CHAPTER VII

A HISTOLOGICAL AND HISTOCHEMICAL STUDY ON THE RETE TESTIS OF SOME SEASONAL MAMMALS

The seminiferous tubules in the mammalian testis are connected with the efferent ducts by the rete testis, which is known to develop from the anterior part of the mesonephros or the Wolffian body during embryonic life (Wiedersheim, 1897; Romer, 1950; Arey, 1954). Allen (1904), who studied the development of the gonads of the pig and the rabbit, observed that the rete tissue first develops at the anterior pole of the developing testis and later grows rapidly beneath the testis over half its lenght so as to occupy space between the developing seminiferous tubules. He also described that the boundaries of the anastomosing rete elements form a single cavernous mass from which the tubuli recti pass out toward the proximal ends of the seminiferous tubules.

The rete testis has been regarded merely as an anatomical structure serving as a passage for the spermatozoa from the testis to the excurrent duct system and no other function has been attributed to it. Marin-Padilla (1964) suggested that this organ might serve some function other than just as a "transit structure for mature spermatozoa".

During the course of the histochemical investigations on the testes of some mammals, it was observed that the

distinct cavernous tissue in the mediastinum testis appears to be a characteristic feature of the seasonal mammals. It was, therefore, thought desirable to investigate the functional significance of the rete testis, other than that of merely being a passage for the spermatozoa, in seasonal mammals. As a prerequisite to such an understanding, the histology of the rete testis with reference to certain seasonal mammals, was reinvestigated. This was followed by histochemical observations with a view to throw some light on its functional aspects.

MATERIAL AND METHODS

Detailed observations were made on the rete testis of adult male rabbits and for comparison the following seasonal mammals were also studied : cat (*Felis domesticus*), dog (*Canis familiaris*), sheep (*Ovis sp.*), bull (*Bos indicus*), Indian palm squirrel (*Funambulus palmarum*) and the rhesus monkey (*Macacus rhesus*).

The testes of the sheep and bull were obtained from the slaughter house. The glands were taken out within 20 minutes after slaughter of the animals and were brought to the laboratory, in an ice container, within an hour. The testes from the cat, dog, rabbit and monkey were removed under anaesthesia, and that of the squirrel from animals freshly killed by decapitation. In every case the glands were quickly frozen and thin slices were cut out, which included the mediastinum, and were fixed in various fixatives maintained at $4^{\circ}C$.

For histological observations the tissue was fixed in Bouin's fluid for 48 hours, washed, dehydrated with graded series of ethanol, cleared in xylene, and embedded in paraffin wax. 5 μ thick sections were cut and stained with haematoxylin and eosin. To study the distribution of lipids, Baker's calcium chloride- formalin mixture was used as the fixative and after required period of fixation and washing, the tissue was embedded in gelatin. 20 μ thick sections were cut on a freezing microtome and stained with Sudan black B by the method described by Pearse (1960). Lipase activity was demonstrated by Gomori's "Tween" method; as modified by George and Iype (1960), using "Tween 85" as the substrate, which has been shown to be a more specific substrate for lipase (George and Ambadkar, 1963). Acid- and alkaline phosphatase activities were studied in the tissues fixed in cold 10% neutral formalin for 16 hours, and processed by Gomori's techniques (Pearse, 1960). For the study of the localization of the Golgi bodies the tissue was fixed <u>in situ</u> by vascular perfusion with neutral formalin containing cadmium chloride. After perfusing for 20 minutes the testes were dissected out and cut into pieces to be fixed again for 10 hours in formalin-cadmium chloride fixative. The tissue was impregnated with silver by Ayoma's method (Baker, 1960), embedded in paraffin wax and 7 μ thick sections were cut. Some of the sections were examined after deparaffinization while others were differentiated with gold chloride before examination.

