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Structural differentiation of the male genital ducts of the echidna (*Tachyglossus aculeatus*)

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INTRODUCTION

Until the recent reports by Glover & Nicander (Glover & Nicander, 1971; Nicander & Glover, 1973) there was little interest in comparative studies of the mammalian epididymis. Benoit (1926) extended the earlier work of Becker (quoted by Reid & Cleland, 1957) by studying a number of laboratory and domestic animals and confirmed that in all the animals studied there was an 'initial segment' in which the epithelium was higher than elsewhere, the stereocilia were long and there were relatively few spermatozoa in the duct lumen. Later workers recognized up to eight major histologically distinct regions in the epididymis of various scrotal mammals (Laurent, 1933; Nicander, 1957, 1958; Reid & Cleland, 1957; Hoffer & Greenberg, 1978), but there was little attempt to make comparisons between species of the structure and location of these regions. This may be because species from only a few orders of eutherian mammals were examined and there are no detailed studies of the structure of the epididymis of primitive mammals which might provide a basis for comparison. For this reason we have begun studies on the structure and function of the excurrent ducts from the testes of the echidna and this initial report is mainly concerned with organ and tissue differentiation.

MATERIALS AND METHODS

Six male echidna were used for these studies. They were collected during the mating season (July, 1976 and 1977, September, 1977 and 1978 and August, 1978 and 1979) and each animal was used in a number of studies. In order to confirm that their testes were producing spermatozoa, histological sections of a testis from each animal were prepared and examined under the light microscope.

Anatomy and histology

The anatomy of the genital ducts was examined in fresh (3 animals) and fixed (3 animals) specimens with the aid of a dissecting microscope. The ductuli efferentes were further examined in one animal by digesting them free of connective tissue stroma, using an aqueous solution of 20% (v/v) hydrochloric acid and 72% (v/v) ethanol for 3 hours, and then teasing them apart.

Material from 2 animals was fixed in Bouin's fluid for histological studies and material from another animal was fixed in 10% formal saline for both histological and histochemical studies. The entire length of ducts was prepared by cutting the cord formed by the ducts within their connective tissue stroma into blocks approximately 10 mm long and processing the blocks into paraffin wax. Longitudinal serial

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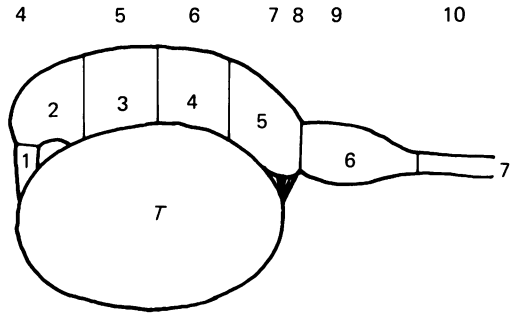


Fig. 1. Diagram showing a lateral view of a testis (*T*) and its genital ducts. The latter are divided into the seven parts used to determine the distribution of extragonadal spermatozoa (Table 1). Part 1 is the ductuli efferentes, parts 2 to 5 are the proximal region of the epididymis, part 6 is the distal region of the epididymis and part 7 is the ductus deferens. The numerics directly above the diagram indicate the sites where measurements of the duct dimensions were made (Figs. 5–10): sites 1, 2 and 3 were respectively at the proximal, middle and distal end of the ductuli efferentes (see text).

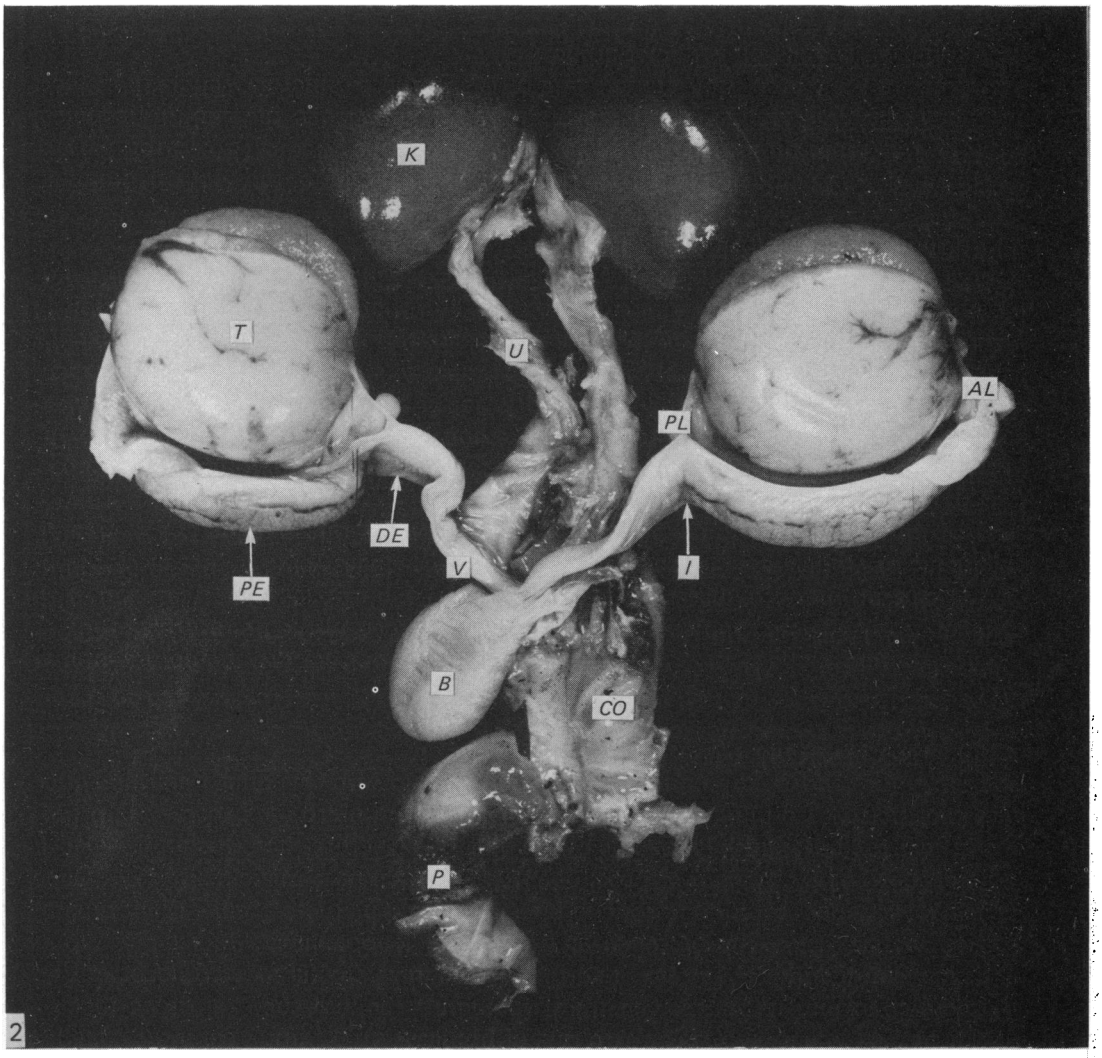
sections of the blocks (5 μm thick) were cut and every twentieth section was prepared for microscopic examination. Sections for general histological observations were stained with haematoxylin and eosin. A combination of Verhoeff's and Van Gieson's stains (Humason, 1972) was also used to prepare some sections in order to differentiate between elastic fibres, collagen and smooth muscle.

