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Morphometric Analysis of Coated Pits and Vesicles in the Proximal and Distal Caput Epididymidis

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ABSTRACT

A morphometric analysis of coated and uncoated structures found in the apical portion of principal cells from both the proximal and distal caput epididymidis has been carried out. Almost all endocytic, coated vesicles are found within 1 μm of the luminal surface of principal cells and the volume fraction of these and of uncoated vesicles is much greater in the proximal caput epididymidis. A serial section analysis indicated that many coated "vesicles" are tangentially sectioned coated pits and that a complex network of interconnected vesicular and tubular structures exists in the apical cytoplasm. Efferent duct ligation has no effect on the number or size of large coated and uncoated vesicles in either the proximal or distal caput epididymidis, indicating that substances delivered to principal cells from the lumen are not required to maintain the endocytic machinery. However, this treatment does result in a considerable increase in the number of large coated vesicles associated with the basal surface of principal cells from the proximal but not the distal caput epididymidis. The volume fraction of small, presumably exocytic, coated vesicles is significantly greater in the apical cytoplasm of cells from the distal caput epididymidis in control animals. Efferent duct ligation results in a significant increase in the volume fraction of these vesicles in the proximal but not distal caput epididymidis. These results show that there are marked differences in structure among principal cells from these two regions of the epididymis and that this may reflect differences in control and function.

INTRODUCTION

It is well established that the secretory and absorptive functions of the epididymis alter the molecular composition of the fluids bathing spermatozoa in the epididymal lumen, and thereby presumably aid sperm maturation (Orgebin-Crist et al., 1975; Bedford, 1975; Wong and Yeung, 1978; Jones et al., 1983). Work in two laboratories (Pelliniemi et al., 1981; Attramadal et al., 1981) suggests that principal cells, the predominant epithelial cell type in the epididymis, are responsible for the endocytosis of most of the androgen binding protein (ABP) found within the lumen. These immunocytochemical studies show extensive uptake of the protein by principal cells of the proximal caput epididymidis, and little uptake by cells of the adjacent distal caput epididymidis or regions further along the duct. Uptake within a specific region of the epididymis despite the presence of luminal ABP along the entire duct (Pelleniemi et al., 1981) indicates that the endocytosis may be a specific, receptor-mediated event. The proximal regions of the caput epididymidis may also be dependent upon substances, for example ABP, delivered to them from the lumen (Danzo et al., 1977; Fawcett and Hoffer, 1979).

Many proteins have been shown to be internalized by cells through receptor-mediated endocytosis, a process in which the protein binds to a specific receptor on the cell surface and is subsequently internalized through clathrin-coated pits along the plasma membrane (Goldstein et al., 1979; Pastan and Willingham, 1981; Pearse and Bretscher, 1981; Steinman et al., 1983). In order to better understand the difference in ABP uptake between the proximal and distal caput epididymidis, we undertook a morphometric analysis of principal cells from the two regions, concentrating on coated and uncoated vesicles near the luminal surface.

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MATERIALS AND METHODS

Fixation and Microscopy

Mature male Sprague-Dawley rats (90 to 110 days of age) were anesthetized by intraperitoneal injection of pentobarbital sodium, 5 mg/100 g body weight (Nembutal, Abbott Labs., North Chicago, IL), and fixed by retrograde perfusion through the abdominal aorta (Vitale-Calpe et al., 1973). An initial 10-sec flush with saline was followed by the perfusion fixative 5.0% glutaraldehyde (TAAB, Marivac, Halifax, Canada) in 0.2 M s-collidine (TAAB) buffer, pH 7.4. Perfusion was continued for 20–25 min, after which 1-mm blocks were dissected from various regions of the epididymis and fixed for another hour. Blocks from the proximal and distal caput epididymidis corresponded to Zones 2 and 3, respectively, as defined by Reid and Cleland (1957; Fig. 1). Blocks were post-fixed for 1.5–2 h in 1.5% osmium tetroxide in 0.2 M s-collidine buffer or in a 1:1 solution of 2% osmium tetroxide and 3% potassium ferrocyanide on ice, dehydrated in a graded series of alcohols, cleared in propylene oxide and embedded in Epon 812 (TAAB). For light microscopy, 1-μm sections were cut on an LKB ultramicrotome, stained with toluidine blue, and examined with a Zeiss light microscope. For electron microscopy, silver sections were cut, stained with uranyl acetate and lead citrate, and viewed with a JEOL 100S electron microscope.

For surgical procedures, Sprague-Dawley rats 80–90 days of age were anesthetized with pentobarbital sodium. Testes and epididymides were exposed through an abdominal incision and viewed through a dissecting microscope with illumination both above and below the efferent ductules. The ductules were ligated with 6–0 silk suture, and great care was taken to avoid ligature of blood vessels within the fat tissue surrounding the efferent ductules. We were particularly careful to avoid the anastomosis between testicular surface veins and efferent ductule veins (Chubb and Desjardin, 1982). Operated and sham-operated epididymides were reinserted into the scrotum. Five weeks after the operations, animals were perfused as described above. All ligatures were intact at that time.

