# Structural Differentiation and Fluid Reabsorption in the Ductuli Efferentes Testis of the Rat

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# Abstract

The ductuli efferentes testis of the rat form a cord which is embedded in adipose tissue. The cord is anatomically differentiated into a proximal cylindrical region, the initial zone, and an ampulla, the coni vasculosi. The initial zone contains six or seven ductuli which leave the rete testis and run in a sinuous path, roughly parallel with one another. However, the ductuli in the coni vasculosi are more sinuous than in the initial zone and they anastomose; pairs join together to form ultimately a single, common ductulus efferens. Stereological studies of paraffin sections and electron micrographs showed that the differentiation of the ductuli into two parts can be recognized at tissue and cellular levels of organization. Stereological and micropuncture studies showed that the ductuli efferentes reabsorb most of the fluid leaving the testis and it was concluded that most reabsorption occurred in the initial zone. It was estimated that the rate of fluid absorption is greater in the ductuli efferentes than in the proximal convoluted tubules of the kidney. The mechanism of fluid transport across the mucosa of the ductuli is considered in the Discussion. It is concluded that transport in vesicles and vacuoles could not account for the rate of fluid reabsorption and that the main mechanism of transport probably involves the coupling of water and active salt transport.

### Introduction

A number of workers have recognized that the ductuli efferentes testis of mammals are structurally differentiated along their length (Benoit 1926; Young 1933; Reid and Cleland 1957; Holstein 1969; Hamilton 1975; Flickinger *et al.* 1978; Jones and Brosnan 1981). Reid and Cleland (1957) referred to two anatomically distinct parts, the initial zone and the coni vasculosi. However, there are no systematic studies relating this anatomical differentiation to the structural differentiation observed at other levels of organization.

Little is known of the functions of the ductuli efferentes. It has been shown that they reabsorb proteins from the testis in the rat (Koskimies and Kormano 1975), elephant (Jones 1980) and echidna (Djakiew and Jones 1983) and a variety of introduced substances such as peroxidase (Sedar 1966; Hermo and Morales 1984; Hermo *et al.* 1985), trypan blue and Indian ink (Mason and Shaver 1952), colloidal mercuric sulfide (Burgos 1964; Montorzi and Burgos 1967) and ferritin, conconavalin-A-ferritin and albumin (Hermo and Morales 1984; Hermo *et al.* 1985). It is also generally recognized that the ducts are involved in the reabsorption of fluid (Young 1933; Mason and Shaver 1952; Jones 1980; Djakiew and Jones 1983; Hermo and Morales 1984; Hermo *et al.* 1985). However, there is a problem in quantitating this activity since micropuncture samples from the proximal end of the ductus 0004-9417/87/010079\$02.00

epididymidis are contaminated with stereocilia from the duct epithelium (Hinton 1979; Djakiew and Jones 1983). Consequently, micropuncture studies of the rat epididymis have involved sampling from the rete testis and mid-caput epididymidis so that it is not possible to assess the separate absorptive effects of the ductuli efferentes and initial segment of the ductus epididymidis (Levine and Marsh 1971; Hinton *et al.* 1980; Turner 1984; Turner *et al.* 1984).

There are also problems in interpreting studies on the mechanism of fluid transport across the mucosa of the ductuli efferentes. For example, Hohlbrugger (1980) reported micropuncture studies of the ductuli efferentes, but subsequently indicated that he studied the initial segment of the ductus epididymidis (Hohlbrugger *et al.* 1982). Structural studies may also have been misinterpreted. In this context it has been shown (Ladman and Young 1958; Morita 1966; Holstein 1969; Yokoyama and Chang 1971; Hoffer *et al.* 1975) that there is extensive development of vesicles and vacuoles in the apical cytoplasm of the mucosa and Flickinger *et al.* (1978) suggested that these would provide the main method of fluid transport across the epithelium. However, Flickinger *et al.* (1978) do not show the presence of organelles such as microtubules which would be involved in the transport of the vacuoles across the epithelium or provide quantitative data to support their proposed mechanism of fluid transport.

The studies described in this paper were carried out to relate the anatomical differentiation of the ductuli efferentes in the rat to the corresponding tissue and cellular differentiation. Stereological data was determined to characterize the structural differentiation and to help in the interpretation of subsequent physiological studies. In this report the stereological data is used to assess the magnitude of fluid absorption along the ductuli. For comparison, fluid absorption was also determined using micropuncture procedures. Samples were collected near each end of the ductuli and sperm concentration was counted in a haemocytometer in order to avoid the problems mentioned above of contamination with stereocilia. The stereological data was also used to assess possible mechanism(s) of fluid transport across the mucosa of the ductuli.