After a preliminary examination of the histological sections, 10 sites were identified along the genital ducts in order to describe their main characteristics (there was no difference in the magnitude of the measurements between the two organs fixed in Bouin's fluid and the one fixed in formalin). Figure 1 indicates the location of the sites chosen to describe the main characteristics of the ducts. Sites 1 and 2 were, respectively, located at the proximal and distal ends of the proximal part of the ductuli efferentes and site 3 was located in the middle of the distal part of the ductuli efferentes. Sites 4 to 7 were located along the wide, proximal region of the epididymis, sites 8 and 9 were located in the narrow, distal region of the epididymis and site 10 was in the ductus deferens. Cross sections of ducts were identified under the light microscope and a calibrated eyepiece micrometer was used to make the following measurements: epithelial height, length of epithelial stereocilia (microvilli), shape of epithelial nuclei (calculated as ratio of length/width), duct diameter (between opposing basement membranes of the epithelium), length of epithelial folds into the lumen of the duct, and thickness of smooth muscle surrounding the duct (periductal muscle). Scores of the concentration of spermatozoa within

Fig. 2. Photograph of the urogenital system of a male echidna. *T*, testis; *AL*, anterior gonadal ligament; *PL*, posterior gonadal ligment; *PE*, proximal region of the epididymis; *I*, isthmus of epididymis; *DE*, distal region of epididymis; *V*, ductus deferens; *B*, bladder; *K*, kidney; *U*, ureter; *CO*, coprodeum; *P*, penis. × 0·77.

Fig. 3. Photograph of the testis and genital ducts of the male echidna dissected free of mesorchium. *T*, testis; *ED*, ductuli efferentes; *S*, septum separating ductuli efferentes into proximal and distal parts; double arrow showing distal end of ductuli efferentes; *PE*, proximal region of epididymis; *I*, isthmus of epididymis; *DE*, distal region of epididymis; *V*, ductus deferens. × 0·66.

Fig. 4. Photograph of the ductuli efferentes, dissected from the connective tissue stroma, showing how they radiate from the testis (left side) and join the ductus epididymidis (arrow shows proximal end). × 1·5.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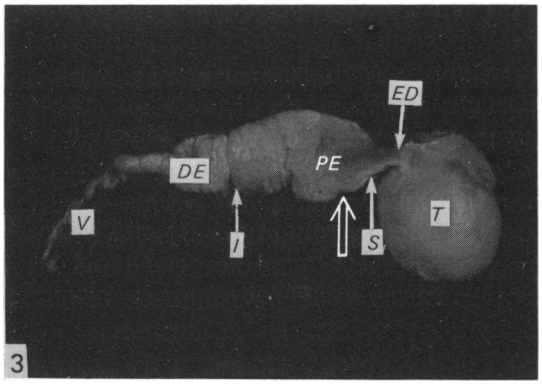
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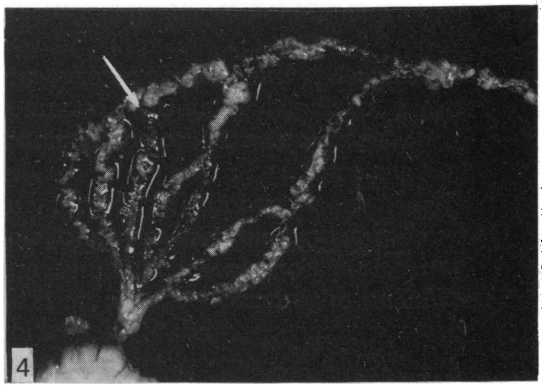
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Table 1. *Distribution of spermatozoa in the genital ducts of the male echidna (means from three animals)*

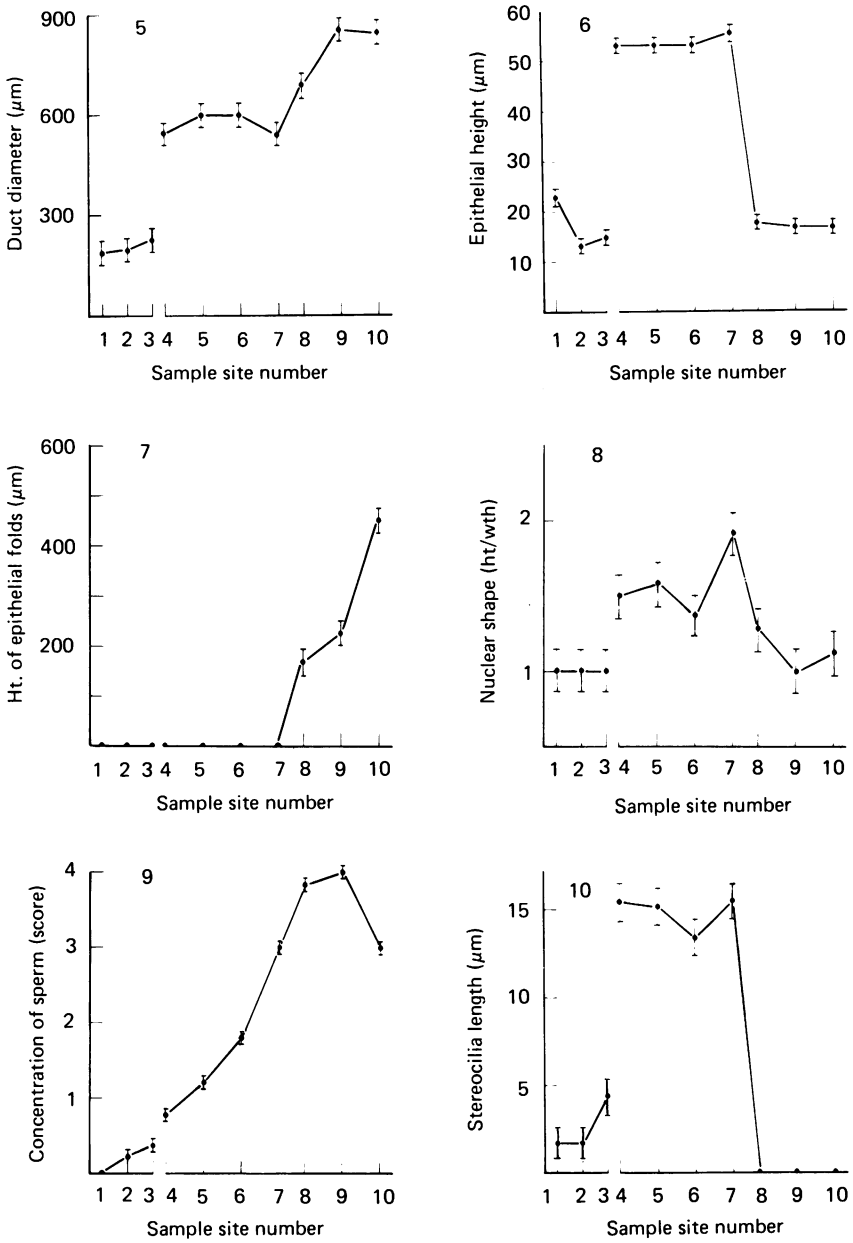
	Part of genital duct (Fig. 1)	Weight of tissue (g/animal)	Number of spermatozoa ($\times 10^6$/animal)	Concentration of spermatozoa ($\times 10^6$/g)	Percentage of total extragonadal spermatozoa
Ductuli efferentes	1	0·33 ± 0·10	2·17 ± 5·75	6·58 ± 0·72	0·14
Epididymis					
Initial segment	2	1·23 ± 0·23	87·70 ± 15·73	71·30 ± 6·32	
	3	1·67 ± 0·39	301·87 ± 86·53	180·76 ± 40·99	70·40
	4	1·38 ± 0·19	301·00 ± 73·59	218·12 ± 44·45	
	5	1·05 ± 0·15	410·49 ± 127·57	390·94 ± 116·40	
Terminal segment	6	0·76 ± 0·10	409·90 ± 127·25	539·34 ± 166·57	26·20
Ductus deferens	7	0·37 ± 0·04	50·96 ± 11·38	137·73 ± 45·28	3·26
Total		6·79	1564·09	230·35	100
P(1 *vs.* mean of 2–7):		***	***	***	
P(mean 2–6 *vs.* 7):		***	***	N.S.	
P(mean 2–5 *vs.* 6):		**	N.S.	**	
P(between parts 2–5):		N.S.	N.S.	*	
P(6 *vs.* 7):		N.S.	**	**	

