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Studies of the deferent ducts from the testis of the African elephant, *Loxodonta africana*. II. Histochemistry of the epididymis

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INTRODUCTION

The epididymis of the elephant is of particular interest since among mammals it is considered to be primitive (Short, Mann & Hay, 1967). Structural studies (Jones & Brosnan, 1979) indicate that the duct is differentiated along its length and that, based on structure and function, it may be classified into three main regions. Some of the characteristics of these regions are quite different from those described for scrotal mammals, but many are similar to the initial, middle and terminal segments described by Glover & Nicander (Glover & Nicander, 1971; Nicander & Glover, 1973) in their proposed classification of the regions of the mammalian epididymis. The purpose of this study was to examine the differentiation of the epididymis using cytochemical methods in order to assess further the possible functions of the various parts of the duct.

MATERIALS AND METHODS

Tissue was sampled from elephants in the Kruger National Park, South Africa, which were being culled to avoid overstocking. Nine mature bulls, either solitary animals or animals from bachelor herds, were immobilized by Scoline (Glaxo Laboratories Ltd., Middlesex) before being shot (see Jones, Rowlands & Skinner, 1974). Tissue samples were fixed or frozen within 30 minutes of death.

Samples from five elephants were fixed in phosphate-buffered formalin at 4 °C for 24 hours, then transferred to cold gum-sucrose in which they were stored and transported to England for histochemical study.

Tissues from a further four elephants were quenched in liquid-nitrogen-cooled isopentane, transferred to plastic vials, and stored in liquid nitrogen until required for further processing.

All the samples were sectioned at 8 μm using a cryostat.

Enzyme histochemistry

Table 1 lists the enzymes which were studied and summarizes the methods used to demonstrate their activity. Acid phosphatase and non-specific esterase were demonstrated in fixed and fresh frozen tissues; all the other methods were used on

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Table 1. *Enzymes studied and methods used*

Enzyme	Substrate (pH of medium)	Inhibitor	Reference
ATPase	ATP (7·2)	—	Wachstein *et al.* (1960)
Alkaline phosphatase	Naphthol-AS-MX-phosphate (9·2)	L-Cysteine 10^{-3} M	Burstone (1961)
Succinate dehydrogenase	Sodium succinate (7·0)	Sodium malonate 5×10^{-2} M	Jones & Holt (1974)
Glucose-6-phosphate dehydrogenase	Glucose-6-phosphate (7·0)	—	
NADH diaphorase	NADH (7·0)	—	Chayen, Bitensky, Butcher & Poulter (1969)
NADPH diaphorase	NADPH (7·0)	—	
5-ene-3β-hydroxysteroid dehydrogenase	Dehydroepiandrosterone (200 μg/ml) (7·0)	—	Baillie, Ferguson & Hart (1966)
17-β-hydroxysteroid dehydrogenase	Testosterone (200 μg/ml) (7·0)	—	
Acid phosphatase	Naphthol-AS-BI-phosphate (5·2)	NaF 10^{-2} M	Barka & Anderson (1962)
Acid phosphatase	Sodium-β-glycerophosphate (5·2)	NaF 10^{-2} M	Lake (1966)
Non-specific esterase	5-Bromo-4-chloro-indoxyl acetate (7·0)	Eserine sulphate 10^{-5} M	Holt (1958)

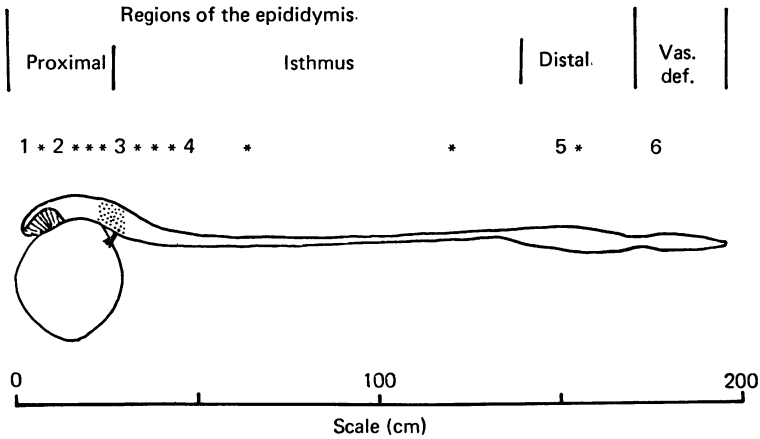


Fig. 1. A scale diagram showing a lateral view of a testis and its efferent ducts as they appeared *in situ* in a 26 years old African elephant. The efferent ducts are indicated by the shaded area above the testis. The classification of the epididymis is that of Jones & Brosnan (1979). Anatomically it is divided into wide proximal and distal regions joined by an isthmus; histologically there is an initial and terminal segment joined by a transitional zone (the middle segment, indicated by speckling. The numerals show the location of the sampling sites used in the 1974 study, and correspond to those used by Jones & Brosnan (1979). Sites 5, 6 and 8 were examined in 1972, and sites 4, and those shown by the asterisks, were examined in 1975.