# **Materials and Methods**

Sexually mature, male Sprauge-Dawley rats weighing 300-400 g were used.

The arrangement of the ductuli was examined in three animals by dissecting the ductuli from their supporting stroma using jeweller's forceps after acid digestion in a mixture of 80 parts of 90% (v/v) ethanol and 20 parts of concentrated hydrochloric acid (Djakiew and Jones 1981).

#### Paraplast Sections

One testis and epididymis from each of three animals was fixed by immersion in Bouin's fixative for 36 h, then dehydrated in ethanol, cleared in xylene and embedded in Paraplast (Lancer, St Louis, U.S.A.). Serial sections (5  $\mu$ m thick) of the ductuli efferentes were cut in a sagittal plane to help identify the different regions. The sections were stained with haematoxylin and eosin.

#### Araldite Sections

Three animals were fixed by vascular perfusion via the thoracic aorta (Forssmann *et al.* 1977) after deep anaesthesia with an intra-peritoneal injection of pentobarbitone sodium (Nembutal: Abbotts Laboratories, Sydney) at a dose of 50 mg/kg. Samples of the initial zone, coni vasculosi and common ductulus efferens were excised (Fig. 1) and minced. They were stained with osmium, dehydrated in ethanol and embedded in Araldite.

Glass knives were used in an ultramicrotome (8800 Ultratome III, L.K.B., Sweden) to prepare thick (1-2  $\mu$ m) sections for light microscopy and glass knives or a diamond knife were used to cut thin

(silver to gold interference colours) sections for electron microscopy. The thick sections were stained with an aqueous solution of 1% (w/v) toluidine blue and 0.5% (w/v) borax, and the thin sections were stained with uranyl acetate and lead citrate (Reynolds 1963).

### Estimation of Volumes

The density of the ductuli efferentes (mean  $\pm$  s.e. of eight freshly collected organs from four animals =  $0.793 \pm 0.043$  g/ml) was determined by weighing, and determining the volume of Ringer displaced (Umbreit *et al.* 1972) in a graduated 1.0 ml pipette. Subsequently this value was used with their fresh weights to calculate the volume of each region.

## Estimation of Shrinkage

Shrinkage due to fixation and embedding was calculated as the ratio of the mean duct diameter in frozen sections divided by the mean duct diameter of Paraplast-embedded sections (Aherne and Dunnill 1966). The shrinkage factor was the same for the different parts of the ductuli so a mean value (mean  $\pm$  s.e. linear shrinkage factor for 10 measurements on each of three animals =  $1 \cdot 19 \pm 0.05$ ) was used to correct all values determined from the paraffin sections. After correction for shrinkage the duct dimensions for material embedded in paraffin were about the same as values measured in Araldite sections indicating that there was little shrinkage of tissue embedded in Araldite.

### Morphometry of Tissue

The use of stereological methods was described in an earlier paper (Djakiew and Jones 1982). Estimates of spermatocrits (S) were obtained using the following formula:

$$S = 100 V_{\rm S} / (V_{\rm S} + V_{\rm L}),$$

where S = luminal volume (%) occupied by spermatozoa,  $V_S =$  volume ratio of sperm and  $V_L =$  volume ratio of lumen unoccupied by sperm. Estimates of fluid reabsorption between two regions were calculated as:

$$R = 100(1 - S_1/S_2),$$

where R = reabsorption (%) and  $S_1$  and  $S_2$  are spermatocrits for the proximal and distal sites respectively. Diameter of the ductuli and epithelial height were measured in Paraplast sections, and cilial length was measured in thick Araldite sections. Ten profiles were measured per region per animal. Cilia in the common ductulus efferens were not measured since it was not possible to determine their extent amongst the spermatozoa.

Four regions of the ductuli efferentes were examined (Fig. 1): the proximal and distal halves of the initial zone, the coni vasculosi and the common ductulus efferens. Ten Paraplast sections of each region were selected at random from each of the three sets (animals) of serial sections. For the stereological analyses images of the sections were projected (magnification =  $\times 135$ ) onto a 108 by 109 mm Multipurpose test system M168 (Weibel 1979) using a Vickers projection microscope (Cooke, Troughton and Simms Ltd, York, U.K.).

