CHAPTER VI

CERTAIN OBSERVATIONS ON A MODIFIED REGION OF CAUDA EPIDIDYMIS OF THE RABBIT

The mammalian spermatozoa attain motility and fertilizing capacity as they pass down the epididymal tract. The epididymis is divisible histophysiologically into several zones (vide chapter II). It has been shown that the proximal part of the epididymal tract is absorptive in function (Mason and Shaver, 1952), and both caput and cauda epididymis synthesize a phospholipid viz. - glycerylphosphorylcholine (GPC) (Martan and Risley, 1963; Scott et al., 1963) which is believed to be utilized as fuel by the spermatozoa in the epididymis of the ram (Dawson and Scott, 1964). In the light of these observations and also those discussed in chapter II of this thesis, the histochemical investigations on the epididymides in some mammals was undertaken. In the rabbit epididymis, it was observed that at the posterior pole of the cauda epididymis there is a modified region of the duct system. The diameter of this part was found to be 10 to 12 times wider than that of the epididymal duct proper, and its inner epithelial lining was observed to be produced into many branching and anastomosing folds. This stricking difference in structure, as far as the author is aware, has not been noted by the earlier investigators and hence the justification for the present study.

In order to obtain a clear picture of the structural

characteristics of the modified region the entire epididymal tract of the rabbit was studied. Certain histochemical observations on this modified region were also made.

MATERIAL AND METHODS

Adult healthy male rabbits were selected from a well maintained laboratory colony. The animals were dissected under ether anaesthesia and the epididymides were removed, free of adipose tissue, and were frozen quickly to -10°C. The epididymides were divided into the following four regions - proximal caput (E_1), distal caput (E2), proximal cauda (E3), and distal cauda (E4) as shown in text figure 1. Thin slices of frozen tissue were immersed in chilled fixatives, which were slowly brought to room temperature wherever necessary. For histological examination, the tissue was fixed in Bouin's fixative for 48 hours at room temperature. After the required period of fixation the tissue was embedded in paraffin. The sections were cut at 10 µ and were stained with haematoxylin and eosine.

Sections were processed for histochemical examination of lipids, lipase, alkaline- and acid-phosphatases by the methods given in Chapter I.

In order to study the Golgi bodies, the tissue was fixed by vascular perfusion <u>in situ</u> with formalin containing cadmium chloride, for 20 minutes and then the tissue was removed from the body. After this the tissue was cut into thin

slices, which were fixed again for 12 hours in the fixative used for perfusion. The tissue was embedded in paraffin after impregnation with silver, by Ayoma's method as described by Baker (1960). A few sections were mounted in Canada balsam after clearing in xylene, while others were mounted after differentiating with gold chloride.

RESULTS

HISTOLOGICAL :

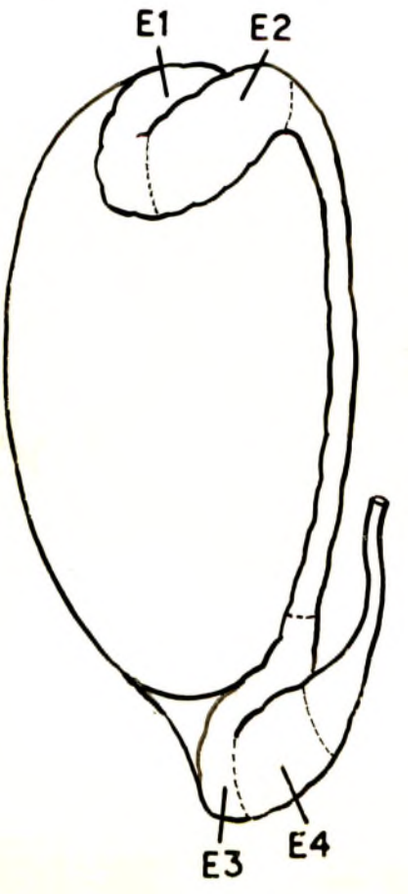
Comparative histological observations on the various regions of the epididymal tract of the rabbit are presented in Table I. The values in the table give the diameter of the tubules in cross sections, the height of the epithelial cells, and the diameter of the nuclei along their longer axes. From the table it is seen that as the diameter of the tubules increases the height of the epithelial cells decreases and the diameter of the nuclei is increased with increase in nuclear size. In proportion to the height of the epithelial cells, the nuclei of the E3 region are the largest. This is clearly observable in the epithelial cells of the modified region of the epididymal tract (Fig. 1). It should, however, be noted that along with this modified region, ducts with the usual diameter and non-folding epithelial lining, are also present side by side (Fig. 2). The modified region was observed to extend also in the E4 region. This region did not represent the beginning of the vas deferens but was a part of the epididymis itself.

