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The ampulla of the ductus deferens in man: morphological and ultrastructural aspects

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ABSTRACT

In order to compare the histology of the ampulla of the ductus deferens with that of the other segments of the duct in man, the seminal vesicle and the adjacent 13–15 cm of the ductus deferens were obtained during cystectomy from 15 adult men, and were processed for light and electron microscopy. Each ductus deferens specimen was divided into 3 segments: segment A or initial segment (the most proximal to the testis) showing a smooth outer surface and, on section, a uniform lumen and absence of mucosal invaginations; segment B (1.5–4 cm) showing a smooth outer surface and, on section, small cavities in the mucosa; and segment C or ampulla (3–4 cm), which was easily recognisable because of the cerebriform pattern on its outer surface. Segment A showed the usual histological pattern reported in studies of the human ductus deferens. Segment B consisted of mucosa, muscularis mucosae, submucosa, muscular coat and adventitia. The epithelial lining formed multiple branched invaginations in the lamina propria and submucosa giving rise to glandular structures. The lumen of the duct and the glands were lined by the same cell types: (1) basal cells; (2) mitochondrion-rich cells; and (3) columnar cells with the ultrastructural features of glycoprotein-secreting cells. The latter cells could be classified into 3 subtypes suggesting different stages of development: (*a*) with abundant mitochondria; (*b*) with abundant rough endoplasmic reticulum; and (*c*) with abundant secretory granules. Segment C or the ampulla showed the same histology as segment B except for the presence of many diverticula in the ampulla. Spermatozoa were infrequent in all 3 segments of the ductus deferens examined. The lumen of the ductus deferens was significantly larger and the muscular coat thinner in segments B and C than in segment A. The surface area per cross-sectioned duct occupied by the glands and diverticula was greater in segment C than in segment B. The present findings suggest that rather than being a sperm reservoir as in other mammals, the human ampulla is a secretory structure which begins in the preceding segment.

INTRODUCTION

The structure of the mammalian ductus deferens has been studied in several species, principally the rat (Flickinger, 1975; Hamilton & Cooper, 1978; Kennedy & Heidger, 1979; Clermont & Hermo, 1988). Most studies on the human ductus deferens only concern its initial portion (closest to the testis) and have been undertaken on vasectomy specimens (McLeod et al. 1973; Popovic et al. 1973; Friend et al. 1976; Silber et al. 1977). The more distal portions of the ductus deferens have been little studied because of difficulties in obtaining well preserved specimens from these portions. A study of the distal segments of the human ductus deferens is of interest because, as in the rat (Flickinger, 1975; Hilton & Cooper, 1978), regional variations along the length of the duct have been reported from autopsy material (Paniagua et al. 1981, 1983). This distal portion of the ductus deferens contains the anatomical structure designated as the ampulla ductus deferentis. The descriptions of this structure have emphasised the presence of a dilated lumen and deep, branched invaginations of the mucosa (Aumüller & Brühl, 1977; Cossu et al. 1978; Riva et al. 1979, 1982). The few ultrastructural studies on the ampulla (Riva et al. 1979, 1982) have described

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2 epithelial cell types (secretory and basal cells), similar to those of the seminal vesicles (Brewster, 1985).

The present report provides new information concerning the structure of the human ampulla, including the ultrastructural characteristics of the epithelial cells and the differences from the other portions of the ductus deferens.

MATERIAL AND METHODS

Surgical specimens obtained from cystectomy of 24 adult men (aged 40–65 y) with carcinoma of the seminal vesicle were examined. After rejection of 9 specimens that showed either carcinomatous infiltration of the spermatic cord or local ischaemia due to localised thrombosis or vascular steal by the tumour, the remaining 15 specimens were used for this study. These specimens included the prostate,seminal vesicle, and the adjacent 13–15 cm of the ductus deferens. The remaining portion of the ductus deferens (the initial 20–24 cm adjacent to the testis) was not collected. After its removal, each ductus deferens specimen and the seminal vesicle were fixed by intraluminal perfusion with a 4% paraformaldehyde solution in PBS at pH 7.4, using a perfusion pump (Cabotron, Barcelona, Spain) at a constant pressure of 563 kg/cm^2, and a flow rate of 12.6 ml/min. Afterwards, each ductus deferens was sectioned into 3 portions (Fig. 1), as follows.