the duct lumen were made using a scale of 0 to 4 where: 0 = absent, 1 = very few, 2 = sparse, 3 = moderately dense, 4 = very dense (ten separate ducts at each site were scored and used to calculate a mean score for each site).

Araldite sections were prepared with material from three animals. Tissue from one animal was fixed by immersion in picric acid-formaldehyde-glutaraldehyde (Jones, 1973) for 30 minutes at room temperature, followed by 2–3 hours at 5 °C. The other two animals were fixed by vascular perfusion (Forssman *et al.* 1977). The tissue was washed in sucrose-cacodylate, post-fixed in 1 % (w/v) osmium tetroxide, dehydrated in ethanol and embedded in Araldite. Araldite sections (0·5–1 μm) were cut for light microscopy and stained with Mallory's Azure II (Richardson, Jarett & Finke, 1960), or 1 % toluidine blue in 0·5 % borax.

Distribution of extragonadal spermatozoa

One of the fixed genital ducts from each animal was divided into seven parts (Fig. 1) to determine the distribution of extragonadal spermatozoa (Jones, Rowlands & Skinner, 1974). Part 1 was the ductuli efferentes, parts 2–5 were the proximal region of the epididymis, part 6 was the distal region of the epididymis and part 7 was the ductus deferens. Each sample of tissue was weighed and transferred to 20 ml of a citrate solution (100 mM sodium citrate, 1 μl/ml of Triton X-100 and 1 % (v/v) formalin) where it was minced and homogenized for 10 minutes at 16000 rpm in a Sorvall omnimixer homogenizer (Dupont Instruments, Newtown, Connecticut, U.S.A.). After dilution to an appropriate volume a sub-sample of the homogenate was taken to estimate the concentration of spermatozoa using a Neubauer haemocytometer. Determinations of the total number of spermatozoa in each part were carried out on the genital ducts from one side of each animal and the value was subsequently doubled to express the results as the total number of spermatozoa per animal.



Figs. 5–10. Biometric characteristics of the genital ducts. Measurements of 3 animals; bars indicate standard errors. The location of the ten sample sites shown on the abscissa are described in Fig. 1. The ordinates are: Fig. 5, duct diameter; Fig. 6, height of the duct epithelium; Fig. 7, the height of epithelial folds; Fig. 8, ratio of length: width of epithelial nuclei; Fig. 9, score of concentration of spermatozoa in duct lumen; Fig. 10, length of epithelial stereocilia.

Statistical analyses

Untransformed data (except as stated below) were examined by carrying out analyses of variance. Only differences which were statically significant in the analyses ($P < 0{\cdot}05$) are described in the Results. The standard errors shown in Figures 5–10 were calculated from the residual error mean squares in the analyses of variance, i.e. the interaction of sampling sites and replicates (animals). However, for the estimates of numbers of extragonadal spermatozoa the magnitude of the variance attributable to differences between animals varied considerably between different parts of the genital duct. Consequently, the data were transformed to logarithms for statistical analyses and the standard errors shown in Table 1 for these data (values correspond to untransformed data) were calculated from the between animal variance at each site: in order to achieve consistency in the Table, the standard errors relating to mean tissue weights were calculated in the same way.

RESULTS

Anatomy

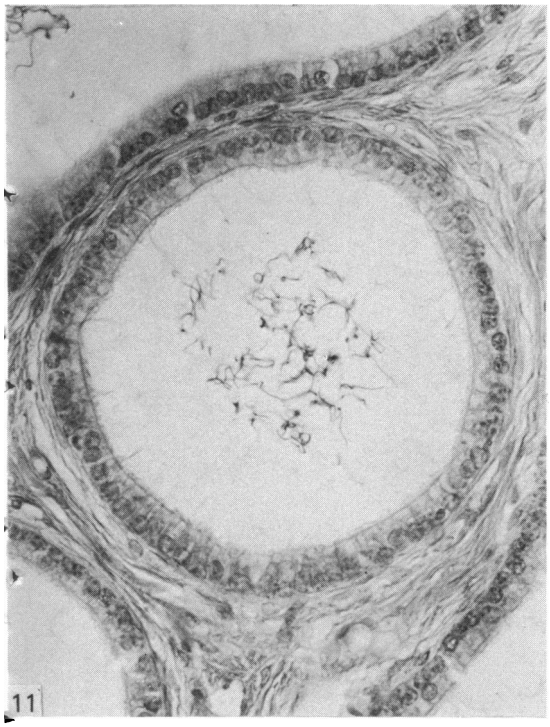
Figure 2 shows the anatomy of the urogenital system of a male echidna. Each testis was suspended by the mesorchium medial and just caudal to the kidneys. The ductuli efferentes were convoluted; within their connective tissue stroma they formed a cord which was divided into two parts (Fig. 3) by a septum of connective tissue. The proximal part was slightly pigmented (brown) and the individual ductuli efferentes coursed through it in separate bundles parallel with one another. The distal part widened towards the epididymis, and the individual ducts, no longer arranged in bundles, coursed at an angle of 40 to 60 degrees from the longitudinal plane. When dissected from their connective tissue stroma, seven convoluted ductuli efferentes were found to radiate from the surface of the testis (Fig. 4). Three adjacent pairs of these ducts joined to form three single ducts, and the four resulting ducts then joined the epididymis. The ductus epididymidis was highly convoluted and enmeshed in a connective tissue stroma composed of collagen and elastic fibres. The cord formed by the duct within the stroma was differentiated into a large proximal region, which coursed over the surface of the testis, and a smaller distal region caudal to the testis (Fig. 3). The two regions were separated by a narrow isthmus which was clearly distinguished by a fold (through 180 degrees) of the epididymal cord where the epididymis left the testis (i.e. between sites 7 and 8, opposite the posterior gonadal ligament; Fig. 2). The duct was narrow in the proximal region, and wide and swollen with spermatozoa in the distal region. The distal