Morphometry of Principal Cells

Silver sections were cut from five to eight 1-mm blocks from both the proximal and distal caput epididymidis in each of four animals. Cross-sections of tubules displaying cells cut in a median sagittal plane were located. Cells from these regions were randomly selected and the apical portion of these cells photo-

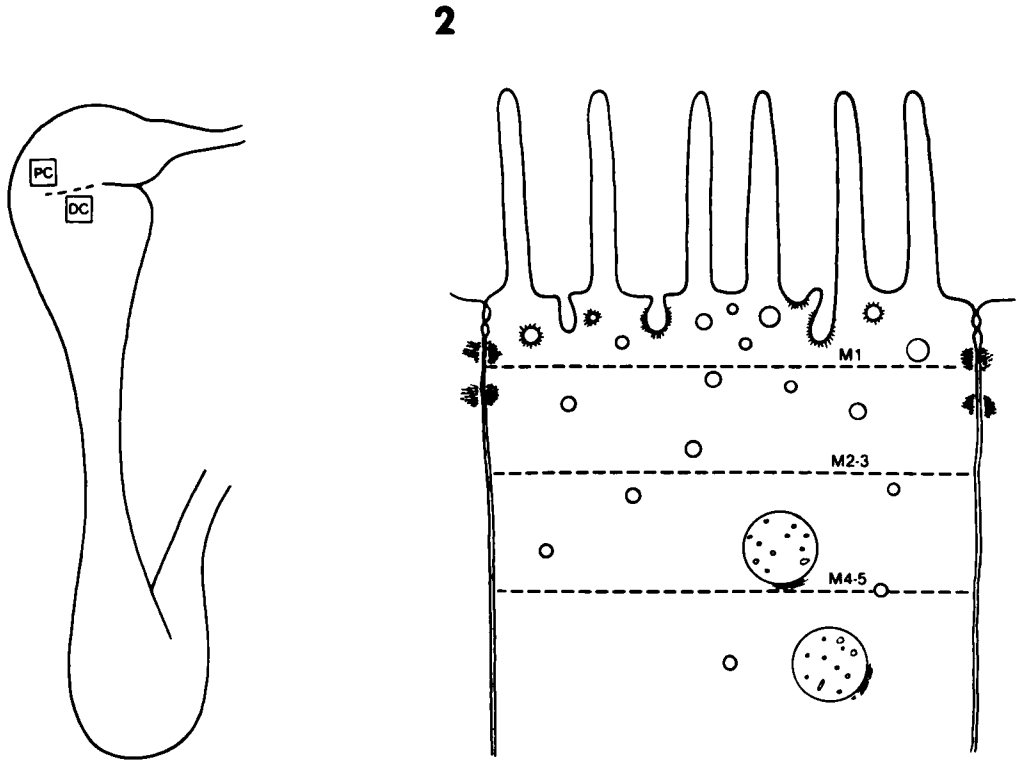

FIG. 1. Divisions of the caput epididymidis. *PC* and *DC*, corresponding to Zones 2 and 3, as defined by Reid and Cleland (1957), were selected as proximal and distal caput epididymidis in this study.

FIG. 2. Drawing of the luminal portion of an epididymal principal cell illustrating the regions *M1*, *M2–3* and *M4–5* used in the quantitation.

graphed. Negatives were printed at a final magnification of 15,000 to 25,000X. Eighty cells, 40 each from both the proximal and distal caput epididymidis, were morphometrically analyzed in each control rat. For a set of 40 cells in any one animal, cells from at least 12 tubule profiles were included in the set of measurements, and never more than 4 cells from the same tubule. In efferent duct-ligated animals, 60 cells in each of three rats were quantitated.

Only the most apical 5 μm of each cell were analyzed. Preliminary experiments led us to trisect this area into the micron closest to the lumen (M1), the second and third micron (M2–3), and the fourth and fifth micron (M4–5) (Fig. 2). Photographs were analyzed on a Hipad digitizer attached to an Apple II computer equipped with a digitizing software package (Bioquant II from R+M Biometrics, Nashville, TN). The cross-sectional areas of all coated vesicles, uncoated vesicles, multivesicular bodies, and mitochondria within the 5-μm area were measured. From these values, volume fractions could be determined by applying the Delesse principle, which states that the planimetric fraction of a section occupied by a given component corresponds to the fraction of tissue volume occupied by that component (Weibel et al., 1966).

Coated vesicles were identified as those spherical structures exhibiting no apparent connection to the luminal surface and possessing a bristle-coated membrane. Uncoated vesicles were distinguished from tubules of smooth endoplasmic reticulum on the basis of their consistently spherical shape, more darkly staining membrane, and electron-lucent interior. The length and frequency of coated pits were also measured with the Bioquant II system.

All other quantitations, statistics, and correlations were performed with the digitizing system. Data were analyzed by analysis of variance and significant differences were tested by Student's *t* test. Morphometry at the light microscope level was performed directly on the Zeiss microscope with the aid of a camera lucida positioned over the Hipad digitizer.

Serial Sectioning

Five blocks each from the proximal and distal caput epididymidis were randomly chosen from a pool of three rats and trimmed to yield block faces less than 0.2 mm^2. Uninterrupted ribbons of dark gray sections were collected on formvar-coated loops, then transferred directly onto single-slotted copper grids and stained with uranyl acetate and lead citrate. Sections were approximately 325–350 A thick, as determined both by interference colors and the number of serial sections needed to fully pass through vesicles of known diameter. Randomly selected cells were photographed through an entire series of at least 16 continuous sections. The middle section in each ribbon was located, and all apparent coated vesicles were followed from this point in both directions to determine whether they were coated vesicles or pits connected to the apical surface. A total of 20 cells were analyzed in this way, 2 cells from each of five blocks in both the proximal and distal caput epididymidis.