#### Stereology of Electron Micrographs

The ultrastructure of the non-ciliated principal cells in the initial zone and coni vasculosi were compared. Electron micrographs (negative magnification =  $\times 3300$ ; print magnification =  $\times 9430$ ) of longitudinal sections of cells (10 cells per region for each of two animals) showing the nucleus, and apical, lateral and basal borders were each overlayed with a transparent sheet containing a double square lattice test system D64 (Weibel 1979) and volume ratios were determined for vesicles (diameter less than  $0.231 \mu m$ ), vacuoles, mitochondria, light electron-dense bodies, dark electron-dense bodies and nuclei in each region of the cell.

#### Micropuncture Procedures

Rats were anaesthetized with 5-s-butyl-5-ethylthiobarbituric acid (Inactin: Byk Gulden Pharmaceuticals, Konstanz, West Germany) at a dose of 50 mg/kg and luminal samples from the rete testis and proximal end of the initial segment of the ductus epididymidis were collected using micropuncture procedures (Djakiew and Jones 1983). The volume of the micropuncture samples was measured in a 1  $\mu$ l Microcap (Drummond Scientific Co., U.S.A.) by laying it over a stage micrometer and measuring the length occupied by the sample. The sample was then dispensed into a conical plastic 1.5 ml tube containing

20  $\mu$ l of 2.9% (w/v) sodium citrate and 0.5% (v/v) Triton X-100, mixed on a vortex mixer and loaded into a haemocytometer for counting.

#### Statistical Estimates

Standard errors, which are given as estimates of dispersion in the text and tables, were calculated from estimates of the variance between animals. Analyses of variance were used to test the statistical significance of differences between the regions shown in Table 1.

In the stereological studies when estimates of absolute dimensions involved calculating the product of the mean regional volume (x) with another mean (y) (e.g. in calculating duct lengths) the appropriate standard errors were calculated using the following formula to estimate the resultant variance (Kendall and Stewart 1963):

Variance = 
$$\bar{y}^2 \cdot S_x^2 + \bar{x}^2 \cdot S_y^2 + S_x^2 \cdot S_y^2$$

where  $S_x^2$  and  $S_y^2$  refer to the between-animal variances for x and y.

# Results

### Anatomy

The ductuli efferentes form a reddish brown cord which courses dorsally from the extra testicular rete testis, through the superior epididymal fat and join the initial segment of the ductus epididymidis. The initial zone of the cord is 7 mm long and 1.5 mm in diameter and it occupies 39% of the volume of the cord (Table 1).

Table 1. Morphometry of the three major regions of the ductuli efferentes testis of the rat Means  $\pm$  s.e. for three animals. Values have been corrected for shrinkage

	Initial zone	Coni vasculosi	Common ductulus efferens	РА
	Values	per region		
Volume $(mm^3)$	$11 \cdot 00 + 1 \cdot 39$	$16.00 \pm 1.30$	$1 \cdot 1 \pm 0 \cdot 09$	<0.001
Epithelial height $(\mu m)$	$26 \cdot 64 \pm 0 \cdot 45$	$30.65 \pm 0.43$	$28 \cdot 13 \pm 0 \cdot 49$	<0.01
Duct diameter (µm)	$171 \cdot 79 \pm 2 \cdot 76$	$130.70 \pm 2.40$	$114 \cdot 58 \pm 2 \cdot 39$	<0.01
Cilial length (µm)	$14 \cdot 55 \pm 2 \cdot 70$	$12\cdot 08\pm 2\cdot 74$		n.s.
Total length of ducts ( $\mu$ m)	$197 \cdot 89 \pm 10 \cdot 56$	$724 \cdot 80 \pm 59 \cdot 68$	$53\cdot 32\pm 3\cdot 56$	<0.001
Width of periductal muscle $(\mu m)$	$2 \cdot 59 \pm 0 \cdot 31$	$4\cdot 06\pm 0\cdot 36$	$5\cdot 46\pm 0\cdot 34$	<0.01
Volume of epithelium				
(mm <sup>3</sup> )	$2 \cdot 75 \pm 0 \cdot 22$	$6 \cdot 08 \pm 0 \cdot 33$	$0\cdot 40\pm 0\cdot 02$	<0.001
Volume of lumen (mm <sup>3</sup> )	$2\cdot 31\pm 0\cdot 03$	$1\cdot 76\pm 0\cdot 16$	$0\cdot 10\pm 0\cdot 001$	<0.001
	Values per unit	volume of tissue		
Length $(mm^{-2})$	$17.99 \pm 0.96$	$45\cdot 30\pm 3\cdot 73$	$48\cdot 47\pm 3\cdot 24$	<0.001
Volume of sperm	$0.007^{B}$	$0.02 \pm 0.003$	$0.053 \pm 0.007$	<0.001
Surface area of luminal				
border (mm <sup>-1</sup> )	$10 \cdot 1 \pm 0 \cdot 79$	$10\cdot 37\pm 0\cdot 18$	$12\cdot 06\pm 1\cdot 83$	n.s.
Ratio of surface area of luminal border:luminal				
volume $(mm^{-1})$	48.10	94.27	131.09	

<sup>A</sup> n.s., not significant.