Fig. 11. Transverse section of ductuli efferentes. Note the sparsity of spermatozoa. Paraffin, H & E. × 355.

Fig. 12. Transverse section of the initial segment of the ductus epididymidis showing the moderate concentration of spermatozoa and the tall epithelium with long stereocilia. Paraffin, H & E. × 144.

Fig. 13. Transverse section of the terminal segment of the ductus epididymidis showing the low epithelium, the high epithelial folds and the densely packed spermatozoa formed into bundles. Paraffin, H & E. × 85.

Fig. 14. Transverse section of the ductus deferens showing the extensive folding of the epithelium, the sparsity of spermatozoa in the lumen and the thick layer of smooth muscle surrounding the duct. Paraffin, H & E. × 55.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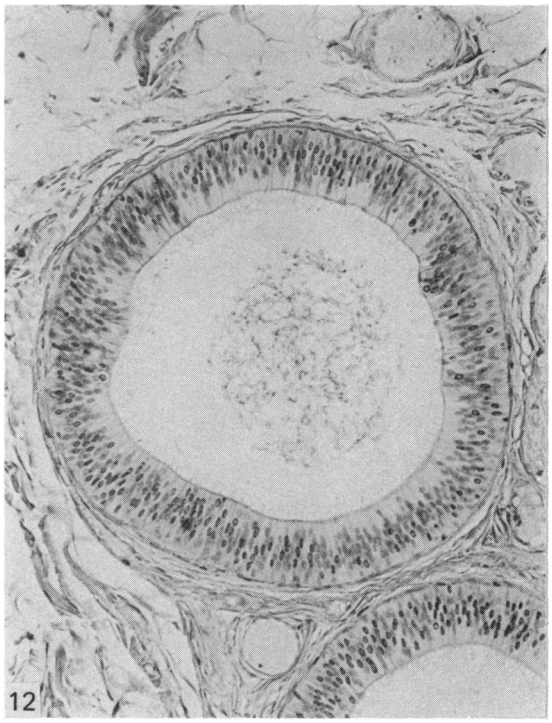