RESULTS

Qualitative Observations (Controls)

The most striking difference at the light microscope level was the 30% greater height of cells from the proximal caput epididymidis compared to the distal caput. Most of this difference could be accounted for by the much greater volume of supranuclear cytoplasm in cells from this region. Low-power electron micrographs (Figs. 3 and 4) showed that the supranuclear area of cells from the proximal caput epididymidis was filled by a more prominent Golgi apparatus and a more extensive smooth endoplasmic reticulum (SER). In addition, tubules of SER appeared more dilated in the proximal caput epididymidis. Multivesicular bodies, which were located near the luminal surface, also appeared more frequently in these cells, but residual bodies and dense lysosomal structures existed in greater numbers in cells from the distal caput epididymidis. In addition, the apical cytoplasm of cells from the proximal caput epididymidis had an empty, vesiculated appearance. Lipid droplets, typically located both immediately above and just below the nucleus, were found much more frequently in cells from the distal caput epididymidis. A striking difference in nuclear shape also existed between the two regions: nuclei of principal cells from the proximal caput appeared smooth and spherical, whereas nuclei in the distal caput epididymidis were deeply notched and lobulated, and often appeared binucleate.

Mitochondria in cells from both regions tended to cluster in three areas: *1*) most notably along the lateral plasmalemma, above the Golgi complex and extending to within 3 μm of the luminal surface; *2*) along the base of the cell; and *3*) less frequently just above the nucleus. The lateral distribution was most obvious in cells from the proximal caput, the basal distribution in cells from the distal caput epididymidis. Although mitochondria in cells from both regions were typically cylindrical in shape and aligned in an apical-basal direction, mitochondria in cells from the distal caput epididymidis were often irregular in outline, appearing curved or barbell shaped.

In addition, fewer cristae were found in mitochondria from cells of the distal caput epididymidis. Serial sections (results not shown) revealed that the lateral clusters of mitochondria in cells from the proximal caput epididymidis were often neighboring extensions

