CHAPTER II

HISTOCHEMICAL OBSERVATIONS ON THE EPIDIDYMIS OF THE RAT

It is fairly well known that the mammalian spermatozoa attain motility and fertilizing potentiality during their passage through the epididymis (Hammond and Asdell, 1926; Young and Simeone, 1930; Branton and Salisbury, 1947; Mukherji and Bhattacharya, 1949; Salisbury, 1956; Blandau and Rumery, 1960). The function of the epididymis was believed to be temporary storage and transport of the spermatozoa. The transport of the spermatozoa from the seminiferous tubules is effected by the fluid produced in the seminiferous tubules which sweeps the spermatozoa to the excurrent duct system (Mason and Shaver, 1952). The cells of the ductuli efferentes are ciliated and the motility of these cilia also helps in driving the sperm cells and the testicular fluid toward distal parts of the epididymal duct system. The ductuli efferentes and the caput epididymis have been shown to resorb the testicular fluid and pass down the concentrated mass of sperm cells (Mason and Shaver, 1952).

The migration of the spermatozoa through the epididymis takes variable periods of time in different species but in any given species the period is more or less constant under normal conditions (Bishop, 1961). One of the notable changes is the movement of the kinoplasmic droplet over the

mid-piece of the spermatozoa and its subsequent loss during passage down the tract (Branton and Salisbury, 1947; Mukherji and Bhattacharya, 1949; Mann, 1954). Spermatozoa with attached kinoplasmic droplet are normally not found in the ejaculated semen. The presence and movement of kinoplasmic droplets on the epididymal spermatozoa as they pass down the epididymal tract have been correlated with a progressive loss of resistance to heat shock by the sperm cells of guinea-pig, rat, and ram (Young, 1929), and also the progressive loss of the resistance to cold shock by the sperm cells of the boar (Lasley and Bogart, 1944) and that of the ram (White and Wales, 1961).

Young and Simeone(1930) observed that the epididymis provides an environment favourable for the storage and functional maturation of the spermatozoa. According to them once the spermatozoa attain maturity there is no influence which can indefinitely preserve their motility and capacity to fertilize. Further they suggested that the entry of the younger spermatozoa from the seminiferous tubules into the epididymal duct and and the exit of mature spermatozoa from the epididymis through ejaculation is a continuous process and even in the absence of ejaculation due to ligation of the vas deferens, this current is maintained as can be seen from the fact that large number of degenerating spermatozoa are found in the distal parts of the vas deferens.

The work of Glover (1962) on rabbits shows that the sperm cells in the epididymis, experimentally maintained in the abdomen, are adversely affected by the higher abdominal temperature. He has also pointed out that the comparatively immature spermatozoa in the caput epididymis react more rapidly to the changed environment than those in the corpus and cauda epididymis. Such an influence indirectly indicates that there are subtle changes occurring in the spermatozoa during their migration down the epididymal tract. According to Young and Simeon (1930) maturation changes undergone by the sperm cells during their passage through the epididymis, are due to the intrinsic properties of the sperm cells. It is not properly understood whether the epididymis has its contributions in the process of sperm maturation. Mason and Shaver (1952) showed that the caput epididymis removes the fluid from the contents of the lumen to pass down a concentrated mass of sperm cells to the distal parts of the epididymis. This attributes an absorptive function to the caput epididymis.

Scott et al. (1963) have shown that the epididymal tissue of the rabbit is capable of incorporating P^{32} into the glycerylphosphorylcholine (GPC) which is a secretory product of the epididymis. The holocrine secretory cells found along with the lining of the epididymal duct have been suggested as the probable sites of the secretion of GPC (Martan and Risley, 1963). Dawson and Scott (1964) in their work on the phospho-

lipids of the ram spermatozoa have hinted at the possibility of the phospholipid secreted in the epididymis, being utilized as metbolic substrate by the epididymal spermatozoa.