<sup>B</sup> Standard error not calculated as two of three values were zero.

Six to seven ductuli course in a sinuous path through the zone and the ductuli are roughly parallel with one another. The ampulla of the cord (coni vasculosi) is about 4 mm long and 2.5 mm wide and occupies 57% of the volume of the cord.



Fig. 1. Longitudinal section of the ductuli efferentes testis showing the differentiation into the initial zone (*iz*) adjacent to the testis (*t*), the coni vasculosi (cv, arrows indicate extent), and the common ductulus efferens (*cde*). *a*, Adipose tissue; *i*, initial segment of the ductus epididymidis. Paraplast, haemotoxylin and eosin. Scale line = 5 cm.

Fig. 2. Ductuli efferentes testis dissected from supporting stroma. Symbols the same as in Fig. 1. Scale line = 5 cm.

The ductuli become highly sinuous and anastomose with one another within the region. Pairs of ductuli join together ultimately to form the common ductulus efferens which courses through the isthmus separating the ductuli efferentes and the ductus epididymidis (Figs 1 and 2).

# Tissue Structure

The ductuli are lined by a pseudostratified, columnar epithelium composed of ciliated cells, non-ciliated principal cells, and a few basal cells and intraepithelial lymphocytes and macrophages. The occurrence of ciliated and principal cells is in the ratio 1:5 in the initial zone and conus vasculosus and 1:2 in the common ductulus efferens. The tunic of smooth muscle increases in thickness towards the epididymis (Table 1). The ductuli in the initial zone are supported by adipose tissue, whereas the coni vasculosi have a loose connective tissue stroma which contains a moderate density of collagen fibres and only a few adipose cells.

Table 1 summarizes the estimates of duct dimensions and stereological parameters. Estimates for the whole initial zone are shown since there were no statistically significant differences between the proximal and distal halves.

The magnitude of many of the stereological estimates differed between the coni vasculosi and the initial zone. It was interpreted that the differences are due to differences in linear dimensions (such as duct diameter and epithelial height), the overall volume of the regions and because the ductuli of the coni vasculosi are more densely packed within the supporting stroma of the coni vasculosi than in the initial zone.

Estimates of spermatocrits calculated from the volume ratios shown in Table 1 for the initial zone and coni vasculosi were respectively  $3 \cdot 0$  and  $15 \cdot 4\%$ . The values correspond to the reabsorption of 81% of the luminal fluid present in the initial zone. There is a further increase in spermatocrit to  $36 \cdot 5\%$  in the common ductulus efferens which corresponds to the reabsorption of 58% of the fluid present in the coni vasculosi, and 92% of the fluid in the initial zone.

# Cell Structure

As the ultrastructure of the epithelium lining the ductuli efferentes of the rat (Fig. 3) is qualitatively similar to that described in other species (Flickinger *et al.* 1978; Jones and Holt 1981; Ramos and Dym 1977), only the pertinent features are described below.

There is little variation in the ultrastructure of the ciliated cells along the ducts. They contain a small Golgi apparatus but unlike the principal cells very few canaliculi are formed by the apical plasmalemma. Vesicles are scarce in the apical cytoplasm and only a few small vacuoles and few electron-dense bodies are present in the supranuclear cytoplasm.

The principal cells are characterized by long stereocilia and the apical plasmalemma between the stereocilia form canaliculi which penetrate the cytoplasm and produce coated vesicles from their basal ends. The apical cytoplasm also contains numerous medium to large vacuoles and an extensive microtubular network. The supranuclear cytoplasm contains a small Golgi apparatus which produces smooth-surfaced vesicles and electron-dense bodies. The latter vary in electron density and are referred to below as light and dark electron-dense bodies. The basal cytoplasm contains a number of large, clear membrane-bound vacuoles and the basal



**Fig. 3.** Electron micrograph of stereociliated principal cells in the epithelium lining the coni vasculosi of the ductuli efferentes showing the occurrence of apical vesicles (V), dense bodies (b) and vacuoles (Va). The lines across the cell show the division of cells into apical, supranuclear and basal regions to estimate the stereological parameters shown in Table 2. Scale line =  $1 \mu m$ .

plasmalemma shows some pinocytotic activity. There is some dilation of the intercellular space of the basal third of the epithelium. In the coni vasculosi the space is partly occupied by interdigitating cytoplasmic protrusions from adjacent cells.

