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HISTOLOGICAL DIFFERENTIATION ALONG TURTLE DUCTUS EPIDIDYMIDIS, WITH A NOTE ON SECRETION OF SEMINAL PROTEINS AS DISCRETE GRANULES

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SUMMARY

Histological analysis of the male reproductive tract of the peninsular flap-shelled turtle *Lissemys p. punctata* revealed that several minute ductuli efferentes reach the epididymis to form into a large thin – walled duct which probably, forms a temporary storage region of sperm arriving from the testis. Originating from this duct, the single long ductus epididymidis takes a highly tortuous course when it differentiates along its length, in terms of diameter and epithelial organization, into four regions, viz., the initial segment, caput, corpus and cauda. Turtle ductus epididymidis differs from that of lizards in the extensive pattern of folding of the epithelium of caput, corpus and cauda regions. The differentiation along the ductus epididymidis of the turtle signifies different functional attributes to the different regions. The epithelium of the initial segment of the turtle epididymis secretes large glycoprotein granules (4-8 µm) and that of the caput secretes minute granules (1-2 µm). The large granules possess a central core and a peripheral coat, whereas the minute granules are uniformly dense. Thus, turtle ductus epididymidis differentiates into initial segment, caput, corpus and cauda regions, and the initial segment and caput secrete seminal proteins in the lacertilian pattern as discrete granules.

Key words: Epididymis; glycoprotein granules; seminal protein

INTRODUCTION

The mammalian ductus epididymidis differentiates into initial segment, caput, corpus and cauda, distinguishable in terms of tubule morphology and histology. Each region has unique functional significance in relation to maturation of sperm (1,2). However, information on the epididymis of reptiles, the first of the amniotes, is scanty. Anatomical descriptions of reptilian epididymis are far too limited (2,3) and such descriptions are incidental to studies on aspects

of reproductive physiology. In connection with the histochemical localization of hydoxysteroid dehydrogenases in the epididymis of the lizard *Psammophilus dorsalis*, it was proposed that the epididymis is tentatively distinguishable into regions comparable to mammalian caput, corpus and cauda (4). In a study on the initiation of sperm motility in the lizard *Lacerta vivipara*, it was reported that its epididymis is histologically distinguishable into these three regions (5); however, according to Mesure *et al.* (6), the epididymal duct of this lizard is rather uniform throughout its length, but the height of the epithelium decreases from head to cauda rendering it possible to distinguish caput, proximal corpus, distal corpus and cauda regions. When analysing the influence of epididymal duct fluid on motility of spermatozoa in the lizard *Hemidactylus flaviviridis*, it was found that fluid from the most posterior part is the most potent (7). However, the epididymis of the lizard *Calotes versicolor* does not clearly demarcate into regions as above, but the duct along its length differentiates into five zones, named tentatively as E1 – E5, and in description these regions are comparable to initial segment, proximal caput, distal caput, corpus and cauda (8). Therefore, there is pertinent need to look into the anatomy and histomorphology of the ductus epididymidis of several more representative species of reptiles.

Further, though several reports had suggested secretion of some granules by the reptilian epididymis (9-16), it was proved unequivocally only in the lizard (17-19). The granules thus secreted appear to differ among different lizards (19) and the secretion is confined to the caput epididymidis (18). Small granules were found in the lumen of the epididymal duct of the turtle *Emys orbicularis*, but the cellular origin of the granules was at the ductuli efferentes (10). The present paper reports differentiation of the ductus epididymidis of the peninsular flap – shelled turtle *Lissemys p. punctata* into regions comparable to the initial segment, caput, corpus and cauda of the mammals, and secretory granules comparable to those in *L. vivipara* (17) and *C. versicolor* (18).

MATERIALS AND METHODS

The turtles were collected from the ponds during the month of September, the breeding phase (15), and brought to the laboratory in storage tanks and fed *ad libitum* with small fish and frog. Adult turtles weighing 750 g and above and measuring 20 cm and more of carapace length were used for the study. Male turtles were identified from the pointed tail; identification was made under mild ether anaesthesia.