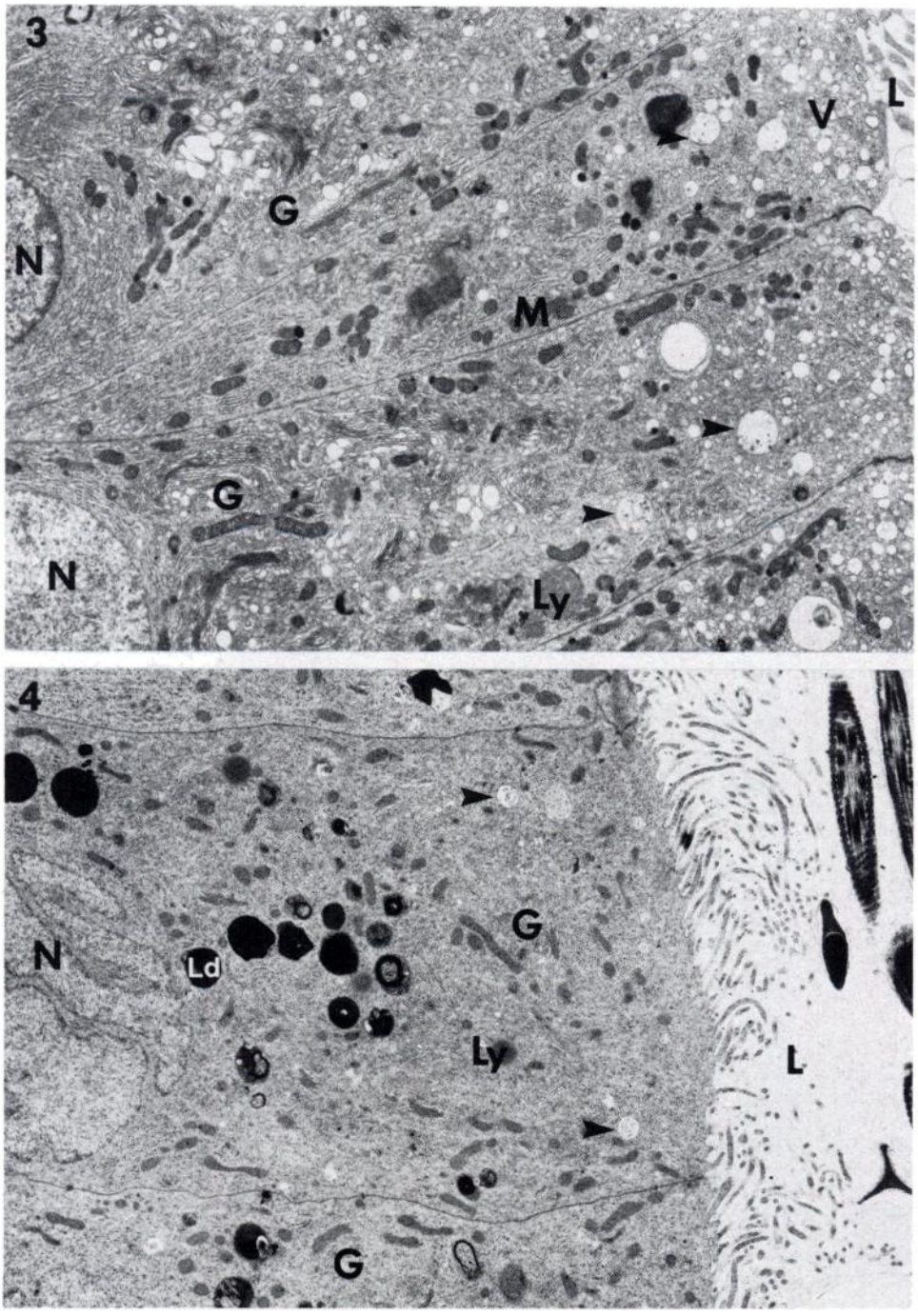

FIG. 3. Low-power electron micrograph of principal cells from the proximal caput epididymidis (Zone 2). These are tall columnar cells with round nuclei (*N*), and extensive and dilated Golgi apparatus (*G*), multivesicular bodies (*arrows*), and vesicles (*V*) close to the lumen (*L*). Mitochondria (*M*) occur most frequently along the lateral borders of the cells and tend to align in an apical-basal direction. ×2000.

FIG. 4. Low-power electron micrograph of principal cells from the distal caput epididymidis (Zone 2). These cells are shorter than cells from the proximal caput epididymidis, have fewer vesicles (*V*) and multivesicular bodies (*arrows*). Nuclei (*N*) are deeply notched and lobulated. Elements of Golgi (*G*) are scattered throughout the supranuclear cytoplasm and lipid droplets (*Ld*), rarely found in cells from the proximal caput, are commonly found surrounding the nucleus. ×2000.

of one or a few highly branched mitochondria. Such complex, convoluted mitochondria occurred less frequently in cells from the distal caput epididymidis.

Qualitative Observations (Efferent Duct-Ligated)

Five weeks after efferent duct ligation the epididymal lumen was completely cleared of spermatozoa. Quantitative analysis revealed that cells of the initial segment regressed approximately 50% in height (results not shown), but that cells in both the proximal and distal caput epididymidis were similar in height to controls (see Table 2). These results confirmed those reported earlier by Fawcett and Hoffer (1979). However, cell height along a tubule cross-section varied greatly. This was especially true in the distal caput, where periodic troughs in the epithelium plunged to a height of less than 15 μm. In both regions, a 60-μm reduction in luminal width was observed (see Table 2). Sham-operated epididymides appeared identical to controls, as seen under both the light and electron microscope.

Very few changes were noted at the ultrastructural level. Most differences seen between principal cells from the proximal and distal caput epididymidis in normal epididymides were maintained in the ligated animals, although the extensive supranuclear SER appeared to be reduced in cells from the proximal caput epididymidis (results not shown). One change associated with ligation occurred at the base of cells (Figs. 5 and 6). In control epididymides, the base of principal cells smoothly abutted a thin straight basal lamina, but in epididymides from efferent duct-ligated animals this apposition took on a highly irregular contour, complete with deep invaginations and protrusions. This was presumably a result of tubule shrinkage. The basal lamina itself more than doubled

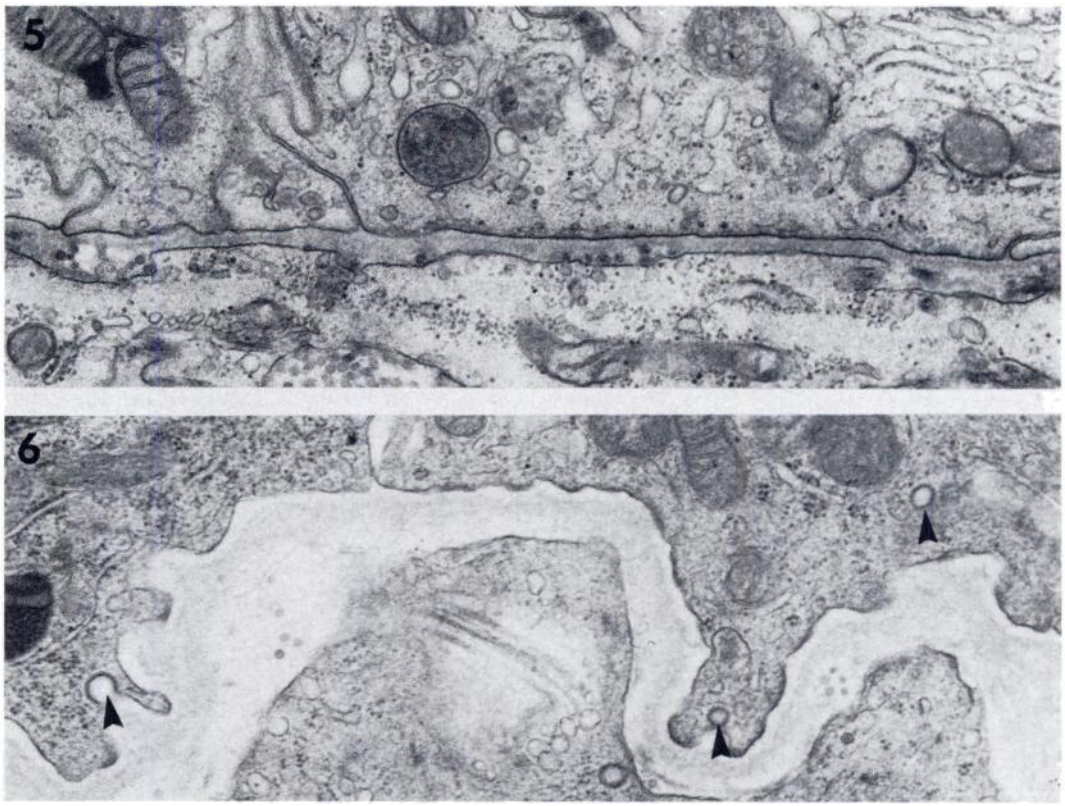

FIG. 5. An electron micrograph of the basal surface of a principal cell from the proximal caput epididymidis. Note the straight apposition to the basal lamina and the lack of large coated pits and vesicles. X23,000.