There is considerable variation in structure of the principal cells between the different regions of the ductuli (Table 2). Volume ratios of the main cellular organelles show that there are about six times the volume of vacuoles in the apical cytoplasm of the cells in the initial zone than the coni vasculosi. The incidence of dense bodies in the supranuclear cytoplasm of the cells is much the same for both regions of the ductuli. However, whereas there are approximately equal volumes of light and dark dense bodies in the initial zone there is about seven times the volume of dark than light dense bodies in the initial zone is about seven times the volume of vacuoles in the basal cytoplasm in the initial zone is about seven times the volume in the coni vasculosi.

Table 2. Estimates of volume of organelles per unit volume of the apical, supranuclear and basalparts of the principal cells, and estimates of absolute volumes of organelles within a principal cellResults are means ± s.e. for eight (proximal region) or 10 (coni vasculosi) cells; the s.e. was computedfrom the variance between cells and includes the variance between (two) animals

	$10^2 \times \text{Volume ratio of organelle}$			$10^8 \times \text{Volume of}$
	Apical	Supranuclear	Basal	organelle per cell (μl) <sup>A</sup>
		Initial zone		
Vesicles	$7 \cdot 0 \pm 1 \cdot 71$	0	$0\cdot 3\pm 0\cdot 09$	8.3
Vacuoles	$6 \cdot 4 \pm 2 \cdot 47$	0	$15.7 \pm 0.04$	25.3
Electron-dense bodies:				
Light	0	$9 \cdot 9 \pm 1 \cdot 62$	0	11.3
Dark	0	$12 \cdot 0 \pm 1 \cdot 85$	0	13.7
Mitochondria	$7 \cdot 8 \pm 1 \cdot 17$	$6.6 \pm 0.50$	$7 \cdot 6 \pm 0 \cdot 54$	25.0
Nucleus	0	0	$26 \cdot 9 \pm 6 \cdot 30$	30.8
	С	oni vasculosi		
Vesicles	$7 \cdot 3 \pm 1 \cdot 15$	0	$0.5 \pm 0.11$	10.3
Vacuoles	$1 \cdot 1 \pm 0 \cdot 89$	0	$2 \cdot 4 \pm 0 \cdot 97$	4.6
Electron-dense bodies:				
Light	0	$2.5 \pm 0.85$	0	3.3
Dark	0	$18 \cdot 2 \pm 2 \cdot 76$	0	23.9
Mitochondria	$9.0 \pm 0.77$	$9 \cdot 2 \pm 0 \cdot 86$	$8 \cdot 0 \pm 0 \cdot 57$	34.5
Nucleus	0	0	0	55.0

<sup>A</sup> Estimated assuming the diameter of the cells is  $7 \cdot 39 \pm 0 \cdot 45 \mu m$  (mean  $\pm$  s.e. of 10 cells in electron micrographs) and the height of the cells is as shown in Table 1.

The principal cells in the common ductulus efferens are structurally similar to the principal cells in the coni vasculosi except that the former rarely contain vesicles and no vacuoles were seen.

# Micropuncture

Sperm concentrations (millions of sperm per millilitre) for five animals were (means  $\pm$  s.e.) respectively 155  $\pm$  97 and 1009  $\pm$  218 for samples from the rete testis and proximal initial segment of the ductus epididymidis. This corresponds to the ductuli efferentes reabsorbing  $83 \pm 8\%$  of the fluid leaving the rete testis.

### Discussion

Our findings on the anatomical differentiation and arrangement of the ductuli efferentes testis of the rat is in agreement with earlier reports (Benoit 1926; Reid and Cleland 1957; Hamilton 1975). Indeed, Reid and Cleland (1957) recognized, but did not clarify, a relationship between the anatomy, the arrangement of the ductuli and the histology of their mucosa. Variation in the ultrastructure of the principal cells along the ductuli have also been noted in the hamster (Flickinger *et al.* 1978), echidna (Bedford and Rifkin 1979) and elephant (Jones and Holt 1981). However, some workers (Morita 1966; Holstein 1969; Ramos and Dym 1977) have not related such differences to their location in the ductuli.