Turtles were sacrificed applying vapour of chloroform. The reproductive system was dissected, and testis and the entire male tract were removed, blotted free of mucous and blood, rinsed in physiological saline (0.9% sodium chloride) and fixed for 24 hr in Hollande-Bouin fixative. The material was paraffin – embedded and serial frontal (whose epididymis) or transverse sections were obtained at 6 µm thickness. Sections were stained in Delafield's haematoxylin, with eosin as the counterstain, and mounted in DPX mountant (20). Diameter of the duct, height of the epithelium and diameter of nuclei of the latter were measured using

a calibrated ocular micrometer (for each ductal region, atleast 50 measurements were made, in the organ collected from 10 turtles). The quantitative parameters were used to calculate the mean and the standard deviation.

Also, the sections were scanned critically to localise granules in the intracellular as well as luminal disposition. Granules, when localized, were measured using a calibrated ocular micrometer. Slices of the epididymis were fixed in neutral buffered – formalin and paraffin sections were tested histochemically for proteins (mercuric bromophenol blue, ninhydrin-Schiff, performic acid-Schiff methods), carbohydrates (periodic acid – Schiff, toluidine blue O methods) and lipids (Sudan black B method), adopting appropriate controls (21).

RESULTS

Differentiation along the ductus epididymidis

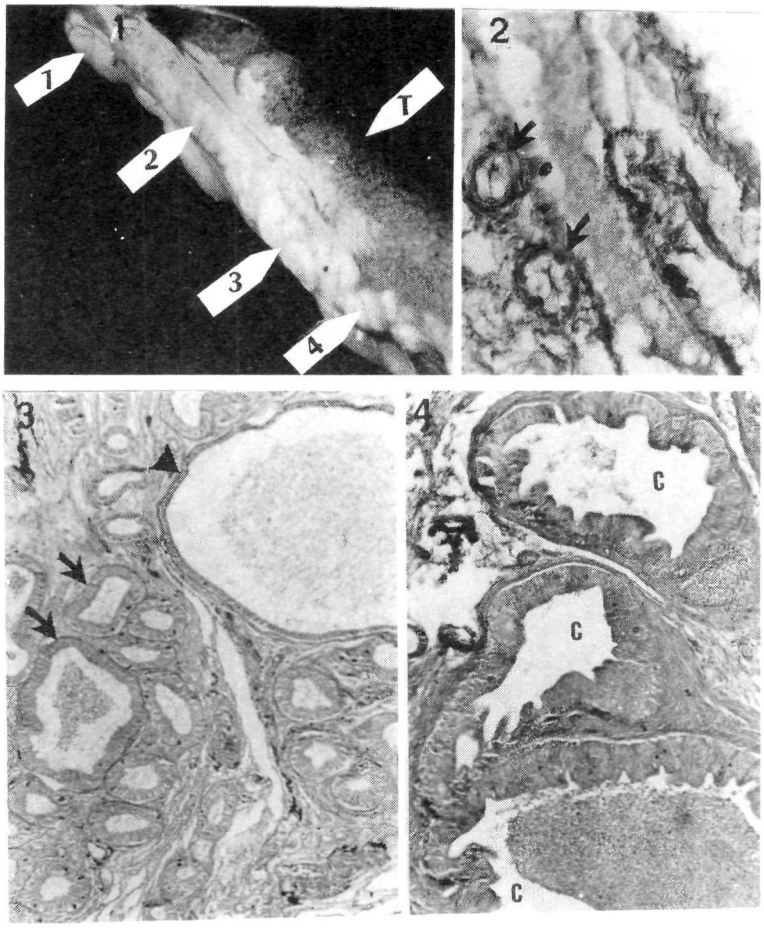
In the epididymis of *Lissemys p. punctata*, the duct is visible through the capsule; in the anterior part the duct is translucent, in the middle part blood-tinged and in the posterior part milky white (Fig. 1).

Critical screening of the transverse sections of the epididymis at different levels revealed the presence of ductuli efferentes (Fig. 2; Table 1) as narrow tubules lined by a single layer of cuboidal epithelium, surrounding a lumen. The epithelium of the ductuli possesses tall microvilli which almost meet at the centre of the lumen.

A large, thin-walled duct is seen in the sections of the anterior part of the organ (Fig. 3). It is aligned towards the face of the organ closer to the testis. It is lined by short cuboidal epithelium (Table 1) possessing short microvilli. The luminal content is exclusively sperm; granules are totally absent.