TABLE 1

Showing the diameter of the tubules, height of the epithelial cells and nuclear diameter in the four different regions of the epididymis of the rabbit

Region	E1	E2	E3	E4
Diameter of duct	140 μ (95 -185)	175 μ (130 -230)	2450 μ (1500 -3000)	460 μ (305 -670)
Height of epithelial cells	40 μ (30 -60)	44 μ (36 -50)	21 μ (20 -22)	28 μ (18 -30)
Nuclear diameter	8 μ (5-12)	9.75 μ (7.5-12.5)	9 μ (7.5-10)	9.25 μ (7.5-12.5)

Figures in paranthesis denote the range.



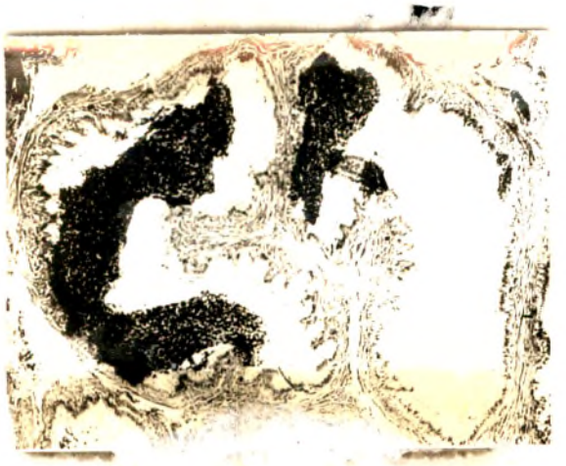
Text-figure 1. Showing the four different regions of the rabbit epididymis - E1 to E4.

The tendency to form folds in the lining of the epididymal tract was seen to begin in the E3 region and it continued down the region with more complication of branching. Trabaculae of connective tissue were observed to extend into the lumen and these were covered with epithelial cells (Fig.3). Such folds were found to run across the lumen to meet with the similar folds extending from the opposite side. The folds also were seen to branch and anastomose.

The histological features of the four regions are given below.

E1 : The diameter of the lumen varies between 95 to 185 μ. The height of the columnar epithelial cells is maximum in this region and varies from 30 to 60 μ. The diameter of the nuclei was 8 μ on an average along their longer axes. The diameter of the nucleus was approximately 1/5 of the height of the epithelial cells. Nuclei were oval in shape and stained homogenously, and were situated very close to the basal parts of the epithelial cells with their longer axes parallel to the height of the cells. Holocrine secretory cells were sparse in this part of the epididymis. Cytoplasm was granular and the granules were concentrated more in the apical region of the cells, forming a sort of apical band. Stereocilia were clearly observable (Fig. 4).

E2 : The diameter of the duct was slightly greater in this region. The average height of the epithelial cells was



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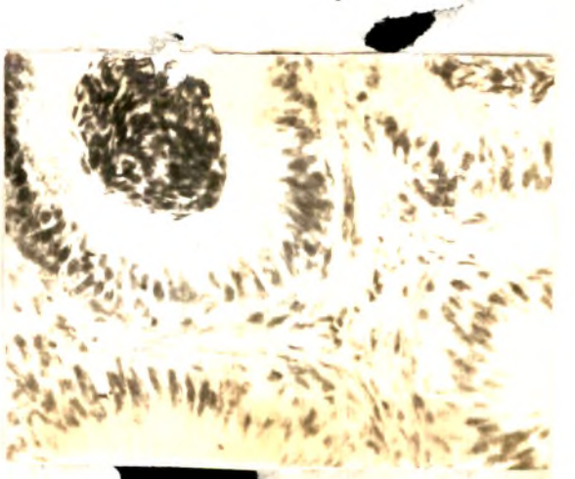