FIG. 6. An electron micrograph of the basal surface of a principal cell from the proximal caput epididymidis of an efferent duct-ligated animal. Note the convolutions of the plasmalemma and basal lamina. Large coated pits and vesicles (*arrows*) are more common. X23,000.

in thickness. These changes were much more pronounced in the proximal caput epididymidis.

Quantitative Analysis (Controls)

Tables 1 and 2 present results of a quantitative analysis of the apical 5 μm of principal cells from control and efferent duct-ligated epididymides. A preliminary quantitation of coated vesicle size indicated that two clearly defined classes of coated vesicles existed, hereafter referred to as "large" and "small" coated vesicles. In control animals the volume fraction occupied by large coated vesicles within the first micron (M1) was significantly greater ($P<0.01$) in cells from the proximal caput than in cells from the distal caput epididymidis (2.96 vs. 0.66) (Table 1). More than 98% of large coated vesicles were found in Region M1 in cells from both regions, while small coated vesicles were common throughout the 5-μm area. The volume fraction occupied by small coated vesicles in Region M1 was significantly higher in the distal caput epididymidis (0.83 vs. 0.29), although this density varied considerably between cells and animals (Table 1). It should be stressed that small coated vesicles were found throughout the supranuclear cytoplasm, and probably arose from the Golgi complex. Neither the number of coated pits per micron of luminal surface nor their length differed significantly in the two regions (Table 1).

Table 1 also shows that the cross-sectional area of large coated vesicles was greater in the proximal caput epididymidis (2.7 vs. 1.30). Although the size distribution of coated vesicles from the distal caput epididymidis approximated a normal distribution skewed slightly to the right, the distribution of these vesicles from the proximal caput epididymidis was skewed far to the right in both control and efferent duct-ligated animals (Fig. 7). We suspect that many "vesicles" landing in the upper tail of this distribution were probably profiles of very large coated pits continuous with the luminal surface, rather than enclosed vesicles. In this case the modal value of the distribution was a better estimate of mean vesicle profile size than mean vesicle size itself. Modal values for the proximal and distal caput epididymidis were 22,000 nm^2 and 12,000 nm^2, respectively (Fig. 7). These modal values yielded rough

TABLE 1. Differences in volume fraction, size and density of coated structures in principal cells from the proximal and distal caput epididymidis of control and efferent duct-ligated animals.

	Control (N=4)		Efferent duct-ligated (N=3)	
	Proximal	Distal	Proximal	Distal
Large coated vesicles (apical region)				
Volume fraction	2.96[a] ± 0.18	0.66 ± 0.06	2.80[b] ± 0.40	0.71 ± 0.10
Cross-sectional area ($nm^2 \times 10^{-4}$)	2.70[a] ± 0.05	1.30 ± 0.07	3.40[b] ± 0.40	1.30 ± 0.16
Coated pits (apical region)				
Number/μm	0.43 ± 0.06	0.40 ± 0.05	0.51 ± 0.06	0.52 ± 0.08
Length (nm)	480.0 ±40.0	400.0 ± 35.0	Not quantitated	
Coated structure density (apical region)				
Number of pits and vesicles/μm of luminal surface	1.56[a] ± 0.08	0.92 ± 0.04	1.50[b] ± 0.04	1.13 ± 0.20
Coated structure density (basal region)				
Number of pits and vesicles/ 100 μm of basal surface	2.60[a] ± 1.20	6.60 ± 1.70	13.6[bc] ±2.30	7.40 ± 1.50
Small coated vesicles (apical region)				
Volume fraction				
(M1)	0.29[a] ± 0.07	0.83 ± 0.14	0.65[c] ± 0.14	1.28 ± 0.38
(M2–3)	0.53 ± 0.11	0.67 ± 0.13	1.12 ± 0.11	0.81 ± 0.14
(M4–5)	0.62 ± 0.05	0.60 ± 0.07	1.14[c] ± 0.08	0.75 ± 0.04

[a]Significantly different from control distal caput epididymidis ($P<0.01$).

[b]Significantly different from ligated distal caput epididymidis ($P<0.01$).

[c]Significantly different from control proximal caput epididymidis ($P<0.01$).

TABLE 2. Differences in uncoated vesicle volume fraction and size, the volume fraction of multivesicular bodies, tubule diameter and cell height in the proximal and distal caput epididymidis of control and efferent duct-ligated animals.