Histochemical studies of the epididymis (Allen and Slater, 1957, 1958 & 1959; Nicander, 1957 & 1958) have shown that the epididymis has a zonal or segmental organization. Allen and Slater (1957) divided the epididymis of the mouse into eight segments or lobes depending on the histochemical properties of the epididymal tract. Reid and Cleland (1957) divided the epididymis of rat into six zones on the basis of histological characters of the epithelial cells of the epididymal duct. According to Reid and Cleland (1957) zones 1 to 3 and the proximal part of zone 4 go together to form the caput epididymis or the head. The distal portion of the fourth zone makes up the corpus epididymis or the isthmus and the fifth and sixth zones constitute the cauda epididymis or the tail. Regional differentiation of the epididymal duct has been correlated with the differential needs of the maturing spermatozoa (Allen and Slater, 1957).

It is known that the histochemical and the functional organization of the epididymis is under the control of the circulating androgens (Cavazos, 1958; Feagans, Cavazos and Ewald, 1961; Gohary, Cavazos and Manning, 1962; Allen and Slater, 1957, 1958 & 1959).

The literature cited above suggests that the epididymis might have some significant role in the maturation process of the spermatozoa. The present histochemical investigation was undertaken as an attempt to elucidate the functions of the epididymis.

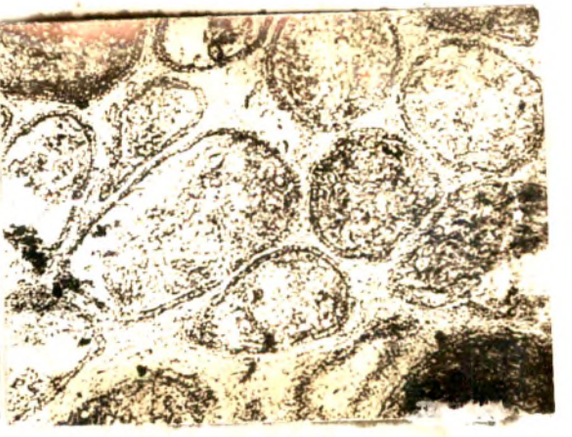
MATERIAL AND METHODS

Epididymides from healthy adult male rats, Rattus norvegicus albinus (Haffkine Institute strain), were removed after killing the animals by decapitation. The adipose tissue was carefully removed off the epididymides. The whole of the epididymis was divided into caput and cauda regions and both these parts were studied separately. Thin slices of the caput- and cauda-epididymis were cut and processed by the methods given earlier (Chapter I) for the study of the following : Lipids, Lipase, Acid- and Alkaline-phosphatases, Succinic dehydrogenase (SDH), Malic dehydrogenase (MDH), Lactic dehydrogenase (LDH), and β-hydroxy butyric dehydrogenase (BDH).

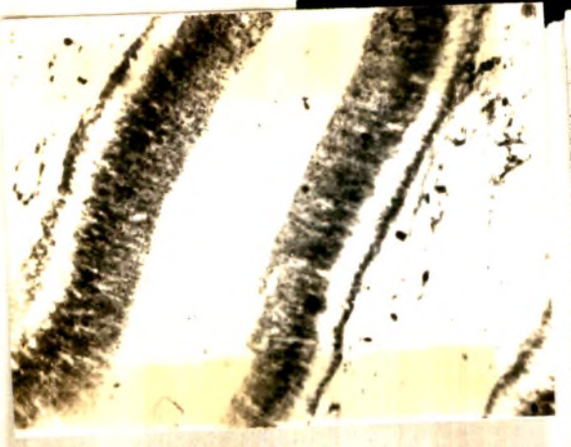
RESULTS

Lipase : This enzyme gave positive reaction of considerable intensity throughout the cytoplasm of the cells lining the initial segment or zone 1. The tall columnar epithelial cells showed a band of comparatively stronger reaction