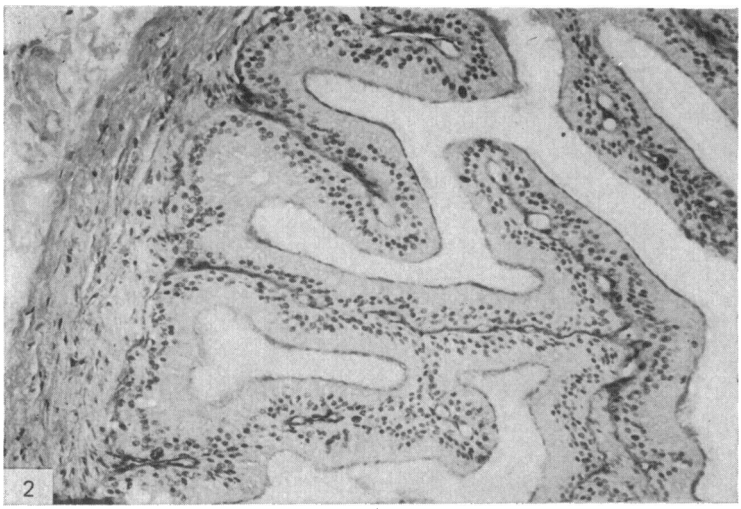
fresh frozen material. In all the studies 'control sections' were incubated in medium containing no substrate or, when this was inappropriate (e.g. when the substrate was a chromagen), specific inhibitors were added to the complete medium (Table 1). However, both forms of control were used in the glycerophosphate method for acid phosphatase and in the succinate dehydrogenase method. Eserine sulphate was used in conjunction with the non-specific esterase technique to distinguish cholinesterase and acetylcholinesterase from non-specific esterase. In order to inhibit diffusion of glucose-6-phosphate dehydrogenase from the cells during the reaction with TNBT the tissue was immersed in 1 % v/v glutaraldehyde in 50 mM tris buffer (pH 7·0) for 1 minute prior to incubation, and 7·5 % w/v polyvinylpyrollidone was added to the incubation medium.

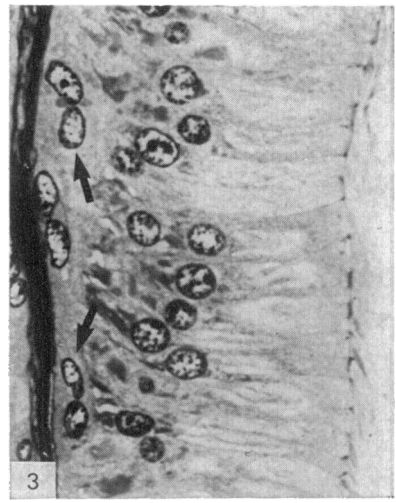
Lipid histochemistry

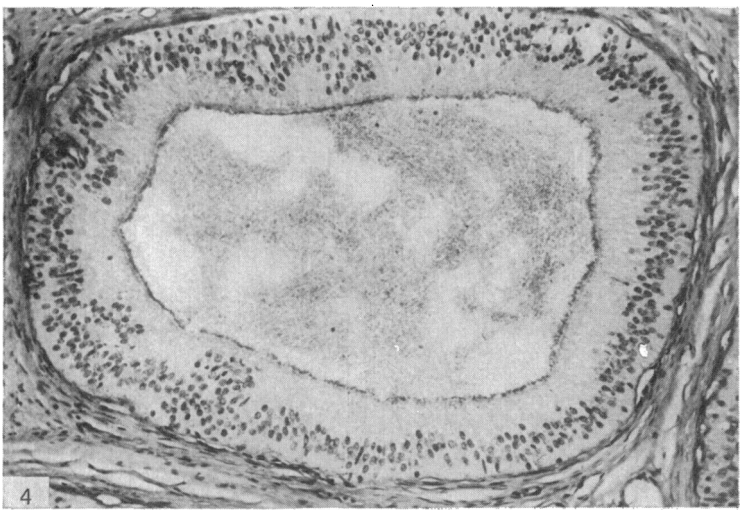
Neutral lipids were studied using Sudan black B in conjunction with acetone extraction. Phospholipids were demonstrated in the unfixed tissues with ferric haematoxylin in combination with an extraction procedure using acetone and a 1:1 mixture of chloroform and methanol (Elleder & Lojda, 1973).

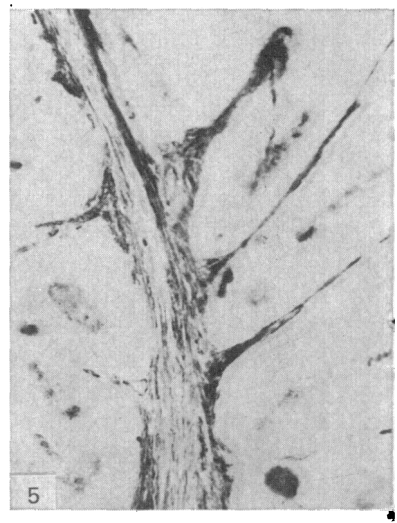
Glycoprotein and carbohydrate

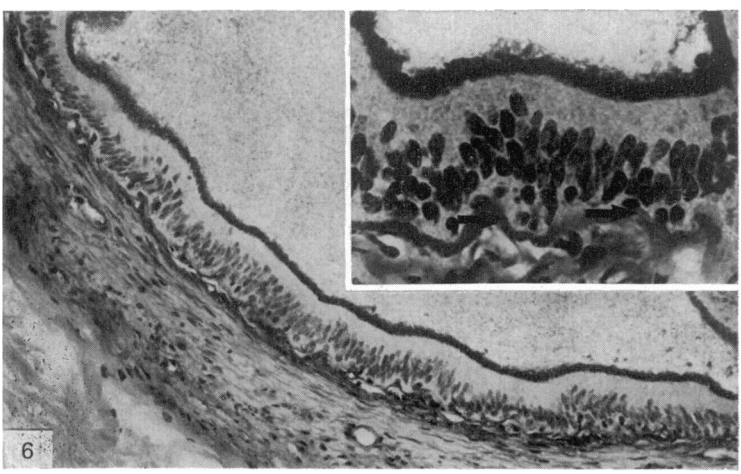
These were studied in the fixed tissues using the combined alcian blue (pH 2·5) periodic acid–Schiff method (AB-PAS, Pearse, 1968). Control sections were incubated in diastase (diluted 1:1000 in water) for the identification of glycogen; and in neuraminidase (1·5 hours at 37 °C in 125 units/ml of 50 mM acetate buffer, pH 5·5, using the *Vibrio cholerae* enzyme (Hoechst Pharmaceuticals)) for the identification of sialic acid.