Segment A. The initial segment (7–8 cm in length) of the removed portion. This showed a smooth outer surface and, on section, a uniform lumen and absence of mucosal invaginations.

Segment B. The following segment (1.5–4 cm in length). This showed a smooth outer surface and, on secretion, small cavities in the mucosa.

Segment C. The more distal segment (3.5–4 cm), extending from the distal border of the seminal vesicle to the ejaculatory duct and corresponding to the ampulla. This segment is easily recognisable because its outer surface shows a cerebriform pattern which is more prominent at the junction with the seminal vesicle. On section small cavities within the mucosa are also observed.

The 3 segments from each subject were cross-sectioned into 0.5 cm slices which were further fixed in the same fixative for 24 h at 4 °C. One out of each 3 consecutive slices was cut into 1 mm^3 blocks that were postfixed in 2% osmium tetroxide, dehydrated and embedded in Epon. Semithin sections (1 μm) were stained with toluidine blue. Ultrathin sections were double stained with uranyl acetate and lead citrate and examined with an electron microscope. Two out of each of the 3 consecutive slices were dehydrated and embedded in paraffin. In most of the paraffin-embedded slices, 10 nonconsecutive, transverse sections (6 μm in thickness) were performed and stained with haematoxylin and eosin, periodic acid–Schiff (PAS), Masson's trichrome or orcein. Some paraffin-embedded slices were longitudinally sectioned and stained in the same way.

On 3 randomly selected paraffin-embedded slices from each ductus deferens segment, 5 haematoxylin–eosin-stained sections were selected at random and the following were calculated for each section: (1) surface areas occupied by (*a*) the ductus deferens lumen, (*b*) the glandular and diverticular structures, and (*c*) the muscular layers using an image analyser (Videoplan, Kontron, Oberkochen, Germany); and (2) the number of epithelial cell nuclei per cross-sectioned duct. From the average values for each man and segment, the means and S.D. for each ductus deferens segment were calculated. After verification that the values were normally distributed, the differences between each pair of means were evaluated by Student's t test.

RESULTS

Segment A

Segment A showed the usual structure reported in studies of the human ductus deferens (Hoffer, 1976; Popovic et al. 1973; Paniagua et al. 1981). This comprised 3 layers: mucosa, muscular coat, and adventitia (Fig. 2). The epithelial lining was scalloped and pseudostratified. In addition to small basal cells, 3 types of columnar cells were seen: (1) principal cells with long stereocilia and numerous lysosomes suggesting a resorptive function; (2) dark or pencil cells with a dark nucleus and cytoplasm as well as cytoplasmic lipid vacuoles suggesting degenerative stages of the principal cells; and (3) mitochondrion-rich cells which might be involved in electrolyte transport through the mucosa of the ductus deferens. No glandular formations were observed. The muscular coat comprised 3 layers: inner longitudinal, middle oblique, and outer longitudinal.

Segment B

The histological structure of segment B consisted of 5 layers; mucosa, muscularis mucosae, submucosa, muscular coat and adventitia (Fig. 3). The epithelium formed deep multiple-branched invaginations in the lamina propria giving rise to glandular structures. Some of these glands penetrated through the muscu-