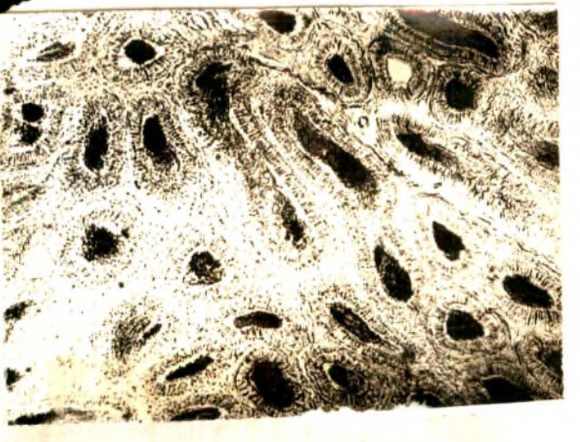
1 10×



2 35×



3 50×

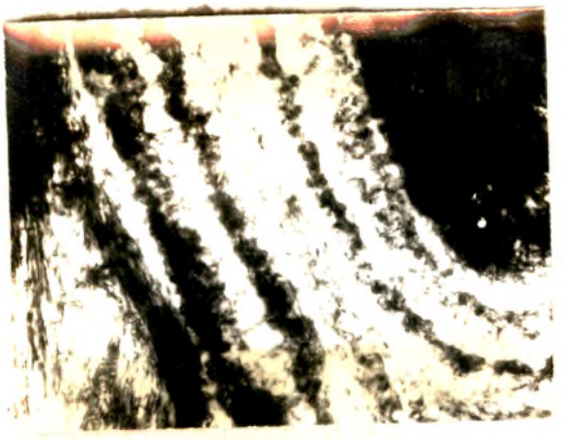


4 10×

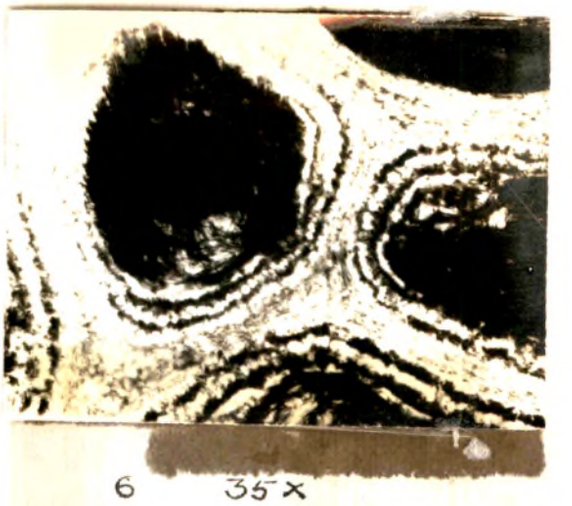
Photomicrographs of the sections of the rat epididymis.

Figs. 1 to 3. Showing lipase activity in the proximal caput, caput proper and the cauda epididymis, respectively.

Fig. 4. Section of the initial segment of the caput epididymis showing alkaline phosphatase activity.



5 65×



6 35×

7 10×

8 10×

Photomicrographs of the sections of the rat epididymis.

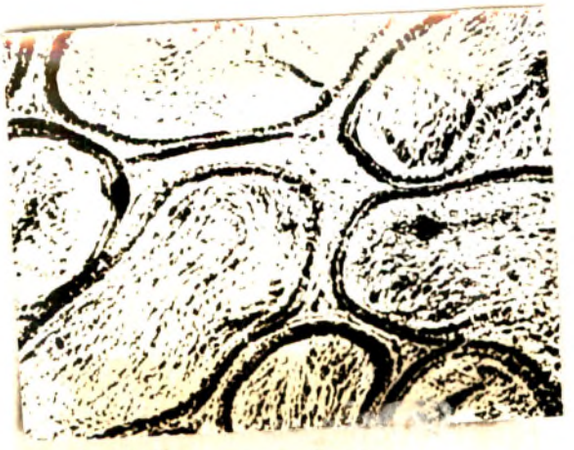
Figs. 5 & 6. Showing the alkaline phosphatase activity in distal caput and the cauda epididymis, respectively.

Figs. 7 & 8. Showing the acid phosphatase activity in the initial segment and the distal caput epididymis, respectively.