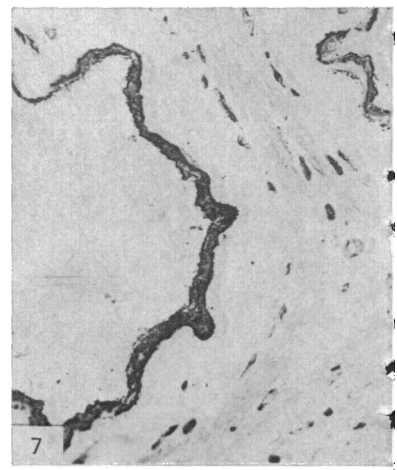


Table 2. *Summary of the enzyme activities* detected histochemically in the epididymis of the African elephant*

Enzyme	Epithelium†									Spermatozoa‡
	Initial segment			Middle segment			Terminal segment			
	M	*S*	*B*	*M*	*S*	*B*	*M*	*S*	*B*	
ATPase	3	1	1	3	1	1	4	1	1	2
Alkaline phosphatase	2	0	3	1	0	3	2	0	3	2
Succinic dehydrogenase	0	0	0	0	3	3	0	4	4	2
Glucose-6-phosphate dehydrogenase	0	3	3	0	3	4	0	4	4	3
NADH / NADPH diaphorase	0	2	2	0	3	4	0	3	4	3
Hydroxysteroid dehydrogenases	0	0	0	0	0	0	0	0	0	0
Acid phosphatase	0	3	3	0	3	4	0	0	1	0
Non-specific esterase	0	1	3	0	2	3	0	4	4	3

* Scored on a scale 0–4 where: 0 = no reaction, 1 = weak reaction, 2 = moderate reaction, 3 = strong reaction, 4 = intense reaction.

† M, microvilli; S, supranuclear region; B, basal.

‡ Each reaction showed much the same intensity in all segments.

RESULTS

The three regions of the epididymis proposed by Jones & Brosnan (1979) are shown diagrammatically in Figure 1 (see legend). The duct was lined by a pseudostratified columnar epithelium composed mainly of principal and basal cells. In some regions the epithelium formed folds which projected into the lumen of the duct. The folds were well vascularized with blood capillaries which showed considerable ATPase activity. Collagen fibres were prominent in the stroma within the folds. The whole length of the duct was surrounded by a sheath of smooth muscle and connective tissue which thickened progressively in the distal segment. The muscle fibres exhibited ATPase, alkaline phosphatase, glucose-6-phosphate dehydrogenase, diaphorase (NADH and NADPH) and succinic dehydrogenase activities.

The cytochemistry of spermatozoa is shown in Table 2. It was much the same in each segment, so is not described below. The histochemistry of the epididymis is described for each of the segments, and a summary of the main enzyme histochemical findings is given in Table 2. Hydroxysteroid dehydrogenase was not detected in any of the epididymal samples examined; this indicates that it was in fact absent, since the enzymes were demonstrated in sections of rat ovary and liver which, for comparison, were processed with the epididymal material.

Fig. 2. Alcian blue and PAS reaction in the initial segment of the epididymis. × 120.

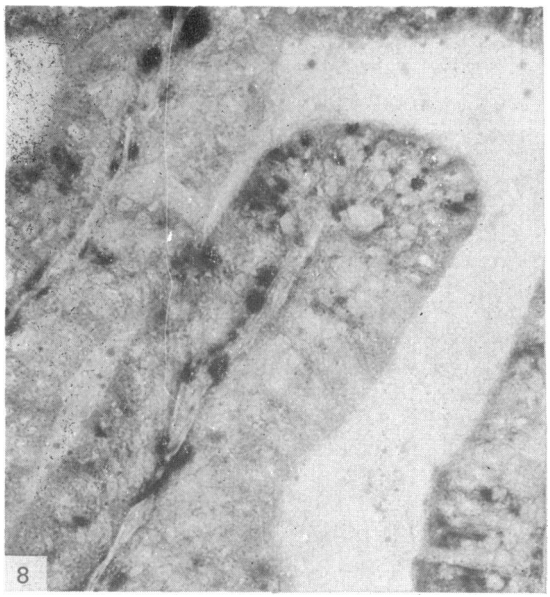
Fig. 3. Epithelium of the initial segment showing basal cells indicated by arrows. × 750.

Fig. 4. Alcian blue and PAS reaction in the middle segment. × 120.

Fig. 5. ATPase reaction in the initial segment. × 72.

Fig. 6. Alcian blue and PAS reaction in the terminal segment. 'Halo cells' are shown in insert. × 120.