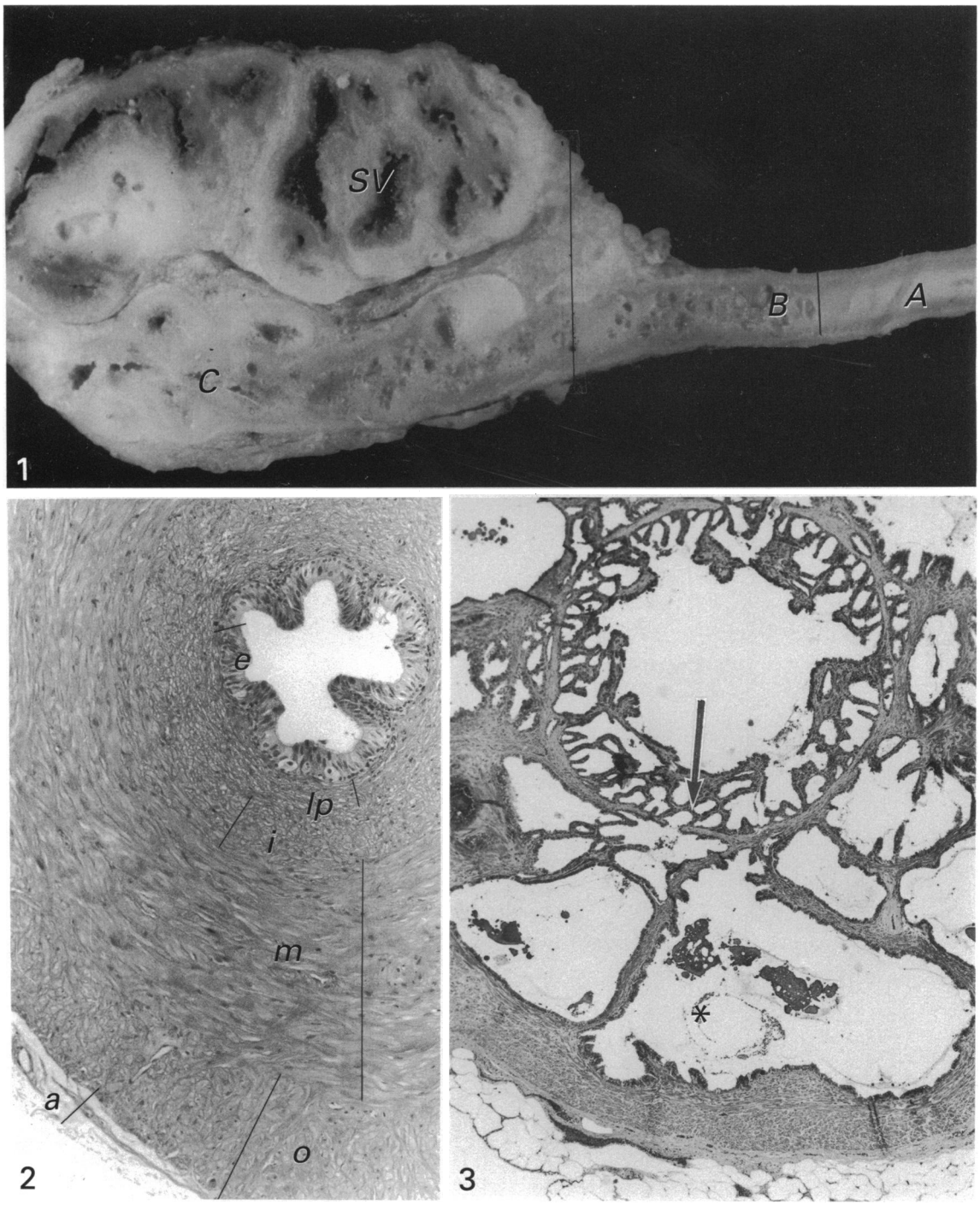

Fig. 1. Ductus deferens portion removed from a 65-year-old man before section into its 3 histologically different segments *A*, *B* and *C*. Segment *C* corresponds to the ampulla. *SV*, seminal vesicle.

Fig. 2. Cross-section of the initial segment of the ductus deferens (segment A) from a 59-year-old man. *e*, epithelium; *lp*, lamina propria; *i*, inner muscular layer; *m*, middle muscular layer; *o*, outer muscular layer; *a*, adventitia. H & E. ×125.