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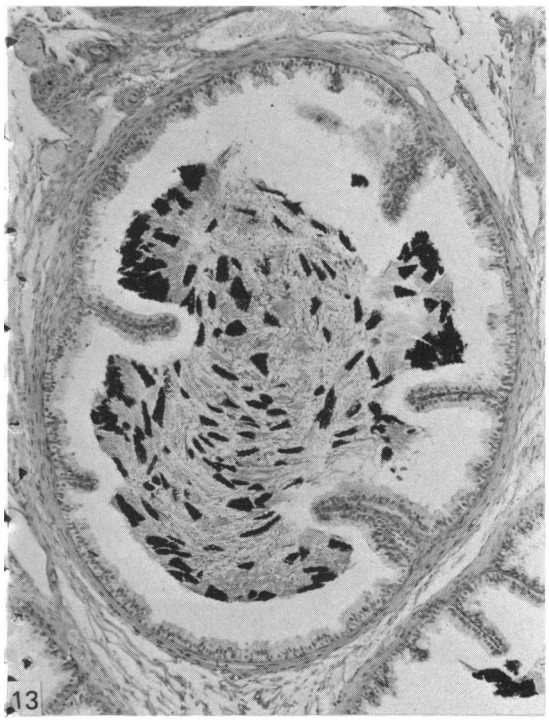
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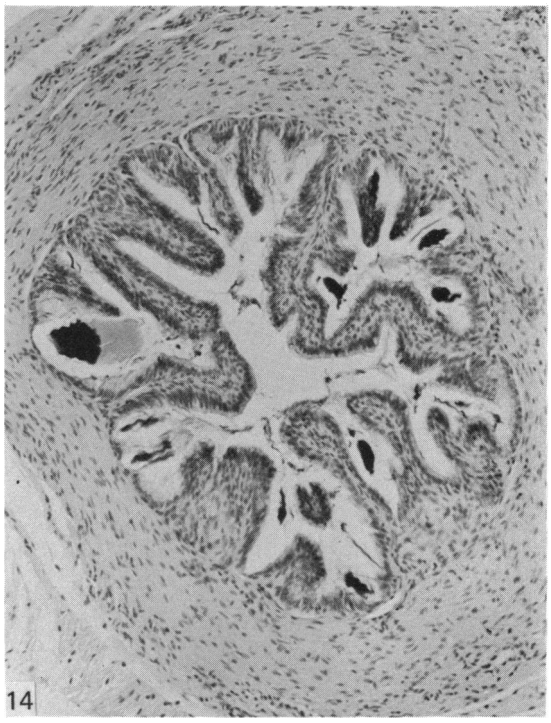
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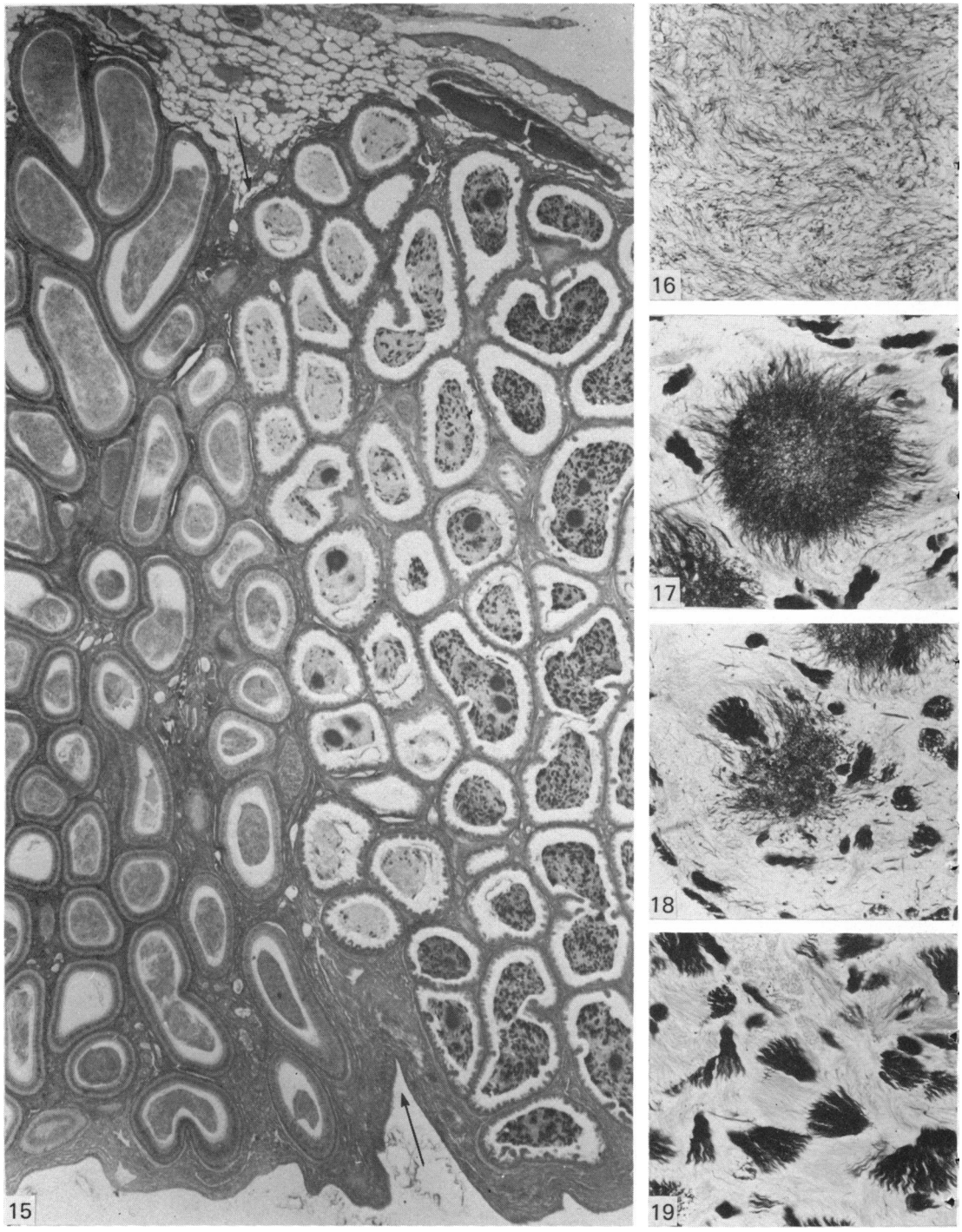
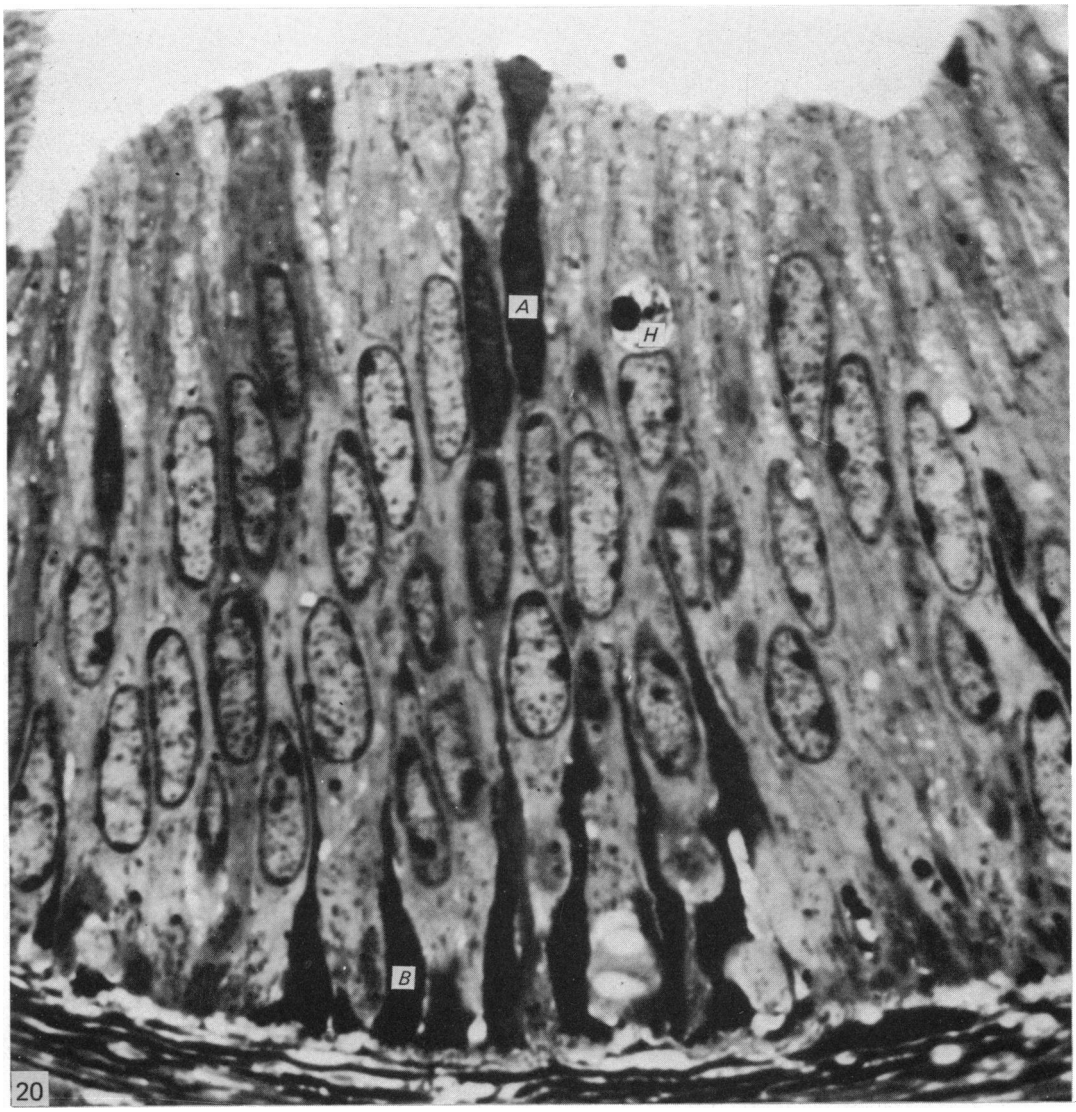
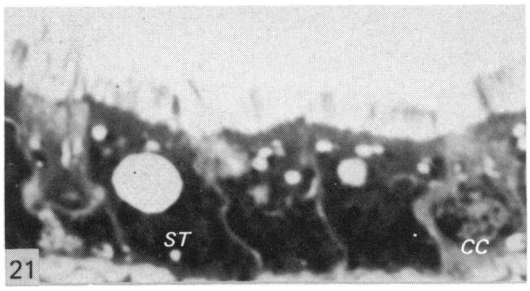


Fig. 15. Longitudinal section through the anatomical isthmus (arrows) of the epididymis showing the abrupt transition (in epithelial height, development of folds and arrangement of spermatozoa) from the initial segment (left) to the terminal segment (right). Paraffin, H & E. × 24.