Fig. 7. ATPase activity in the middle segment. × 72.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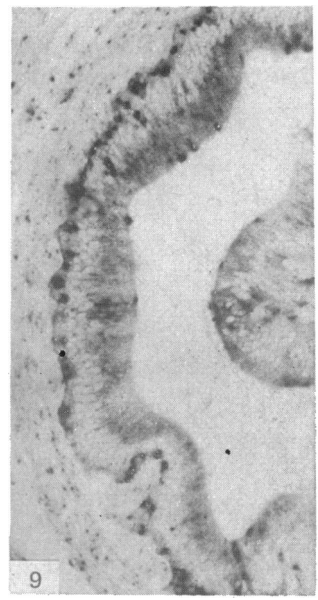
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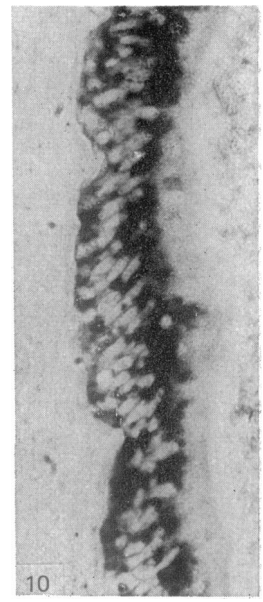
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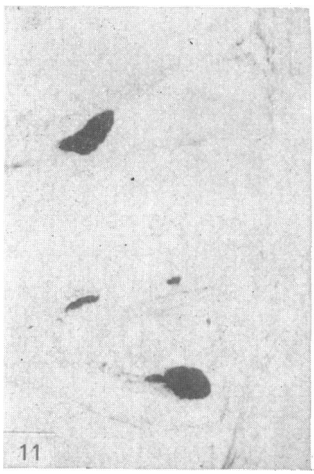
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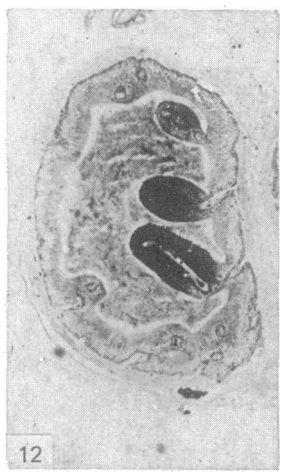
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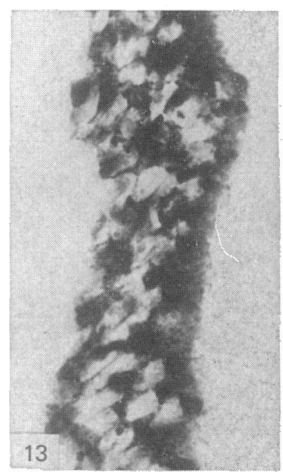
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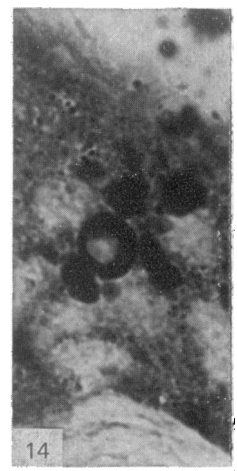
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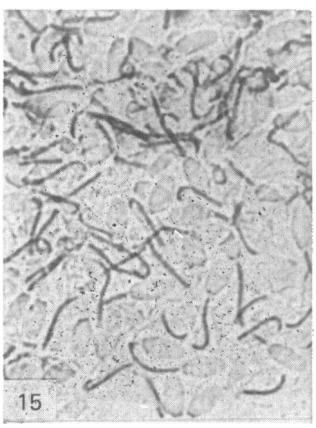
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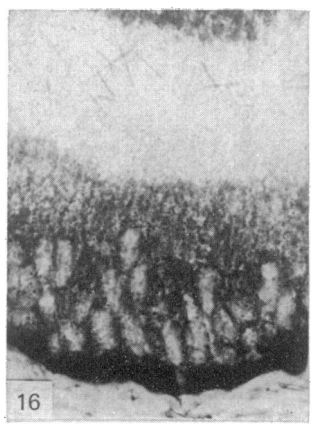
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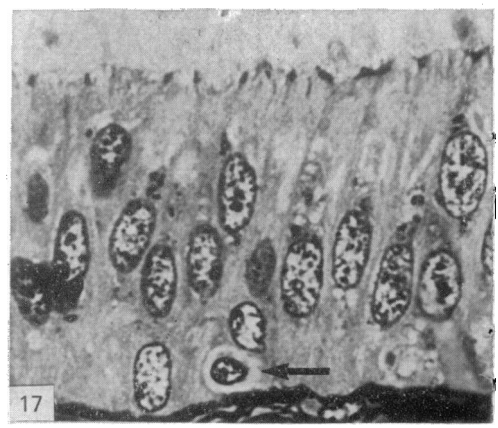
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Initial segment (Figs. 2, 3, 5, 8, 11)

The duct in this region was wide and contained relatively few spermatozoa. The lining epithelium was tall and folded, particularly in the proximal part of the segment (Fig. 2). The principal cells contained large round nuclei each with one or two nucleoli. A discontinuous layer of basal cells, which were mostly in pairs (Fig. 3, arrows), was present adjacent to the basement membrane.

The stereocilia were rich in acidic, alcian blue-positive, neuraminidase-resistant glycoprotein (Fig. 2). The cytoplasm of the principal cells contained a small quantity of glycogen, shown as a diffuse, weakly PAS-positive reaction inhibited by pre-treatment with diastase. Some principal cells (especially those adjacent to capillaries, or near the apex of an epithelial fold) contained accumulations of diastase-resistant, PAS-positive granules in the basal region. Other principal cells contained supra-nuclear aggregates of diastase-resistant, PAS-positive granules. Further, some basal cells were packed with diastase-resistant, PAS-positive granules. These were sometimes associated with alcian blue-positive granules.

No sudanophilic lipids could be detected in the epithelium, but a high concentration of phospholipids was demonstrated in the principal cells as a strong diffuse reaction with ferric haematoxylin. However, the ferric haematoxylin reaction was very weak in sections extracted with methanol–chloroform prior to staining.

There were moderate to strong reactions for ATPase and alkaline phosphatase in the stereocilia, but the reactions varied in intensity between animals. The reactions were so intense within the crypts of the epithelial folds (Fig. 11) that it is considered unlikely that the intensity of the reaction was simply due to the stereocilia being concentrated in these regions. ATPase showed a weak uniform reaction throughout the cytoplasm of the principal cells. However, alkaline phosphatase showed a strong reaction around the basement membrane of these cells, and no reaction in the supra-nuclear cytoplasm (Fig. 11).