Fig. 3. Cross-section of the ductus deferens from a 63-year-old man sectioned at the level of segment B showing mucosal (arrow) and submucosal (asterisk) glands. H & E. ×40.

laris mucosae forming submucosal glands which were less numerous and more dilated than the mucosal glands (Fig. 3). The epithelial cells lining the lumen of the duct, together with the mucosal and submucosal glands, formed a columnar pseudostratified epithelium in which basal and columnar cells could be distinguished. On light microscopy the columnar cells could be classified by the staining affinity of their nuclei and cytoplasm into pale, intermediate and dark cells (Fig. 4). The nuclei of columnar cells varied in

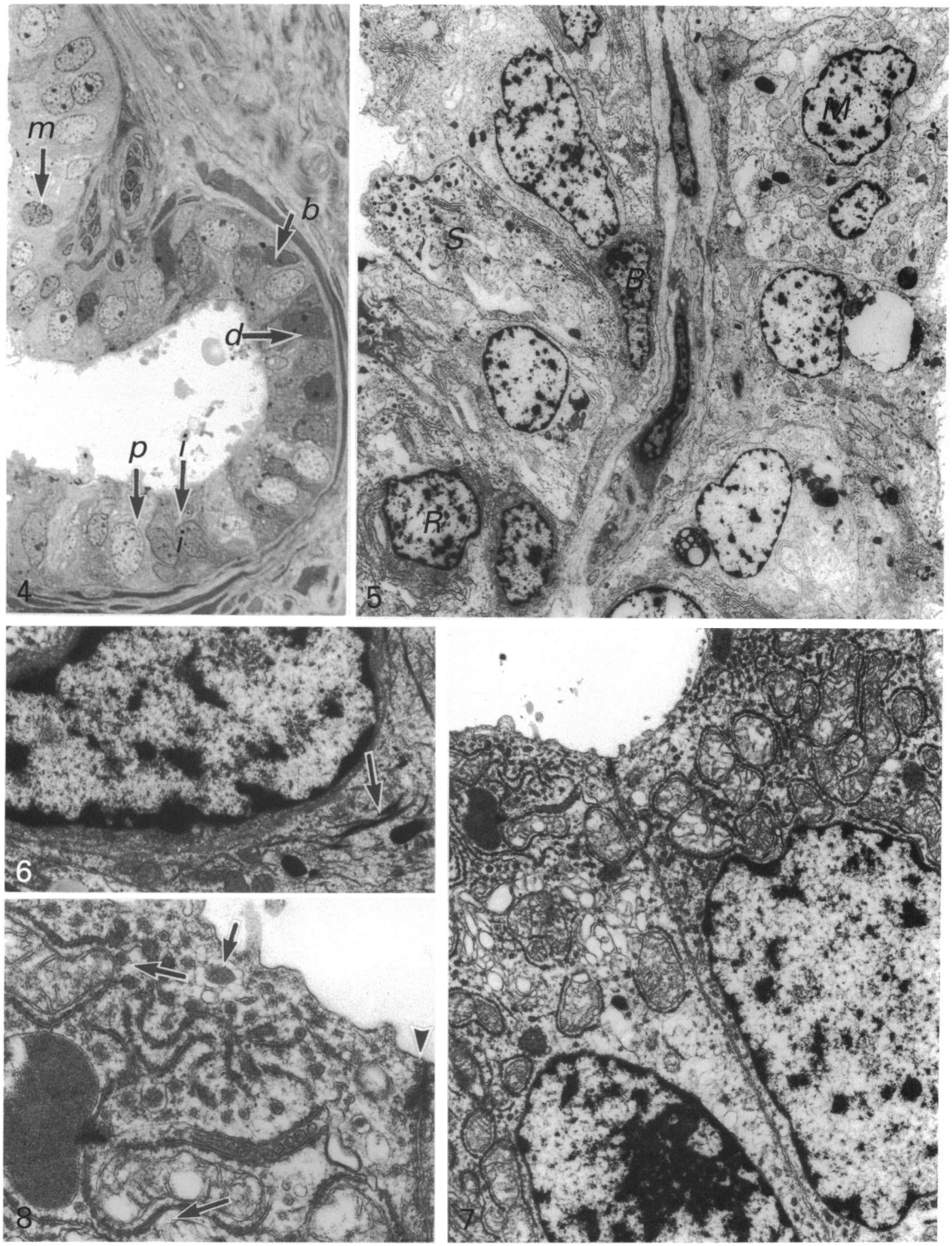

Fig. 4. Semithin section of the epithelium of the ductus deferens at the level of segment B showing basal cells (*b*), mitochondrion-rich cells (*m*), and 3 types of columnar secretory cells: pale cells (*p*), intermediate cells (*i*) and dark cells (*d*). Toluidine blue. ×950.