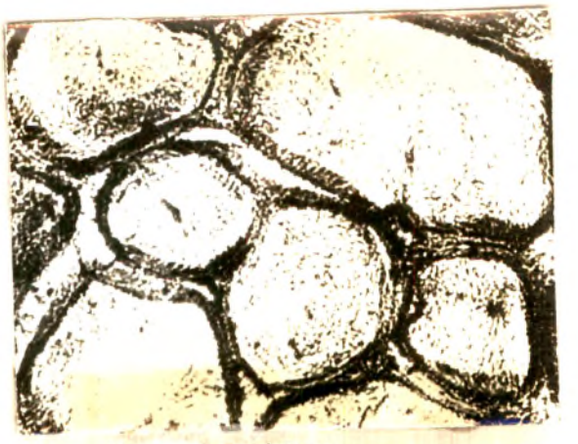
appearing as a circular band at the basal region of the cells, in the initial segment (Fig. 1). The test of the caput epididymis gave a diffused cytoplasmic localization of the lipase activity (Fig. 2). The cells of the cauda epididymis gave a similar diffuse cytoplasmic reaction. An interesting feature was that a stronger reaction band was localized in the apical region of the epithelial cells in contrast to the basal ring in the initial segment (Fig. 3). The holocrine secretory cells from all parts of the caput epididymis gave a positive reaction for lipase.

Alkaline phosphatase : A weak reaction was obtained in the initial segment (Fig. 4) and the distal parts of the caput epididymis. The enzyme activity was localized as a weak and diffuse reaction in the cytoplasm and comparatively a stronger band of activity in the apical regions of the cells of caput epididymis (Fig. 5). In the cauda epididymis the lining cells showed a very weak reaction in general but two bands of stronger reaction in the basal and the apical portions giving apparently a double-tier picture was observed (Fig. 6). The holocrine secretory cells were not distinguishable on the basis of the reaction for alkaline phosphatase activity.

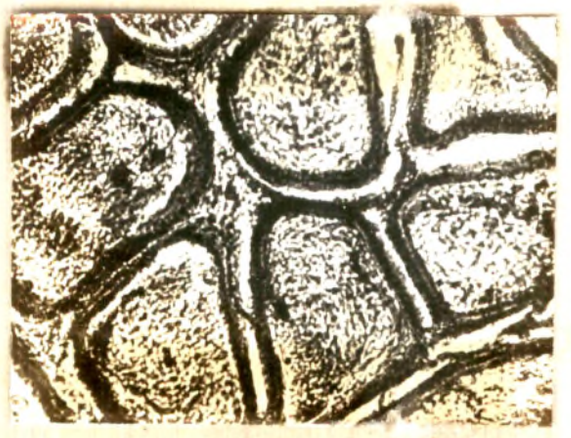
Acid phosphatase : The epithelial cells of the initial segment of the caput region gave a considerably strong reaction. The phosphatase activity was seen as a diff-



9 10×



10 10×



11 10×

12 80×

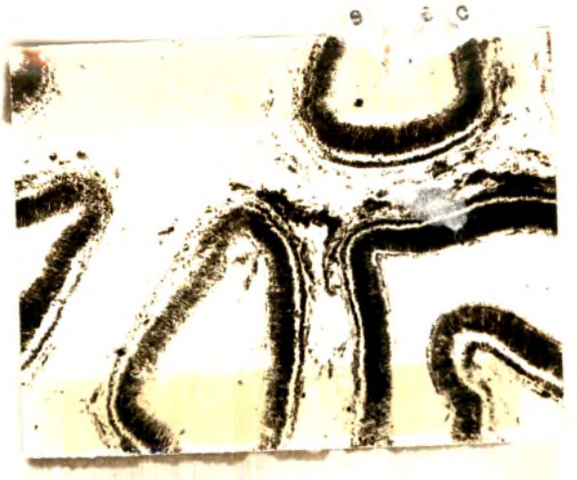
Fig. 9. Section of the rat epididymis showing the acid-phosphatase activity in the cauda region.

Figs. 10 to 12. Sections of the rat epididymis showing SDH, BDH and LDH activities, respectively.

use cytoplasmic reaction. The holocrine secretory cells stand out clearly due to their stronger reaction in the initial segment (Fig. 7). The cells of the distal parts of the caput epididymis also showed a positive reaction like those of the initial segment but in this region the holocrine secretory cells were not distinguishable from the rest of the cells, unlike that in the preceeding segment (Fig. 8). In the cauda epididymis the cells showed a very weak reaction and this is confined to the apical cytoplasm above the nuclei of the cells but the holocrine secretory cells showed a considerably strong reaction (Fig. 9).