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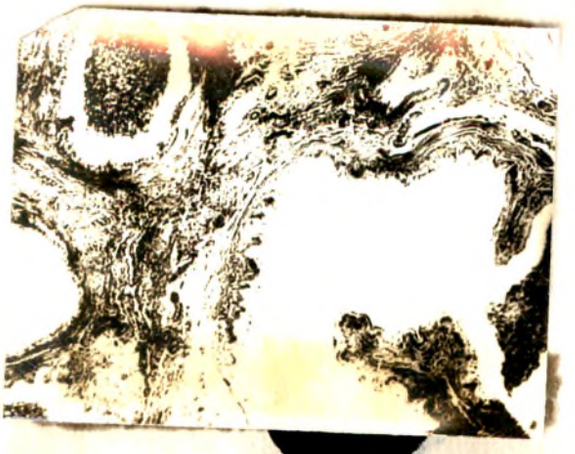

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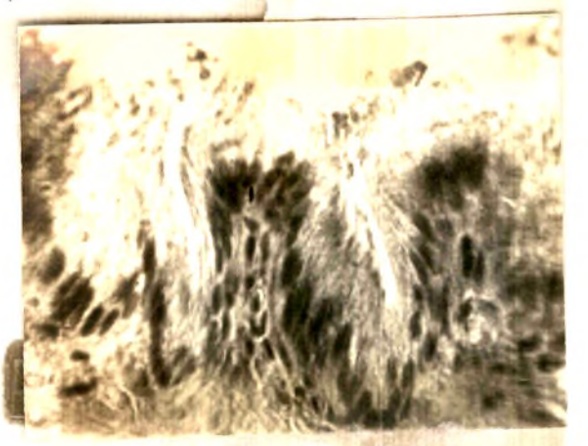
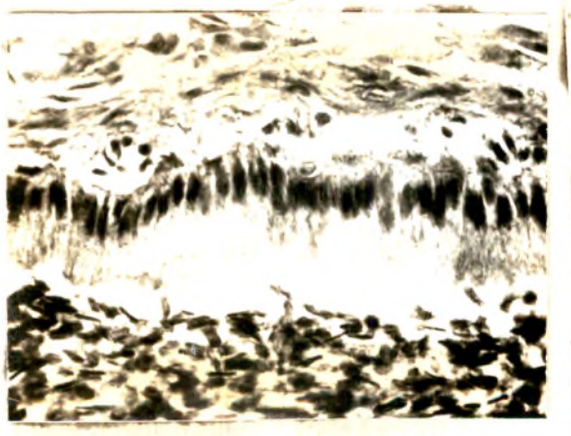


4 80×

Fig. 1. Photomicrograph of the modified region of the rabbit epididymis. Note the size of the nuclei. Haematoxylin-eosin.
Fig. 2. Showing the modified region of the epididymis along with the normal epididymal ducts.
Fig. 3. Showing the modified region with its folds.
Fig. 4. Showing the histological details of the E1 region.



5 35×

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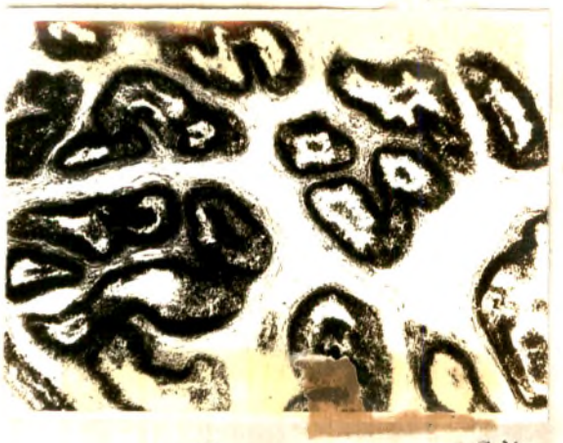