Fig. 5. Low-power electron micrograph of the epithelial lining of the ductus deferens at the level of segment B showing basal cells (*B*) and the 3 subtypes of columnar secretory cells: cells with numerous mitochondria (*M*), cells with abundant rough endoplasmic reticulum (*R*) and cells with numerous secretory granules (*S*). ×2750.

Fig. 6. Basal cell from segment B showing scanty cytoplasmic organelles and abundant microfilaments (arrow). ×16000.

Fig. 7. Columnar cells with numerous mitochondria ×16000.

Fig. 8. Higher magnification of the apical cytoplasm of a columnar cell with numerous mitochondria. Each mitochondrion is surrounded by a rough endoplasmic reticulum cisterna (large arrows). Apical secretory granules (small arrow) and a junctional complex (arrowhead) can also be seen. ×44500.

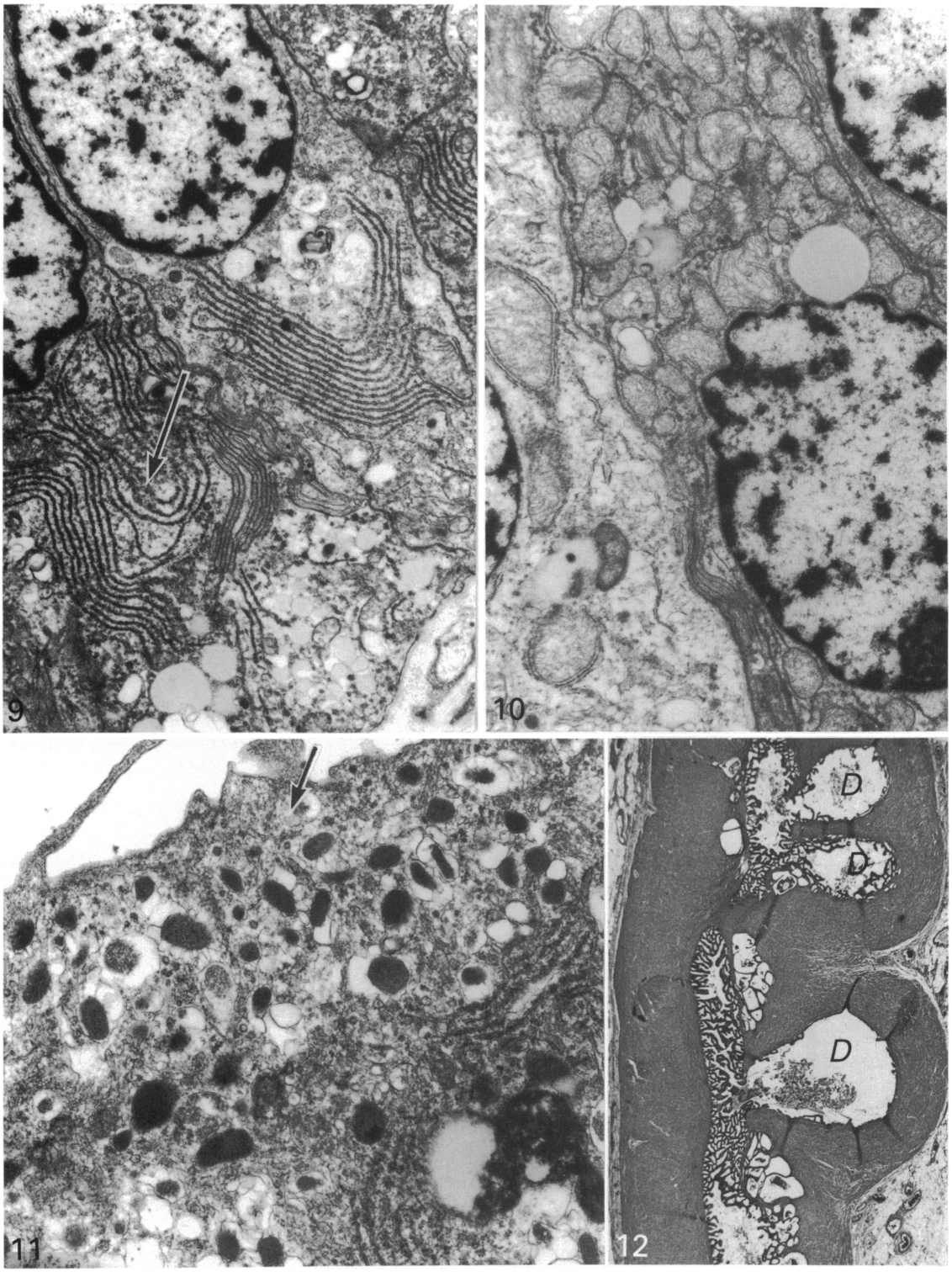

Fig. 9. Columnar cells with abundant rough endoplasmic reticulum cisternae forming fingerprint-like structures (arrow). ×16000.
Fig. 10. Apical portion of a columnar cell with numerous membrane-bound secretory granules consisting of a dense core surrounded by a clear halo (arrow). ×22000.
Fig. 11. Mitochondrion-rich cell with the cytoplasm almost filled by closely packed mitochondria. This cell type differs from the columnar cell with abundant mitochondria which are surrounded by rough endoplasmic reticulum (arrow). ×22000.
Fig. 12. Longitudinally sectioned ductus deferens from a 49-year-old man at the level of segment B showing diverticula (*D*) which are responsible for the irregular outline of the outer surface. H & E. ×32.

outline and binucleate cells were sometimes seen. On electron microscopy (Fig. 5), the characteristics of these cell types could be established.