It is not known why there is a lower volume of supranuclear vacuoles and light electron-dense bodies in the principal cells lining the coni vasculosi than the initial zone. The endocytotic activity of the luminal border in both regions appears to be equal. Since the endocytosis is at least partly concerned with heterophagic digestion involving the formation of vacuoles and dense bodies (Montorzi and Burgos 1967; Yokoyama and Chang 1971; Jones and Holt 1981), it is suggested that the difference in occurrence of vacuoles and dense bodies may be due to the greater concentration of macromolecules in the lumen of the coni vasculosi than initial zone and the consequent more rapid transformation to electron-dense bodies.

Micropuncture studies of the epididymis of the echidna (Djakiew and Jones 1983) supported an earlier proposal (Jones 1980) that the mammalian ductuli efferentes reabsorb most of the fluid leaving the testis. Both of the methods of determining fluid reabsorption which were used in this paper also support the proposal. The agreement between the two methods is satisfactory considering that the location of the distal samples was not the same for the different techniques, there is a problem in distinguishing cilia and sperm tails in the common ductulus efferens and the sampling techniques involved in micropuncture may introduce some bias.

The stereological data provides a basis for estimating the volume of fluid reabsorbed by the ductuli efferentes. When the estimates of percentage of fluid absorbed and surface area of luminal border ( $2 \cdot 77 \text{ cm}^2$ , Table 1) are used with Free and Jaffe's (1979) estimate of fluid output by the testis ( $38 \cdot 37 \mu l h^{-1}$ ), calculations show that the rate of fluid absorption by the ductuli efferentes of the rat ( $12 \cdot 74 \mu l \text{ cm}^{-2} h^{-1}$ ) is much higher than for their homologous structure in the kidney, the proximal convoluted tubules (reabsorbs  $2 \cdot 2 \mu l \text{ cm}^{-2} h^{-1}$ , House 1974).

In their report on ultrastructural studies of the ductuli efferentes in the hamster Flickinger *et al.* (1978) concluded that the main method of fluid absorption across the mucosa is by membrane-bound vesicles and vacuoles. This proposal is consistent with the occurrence of an extensive microtubular network in the epithelial cells. However, our studies in the elephant (Jones 1980) and echidna (Djakiew and Jones 1983) indicate that the vesicles and vacuoles could only account for part of the fluid transport since about twice as much fluid as protein is reabsorbed. Our calculations for the rat also indicate that vesicles and vacuoles could not transport fluid rapidly enough to account for all of the fluid which is reabsorbed. The calculations assume that the ductuli efferentes absorb  $35 \cdot 3 \mu l/h$  (Free and Jaffe 1979). From the estimates of volume ratios of vesicles and vacuoles, cell dimensions and epithelial volume, in Tables 1 and 2, and the ratio of ciliated to principal cells

(see text), it was calculated that the total volume of vesicles and vacuoles in the initial zone and coni vasculosi is 0.674 and  $0.573 \mu$ l respectively. Consequently, the rate of passage of these bodies across the epithelium would be  $2 \cdot 1 \min$  (i.e.  $1.247 \times 60/35.3$ ) a rate which is faster than the uptake of fluid into the cells (Hermo and Morales 1984). An alternative mechanism of fluid transport across the mucosa of the ductuli would be by the filtration process involved in transporting fluid across the endothelium of blood capillaries (Starling-Landis hypothesis; Wiederhielm 1968). However, it is unlikely that this mechanism would be important since the luminal hydrostatic pressure at the proximal end of the ductuli  $(2 \cdot 2 \text{ mm})$ Hg; Free and Jaffe 1979) is about the same as in the interstitium (Wiederhielm 1968), and the protein concentration of the luminal fluid (1 · 1 mg/ml; Hinton 1979) is about the same as for interstitial fluid (Wiederhielm 1968). Consequently, it is suggested that the main mechanism of fluid transport across the duct epithelium probably involves the coupling of water and active solute transport (Diamond 1971; Hill 1975). This proposal is consistent with the occurrence of some dilation of the lateral intercellular species between the mucosal cells. Also, it is in agreement with the recent findings of Hermo and co-workers (Hermo and Morales 1984; Hermo et al. 1985) which indicate that the products of fluid-phase and absorptive endocytosis in the ductuli epithelium are destined for the lysosomal apparatus of the cell.