Oxidative enzymes : The epithelial cells of all the regions of the epididymis showed the presence of all the oxidative enzymes studied, in the form of fine granules localized throughtout the cytoplasm of the cells. In every case the nuclei were negative (Figs. 10-13). As far as the oxidative enzyme activities studied here are concerned, the holocrine secretory cells were not distinguishable from the rest of the cells.

Lipids : All the segments of the epididymal tract were strongly sudanophilic (Figs 14 & 15). The epithelial cells of the proximal part of the caput epididymis and those of the distal part of the cauda epididymis were observed to contain stronger sudanophilic cytoplasm when compared to the cells



13 35×

14 35×

15 35×

Fig. 13. Section of the rat epididymis showing MDH activity.

Fig. 14. Section of the proximal caput (initial segment) epididymis of the rat showing the lipids stained with Sudan black B.

Fig. 15. Section of the cauda epididymis of the rat showing the distribution of lipids stained with Sudan black B.

from the other parts of the tract.

DISCUSSION

Allen and Slater (1957) studied the distribution of non-specific ali-esterase activity in the epididymis of the mouse. They found that intense ali-esterase activity was present in the body and tail of the epididymis, which was localized in the epithelial cells. In the present work, which deals with a specific ali-esterase viz. lipase, the distribution pattern of the enzyme activity was quite different. The epithelial cells of the initial segment were strongly positive while the cells from rest of the caput epididymis were moderately positive. A basal band of comparatively strong lipase activity was observed in the epithelial cells of the caput epididymis, whereas in the cauda epididymis such a band was present in the apical region of the cells. Such a histochemical regionalization in the enzyme activty of the epithelial cells in the different parts of the excurrent duct system, clearly indicates, a metabolic adaptation for the secretory / absorptive activities.

It was observed that there is no alkaline phosphatase activity in the corpus and cauda epididymis of the mouse (Allen and Slater, 1957). During the course of the present investigation, it was found that the non-specific alkaline glycerophosphatase activty showed an interesting pattern of localization in the cells of the cauda epidi-

dymis, which was observable in the form of two bands, and this pattern of enzyme distribution substantiates the suggestion made regarding the possible role of lipase in the secretory / absorptive activities.

In general, the acid phosphatase activity is more than that of alkaline phosphatase and this suggests that glycolytic metabolism may be predominant in the epididymal tissue(Moog,1946). In this connection it may be mentioned that glycogen is "secreted" in the epididymis of the rabbit (Nicander, 1954). Maneely (1958) demonstrated a glycoproteinaceous secretory product in the lumen of the rat epididymis. The work of Dawson and Rowlands (1959) pointed out yet another secretory product of the epididymis viz. GPC. Martan and Risley (1963) suggested that the holocrine secretory cells are the probable sites of GPC secretion. These cells were also found to contain strong acid phosphatase activity. In the present work also similar results were obtained. Though Allen and Slater (1958), have not recognized these as holocrine secretory cells, they did describe them as showing high acid phosphatase activity. According to these authors (1958) acid phosphatase activity in the epithelial cells of the mouse epididymis is confined to the Golgi zone, thereby suggesting the role of acid phosphatase in the secretory mechanism of the cells. The present investigations point out distinctly that the epithelial cells of the initial

segment and those of the cauda epididymis are more active than the cells from the other parts of the epididymal tract thereby suggesting a comparatively higher rate of metabolic activities at these sites. This can be correlated with the absorptive function in the initial segment, as suggested in the studies of Mason and Shaver (1952) as well as with the secretory functions of the caput and cauda epididymis as suggested by Nicander (1954), Maneely (1958), and Dawson and Rowlands (1959).

The presence of intense SDH and MDH reactions indicates the degree of oxidative activity of the tricarboxylic acid cycle in the epididymal tissue and LDH activity, which is also equally intense, shows the level of glycolytic activity. When it is taken into consideration that lipase and BDH activities are also high and that lipids are present in the epithelial cells, it is probable that the lipids might be utilized as metabolic substrates when the glycolytic metabolism is unable to meet the demand for a continuous source of energy needed in the secretory processes. However, it should be mentioned that the band-wise distribution of the enzyme activities in the cytoplasm of the epithelial cells of the rat epididymis, calls for further investigations in order to obtain a better understanding of its metabolic significance at the sub-cellular levels.