The principal cells exhibited moderate reactions (less intense than in the other segments) for glucose-6-phosphate dehydrogenase and the two diaphorase enzymes. The glucose-6-phosphate dehydrogenase was distributed uniformly throughout the cytoplasm. However, both the diaphorases showed a slightly more intense band of activity in the juxtaluminal cytoplasm than elsewhere. It was concluded that succinic dehydrogenase activity was not demonstrated within the epithelial cells since the sparse formazan deposit that formed in the presence of substrate was much the same as that observed in sections incubated in the absence of substrate.

Fig. 8. Non-specific esterase activity in the initial segment. × 300.

Fig. 9. Non-specific esterase activity in the middle segment. × 120.

Fig. 10. Non-specific esterase activity in the terminal segment. × 300.

Fig. 11. Alkaline phosphatase activity in the initial segment. × 72.

Fig. 12. Neutral lipid demonstrated with Sudan black in the middle segment. × 35.

Fig. 13. Succinic dehydrogenase activity in the terminal segment. × 550.

Fig. 14. Neutral lipid demonstrated with Sudan black in the cytoplasm of a 'halo cell'. × 1600.

Fig. 15. NADH diaphorase activity in the middle piece of elephant spermatozoa. × 550.

Fig. 16. Glucose-6-phosphate dehydrogenase activity in the terminal segment. × 550.

Fig. 17. Epithelium of terminal segment. A 'halo cell' is shown by arrow. × 750.

Acid phosphatase was demonstrated as a moderately strong, continuous band of activity in the supranuclear region of the principal cells. It was diffuse in the azo dye preparations, but both diffuse and granular in sections prepared by the metal precipitation technique. Non-specific esterase was also present in the supranuclear region, but only as a weak and diffuse reaction (Fig. 8). Both the enzymes exhibited considerable activity in the cytoplasm of the basal cells (Fig. 8).

The periductal smooth muscles in this segment showed greater cytochemical activities for some enzymes than in the other segments. Both glucose-6-phosphate dehydrogenase and alkaline phosphatase showed greater activities than elsewhere, and the intense activity for ATPase was particularly marked.

Middle segment (Figs. 4, 9, 12)

The duct was narrower, the epithelium taller, and there were more basal cells, with more extensive cytoplasm, than in the other segments. However, there were very few epithelial folds and the lumen was densely packed with spermatozoa (Fig. 4).

The stereocilia stained with alcian blue (Fig. 4) indicating the presence of acidic glycoprotein; prior treatment with neuraminidase did not affect the result. A narrow band of diastase-resistant, PAS-positive material was demonstrated at the base of the stereocilia. There was some variation between animals in the content of PAS-positive, diastase-resistant material within the principal cells. In sections from some animals only a diffuse, weak PAS reaction could be observed in the cytoplasm of the apical cells, while in sections from other animals the basal, or supranuclear granular material (similar to that seen in the initial segment) was present in considerable amounts. Cells containing the granular material were usually adjacent to blood capillaries and associated with basal cells; their cytoplasm was packed with granules. Alcian blue-positive granules were also present in some basal cells, usually in association with the PAS-positive granules.

The principal cells were rich in phospholipids and there were localized regions containing large quantities of acetone-soluble, Sudan black-positive, neutral lipid droplets (Fig. 12). These were distributed in discrete groups just above and below the nuclei, and in parts of the apical cytoplasm near the lumen.

There was a weak ATPase activity throughout the principal cells, and a moderate activity in the stereocilia. Alkaline phosphatase activity was mainly demonstrated as a moderate reaction in the infranuclear cytoplasm adjacent to the basement membrane, and a weak reaction in the stereocilia.

Succinic dehydrogenase, glucose-6-phosphate dehydrogenase, and the diaphorase enzymes, all showed strong to intense reactions and similar distributions within the cytoplasm of the principal cells. They all showed a uniform, strong reaction throughout the cytoplasm, and a very intense narrow band of activity adjacent to the basement membrane. Succinic dehydrogenase also showed a supranuclear band of intense activity.

Non-specific esterase and acid phosphatase showed moderate to intense reactions in both the principal and basal cells of the epithelium. In general, the reactions were stronger in the basal cytoplasm. However, the non-specific esterase reaction tended to be most intense in either the infra- or supranuclear cytoplasm (Fig. 9).

Terminal segment (Figs. 6, 10, 13, 14, 16, 17)

The epithelium (Figs. 6, 17) was less tall than elsewhere in the duct, it was considerably folded, and the lumen was wide and packed with spermatozoa. Basal cells

were sparsely distributed and, unlike the basal cells of the more proximal regions, few contained PAS- or alcian blue-positive granules. 'Halo cells' (Reid & Cleland, 1957) were also observed throughout the epithelium and among spermatozoa in the lumen (Figs. 6 (inset), 17). Structurally they resembled lymphocytes, but contained neutral lipid which was demonstrated with Sudan black (Fig. 14).

The stereocilia stained intensely with alcian blue (Fig. 6), indicating the presence of many acid glycoproteins. Neuraminidase failed to affect the alcian blue staining. A pink diffuse staining with PAS was observed in all sections after diastase treatment. However, there was little of the granular material which was observed in the more proximal segments.

Ferric haematoxylin staining was more intense than in the more proximal segments, and there was a low but persistent affinity for the stain in sections stained after solvent extraction. Neutral lipids were observed in the epithelium, mainly as supranuclear accumulations of lipid droplets (Fig. 14).

The alkaline phosphatase reaction in the stereocilia was about the same intensity as that observed in other regions of the epididymis. The ATPase reaction was intense, both among and at the base of the stereocilia (Fig. 7).