(1) *Basal cells* showing a triangular outline on section with scanty cytoplasmic organelles and numerous microfilaments (Fig. 6). The nucleus was irregular and contained abundant heterochromatin masses (Figs 5, 6).

(2) *Columnar cells* with abundant cytoplasmic organelles, including cisternae of rough endoplasmic reticulum, polysomes, a well developed supranuclear Golgi complex, mitochondria, membrane-bound secretory granules (150–200 nm) that have an electron-dense content which does not completely fill the vesicle, lipid droplets and lipofuscin. The secretory granules stained positively with the PAS technique. These cells displayed short microvilli in the adluminal surface and were connected to the adjacent cells by junctional complexes and interdigitations (Fig. 5). Within this cell type, 3 subtypes could be distinguished. (2*a*) *Pale cells* with numerous mitochondria (Figs 7, 8). Each mitochondrion appeared partly surrounded by a rough endoplasmic reticulum cisterna (Fig. 8). The Golgi complex was highly developed. Secretory granules were scanty and appeared confined to the apical portion of the cytoplasm (Fig. 8). These pale cells were usually shorter than the other columnar cell subtypes. (2*b*) *Intermediate cells* with abundant cisternae of rough endoplasmic reticulum arranged in a fingerprint-like fashion throughout the whole cytoplasm (Fig. 9). (2*c*) *Dark cells* with abundant secretory granules (Fig. 10). The nucleus and cytoplasm of these cells were darker than those of the other columnar cell subtypes. In addition, the nuclei of the dark cells were irregularly outlined and displayed deep indentations. The apical portion of the cytoplasm protruded into the lumen and exocytosis of the secretory granules could often be seen. The rough endoplasmic reticulum was confined to the basal portion of the cell. Lipofuscin granules were large and numerous.

