HISTOCHEMICAL STUDY OF THE EPIDIDYMIS IN RAT
(RATTUS NORVEGICUS)

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ABSTRACT
Histochemical study of the epididymis in rat carried out in both prepubertal and postpubertal age groups. Age related changes in the presence of carbohydrates, basic proteins, tyrosine and lipids were recorded in the three regions of epididymis. The peritubular connective tissue and lining epithelium contained lipids which increased with age.

INTRODUCTION
Histochemistry of the epididymis has been described by Oke et al. (1988) in giant rat, Singh and Dhingra (1971), Singh (1989) and Gayake et al. (1999) in buffalo. However literatures regarding the age changes in the histochemical characters of the three regions of the epididymis in rats are lacking. Hence the present investigation had been conducted.

MATERIAL AND METHODS
The materials for the present study were obtained from thirty six prepubertal male rats of day-old to four weeks of age and eighteen postpubertal male rats of six weeks to ten weeks of age. The tissue pieces were fixed in 10% neutral buffered formalin, Helly's fluid and Zenker's fluid. The tissues were processed by routine paraffin technique. Sections of 4-6 μm thickness were cut for different staining techniques. For the study of lipids, fresh frozen sections were cut at 15-20 μm thickness.

The following methods were adopted for histochemical staining of tissues. Periodic Acid Schiff (PAS) for mucopolysaccharides, Alcian blue pH 2.5 for mucosubstances, oil red O in propylene glycol for lipids (Luna, 1968), Mercury Bromphenol blue method for proteins (Stoward and Pearse, 1985), Million reaction for tyrosine, Performic acid-Alcain blue method for disulphide and sulphhydril linkages and Feulgen reaction for deoxyribo nucleic acid (DNA) (Bancroft and Stevens, 1996).

RESULTS AND DISCUSSION
Carbohydrate: The basement membrane of the tubule, stereocilia and peritubular connective tissue of the epididymis were PAS reactive (Fig. 1) which is in concurrence with the findings of Singh and Dhingra (1971) in buffalo. The authors also opined that PAS positive material may indicate a readily available source of energy for the secretory activity of the cells. The luminal border of the lining cells in all the three regions of the epididymis was PAS reactive for carbohydrates.

Protein: The capsule, the epididymal tubules and the peritubular connective tissue were reactive for basic proteins as recorded by Singh (1989) in buffalo (Fig. 2). Basic proteins were low in prepubertal animals and the intensity of reaction increased as the age advanced. The presence of basic proteins may indicate the lipoprotein nature of the cell membranes.

A mild reaction was recorded for tyrosine and disulphide linkages of protein. Pyne and Sinha (1989) opined that tyrosine may help in various enzymic functions. In the epididymis, the nuclei of lining cells and sperm heads of lumen were Feulgen reactive (Fig. 3). Feulgen
Fig. 1. Photomicrographs of the testis and the epididymis from an eight-week-old rat showing PAS reaction (arrow). T-Testis, E-Epididymis - PAS x 100.

Fig. 2. Photomicrograph of the middle segment of the epididymis from a four-week-old rat showing basic proteins (arrow) - Mercury-Bromophenol blue x 200.
Fig. 3. Photomicrograph of the middle segment of the epididymis from a ten-week-old rat showing Feulgen reaction. E-Nuclei of epithelial cells; S-Nuclei of sperms - Feulgen reaction × 200

Fig. 4. Photomicrograph of the cryosection of the epididymis from a four-week-old rat showing lipids (arrow) - Oil red 0 × 400
reaction demonstrates DNA inside the cells.

- Lipid: In the epididymis, the peritubular connective tissue of the tubules and the lining epithelium contained lipid (Fig. 4) as reported by Singh (1989) in buffalo. More intense reaction was noticed in post-pubertal animals.

REFERENCES