RESULTS

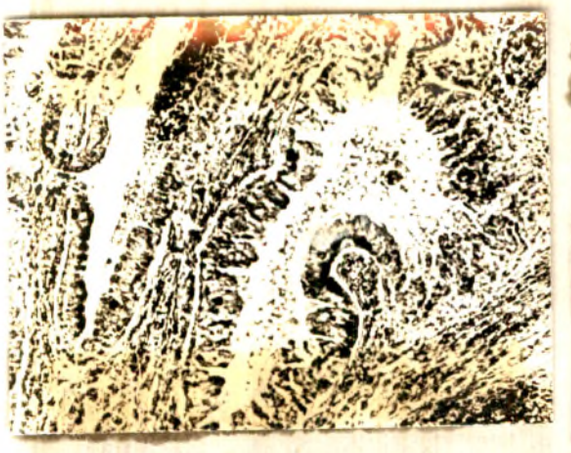
Histological :

The mediastinal rete testis form a single wide lumen in the central region of the rabbit testis. From the walls of the lumen many digitate processes were observed to extend into the lumen. These processes possessed a compact core of connective tissue over which the epithelium of the lumen was continuous. The epithelial cells were cuboidal in shape and possessed prominent nuclei showing scattered granular chromatin material. The nucleoli were also observed in some nuclei but not in all. The cytoplasm was more or less hyaline in nature. The tubuli recti were found to open into the lumen of the rete testis but the spermatozoa were rarely observed in the lumen (Figs. 1 & 2).

The rete testis of the squirrel was found to be similar to that of the rabbit. Cat, dog, sheep and monkey were found to possess a rete testis consisting of many narrow rete tubules forming a net-work,as is generally described in the text-books on histology. The rete testis of the bull, which occupied a considerable area of the mediastinal substance of the testis, was found to be very much different in structure, from the rest of the mammals studied. The wall of the lumen in the bull rete testis was found to extend into profusely branching folds projecting into the lumen. These folds are covered with large epithelial cells possessing large nuclei. The mediastinal rete tissue was found to be abundantly supplied with numerous blood vessels. Several

1 40×

2 40×



3 35×

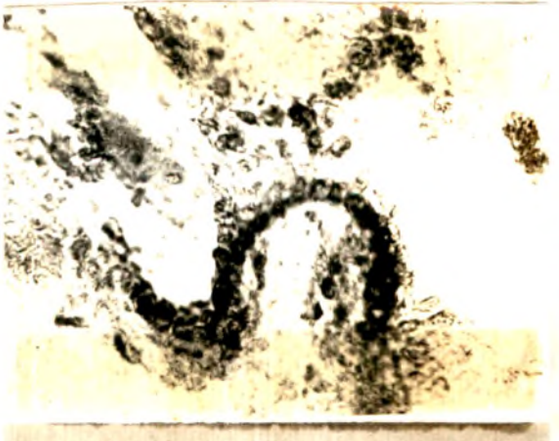
4 35×

Figs. 1 & 2. Sections of the rabbit testis passing through rete testis showing the tubuli recti opening into the central large lumen of the rete. Haematoxylin-eosin.

Figs. 3 & 4. Sections of the bull rete testis showing the lipids.

5 10×

6 10×

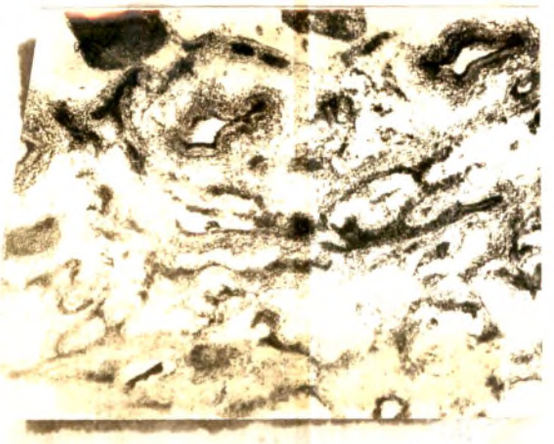


7 10×

8 35×

Photomicrographs of the sections of the rete testis of the rabbit.

Fig. 5. Showing lipase activity.
Fig. 6. Showing alkaline phosphatase activity.
Fig. 7. Showing acid phosphatase activity.
Fig. 8. Showing the localization of Golgi elements.