Figs. 16–19. Spermatozoa in the lumen of the ductus epididymidis showing their random arrangement in the initial segment (Fig. 16) and the successive stages of rearrangement to form spheres (Fig. 17), then a transitional stage (Fig. 18) in the formation of bundles (Fig. 19) as they enter the terminal segment. Paraffin, H & E. × 270.





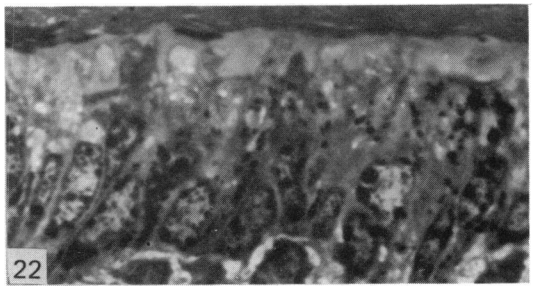


Fig. 20. Longitudinal section of the epithelium lining the initial segment of the ductus epididymidis. Note the 'halo cells' (*H*), apical cells (*A*) and the more intensely stained basal cells (*B*). Stereocilia were present at the luminal surface of the principal cells, but due to their low contrast in the photomicrograph, they are not shown. Araldite, toluidine blue. × 1820.

Fig. 21. Longitudinal section of the epithelium lining the ductuli efferentes showing stereociliated cells (*ST*), which contained large supranuclear vacuoles, and ciliated cells (*CC*) which stained less intensely than the stereociliated cells. Araldite, toluidine blue. × 2420.

Fig. 22. Longitudinal section of the epithelium lining the ductus deferens. Araldite, toluidine blue. × 1820.

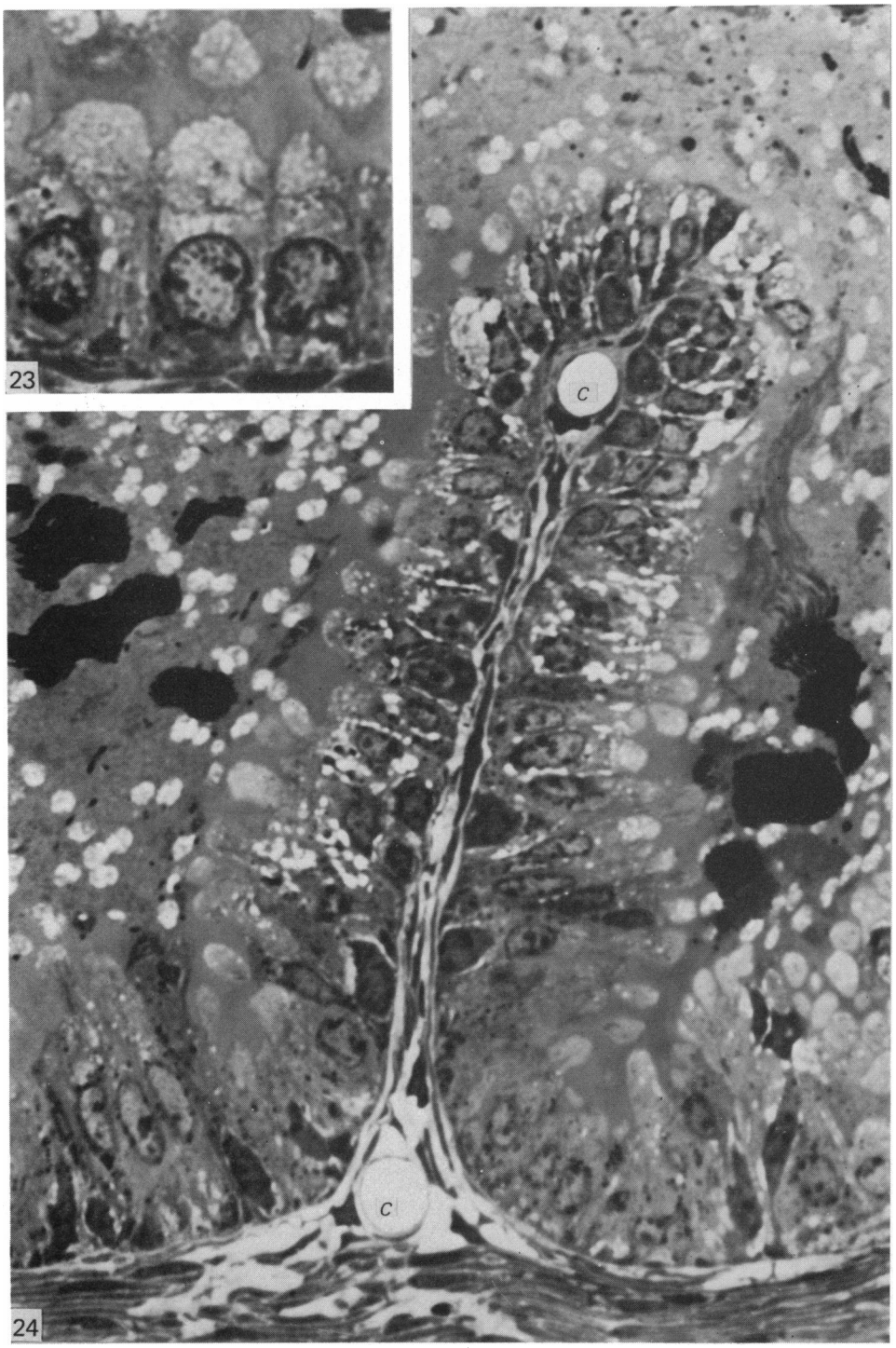


Fig. 23. Longitudinal section of the epithelium lining the terminal segment of the ductus epididymidis showing the active apocrine secretion. Araldite, azure II. × 1950.

Fig. 24. Section of the terminal segment of the ductus epididymidis showing an epithelial fold. Only blood capillaries (*C*) and loose connective tissue are present between the opposing walls of the epithelium of the fold. The lumen is filled with bundles of spermatozoa and the apocrine secretion. Araldite, toluidine blue . × 780.

region of the epididymis merged with a short ductus deferens which was convoluted at its proximal end and straight at its distal end (Fig. 3). Although the ductus deferens had a thicker, more rigid wall than the ductus epididymidis, it was not possible to distinguish a junction between the two.

Light microscopy

The main biometric characteristics of the genital ducts are summarized in Figures 5–10.