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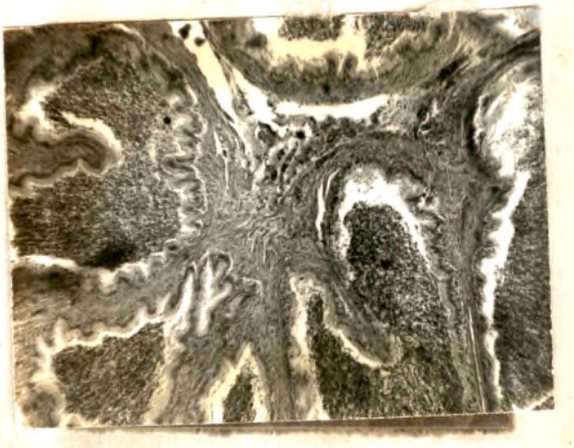


8 10×

Figs. 5 to 7. Microphotographs of the sections of the rabbit epididymis showing the histological details of regions E2, E3 and E4 respectively.

Fig. 8. Section of the ductuli efferentes showing the lipids.

more or less same as that of E1. Nuclei occupied approximately the central region of the cells and were homogenously stained.

In the distal parts of this region the epithelial lining showed a festooned appearance, different from the folds of the modified region which is due to unequal thickening of the stroma covered with common epithelial cells and glandular cells (Fig. 5). The nuclei in the glandular cells on such thickenings were observed to be located more towards the luminal ends of the cells. The staining pattern of such nuclei was different from that of the others. Here the nuclei showed a lighter homogenous staining with clumps of strongly staining chromatin material close to the inner surface of the nuclear membrane. Cytoplasm was as in E1.

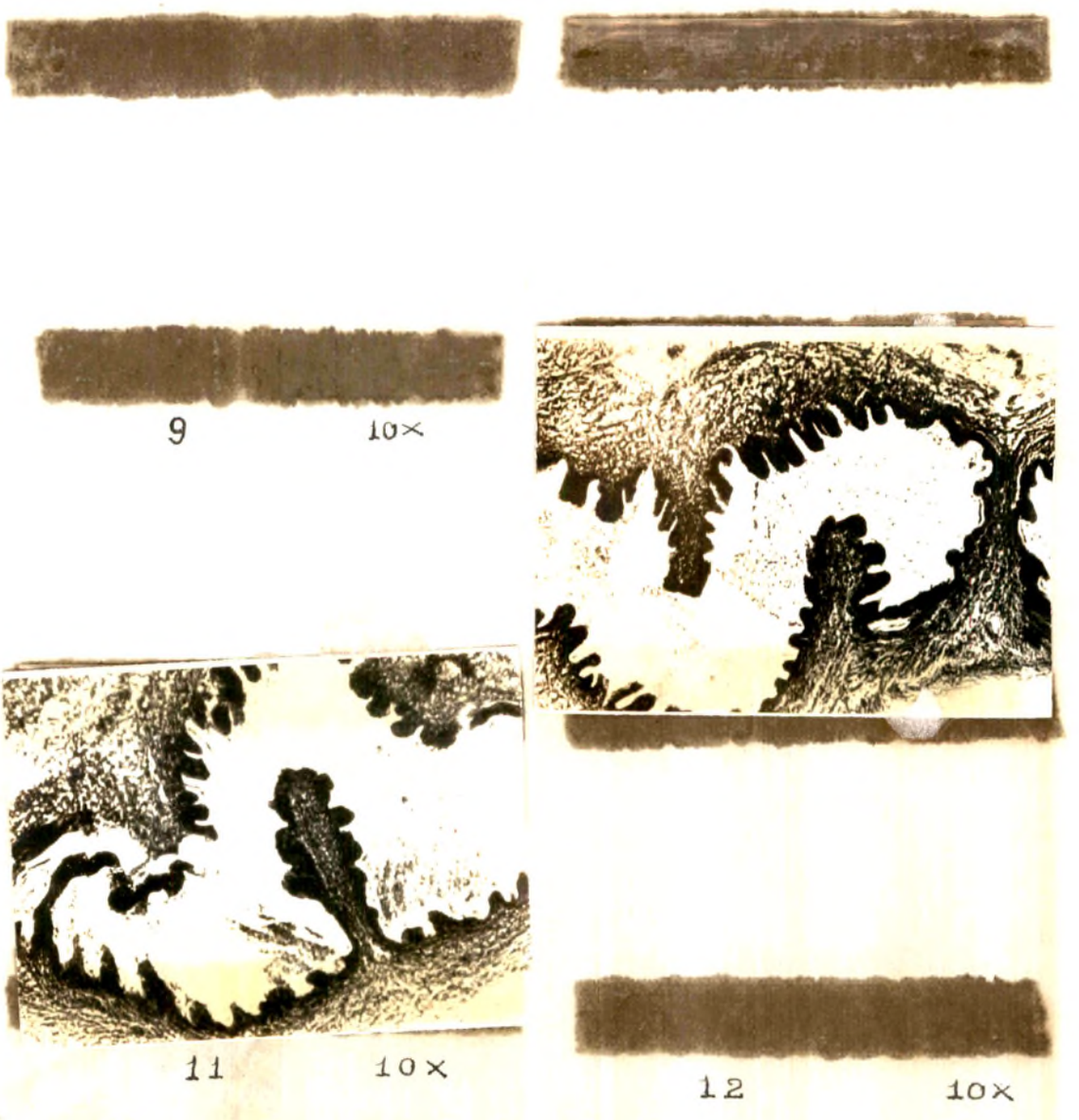
E3 : The diameter of the tubules, in this region, was observed to be about 10 to 12 times more than that of E1 and E2 regions. The tendency to form folds, branch, and anastomose, was observed to be strikingly high, leading to the compartmentalization of the luminal cavity at many places. The height of the epithelial cells was observed to be reduced to the minimal levels (average height 22 μ), but relatively the size of the nuclei was increased (1/2.2 of the cell height, cf. Table 1). In this region the nuclei were spherical and stained darkly and evenly. Perinuclear cytoplasm was observed to be hyaline but the rest of the cytoplasmic part presented a granular appearance. Such granules were abundant in the basal and