Table : 1 Measurments of the tubule diameter, epithelial height and epithelial nuclear diameter along the epididymal duct of *Lissemys p.punctata* (values are mean ±SD of 50 measurements each made from sections of the epididymis of five turtles)

Tubular region	Tubule diameter (µm)	Epithelial height (µm)	Nuclear diameter (µm)
Thin-walled duct	463.6 ± 10.8	8.6 ± 1.9	3.1 ± 0.6
Initial segment	128.3 ± 7.3	25.2 ± 2.2	7.13 ± 0.5
Caput	752.4 ± 16.3	51.1 ± 8.5	5.2 ± 0.3
Corpus	572.3 ± 12.5	37.9 ± 9.7	5.0 ± 0.5
Cauda	1074.6 ± 21.7	16.2 ± 2.3	3.4 ± 0.3.



Figures 1 - 4 : History of the Testis and epididymis of *Lissemys p. puncata*

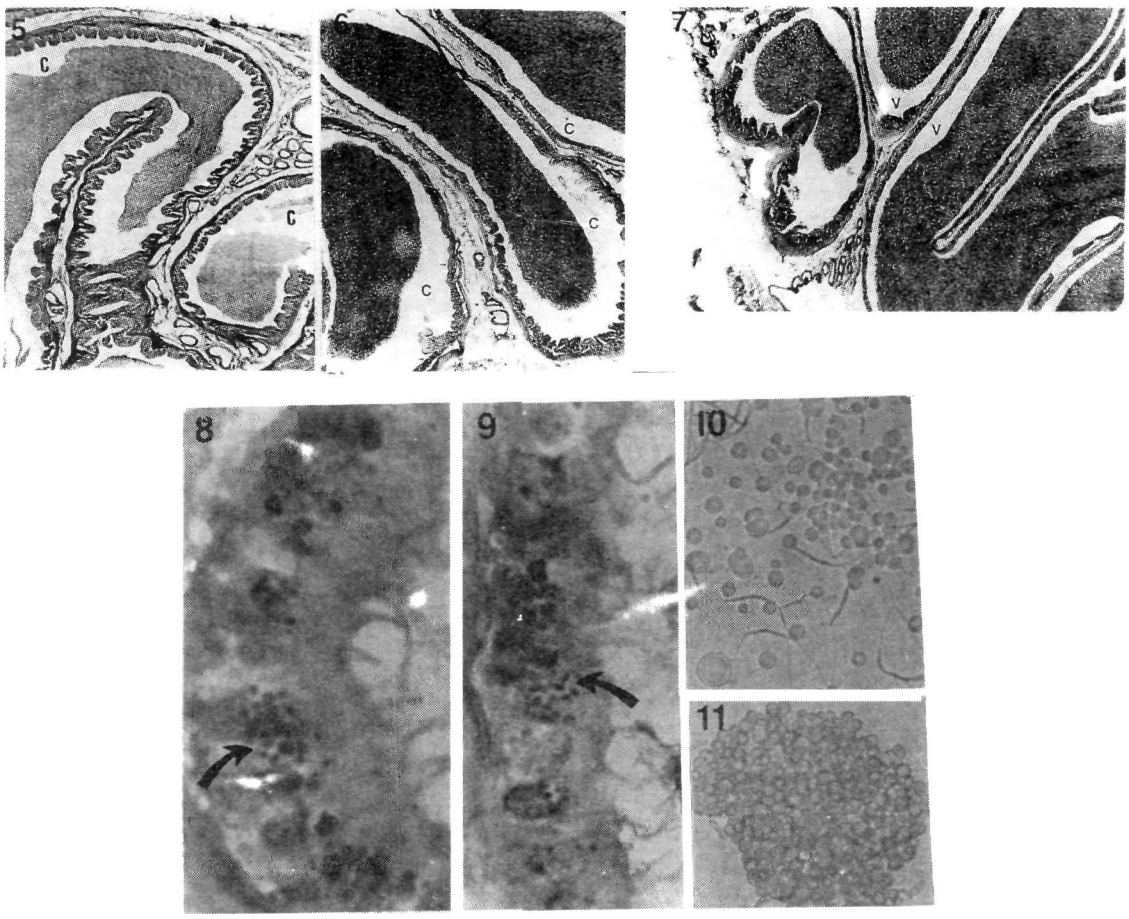
Fig. 1. Differentiation along the epididymis (whole mount) x 4

T-testis; 1-4-epididymis. 1. Transluscent; 2. Blood-tinged; 3. Milky white; 4. Vas deferens.

Fig. 2. Section through the epididymis of *Lissemys p. punctata* showing ductuli efferentes in T.S. (arrow) and L.S. (h & e) x 200.