The ductuli efferentes (Fig. 11) were narrow (mean diameter of 202·5 μm). They were lined by a low columnar epithelium which decreased in height from site 1 to 2 and the lumen contained few spermatozoa. The epithelium was composed of ciliated and stereociliated cells with basal, spherical nuclei containing an uneven distribution of chromatin. The supranuclear cytoplasm often contained large vacuoles (Fig. 21). The ducts were surrounded by a single layer (mean of 3·0 μm wide) of slender smooth muscle cells.

The ductus epididymidis was wider than the ductuli efferentes, contained more spermatozoa, and was lined by a pseudostratified, stereociliated, columnar epithelium containing oval nuclei. There were two histologically distinct segments of the epididymis, the initial and terminal segments, which were abruptly separated at the anatomically distinct isthmus (Fig. 15). Consequently, the two segments, respectively, correspond precisely to the proximal and distal regions which were described above.

The initial segment was characterized by a wide duct (mean diameter of 571·3 μm; Fig. 12), high epithelium (mean of 53·7 μm) and a narrow lumen containing a low concentration of spermatozoa. The spermatozoa were evenly distributed and randomly orientated (Fig. 16) within the duct and increased in concentration in a proximodistal gradient along the duct. The epithelium (Fig. 20) was composed of principal, apical, basal and 'halo' cells (Reid & Cleland, 1957). The nuclei of principal cells were oval with a heterogeneous distribution of chromatin and about five nucleoli. In some sections the nuclei were wider than the surrounding cytoplasm. The supranuclear cytoplasm contained a region of Golgi apparatus which stained less intensely than the surrounding cytoplasm. Apical cells stained more intensely than the principal cells. Their nuclei contained a homogeneous distribution of chromatin and about four nucleoli. The basal cells also stained more intensely than the principal cells: they contained relatively little cytoplasm and it was elongated toward the lumen. The basal cell nuclei contained a homogeneous distribution of chromatin and about three nucleoli. The 'halo' cells were seen throughout the epithelium, but mainly in the basal half. Their cytoplasm was yellow in both paraffin and Araldite sections and was dominated by numerous small and large round vacuoles. The nuclei were irregularly shaped and intensely stained. The duct was surrounded by a thin (mean of 8·5 μm wide) layer of slender smooth muscle cells.

The temrinal segment was characterized by a very wide duct (mean diameter of 690 μm at site 8 and 855 μm at site 9), a low epithelium with very short microvilli and a wide lumen interrupted by numerous folds (Fig. 13). It contained a high concentration of spermatozoa and was surrounded by a thick layer of periductal muscle (Fig. 13). In the most proximal part of the terminal segment, adjacent to the isthmus (Fig. 15), spermatozoa formed bundles (Figs. 17–19). In the proximal part of this segment, their heads congregated into spherical aggregates in which the spermatozoa radiated from the centre (Fig. 17). More distally, they attached laterally (Fig. 18) and formed bundles of 20 to 25 spermatozoa orientated parallel to one another

(Figs. 19, 24). The duct epithelium of the terminal segment contained no apical cells, and there were fewer basal and halo cells than in the initial segment. In Araldite sections the principal cells showed signs of considerable apocrine, secretory activity (Fig. 23). The secretions were filled with numerous vesicles and vacuoles and appeared to disintegrate in the duct lumen. The folds were well vascularized (Fig. 24) and were not penetrated by the periductal muscle. Their frequency and length increased towards the distal end of the duct. The epithelium on the crests of the folds was slightly lower (approximately 2 μm) than in the 'crypts' between the folds, but no other differences were identified. The periductal muscle increased in thickness along the duct (mean of 16·1 μm at site 8 and 104·9 μm at site 9) and was composed of two types of cell. A single layer of slender smooth muscle cells (like the cells in the ductuli efferentes and initial segment) lay adjacent to the basement membrane and this was surrounded by a layer of thicker smooth muscle cells which increased in numbers in a proximodistal gradient.

The ductus deferens was characterized by a very wide duct (mean diameter of 850 μm), a low columnar epithelium (mean of 13·1 μm), a lumen almost obliterated by numerous long folds and a very thick (mean of 111·1 μm) layer of periductal muscle composed of the slender and thick cells found in the terminal segment of the epididymis (Fig. 14). The lumen contained only a few bundles of spermatozoa and some single spermatozoa. The duct epithelium was composed of principal and basal cells only. In Araldite sections (Fig. 22) the cytoplasm of the principal cells contained round vesicles and intensely stained dense bodies. The nuclei of the principal cells were irregularly shaped, contained an uneven distribution of chromatin and about four nucleoli.

Distribution of extragonadal spermatozoa

Table 1 shows the distribution of extragonadal spermatozoa in the genital ducts and the weights of the seven parts which were examined. The ductuli efferentes were much lighter than the derivatives of the mesonephric ducts. Within the mesonephric duct the epididymis was much heavier than the ductus deferens, and the initial segment of the epididymis (proximal region) was heavier than the terminal segment (distal region).

There was a progressive increase in the concentration of spermatozoa from the ductuli efferentes to the terminal segment of the epididymis, but the concentration was lower in the ductus deferens than in the terminal segment of the epididymis. The greatest increase (tenfold) in concentration was between the ductuli efferentes (part 1) and the most proximal part of the initial segment (part 2). Although the terminal segment of the epididymis contained the highest concentration of spermatozoa, it contained only 26·2% of all the extragonadal spermatozoa; the majority (70·4%) were found in the initial segment of the epididymis.

DISCUSSION

The structure of the echidna ductuli efferentes is similar to that described for marsupials (Ladman, 1967; Martan, Hruban & Slesers, 1967) and eutherian mammals (Burgos, 1957; Ladman & Young, 1958; Burgos, 1960; Morita, 1966) in that numerous relatively long narrow ducts are arranged in parallel, the ducts are lined by a low columnar epithelium composed of ciliated and stereociliated cells and there are very few spermatozoa in the lumen. Our findings (Fig. 9; Table 1) also indicate

that the ductuli are involved in a considerable amount of fluid absorption from the lumen of the duct as are the ductuli efferentes of eutherian mammals (Crabo, 1965; Tuck, Setchell, Waites & Young, 1970; Levine & Marsh, 1971). The anatomical differentiation of the ductuli efferentes into two parts is similar to the arrangement described in the rat (Reid & Cleland, 1957; Hamilton, 1975) where the distal part corresponds to the conus vasculosa. It probably also corresponds to the two parts described by Bedford & Rifkin (1979). In the studies described in this report it was noted that there was a difference in pigmentation between the proximal and distal parts but it was not possible to distinguish any consistent difference between these parts in the paraffin sections. As Araldite sections were prepared only from the proximal part of the ductuli efferentes it is not possible to confirm Bedford & Rifkin's (1979) proposal that there is a difference in the cytology of epithelium from the proximal and distal parts.