The distributions of the diaphorases and glucose-6-phosphate dehydrogenase were much the same as those described for the middle segment, and the diaphorase reactions were equally as intense. The glucose-6-phosphate dehydrogenase (Fig. 16) and succinic dehydrogenase (Fig. 13) reactions were also intense, and stronger than elsewhere in the epididymis. The succinic dehydrogenase reaction was predominantly granular, and mainly in the supranuclear cytoplasm. However, this enzyme and the other three oxido-reductases showed an increased concentration of stain around the basement membrane.

Non-specific esterase was present in most of the principal cells (Fig. 10), and the staining was more intense than elsewhere in the epididymis. Two regions of activity were recognized: a very intense granular staining immediately above and below the nucleus, and a weaker, diffuse reaction in the apical third of the cytoplasm. Acid phosphatase showed a weak cytochemical reaction which was mainly confined to the infranuclear cytoplasm.

DISCUSSION

No significant variation was observed between the histochemical properties of spermatozoa from different parts of the epididymis. The most intense enzyme activities observed were those for non-specific esterase, glucose-6-phosphate dehydrogenase and the diaphorases. The high esterase activity contrasts with that of the other lysosomal enzyme, acid phosphatase, which could not be detected in the spermatozoa. The localization of glucose-6-phosphate dehydrogenase, diaphorase and succinic dehydrogenase in the middle pieces of elephant spermatozoa (Fig. 15) is in agreement with findings in the ram, bull, cat, dog, guinea-pig, mouse, rabbit and man (Edwards & Valentine, 1963; Balogh & Cohen, 1964; Hrudka, 1965; Bolton & Linford, 1970; Mathur, 1971; Jones & Holt, 1974). The difference in reaction intensity between succinic dehydrogenase and glucose-6-phosphate dehydrogenase is not considered to be significant, as it is probably due to differences in the incubation times used.

It is curious that in this study the cytochemical reaction for succinic dehydrogenase in the initial segment of the epididymis was weak, and of about the same magnitude in sections incubated in the presence and absence of substrate. It is unlikely that this

response was due to an absence of succinic dehydrogenase since it has been demonstrated in an analogous region of the epididymis in other species (Holt, unpublished data), and cytological studies of the elephant epididymis have shown that the initial segment has numerous mitochondria (Jones & Brosnan, 1979). Consequently, the histochemical findings must be due either to the mitochondrial membranes in this segment being impermeable to succinate, or to the inactivation of the enzyme by other cytoplasmic constituents (Loomis & Battaile, 1966).

The most obvious and consistent feature of the carbohydrate content of the elephant epididymis was the layer of acidic, alcian blue-positive glycoprotein which covered the luminal surface of the epithelium. The layer was thicker in the distal region of the duct than elsewhere (compare Figs. 2, 4, 6). The only other alcian blue-positive material was that found in some of the carbohydrate-filled basal cells. These findings may indicate that the epididymal glycoproteins are synthesized within the principal cells, although their exact source is still unclear. Some recent evidence indicates that they may be synthesized in the proximal regions of the epididymis and subsequently transported within the duct (Lea, Petrusz & French, 1978; Olson & Hamilton, 1978). Since neuraminidase did not affect the affinity of these glycoproteins for alcian blue they probably contained a mixture of acidic groups, but sialic acid cannot be excluded as a component, it having been reported in epididymal secretions (Prasad, Rajalakshmi, Gupta & Karkun, 1973). This is noteworthy, as sialic acid may be important in sperm maturation (Bedford, 1963, 1973, 1975; Bey, 1965; Nicolson & Yanagimachi, 1972; Mercado & Rosado, 1973).

The PAS-positive inclusions found in the epididymal epithelium are similar to those found (Nicander, 1957, 1958) in the epididymis of the rabbit, ram, bull and stallion. Their identity was discussed by Martan (1969) and Hamilton (1972). However, without further studies it is not possible to decide whether the material was secreted or ingested.

It is noteworthy that the 'halo cells' found in the elephant epididymis differ from those described in other mammals (Reid & Cleland, 1957; Hoffer, Hamilton & Fawcett, 1973; Dym & Romrell, 1975) in that they are found within the duct lumen as well as within the lining epithelium, and because they are rich in neutral lipids. Indeed, the occurrence of lipid indicates that they resemble macrophages more than lymphocytes.

The staining of the epididymal epithelium for phospholipids was most intense in the terminal segment. This finding is in agreement with biochemical work by Darin-Bennett, Morris, Jones & White (1976), who found an increasing concentration of glycerophosphorylcholine in homogenates of tissue samples taken progressively from the proximal to the distal end of the epididymis.

Other workers have reported the presence of alkaline phosphatase in the stereocilia. Nicander (1957, 1958) found it throughout the rabbit and stallion epididymis, and in the head and tail of the bull and ram epididymis. Roussel & Stallcup (1966) demonstrated the enzyme in stereocilia and apical cytoplasm of principal cells throughout the bull epididymis.

These studies showed a distinct difference in the localization of the two hydrolytic enzymes within the principal cells of the epithelium. The strongest reactions for acid phosphatase were found in the two most proximal segments, whereas the strongest reaction for non-specific esterase was in the terminal segment. These results differ from those of Moniem & Glover (1972), who found that non-specific esterase, acid phosphatase, and β-glucuronidase were most active in the middle

segment of rat, rabbit and ram epididymides, and in the proximal part of the terminal segment in the hamster. However, our findings agree with the proposal (Glover & Nicander, 1971) that the occurrence of these enzymes is related to the presence of multivesicular bodies and vacuoles in the epididymal epithelium. Our findings also agree with Moniem & Glover's (1972) report of intense non-specific esterase activity in the basal cells of the initial segment of the rat epididymis. Indeed, our work supports the suggestion (Suzuki & Glover, 1973) that the basal cells are involved in macrophagic and degradative activity.

