absorptive cells of sucking animals, and the tubule system itself is considered to function to ingest macromolecular materials, such as immunoglobulin, from the lumen (see a review by Yamamoto, 1982). The tubules and ductules in the epididymal cells may be similar in nature to the tubule system in the intestinal cells.

The multivesicular bodies are generally regarded as lysosomal in nature and to be involved in absorptive activities of the cells (Friend, 1969). In the principal cells of the epididymal duct, horse-radish peroxidase, ferritin, or other tracers intraluminally administered are transported by coated vesicles to the multivesicular bodies which contain acid phosphatase (Friend, 1969; Moniem and Glover, 1972; Hoffer et al., 1973; Moore and Bedford, 1979). The appearance of giant multivesicular bodies is explained as an elevated absorptive activity of the cells, and their numerous heterogenous figures may represent the residue of degraded materials.

As seen in the present results, the presence of intermediate forms between large multivesicular bodies and dense bodies suggest that the former may be transformed into the latter. We have previously demonstrated that, when temporal aspermia in the epididymal duct is induced by testicular irradiation, PAS-positive inclusions appear during aspermia and the inclusions disappear with reappearance of spermatozoa (Abe et al., 1983c). This finding indicates the possibility that the contents of the inclusions or multivesicular bodies are degraded and digested.

With the development of the multivesicular bodies, increasing amounts of small vesicles, considered to be derived mainly from the Golgi complex (Friend and Farquhar, 1967), appear rapidly in the bodies. In the epididymal cells with many multivesicular bodies reported in this study, however, the Golgi complex is not as well developed as that in the typical principal cell. Though the functional significance and origin of the
vesicles in the multivesicular bodies are still uncertain, a number of the vesicles are thought to provide so extensive a surface contact with ingested material that they may be presumed to play an important role in digestion of the material.

The giant multivesicular bodies eventually formed contain occasional bundles of fine tubules in addition to various heterogenous figures. Such tubules are not seen in the multivesicular bodies with no heterogenous figures, but they are contained also in the dense bodies in the basal cytoplasm. Thus it is probable that the fine tubules may be formed by condensation or digestion of the ingested luminal material. The tubules are thought to be derived from the luminal material. In the normal mouse, as reported previously (Abe et al., 1983a), the inclusions containing bundles of the fine tubules are also found in the principal cells in Segment IV.

The tubule-containing inclusions are more frequent and larger, having a much denser matrix in the operated mouse than in the normal. Much larger amounts of the ingested material may produce more tubules with residual dense substances. The tubule-containing inclusions are believed to form as a result of the absorptive activity of the principal cells.

In light of the present and previous studies (Abe et al., 1982a, b, 1983c), a possible implication of the morphologic changes in the epididymis after efferent duct cutting is as follows; the cutting of the efferent duct interrupts the influx of spermatozoa into the epididymal duct; the luminal PAS-positive material, which is considered to contain the substance to be utilized by the spermatozoa and to be secreted in the proximal region of the duct, remains unbound to the spermatozoa and is accumulated in greater amounts in Segment IV; the material becomes coagulated and comes into contact with some of the principal cells, this contact results in formation of a micropapilla with ductules most likely for a more active ingestion of the coagulated material by the cells; and the internalized material is then accumulated in the huge multivesicular bodies and degraded. Finally, such giant multivesicular bodies become transformed into smaller dense bodies.

REFERENCES