	Controls (N=4)		Efferent duct-ligated (N=3)	
	Proximal	Distal	Proximal	Distal
Uncoated vesicles				
Volume fraction				
(M1)	7.28[a] ± 1.31	3.24 ± 1.17	8.42[b] ± 0.28	4.82 ± 1.16
(M2–3)	4.80[a] ± 1.32	1.13 ± 0.55	Not quantitated	
(M4–5)	2.40[a] ± 0.60	0.61 ± 0.33	Not quantitated	
Cross-sectional area ($nm^2 \times 10^{-4}$)				
(M1)	2.52 ± 0.19	2.12 ± 0.19	2.91 ± 0.21	2.26 ± 0.46
(M2–3)	3.13 ± 0.08	2.73 ± 0.31	3.32 ± 0.33	3.33 ± 0.73
(M4–5)	3.29 ± 0.15	2.35 ± 0.33	2.79 ± 0.12	3.39 ± 0.56
Multivesicular bodies				
Volume fraction	5.79[a] ± 2.50	1.99 ± 0.25	5.34[b] ± 0.73	2.42 ± 0.24
Tubule diameter (μm)	187.0[a] ± 10.70	323.0 ± 27.20	126.0[bc] ± 1.72	268.0[d] ± 10.80
Cell height (μm)	38.0 ± 1.20	30.5 ± 1.30	35.6 ± 1.79	31.7 ± 2.17

[a]Significantly different from control distal caput epididymidis ($P<0.01$).

[b]Significantly different from ligated distal caput epididymidis ($P<0.01$).

[c]Significantly different from control proximal caput epididymidis ($P<0.01$).

[d]Significantly different from control distal caput epididymidis ($P<0.01$).

estimates of vesicle diameter for the two regions of 170 nm in the proximal and 125 nm in the distal caput epididymidis.

Consequently the average volume of each large coated vesicle from the proximal caput epididymidis was approximately 2.5 times that of each vesicle from the distal caput epididymidis. In contrast, the cross-sectional area of uncoated vesicles was approximately the same in both regions (Table 2). Because almost all

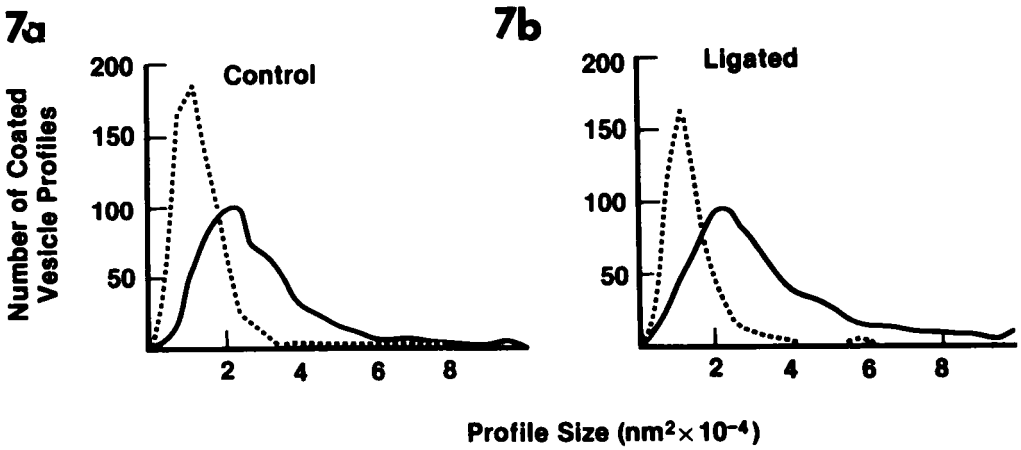

FIG. 7. Frequency distribution of large coated vesicle cross-sectional area in cells from the proximal *(solid line)* and distal caput epididymidis *(dashed line)* from control and efferent duct-ligated animals. Note that the distribution in the proximal caput is skewed to the right.

large coated vesicles were located within 1 μm of the luminal surface, it was reasonable to assume (and serial sections showed) that many of these "vesicles" were actually tangentially sectioned coated pits. We thus pooled all large coated vesicles and pits together under one classification, "coated structures," and then determined a coated structure density along the luminal surface (Table 1). For each of the 320 cells included in the quantitation, the number of coated vesicles and pits in a given cell was divided by the apical width of that cell. As expected, average coated structure density at the luminal surface in cells from the proximal caput was significantly greater ($P<0.01$) than in cells from the distal caput epididymidis (1.56 vs. 0.92). The volume fraction of uncoated vesicles in M1 (Table 2) was also significantly greater ($P<0.01$) in the proximal caput epididymidis (7.28 vs. 3.24). Although uncoated vesicles occurred throughout the apical 5 μm, their volume fraction decreased significantly from the first to the fifth micron ($P<0.025$) (Table 2). The volume fraction occupied by multivesicular bodies (Table 2) was also significantly greater in cells from the proximal caput epididymidis ($P<0.01$).

Quantitative Analysis (Efferent Duct-Ligated)

Although tubule diameter decreased in both the proximal and distal caput epididymidis after efferent duct ligation, there was no significant difference in the height of the epithelial lining (Table 2). Tables 1 and 2 show that most quantitative differences between cells from the proximal and distal epididymidis in normal animals were maintained in ligated animals. The size and volume fraction of both coated and uncoated vesicles were significantly greater ($P<0.05$) in cells from the proximal caput epididymidis. In ligated animals, as well as in controls, coated vesicles were almost 2.5× larger in cells from the proximal caput epididymidis.

Interestingly, the volume fraction occupied by small coated vesicles in cells from the proximal but not distal caput epididymidis was greater in ligated animals ($P<0.05$) when compared to controls.