It is suggested that the main function of the common ductulus efferens is to act as a conduit between the more proximal efferent ducts and the initial segment of the ductus epididymidis. This function is indicated by its length, thickness of the muscular tunic and frequent occurrence of ciliated cells (Winet 1980).

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### References

- Aherne, G. F., and Dunnill, M. S. (1966). Quantitative aspects of placental structure. J. Pathol. Bacteriol. 91, 123.
- Bedford, J. M., and Rifkin, J. M. (1979). An evolutionary view of the male reproductive tract and sperm maturation in a monotreme mammal-the echidna, *Tachyglossus aculeatus*. Am. J. Anat. 156, 207-30.
- Benoit, M. J. (1926). Recherches anatomiques, cytologiques et histophysiologiques sur les voies excretries du testicule, chez les mammifères. Arch. Anat. Histol. Embryol. (Strasbourg) 5, 173-412.
- Burgos, M. H. (1964). Uptake of colloidal particles by cells of the caput epididymidis. Anat. Rec. 148, 517-25.
- Diamond, J. M. (1971). Standing-gradient model of fluid transport in epithelium. Fed. Proc. 30, 6-13.
- Djakiew, D., and Jones, R. C. (1981). Structural differentiation of the male genital ducts of the echidna, *Tachyglossus aculeatus*. J. Anat. 132, 187-202.
- Djakiew, D., and Jones, R. C. (1982). Stereological analysis of the epididymis of the echidna, *Tachyglossus aculeatus*, and Wistar rat. Aust. J. Zool. 30, 865-75.
- Djakiew, D., and Jones, R. C. (1983). Sperm maturation, fluid transport and secretion and absorption of protein in the epididymis of the echidna, *Tachyglossus aculeatus*. J. Reprod. Fertil. 68, 445-6.

Flickinger, C. J., Howards, S. S., and English, H. F. (1978). Ultrastructure differences in efferent ducts and several regions of the epididymis of the hamster. Am. J. Anat. 152, 537-85.