These and concurrent structural studies (Jones & Brosnan, 1979) confirm the earlier suggestion (Jones, Rowlands & Skinner, 1974) that the elephant epididymis is differentiated along its length to carry out similar functions to those of the epididymis of scrotal mammals. The structural findings show that the elephant epididymis is divided into initial and terminal segments, with a transitional middle zone (the middle segment) which is where sperm maturation mainly occurs. It is proposed that the initial segment is the major region in the epididymis for fluid reabsorption. This is confirmed by the distinct cytochemical reactions for ATPase and alkaline phosphatase in the stereocilia of the initial segment. The strong to intense cytochemical reactions for glucose-6-phosphate dehydrogenase in the initial and middle segments also indicate that they may be sites of anabolic activity. The structural findings also indicate that spermatozoa are stored in the terminal segment ready for ejaculation. The intense reactions for succinic dehydrogenase and non-specific esterase in this segment indicate that it is involved in considerable metabolic activity, which may well be associated with the production of metabolic substrates for the spermatozoa. Further, an active tricarboxylic acid cycle would be expected to lead to the formation of considerable amounts of adenosine triphosphates (ATP) which might be used in the transport of metabolites and ions into and out of the lumen. The presence of such transport activity is indicated by the intense ATPase reaction and moderate alkaline phosphatase activity found along the luminal border of the epithelium in this segment (Fig. 7).

SUMMARY

The three main segments of the elephant epididymis were examined for the occurrence, in the spermatozoa and lining epithelium, of carbohydrates, neutral lipids and phospholipids, ATPase, alkaline phosphatase, succinic dehydrogenase, glucose-6-phosphate dehydrogenase, diaphorases, hydroxysteroid dehydrogenases, acid phosphatase and non-specific esterase.

The most distinct feature of the carbohydrate content of the epididymis was a layer of acidic, alcian blue-positive glycoprotein over the luminal surface of the epithelium, particularly in the terminal segment. PAS-positive, diastase-resistant inclusions were also found throughout the epididymis.

Neutral lipid occurred as droplets above and below the nucleus in the epithelium of the middle segment, and as supranuclear accumulations in the terminal segment.

All the enzymes except the steroid dehydrogenases were detected in the epididymal epithelium, and all except the steroid dehydrogenases and acid phosphatase were detected in the spermatozoa. There was considerable variation in the intensity of the cytochemical reactions in the epithelium, but not in the spermatozoa, in different regions of the epididymis. In general, the enzymes involved in active transport showed strongest reactions in the initial and terminal segments, the reactions in the stereocilia being the most intense. The enzymes involved in energy metabolism showed

strongest reactions in the middle and terminal segments, with the activity being fairly evenly distributed throughout the cytoplasm of the principal cells. However, the two lysosomal enzymes which were studied showed quite different distributions: the reactions for acid phosphatase were strongest in the initial and middle segments, whilst the reactions for non-specific esterase were strongest in the middle and terminal segments. It is suggested that the initial segment is involved in absorptive and anabolic activity, the middle segment in anabolic activity, and the terminal segment (where spermatozoa are stored ready for ejaculation) in considerable metabolic activity and active transport of substrates across the epithelium.

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REFERENCES

BAILLIE, A. H., FERGUSON, M. M. & HART, D. McK. (1966). *Developments in Steroid Histochemistry*. London and New York: Academic Press.

BALOGH, K. & COHEN, R. B. (1964). A cytochemical technic for studying oxidative enzyme systems of mammalian spermatozoa in semen smears. *Fertility and Sterility* **15**, 35–39.

BARKA, T. & ANDERSON, P. J. (1962). Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. *Journal of Histochemistry and Cytochemistry* **10**, 741–753.

BEDFORD, J. M. (1963). Changes in the electrophoretic properties of rabbit spermatozoa during passage through the epididymis. *Nature* **200**, 1178–1180.

BEDFORD, J. M. (1973). Components of sperm maturation in the human epididymis. In *Advances in the Biosciences, 10. The Masculine Gender* (ed. G. Raspe & S. Bernhard). Oxford: Pergamon.

BEDFORD, J. M. (1975). Maturation, transport and fate of spermatozoa in the epididymis. In *Handbook of Physiology*, section 7, vol. 5, *Male Reproductive System* (ed. D. W. Hamilton & R. O. Greep), pp. 303–317. Washington: American Physiological Society.

BEY, E. (1965). The electrophoretic mobility of sperm cells. In *Cell Electrophoresis* (ed. E. J. Ambrose), pp. 142–151. London: Churchill.

BOLTON, A. E. & LINFORD, E. (1970). Presence of the dehydrogenases of the pentose phosphate pathway in boar spermatozoa. *Journal of Reproduction and Fertility* **21**, 353–354.

BURSTONE, M. S. (1961). Histochemical demonstration of phosphatases in frozen sections with naphthol AS-phosphates. *Journal of Histochemistry and Cytochemistry* **9**, 146–153.

CHAYEN, J., BITENSKY, L., BUTCHER, R. G. & POULTER, L. W. (1969). *A Guide to Practical Histochemistry*. Edinburgh: Oliver & Boyd.

DARIN-BENNETT, A., MORRIS, S., JONES, R. C. & WHITE, I. G. (1976). The glycerylphosphorylcholine and phospholipid pattern of the genital duct and spermatozoa of the African elephant, *Loxodonta africana*. *Journal of Reproduction and Fertility* **46**, 506–507.

DYM, M. & ROMRELL, L. J. (1975). Intraepithelial lymphocytes in the male reproductive tract of rats and rhesus monkeys. *Journal of Reproduction and Fertility* **42**, 1–8.