Whereas few changes were noted at the apical surface, striking changes occurred at the base of principal cells in ligated animals. Compared to the apical surface, the lateral and basal surfaces of principal cells from control or ligated animals possessed few coated pits and vesicles. In addition, no significant differences occurred in the number of coated pits and vesicles along the lateral surfaces either between cells from the proximal and distal caput epididymidis or between ligated and control animals (data not shown). However, along the base of principal cells, differences in coated structure density between the two regions were found (Table 1). When 40 or more cells from a pool of five rats were quantitated, coated structure densities were significantly greater in the distal caput ($P<0.05$) than in the proximal caput epididymidis (6.4 vs. 2.6 coated structures/100-μm membrane). In both regions, small, presumably exocytic, coated vesicles rarely appeared near the basal surface.

In efferent duct-ligated animals, an interesting change occurred along the base of cells from the proximal caput epididymidis (Table 1). The frequency of coated structures within 1 μm of the basal surface increased dramatically when compared to controls (13.6 vs. 2.6 coated structures/100-μm membrane). No significant increase occurred in cells from the distal caput epididymidis of ligated animals.

Serial Section Analysis

Table 3 shows that in both the proximal and distal caput epididymidis, 25% of apparent coated vesicles appeared to be true vesicles and approximately 50% were deeply invaginated coated pits. Another 25% could not be assessed as either pits or vesicles. In such cases, neighboring coated and uncoated vesicles seemed to

TABLE 3. Serial section analysis of "apparent" coated vesicle profiles.

Region	N	Number of vesicle profiles (per cell)	Coated vesicles (%)	Coated pits (%)	Undetermined (%)
Proximal	10	11.1 ± 0.9	24.3 ± 5.7	51.5 ± 5.2	24.0 ± 3.3
Distal	10	7.6 ± 1.3	22.6 ± 4.2	44.7 ± 4.8	32.5 ± 5.5

adjoin the structure in question by a series of narrowly connected vesicles and tubules to the cell surface (Fig. 8). The serial sectioning of principal cells revealed that the apical cytoplasm of these cells was filled with a complex, interconnected tubular-vesicular network.

DISCUSSION

The present study has demonstrated that a much more extensive population of both large coated and uncoated apical vesicles exists in principal cells from the proximal caput epididymidis than in cells from the distal caput epididymidis, a difference that is unaffected by efferent duct ligation. The higher volume fraction of large coated vesicles in cells from the proximal caput epididymidis is due both to a greater number and greater average size of vesicles in that region. We find these differences particularly striking since such a short distance separates these two regions of the epididymis. However, this study does not demonstrate directly a particular endocytic or exocytic function of these apical vesicles. Hoffer et al. (1973) observed that the ultrastructural characteristics of principal cells from the rat caput epididymidis indicated that these cells perform both secretory and absorptive functions. Moore and Bedford (1979a,b) interpreted a loss in apical vesicles in castrated rats as reflecting reduced secretion by principal cells, and Parr (1982), in a quantitative study of apical vesicles in the uterine endometrium, concluded that these were most likely associated with exocytosis, not endocytosis. These researchers did not restrict their quantitation of the apical 5 μm of the cell, but instead quantitated the entire supranuclear regions of cells. Clearly, in the epididymis at least, some vesicles within the apical 5 μm may be associated with exocytosis; indeed, principal cells are known to secrete many proteins and other molecules (Lea et al., 1978; Brooks and Higgins, 1980; Jones et al., 1980; Kohane et al., 1980; Flickinger, 1981). In addition, the morphological appearance of the Golgi in these cells, as well as the large population of small coated vesicles, suggests that secretion is taking place. However, the apical population of uncoated vesicles in principal cells is probably indicative of endocytosis because the decline in uncoated vesicle volume fraction from the luminal surface to a point 3–5 μm further into the cell body suggests a pathway not from the Golgi to the surface, but rather from the surface into multivesicular bodies and lysosomes. Many multivesicular bodies are found in principal cells, a fact which in itself could be taken as evidence for absorption in these cells. In any

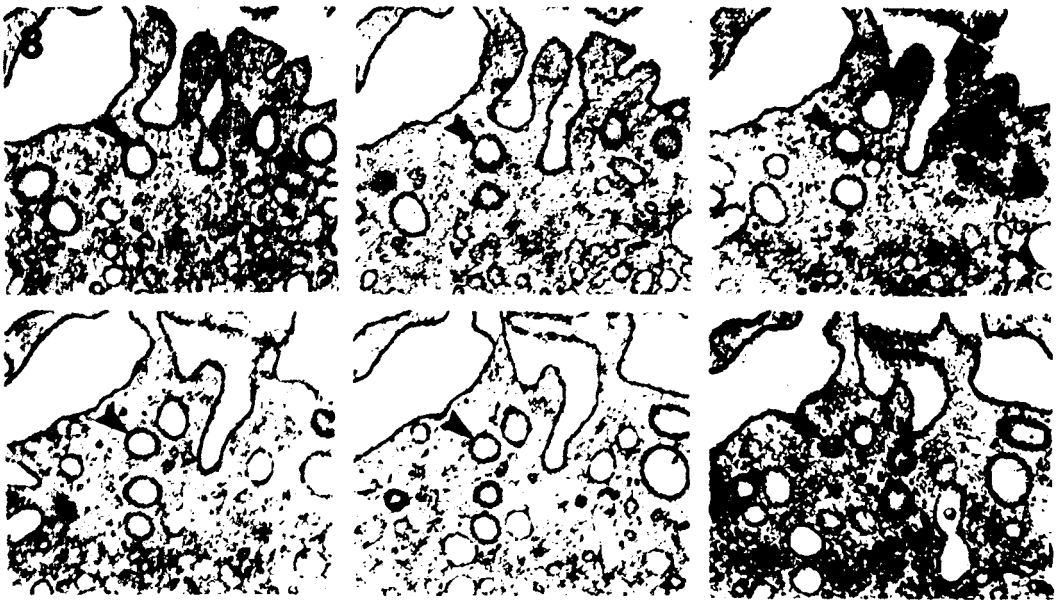

FIG. 8. Serial sections of coated vesicle and pits in the apical region of epididymal principal cells. The coated vesicle indicated (*arrow*) does not exhibit any connection to the apical surface of the cell. ×23,000.