Fig. 3. Section through the epididymis of *Lissemys p. punctata* showing the large thin-walled duct (arrow-head) surrounded by sections of the initial segment (arrows). (h & e) x 100.

Fig. 4. Section through the caput epididymidis (C) of *Lissemys p. punctata.* (h & e) x 50.



Figures 5 - 11 : History of the epididymis of *L. p. punctata*

Fig. 5. **Section through the corpus epididymidis (C) of *Lissemys p. punctata*.** (h & e) x 50.

Fig. 6. **Section through the cauda epididymidis (C) of *Lissemys p. punctata*.** (h & e) x 50.

Fig. 7. **Section through the deferens (V) of *Lissemys p. punctata*.** (h & e) x 50.

Fig. 8. **Section of the epididymal duct of *Lissemys p. punctata* at the initial segment magnified showing the secretory granules of the large variety (arrow).** (h & e) x 1000.

Fig. 9. **Section of the duct at caput epididymidis magnified showing the secretory granules of the smaller variety(arrow).** (h & e) x 1000.

Fig. 10. **Biphasic appearance of the granules of the larger variety of the granules.** (h & e) x 1000.

Fig. 11. **Positive reaction for lipid around the periphery of the granules.** (Sudan black B) x 1000.

The ductus epididymidis, after its origin, becomes highly coiled and contorted along the length of the epididymis when it differentiates in terms of diameter as well as histoarchitecture into four regions, hereafter referred to as the initial segment (IS), caput, corpus and cauda. These regions were seen mixed along the whole organ, though the ductuli, large thin-walled duct, IS and caput were concentrated at the anterior part, corpus at the middle part and cauda at the posterior part; however, excepting for cauda no region is exclusive of any one of the tubular regions.

At the IS the duct (Fig. 3; Table 1) possesses tall columnar epithelium. The cells possess fairly tall microvilli; sperm and spherical granules are present in the lumen, but suspended by the microvilli.

At the caput, the duct (Fig. 4) measures a diameter larger than at the initial segment (Table 1). The epithelium is also taller than at the IS. The epithelium is not of uniform in height, and thrown into folds and crypts (such a pattern is retained at the corpus also, but the height of the folds is reduced) and measures a height of 55 to 60 µm at the tallest points. The nuclei of the epithelium are almost of uniform size, irrespective of the height of the epithelium. Microvilli are extremely short. The lumen contains sperm in admixture with strongly acidophilic small and large spherical granules.

At the corpus, the duct (Fig. 5) is smaller than at the caput with the epithelium as at the caput, but shorter (Table 1). The nuclei are spherical. Microvilli as well as luminal content are as at the caput.

At the cauda, the duct (Fig. 6) is the widest (Table 1). The epithelium is short cuboidal, almost flat, and uniform along the luminal profile; folds and crypts are less prominent and confluent. Microvilli appear to be almost absent. The spacious lumen is fully packed with semen, with either homogenous or particulate appearance. The duct at the cauda continues posteriorly at the ductus deferens (Fig. 7).

Glycoprotein granules

The epididymis of *Lissemys p. punctata*, during the period of study, contained spherical granules in the epithelium as well as lumen. There were two kinds of granules, viz., large (4-8 µm) and minute granules (1-2 µm). The epithelium of the IS, possessing large nuclei was seen to secrete the larger variety (Fig. 8) and the epithelium at caput epididymidis, possessing smaller nuclei, to secrete the smaller variety (Fig. 9). While in the IS the large granules were oriented around the nucleus, in the caput the minute granules were definitely supranuclear. Further, in the former the granules appeared to increase in diameter from the base of the epithelium towards the apex, whereas in the latter the granules were of uniform size and did not appear to show any topographical variation.

The large granules showed a biphasic appearance, containing a dense coat and a less dense core (Fig. 10). Such a distinction was not evident in the smaller variety. The granules were abundant and densely mixed with sperm in the duct from caput epididymidis onwards.

The granules (both the varieties) reacted strongly for protein and carbohydrate, the intensity of the reaction in the larger granules differing in relation to the peripheral coat and the central core. Test for lipid showed positive reaction only around the periphery (outer boundary) of granules (Fig. 11).