- Forssmann, W. G., Ito, S., Weihe, E., Aoki, A., Dym, M., and Fawcett, D. W. (1977). Improved perfusion fixation method for the testis. *Anat. Rec.* 188, 307-14.
- Free, M. J., and Jaffe, R. A. (1979). Collection of rete testis fluid from rats without previous efferent duct ligation. *Biol. Reprod.* 20, 269-78.
- Hamilton, D. W. (1975). Structure and function of the epithelium lining the ductuli efferentes, ductus epididymidis and ductus deferens in the rat. In 'Handbook of Physiology'. Section on Endocrinology. (Eds E. B. Astwood and R. A. Greep.) pp. 259-301. (The American Physiological Society: Bethesda.)
- Hermo, L., Clermont, Y., and Morales, C. (1985). Fluid-phase and adsorptive endocytosis in ciliated epithelial cells of the rat ductuli efferentes. *Anat. Rec.* 211, 285-94.
- Hermo, L., and Morales, C. (1984). Endocytosis in nonciliated epithelial cells of the ductuli efferentes of the rat. Am. J. Anat. 171, 59-74.
- Hill, A. E. (1975). Solute-solvent coupling in epithelia: a critical examination of the standing-gradient osmotic flow theory. *Proc. R. Soc. Lond.* B190, 99-114.
- Hinton, B. T. (1979). The composition and movement of luminal fluids in the testis and epididymis. Ph.D. Thesis presented to the Council for National Academic Awards, Cambridge.
- Hinton, B. T., White, R. W., and Setchell, B. P. (1980). Concentrations of *myo*-inositol in the luminal fluid of the mammalian testis and epididymis. J. Reprod. Fertil. 58, 395-9.
- Hoffer, A. P., Hamilton, D. W., and Fawcett, D. W. (1975). Phagocytosis of spermatozoa by the epithelial cells of the ductuli efferentes after epididymal obstruction in the rat. J. Reprod. Fertil. 44, 1-9.
- Hohlbrugger, G. (1980). A micropuncture study of transepithelial water reabsorption in the rat ductuli efferentes. *Fertil. Steril.* 34, 50-4.
- Hohlbrugger, G., Schweisfurth, H., and Dahlheim, H. (1982). Angiotensin I converting enzyme in rat testis, epididymis and vas deferens under different conditions. J. Reprod. Fertil. 65, 97-103.
- Holstein, A. F. (1969). Morphologische Studien am Nebenhoden des Menchen. Zwangtose Abhandlungenaus dem Gebeit der Normalen und Pathologeschen Anatomie 20, 1–91. (Eds W. Bargmann and W. Doerr.) (G. Thieme, Verlag: Stuttgart.)
- House, C. R. (1974). 'Water Transport in Cells and Tissues.' p. 429. [Edward Arnold (Pubs) Ltd: London.]
- Jones, R. C. (1980). Luminal composition and maturation of spermatozoa in the genital ducts of the African elephant, Loxodonta africana. J. Reprod. Fertil. 60, 87-93.
- Jones, R. C., and Brosnan, M. F. (1982). Studies of the deferent ducts from the testis of the African elephant, *Loxodonta africana*. I. Structural differentiation. J. Anat. 132, 371-86.
- Jones, R. C., and Holt, W. V. (1981). Studies of the deferent ducts from the testis of the African elephant *Loxodonta africana*. III. Ultrastructure and cytochemistry of the ductuli efferentes. J. Anat. 133, 247-55.
- Kendall, M. G., and Stewart, A. (1963). 'The Advanced Theory of Statistics.' Vol. 1. (Charles Griffith and Co. Ltd: London.)
- Koskimies, A. I., and Kormano, M. (1975). Proteins in fluids from different segments of the rat epididymis. J. Reprod. Fertil. 43, 345-8.
- Ladman, A. J., and Young, W. C. (1958). An electron microscopic study of the ductuli efferentes and rete testis of the guinea pig. J. Biophys. Biochem. Cytol. 4, 219-26.
- Levine, N., and Marsh, D. J. (1971). Micropuncture studies of the electrochemical aspects of fluid and electrolyte transport in individual seminiferous tubules, the epididymis and the vas deferens in rats. J. Physiol. 213, 557-70.
- Mason, K. E., and Shaver, S. L. (1952). Some functions of the caput epididymis. Ann. N.Y. Acad. Sci. 55, 585-93.
- Montorzi, N. M., and Burgos, M. H. (1967). Uptake of colloidal particles by cells of the ductuli efferentes of the hamster. Z. Zellforsch. 83, 58-69.
- Montorzi, N. M., and Labiano, S. A. (1970). Uptake of water, sodium and glucose by the ductuli efferentes of the hamster 'in vitro'. Acta Physiol. Lat. Am. 20, 47-51.
- Morita, I. (1966). Some observations on the fine structure of the human ductuli efferentes testis. Archi. Histolo. Japon. 26, 341-65.
- Ramos, A. S., and Dym, M. (1977). Ultrastructure of the ductuli efferentes in monkeys. *Biol. Reprod.* 17, 339-49.

Reid, B. L., and Cleland, K. W. (1957). The structure and function of the epididymis. I. The histology of the rat epididymis. *Aust. J. Zool.* 5, 223-46.

Reynolds, E. C. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17, 208-12.

Sedar, A. W. (1966). Transport of exogenous peroxidase across the epithelium of the ductuli efferentes. J. Cell Biol. 31, 102A.

Turner, T. T. (1984). Resorption versus secretion in the rat epididymis. Reprod. Fertil. 72, 509-14.

Turner, T. T., Jones, C. E., Howards, S. S., Ewing, L. L., Zegeye, B., and Gunsalus, G. L. (1984). On the androgen microenvironment of maturing spermatozoa. *Endocrinology* **115**, 1925-32.

Umbreit, W. W., Burris, R. H., and Stauffer, J. F. (1972). 'Manometric and Biochemical Techniques.' (5th Edn.) p. 146. (Burgess Publ. Co.: Minneapolis.)

Weibel, E. R. (1979). 'Stereological Methods.' Vol. 1. Practical Methods for Biological Morphometry. (Academic Press: London, New York, Toronto, Sydney, San Francisco.)

Wiederhielm, C. A. (1968). Dynamics of transcapillary fluid exchange. J. Gen. Physiol. 52, 29-63. Winet, H. (1980). On the mechanism for flow in the efferent ducts. J. Androl. 1, 304-11.

Yokoyama, M., and Chang, J. P. (1971). An ultracytochemical and ultrastructural study of epithelial cells in ductuli efferentes of Chinese hamster. J. Histochem. Cytochem. 19, 766-74.

Young, W. C. (1933). Die resorption in den ductuli efferentes der maus und ihre bedeutung fur das problem der unterbinding in hoden-nebenhodensystem. Z. Zellforsch. 17, 729-59.

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