case, it is generally accepted that the processes of exocytosis and endocytosis operate to some extent in tandem, the cell thereby avoiding the problem of an incessant accretion or depletion of plasma membrane. The number and appearance of coated vesicles and pits in the proximal caput epididymidis is in fact qualitatively similar to that in mosquito and hen oocytes, which are known to absorb huge quantities of yolk protein through coated structures (Roth and Porter, 1964; Perry and Gilbert, 1979).

Because many proteins are internalized after binding to specific receptors and clustering in clathrin-coated pits (see Steinman et al., 1983 for review), it is tempting to speculate that the additional clathrin-coated membrane in cells from the proximal caput epididymidis facilitates extensive and efficient receptor-mediated endocytosis of one or more proteins from the epididymal lumen. However, our results with efferent duct-ligated animals indicate that substances which are continually transported to the lumen of the ductus epididymis via the efferent ducts—for example, ABP—are not required to induce or maintain the large amount of coated membrane in cells from the proximal caput epididymidis. Therefore, even if such substances are endocytosed by principal cells and are needed for the proper functioning of these cells, they do not induce the assembly of clathrin along the membrane.

In a cell as highly polarized as the principal cell, the paucity of coated vesicles and pits along the basolateral surfaces of the cell is not surprising. However, we were surprised to find an increase in coated pits and large coated vesicles along the base of cells from the proximal caput epididymidis of efferent duct-ligated animals. Because we could not detect an increase in small coated vesicles in the infranuclear cytoplasm, we consider it unlikely that the increase in large coated structures at the base of these cells reflects heightened exocytosis into the interstitium. We suspect, rather, that the increase points to a greater endocytic activity at the base of the cell. This change may be in compensation for luminal endocytosis which has been altered by efferent duct ligation. Although there was no change in the endocytic apparatus of principal cells after efferent duct ligation, it is possible that proteins or other substances no longer received in sufficient quantity at the luminal surface are received instead through the basal surface. For example, ABP is present in the peripheral circulation of rats (Gunsalus et al., 1978). Alternatively, an increased assembly of clathrin along the membrane may not reflect any functional relationship to endocytosis but instead a structural change in response to the increased convolution of the basal surface.

Following ligation of the ductuli efferentes there is an increase in the apical density of small coated vesicles in cells from the proximal caput epididymidis. These vesicles are not usually associated with endocytosis. For example, studies performed in the vas deferens (Friend and Farquhar, 1967) and other tissues (Herzog and Farquhar, 1977) suggest that these vesicles are part of an exocytic pathway, serving as carriers between the rough endoplasmic reticulum, the Golgi and the luminal surface. This may indicate, then, that efferent duct ligation induces principal cells from the proximal caput epididymidis to increase their synthetic and exocytic function.

The serial section analysis suggests that the apical cytoplasm of principal cells is not composed simply of a population of discreet, independent vesicles, but rather of a complex network of narrowly connected vesicular and tubular structures, similar to that described in other cell types (Wall et al., 1980; Ackerman and Walker, 1981). Some investigators have argued that the complete budding of coated vesicles from the plasma membrane, and hence continuous assembly and disassembly of clathrin from the membrane, would be energetically inefficient (Pastan and Willingham, 1981). These workers have suggested that the large majority of "coated vesicles" are actually connected by long narrow necks to invaginations of the plasma membrane, internalization being completed when uncoated vesicles (receptosomes) bud off, allowing the clathrin-coated membrane to return to the surface. Our serial section study shows that some coated vesicles, apparently severed from the plasma membrane, do exist in principal cells. Although this is contrary to the model of Pastan and Willingham, we feel that as all bona fide large coated vesicles were confined to within 1 μm of the apical surface of the cell, these structures should perhaps be viewed as functional extensions of coated pits.

This study has demonstrated that an extensive and diverse population of large coated and uncoated pits and vesicles, as well as small coated vesicles, exists in principal cells from the epididymis. We have shown that there is a

dramatic difference in the volume fraction of coated structures between cells from two adjacent regions. One of these regions, the proximal caput epididymidis, selectively takes up ABP while the distal caput does not, or only in much reduced amounts. The additional coated membrane in cells from the proximal caput indicates that these cells are also more likely to be active in specific (receptor-mediated) endocytosis than cells from the distal caput epididymidis and may reflect this apparently specific uptake of ABP. In contrast to the initial segments of the epididymis (Fawcett and Hoffer, 1979), we did not find that the height of principal cells from the proximal or distal caput epididymidis (Zones 2 and 3; Reid and Cleland, 1957) decreased significantly after efferent duct ligation, nor was there a change in the volume fraction occupied by coated pits and vesicles at the luminal surface. Nevertheless, the fact that those changes that were elicited by this treatment were confined to cells from the proximal caput epididymidis indicates that cells from these two adjacent regions may be under different control.

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