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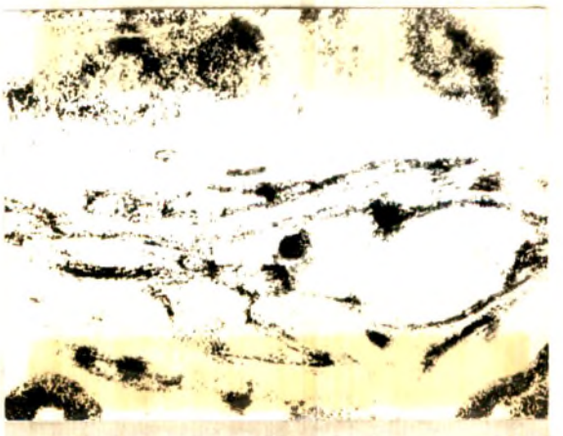
10 10×

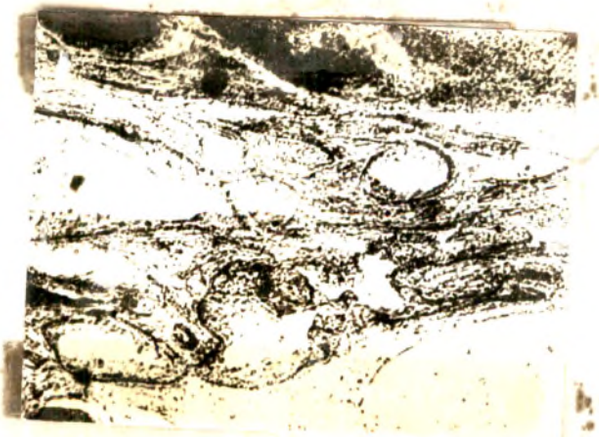


11 10×

12 10×

Fig. 9. Section of the rete testis of the squirrel showing Golgi elements.

Figs. 10 to 12. Sections of the rete testes of cat, dog, sheep and the monkey showing lipase activity.



13 10×

14 10×

Figs. 13 & 14. Sections of the rete testes of squirrel and monkey showing lipase activity.

pigmented globules were found in the lumen of the rete testis.

Histochemical :

The cells lining the rete testis of the mammals under investigation, possessed strong sudanophilic granules and globules in the cytoplasm. The space in between the branching folds of the epithelium of the bull rete testis were found to be filled with many sudanophilic globules (Figs. 3 & 4). The cytoplasm of these cells possessed considerable lipase activity which presented a granular pattern (Fig. 5). Alkaline glycerophosphatase activity was very weak but acid phosphatase activity was found to be very high, and both the enzymes were localized in the cytoplasm (Figs. 6 & 7). The Golgi elements were demonstrated only in the rabbit and squirrel testes. These were found to be present as comparatively small bodies scattered in the cytoplasm, whereas, those in the apical region in the rabbit rete testis appeared aggregated together.

DISCUSSION

The rete testis of the bull, in particular, has a structural plan highly indicative of its being glandular in nature. The profusely branching folds of the epithelium of the rete testis, is perhaps a secondary modification providing a greater surface for secretion. Again, the copious blood supply to this organ further supports the possibility of its having a secretory function. Here it may be mentioned that Harrison (1949-50), in his comparative anatomical studies on blood supply of the mammalian testis, had shown that, in contrast

to other mammals studied by him, the rete of the bull testis is very well supplied with numerous centrifugally and centripetally running blood vessels. This was also observed in the present investigation. The central large lumen and the digitate processes in the rabbit rete testis also appear to be glandular. In this respect both the rabbit and bull rete testes are very similar.

Mason and Shaver (1952), on the basis of their observations on the caput epididymis of the hamster, suggested that the rete testis, ductuli efferentes and proximal ductus epididymis jointly absorb the excess fluid and the extraneous material coming along with the sperms. They (1952) also ascribed a function of restoring the energy-rich substances that might come into the ducts along with the testicular fluid, to the rete testis, ductuli efferentes and the proximal part of the ductus epididymis.

The fact that the cells lining the rete testis are rich in lipids and lipase suggests that the cells are capable of actively metabolizing lipids. The high acid phosphatase activity is indicative of changes in the phosphate pool. In the light of these observations it clear that the cells of the rete testis are metabolically very active. The lipid material present in the lumen of the rete testis may be regarded a secretion product of its cells. If this is true, a secretory function can be attributed to this organ.