It is unfortunate that earlier workers examined only scrotal mammals and so referred to those derivatives of the mesonephric duct which are adjacent to the testis as the epididymis and those parts which are distal to the testis as the ductus deferens (Oudemans, 1892; Weber, 1898). Indeed, it is considered more informative to recognize that derivatives of the echidna mesonephric duct which are not adjacent to the testis may nevertheless be homologous with some parts of the epididymis of scrotal mammals. Consequently, we have adopted Glover's (1968) proposal that the anatomical proximal and distal regions of the derivatives of the testicond mesonephric duct are homologous with the head and tail of the epididymis of scrotal mammals. Only the head of the echidna epididymis is adjacent to the testis; the tail and the ductus deferens are separate and distal. The ductus deferens is clearly adapted mainly for the transport of spermatozoa and is distinguished from the tail of the epididymis by its thick rigid coat of smooth muscle.

It is concluded that the epididymis of the echidna is histologically differentiated into only two segments, the initial and terminal segments. Bedford & Rifkin (1979) concluded that the initial segment of the echidna epididymis could be further subdivided into two regions on the basis of quantitative differences in cellular organelles and the concentration of spermatozoa in the lumen, but our studies with the light microscope do not support this conclusion, nor do our preliminary studies with the electron microscope. Further, we believe that the proximodistal gradient in concentration of spermatozoa along the segment (Fig. 9; Table 1) is due to absorption of fluid along the duct, which is not an appropriate criterion on which to base the proposed classification. Unlike the eutherian mammals which have been studied (Benoit, 1926; Reid & Cleland, 1957; Glover & Nicander, 1971; Nicander & Glover, 1973; Suzuki & Racey, 1976), the transition between the initial and terminal segments in the echidna (Fig. 15) is abrupt (there is no middle segment), and the histological differentiation of the echidna epididymis corresponds quite precisely with the anatomical differentiation. Consequently, as the structure and function of the different parts of the epididymis may be related to its vasculature (Kormano, Suoranto & Reijonen, 1973; Kormano & Reijonen, 1976; Setchell, Waites & Till, 1964; Brown & Waites, 1972), it may be informative for comparative purposes to compare the vasculature of the epididymis of the echidna and scrotal mammals.

The initial segment of the echidna ductus epididymidis is histologically and ultrastructurally (Bedford & Rifkin, 1979) similar to the initial segment of the ductus epididymidis of other mammals which have been studied (mouse, rat, guinea-pig, dog, cat, rabbit, bull, horse, sheep, pig and mole: Benoit, 1926; Nicander, 1958; Suzuki & Racey, 1976; Nicander & Malmqvist, 1977). It is a region of tall pseudo-

stratified epithelium with long stereocilia and relatively few spermatozoa in the narrow duct lumen. The epithelium appears pseudostratified as it is composed of four cell types (principal, apical, basal and 'halo' cells) and because the nuclei of the principal cells are probably wider than the rest of the cell they must be located at varying levels within the epithelium. The estimates of concentration of spermatozoa along the initial segment of the echidna epididymis indicate that it is carrying out the same reabsorptive function as the initial segment of scrotal mammals (Crabo, 1965; Levine & Marsh, 1971). Furthermore, the way in which echidna spermatozoa form bundles (Figs. 15–19) immediately they leave the initial segment is reminiscent of the rouleaux formation of guinea-pig spermatozoa (Fawcett & Hollenberg, 1963) which has been associated with their maturation (Bedford, 1975) which seems to be a function of the initial segment of the epididymis. Indeed, the only unusual feature of the initial segment of the echidna epididymis is that it does, relative to the size of the testis, appear to be larger and to contain a greater proportion of the extragonadal spermatozoa (Table 1) than it does in scrotal mammals such as the rat and sheep (Benoit, 1926; Chang, 1945; Orgebin-Crist, 1968).

The terminal segment of the echidna ductus epididymidis shares a number of characteristics with the terminal segment of scrotal mammals (Glover & Nicander, 1971), i.e. it is a wide duct, lined by a low epithelium, surrounded by thick layers of smooth muscle and contains a high concentration of spermatozoa. These appear to be features of a storage region as the wide duct ensures that spermatozoa pass through it slowly (i.e. $v = F/A$ where v is velocity, F is flow rate of fluid and A is the cross sectional area of the duct), the low epithelium would minimize the diffusion distance for metabolic substrates and products and the thick periductal muscle layer must be present for the spermatozoa to be accessible during ejaculation. However, the epithelium of the terminal segment of the echidna differs from the epithelium in the analogous region of scrotal mammals in that in the former it produces considerable apocrine secretion, and also, it is thrown into folds. The presence of folds indicates that there may be some advantage in the duct being short. For example, it would allow a rapid transport of spermatozoa through the terminal segment. The function of the apocrine secretion in the terminal segment is unknown. Carrick & Hughes (1978) described this activity in the platypus epididymis and suggested that it may be a way of increasing the volume of seminal plasma. This is an intriguing possibility, but there is little reason to conclude that the monotremes should contribute a larger volume of seminal plasma from the ductus epididymidis than therian mammals. Indeed, it is noteworthy that even though there is considerable apocrine secretion in the terminal segment, there is no noticeable reduction in concentration of spermatozoa (Table 1), so that presumably some reabsorption of fluid also occurs.

It is curious that the echidna stores a much smaller proportion (26%) of its extragonadal spermatozoa in the tail of the epididymis than most scrotal mammals (about 50–75%: Chang, 1945; Dott & Skinner, 1967; Orgebin-Crist, 1968; Amann, Johnson, Thompson & Pickett, 1976). This may be because it is a solitary animal which does not need to store a large number of spermatozoa ready for ejaculation. However, the observation (Jones & Djakiew, 1978) that other mammals which store spermatozoa deep within the abdominal cavity also hold few immediately available for ejaculation provides circumstantial evidence for the suggestion (Glover, 1973; Bedford, 1977) that there is an adaptive advantage in storing spermatozoa at a temperature lower than body temperature.

SUMMARY

Seven ductuli efferentes radiate from the testis of the echidna and join the ductus epididymidis either directly or after joining one of their neighbours. They are pigmented brown and appear to be structurally and functionally similar to the ductuli efferentes of therian mammals.

The epididymis is anatomically differentiated into a large head and small tail which appear to be, respectively, larger and smaller than the similar regions of the epididymis of scrotal mammals; they also contain, respectively, larger and smaller proportions of the animals' extragonadal spermatozoa. Only the head of the epididymis is adjacent to the testis: the tail and the ductus deferens are distal to the testis.

The ductus epididymidis is also histologically differentiated into two segments (initial and terminal segments) which correspond precisely with the anatomical differentiation. The initial segment is structurally similar to the initial segment of the epididymis of scrotal mammals (i.e. tall epithelium with long stereocilia, a fairly homogeneous supranuclear cytoplasm containing Golgi apparatus and a low concentration of spermatozoa in the lumen). The terminal segment has adaptations of the duct (as in scrotal mammals) for the storage of spermatozoa such as a wide lumen containing a high concentration of spermatozoa, low epithelium and thick layers of periductal muscle. However, it is peculiar in that the duct epithelium is thrown into folds and it is involved in considerable apocrine secretion.

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