apical regions of the epithelial cells. Some of the cells showed a high degree of vacuolization and the vacuoles were seen to coalesce and occupy a major portion of the cytoplasm. The contents of the vacuoles were non-eosinophilic and a thin layer of strongly eosinophilic cytoplasm was observed around the vacuoles.

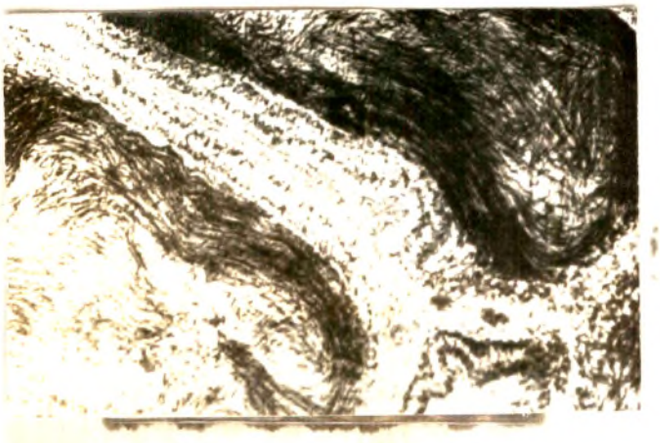
Sections of the normal epididymal ducts with non-folding epithelial lining were present in this region of the epididymis. The epithelial cells of such ducts were similar to those of the E1 and E2 regions. The holocrine secretory cells were found to be rare in the epithelial lining of the modified region.

E4 : The branching of epithelial folds was observed to be less complicated. The ducts were moderately wide (average width 460 μ). The height of the epithelial cells was at a reduced level (cf. Table 1). The nuclei were oval in shape and were strongly basophilic staining homogenously. Many of the epithelial cells were observed to possess large non-eosinophilic vacuoles. The lining membrane of the vacuoles possessed strong eosinophilic granules sticking very closely on the outer side. The cytoplasm was of granular nature. A large number of holocrine secretory cells were present in the epithelial lining.