(3) *Mitochondrion-rich cells*. These differed from the columnar cells with numerous mitochondria in their reverse pyramidal shape, the absence of secretory granules, and the moderate amount of cytoplasmic organelles other than mitochondria, which were more numerous than in the columnar cells and were not surrounded by rough endoplasmic reticulum cisternae (Fig. 11).

The most remarkable feature of the lamina propria was the presence of numerous mast cells. The muscularis mucosa consisted of a layer of circularly arranged smooth muscle cells and elastic fibres. This layer was crossed by the epithelial invaginations forming the submucosal glands. The muscular coat comprised 2 layers of smooth muscle cells with a circular arrangement in the inner layer, and longitudinal one in the outer layer.

Table 1. *Variations in the number of each cell type per cross-sectioned duct in the 3 different segments of the human ductus deferens*

Epithelial cell type	Segment A	Segment B	Segment C (ampulla)
Basal cells	$97.86 \pm 10^{\alpha}$	$40.2 \pm 3^{\beta}$	$41.5 \pm 2^{\beta}$
Mitochondrion-rich cells	$20.97 \pm 3^{\alpha}$	$37.5 \pm 5^{\beta}$	$36.0 \pm 3^{\beta}$
Pencil cells	30.29 ± 4		
Principal cells	76.89 ± 8		
Columnar secretory cells			
with numerous mitochondria		$38.5 \pm 2^{\alpha}$	$37.2 \pm 3^{\alpha}$
with abundant RER		$75.4 \pm 4^{\alpha}$	$85.3 \pm 2^{\alpha}$
with abundant secretory granules		$150 \pm 10^{\alpha}$	$162 \pm 5^{\alpha}$

Data are expressed as mean ± S.D. For each cell type, the significance of differences between segments is indicated by superscript letters. Values with different superscript letters are significantly different ($P < 0.05$).

Table 2. *Variations in the surface area occupied by the lumen, glands plus diverticula, and muscular coat per cross-sectioned duct in the 3 different segments of the human ductus deferens*

	Surface area (mm^2)		
	Segment A	Segment B	Segment C (ampulla)
Ductus deferens lumen	$0.67 \pm 0.43^{\alpha}$	$1.72 \pm 0.90^{\beta}$	$1.80 \pm 0.34^{\beta}$
Glands + diverticula	—	$5.61 \pm 0.11^{\alpha}$	$7.18 \pm 0.88^{\beta}$
Muscular coat	$6.13 \pm 1.6^{\alpha}$	$4.10 \pm 0.06^{\beta}$	$4.53 \pm 0.92^{\beta}$

Data are expressed as mean ± S.D. For each cell type, the significance of differences between segments is indicated by superscript letters. Values with different superscript letters are significantly different ($P < 0.05$).

Segment C

The same histological structure described in segment B was observed in segment C. In addition, this segment displayed many diverticula consisting of expansions of the mucosa and submucosa towards the adventitia (Fig. 12). Due to these diverticula, the muscular coat forms protrusions on the outer surface of the duct that give rise to the cerebriform structure characteristic of this segment.

Spermatozoa were only occasionally found along the whole length of the ductus deferens. These spermatozoa were not more numerous in segment C.

Morphometric study

For each epithelial cell type, the numbers of cells per cross-sectioned ductus deferens are shown in Table 1. The lumen of the ductus deferens was significantly larger in segments B and C than in segment A. The muscular coat was larger in segment A than in segments B and C. The surface area per cross-sectioned duct occupied by the glandular structures and diverticula was greater in segment C than in segment B (Table 2).

DISCUSSION

The term ampulla has been used to designate a dilated segment at the most distal third of the mammalian ductus deferens. The morphology of the ampulla varies from one species to another (Cooper & Hamilton, 1977; Riva et al. 1979, 1982; Murakami et al. 1986). There have been few reports on the histology of the human ampulla, and the results of the present study provide new data which reveal that the human ampulla differs both from that of other mammals and from the other segments of the human ductus deferens.