DISCUSSION

Differentiation along the ductus epididymis

In reptiles, the thin-walled duct into which ductuli efferentes lead has been reported earlier in the lizard *C. versicolor* alone (8). The terminal ends of the efferent ducts of rat form into a single duct which in turn leads into the ductus epididymidis (22). It could be conceived that in view of the epididymal processing of reptilian sperm in the ductus epididymidis (5, 7, 8, 18), the large thin-walled duct would form a repository of the sperm arriving from the testis, for release towards the epididymal duct in a manner that would provide the quantum of sperm the ductus epididymis would process.

Through three regions were recognised in the epididymis of the lizard *P. dorsalis*, based on the activity of certain enzymes (4), in a view it was categorically stated that reptilian epididymis does not distinguish into the mammalian pattern (15). However, four regions were identified in the epididymis of *L. vivipara, viz.,* caput, proximal corpus, distal corpus and cauda, but the duct was of uniform diameter along its entire length, with only the epithelium showing some degree of differentiation (7). Thus, the pattern of differentiation of the turtle epididymal duct into four discrete regions agrees with the situation in *C. versicolor*, where it increases in size from head to cauda (8); however, in *Lissemys p. punctata*, the duct from caput onwards is larger than in lizard. As regards the epithelium, the pattern appears in general to be common for the turtle and the lizard as far as the IS is concerned; whereas in the lizard the corpus region has the tallest epithelium, in the turtle it is the caput region. The folding of the epithelium at caput, corpus and cauda regions appears to be unique to the turtle, as it has not been reported in the snakes and lizards (15). The differentiation of the epididymal duct in terms of size as well as epithelial height signifies different functional attributes to each of the regions, which remain to be established. The elaborate pattern of folding of the epithelium of the epididymal duct of the turtle caput and corpus regions compare with such folds in the intestinal tract, ureter and vas deferens of mammals, where the folding is meant to increase the surface area. It is generally known that chelonians have a post-nuptial spermatogenesis in which spermatogenesis occurs immediately after the mating season and the sperm, thus produced, are stored for a considerable length of time until the next mating season (23-25). Therefore, the folding pattern of the epithelium in the turtle epididymis is probably meant to store and sustain the sperm for a longer period.

Secretion of glycorprotein granules

In an earlier study, directed towards finding secretion of granules in chelonian epididymis, occurrence of Sudan black B-positive granules in the epididymal canal of *E. orbicularis* was reported (10). Sudan black B-positive granules were noticed in the epididymis of the tortoise *Testudo hermanni hermanni*, and were presumed as lipid droplets (24). In the present study also, the granules reacted around the periphery positively with Sudan black B, suggesting that the granules noticed in *Testudo h. hermanni* (24) were the same as in the present study and that they are not lipid droplets, but only the secretion granules. The granules in these chelonians were suggested or originating from the ductuli efferentes. Actually, ductuli efferentes are tubules smaller than the ductus epididymidis (26). The present study provides direct evidence for the secretion of these granules by the epithelium of the IS and caput of the ductus epididymidis. It is highly probable that the tubule they dealt with in *Testudo h. hermanni* was actually the IS, but wrongly identifies as the ductuli efferentes.

Secretion of two types of granules has so far reported in only one instance, viz., the lizard *C. versicolor* (18). Dufaure and Saint Girons (19) suggested that the granules are produced in the epithelium as minute ones and fuse in the lumen to form larger granules, but the authors failed to trace the cellular origin of the larger granules, and noticed only the smaller granules secreted by the epithelium of the posterior parts of the ductus epididymidis. Apparently, snake epididymis does not secrete seminal proteins as discrete granules (19).

Histochemical characterization of the granules reveals them to be rich in glycoproteins. Positive reaction for lipid around the periphery of the granules suggests them as membrane bound, membrane being lipoproteinaceous, and that the content does not contain lipid (18). In a preliminary observation of the histochemical nature of the epididymal granules of *L. vivipara*, it was reported that they contain acid mucopolysaccharide (17, 27). Lectin-binding studies revealed the presence of carbohydrate moieties in the granules of this lizard (28). Biochemical analyses of the granules of *L. vivipara* (17, 27-30) and *C. versicolor* (26) show them to be rich in proteins and glycoproteins. Glycoproteinaceous nature of the epididymal secretion has been reported in mammals (2), but it is never in the form of granules comparable to those in reptiles. The significance of the epididymal secretion of the seminal proteins as glycoproteins in the form of discrete granules in reptiles, as against the solubilized form in which they are secreted in mammals, is intriguing; the adaptive significance of this condition is under investigation.

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