Very few sections of the duct with non-folding epithelial lining were noticed in this region of the epididymis.



Figs. 9 to 12. Sections passing through the modified region of the rabbit epididymis showing the distribution patterns of lipids, lipase, alkaline- and acid-phosphatases respectively.



13 80×

Fig. 13. Photomicrograph of the section of the rabbit epididymis passing through the modified region showing the localization of the Golgi elements in the epithelial cells.

HISTOCHEMICAL :

Lipids - Lipids were abundant in the epithelial cells lining the entire epididymal tract. Particularly, the cells of the ductuli efferentes and those of the modified region possessed comparatively stronger sudanophilic cytoplasm (Figs. 8 & 9).

Lipase - The epithelial cells lining the modified region showed a fairly strong enzymatic activity in the cytoplasm (Fig. 10).

Alkaline phosphatase - A diffuse and weak activity was obtained in the cytoplasm. The nuclei of the cells showed a still weaker enzymatic activity (Fig. 11).

Acid phosphatase - The activity of this enzyme was the strongest and was localized in the cytoplasm in the form of granules (Fig. 12).

Golgi bodies - The silver impregnated masses of Golgi elements were confined to the apical portions of the epithelial cells above the nuclei. The Golgi bodies appeared as an apical band in the epithelial cells (Fig. 13).

DISCUSSION

The histological features of the modified region of the cauda epididymis in the rabbit described above, clearly indicate that this part is distinctly different from the rest of the epididymal duct. It should be of considerable interest to know its homology. On the basis of the ontogenetic and phylogenetic studies on the origin of the vertebrate urinogenital system and its component elements, it is known that the

anterior mesonephridial components - "the sexual kidney" - (Walter and Sayles, 1949) establish connections between the testis and the Wolffian or archinephric duct, by forming ductuli efferentes (Walter and Sayles, 1949; Romer, 1950). The posterior part of the mesonephros generally does not develop to a functional level and is represented by certain rudimentary structures in the reproductive system of vertebrates. In the male reproductive system of mammals such reminiscent structures of the Wolffian body are the appendix epididymis (representing the anterior tip of the Wolffian duct), paradidymis, and the ductuli aberrantes (both representing the posterior mesonephric nephridial elements). The ductuli aberrantes are found at the caudal end of the ductus epididymis (Walter and Sayles, 1949; Maximow and Bloom, 1961). A ductus aberrantis is found to be communicating with the ductus epididymis at the posterior part where the latter forms the cauda epididymis. On the basis of the present findings, it is suggested that in the case of the rabbit, this aberrant mesonephridial component establishes contact, at both ends, with the cauda epididymis and thus enters into the excurrent duct of the male reproductive system. Such an aberrant duct may be considered homologous with the modified region presently described in the cauda epididymis of the rabbit. It should be mentioned here that embryological evidence from a detailed study of this structure is necessary for the acceptance of this suggestion. If such homology is established it is possible to ascribe to it an absorptive/secretory

function, which would be in accordance with the known primary function of the mesonephridial tubules of regulating the concentrations of substances in circulating blood and in the lumen of the tubules.

The histochemical observations reported here tend to indicate, more specifically, a secretory role to this region of the epididymis. The finding that inspite of the reduction in the size of the epithelial cells the size of the nuclei is increased considerably and that the cytoplasm showed prominent vacuolization, supports the above contention. It has been mentioned already that there are numerous holocrine secretory cells in this modified region, extending in the E4 part, which might be responsible for the secretion of glyceryl phosphoryl choline, such a function has been attributed to the holocrine secretory cells by Martan and Risley (1963). The epithelial cells of this region in the E3 part, where the holocrine secretory cells are few, may be responsible for the elaboration of yet another secretory substance. But unless evidence for any such secretion is available it is not possible to state any thing further.

In this context it would be pertinent to mention that the posterior degenerating elements of the mesonephros are known to form the glands of Leydig (Romer, 1950). The secretion of these glands is believed to stimulate spermatozoa. These glands may be considered, on the basis of embryological evidence, to be equivalent to the ductuli aberrantes, which have been homologized with the modified region of the cauda epididymis, in the present study.