EDWARDS, R. G. & VALENTINE, R. C. (1963). Cytochemical demonstration of succinic dehydrogenase in intact rabbit spermatozoa. *Experimental Cell Research* **31**, 508–516.

ELLEDER, M. & LOJDA, Z. (1973). Studies in lipid histochemistry. XI. New, rapid, simple and selective method for the demonstration of phospholipids. *Histochemie* **36**, 149–166.

GLOVER, T. D. & NICANDER, L. (1971). Some aspects of structure and function in the mammalian epididymis. *Journal of Reproduction and Fertility Suppl.* **13**, 39–50.

HAMILTON, D. W. (1972). Mammalian epididymis. In *Reproductive Biology* (ed. H. Balin & S. Glasser), pp. 268–337. Amsterdam: Excerpta Medica.

HOFFER, A. P., HAMILTON, D. W. & FAWCETT, D. W. (1973). The ultrastructure of the principal cells and intraepithelial leucocytes in the initial segment of the rat epididymis. *Anatomical Record* **175**, 169–202.

HOLT, S. J. (1958). Indigogenic methods for esterases. In *General Cytochemical Methods* (ed. J. F. Danielli), pp. 375–398. New York: Academic Press.

HRUDKA, F. (1965). Cytochemical demonstration of catabolic activity in spermatozoa by the formazan test. *Journal of Reproduction and Fertility* **10**, 15–20.

JONES, R. C. & HOLT, W. V. (1974). The effects of washing on the ultrastructure and cytochemistry of ram spermatozoa. *Journal of Reproduction and Fertility* **41**, 159–167.

JONES, R. C., ROWLANDS, I. W. & SKINNER, J. D. (1974). Spermatozoa in the genital ducts of the African elephant, *Loxodonta africana*. *Journal of Reproduction and Fertility* **41**, 189–192.

JONES, R. C. & BROSNAN, M. (1979). The deferent ducts from the testis of the African elephant, *Loxodonta africana*. 1. Structural differentiation. (In preparation.)

LAKE, B. D. (1966). The histochemistry of phosphatases: the use of lead acetate instead of lead nitrate. *Journal of the Royal Microscopical Society* **85**, 73–75.

LEA, O. A., PETRUSZ, P. & FRENCH, F. S. (1978). Purification and localization of acidic epididymal glycoprotein (AEG): a sperm coating protein secreted by the rat epididymis. *International Journal of Andrology, Suppl.* **2**, 592–607.

LOOMIS, W. D. & BATTAILE, J. (1966). Plant phenolic compounds and the isolation of plant enzymes. *Phytochemistry* **5**, 423–438.

MARTAN, J. (1969). Epididymal histochemistry and physiology. *Biology of Reproduction* **1**, 134–154.

MATHUR, R. S. (1971). Histo-enzymological observations on spermatozoa of inbred strains of mice. *Journal of Reproduction and Fertility* **27**, 5–11.

MERCADO, E. & ROSADO, A. (1973). Structural properties of the membrane of intact human spermatozoa. A study with fluorescent probes. *Biochimica et biophysica acta* **298**, 639–652.

MONIEM, K. A. & GLOVER, T. D. (1972). Alkaline phosphatase in the cytoplasmic droplet of mammalian spermatozoa. *Journal of Reproduction and Fertility* **29**, 65–69.

NICANDER, L. (1957). On the regional histology and cytochemistry of the ductus epididymidis in the rabbit. *Acta morphologica neerlando-scandinavica* **1**, 99–118.

NICANDER, L. (1958). Studies on the regional histology and cytochemistry of the ductus epididymidis in stallions, rams and bulls. *Acta morphologica neerlando-scandinavica* **1**, 337–362.

NICANDER, L. & GLOVER, T. D. (1973). Regional histology and fine structure of the epididymal duct in the golden hamster (*Mesocricetus auratus*). *Journal of Anatomy* **114**, 347–364.

NICOLSON, G. L. & YANAGIMACHI, R. (1972). Terminal saccharides on sperm plasma membranes: identification by specific agglutinins. *Science* **177**, 276–279.

OLSON, G. E. & HAMILTON, D. W. (1978). Characterization of the surface glycoproteins of rat spermatozoa. *Biology of Reproduction* **19**, 26–35.

PEARSE, A. G. E. (1968). *Histochemistry, Theoretical and Applied*, vol. I. London: Churchill.

PRASAD, M. R. N., RAJALAKSHMI, M., GUPTA, G. & KARKUN, T. (1973). Control of epididymal function. *Journal of Reproduction and Fertility Suppl.* **18**, 215–222.

REID, B. L. & CLELAND, K. W. (1957). Structure and function of the epididymis. 1. The histology of the rat epididymis. *Australian Journal of Zoology* **5**, 223–246.

ROUSSEL, J. D. & STALLCUP, O. T. (1966). Histochemical study of the development of alkaline phosphatase in the testicle and epididymis of young bovine males. *International Journal of Fertility* **11**, 215–225.

SHORT, R. V., MANN, T. & HAY, M. F. (1967). Male reproductive organs of the African elephant, *Loxodonta africana*. *Journal of Reproduction and Fertility* **13**, 517–536.

SUZUKI, F. & GLOVER, T. D. (1973). The effect of castration on the epididymal epithelium of the golden hamster (*Mesocricetus auratus*). *Journal of Reproduction and Fertility* **35**, 584–585.

WACHSTEIN, M., MEISEL, E. & NIEDZWIEDZ, A. (1960). Histochemical demonstration of mitochondrial adenosine triphosphatase with the lead-adenosine triphosphate technique. *Journal of Histochemistry and Cytochemistry* **8**, 387–388.