The anatomical configuration of the human ampulla, more dilated than the other segments of the ductus deferens, suggests that, at this level, the lumen of the ductus would be more dilated than in the other portions. This also conforms with the notion that the ampulla is a sperm reservoir, as has been suggested in previous reports on the ampulla in several mammalian (Cooper & Hamilton, 1977; Riva et al. 1979, 1982; Murakami et al. 1986) and nonmammalian (Zalisko & Larsen, 1988) vertebrates. However, no spermatozoon clumps but only isolated spermatozoa were found along the entire length of the ductus deferens, including the ampulla. In addition, the morphometric study revealed that the surface area occupied by the lumen of the ductus in the ampulla is similar to that in the preceding segment, and that this surface is 2.7 times larger than the surface of the lumen in the initial segment of the ductus deferens.

The histological structure of the ampulla (segment C) is similar to that of the preceding segment (segment B) and differs from that of the initial segment of the ductus deferens (segment A) in the presence of muscularis mucosa and submucosa containing numerous glands. Columnar cells showing the ultrastructural features of protein secretory cells (granules and abundant rough endoplasmic reticulum) (Samuel & Flickinger, 1987) are the most abundant cell type lining these structures. The positive staining of these granules with the PAS technique suggests that this secretion also contains carbohydrates. These cells are different from the principal cells located in the initial segment of the ductus deferens, where secretory cells have not been found in man (Hoffer, 1976; Paniagua et al. 1981) or in other mammals (Flickinger, 1975; Hamilton & Cooper, 1978; Kennedy & Heidger, 1979).

The 3 subtypes of columnar secretory cells seem to correspond to 3 consecutive functional stages. The columnar cells with numerous mitochondria might represent a stage of quiescence or an initial phase of the secretory process. Afterwards, these cells become active cells that enlarge their cytoplasm and develop large amounts of rough endoplasmic reticulum that is involved in the synthesis of the secretory product. Finally, the cells become filled with numerous secretory granules which are released by exocytosis to the ductus lumen. The presence of large lipofuscin bodies in the cytoplasm of these cells might be related to the destruction of organelles that are more abundant in previous stages such as mitochondria or rough endoplasmic reticulum.

The function of the mitochondrion-rich cells is unclear. They might represent old or 'exhausted' columnar secretory cells that have undergone mitochondrial hyperplasia and loss of organelles involved in glycoprotein synthesis. However, these cells are also present, in low numbers, in the initial segment of the ductus deferens in man (Hoffer, 1976; Paniagua et al. 1981) and rat (Kennedy & Heidger, 1979). It has been suggested that these cells might be involved in the acidification of the seminal plasma or transport of electrolytes, hydrogen ions and water across the mucosa.

The basal cells in the ductus deferens, like those in other epithelia, are probably undifferentiated cells that are capable of differentiation into columnar cells, replacing the dead sloughing cells (Paniagua et al. 1981; Riva et al. 1982).

The presence of diverticula is the only histological difference between segments B and C. These diverticula are responsible for the anatomical differences between both segments, since they give the outer surface of the ampulla the cerebriform appearance which is lacking in segment B. The structure of these diverticula does not differ from that of the nondiverticular ductus deferens wall, and they also lack spermatozoon masses. The function of the diverticula might be to increase the surface occupied by the epithelium and thus the number of secretory cells.

A spermatophagic role has been attributed to the

ductus deferens epithelial cells in the rat (Cooper & Hamilton, 1977; Kennedy & Heidger, 1979), hamster (Bedford, 1976), rabbit (Flickinger, 1975), dog (Murakami et al. 1986), monkey (Alexander, 1972) and man (Cossu et al. 1978; Amann & Howards, 1980; Riva et al. 1982). However, we have failed to observe phagocytosed spermatozoa in these cells in any ductus deferens segment.

On the basis of the present findings it may be concluded that, unlike in other mammalian species, the human ampulla is not a sperm reservoir but a secretory structure which begins in the preceding segment (segment B). The histological similarities between these secretory segments of the human ductus deferens and the seminal vesicle of several mammals (Takenawa et al. 1981; Samuel & Flickinger, 1987; Chow, 1988; Herr, 1989) suggest that the secretory component might be similar in both structures.

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