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The effects of adrenalectomy on the harderian gland of the Wistar albino rats

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Harderian glands of the Wistar albino rats normal and adrenalectomized were investigated by light microscopy. In normal, these glands have a tubuloalveolar structure. The gland is located in the medio posterior aspect of the orbit. It is lobulated and appears homogeneous in colour and texture. Harderian gland consist of tubules with wide lumina lined by a single layer of columnar epithelial cells surrounded by myoepithelial cells within their basal lamina. It contains porphyrin pigment which is stored as solid intraluminal deposits. The glandular epithelium possesses two cell types, termed A and B. Type A cells are more numerous.

The single excretory duct of the gland is directly continuous with endpieces at the hilus and opens nasally and ventrally to the third eyelid. The excretory duct is accompanied by many acini of small serous glands around it. The tubuloalveoli of the gland is not divided into lobules. There is no branched duct system within the gland.

The secretion seems to be associated with porphyrins, is essentially released by exocytosis, but holocrine secretion also occurs. The single excretory duct is lined by a stratified epithelium. The gland is surrounded by a collagenous capsule.

The adrenalectomy, caused degenerative changes in the glands. Epithelial height was lower than in normal gland epithelium. Most of the acini were completely disorganised. The acinar lumina were filled with porphyrin debris. The results suggest that rat harderian glands are sensitive to adrenal androgen changes in both male and female rats.

Keywords: Harderian glands, adrenalectomy, porphyrin pigment, microscopy.

The harderian gland is an orbital organ present in many vertebrates (1–4). From the time of Harder's discovery of the gland in 1694 in the fallow deer a considerable amount of research on this gland has been reported. The rodent harderian gland has been extensively used as a model for porphyrin biosynthesis ever since 1955 when Cohn showed by histological techniques that the glands contained porphyrin pigment bound to lipid (5). The fine structure of the harderian gland has been detailed and fully

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studied in many vertebrates like mice (1), frogs (6), lizards (11), turkeys (2), mongolian gerbils (7), armadillos (8), hamsters (9), chicken (10), terrapin (11), guinea pigs, dolphins (3), rats (14), (6–14).

The functions of the gland are generally considered to be the protection and lubrication of the cornea (11), a source, and may play role in sexually activities, e.g., participating in local immunological or endocrine related reactions (3). It may be influenced, directly or indirectly, by ovarian hormones, and depending of the age, modifications of the harderian glands reflect alterations in the ovarian function (15). A large amount of investigation has been concentrated on the role of melatonin amount involved with porphyrin pigmentsynthesis in the harderian glandfunction (3, 11, 15, 16).

In rodents it has been shown that sexual dimorphism of the harderian glands is dependent upon the endocrine activity of the gonads; androgens can prevent the morphological and biochemical changes induced by castration (17). The previous histological observations failed to demostrate any effect of gonadectomy. Mast cell numbers in the male golden hamster increased greatly after casration (17). Following adrenalectomy we studied wether the lack of adrenal androgen had an effect on the glandular structure. Present study demonstrates the normal and adrenalectomised structure of the harderian glands in Wistar albino rats.

Materials and Methods

Twelve to 200 g weighing male and female Wistar rats were used in the study. Animals were maintained with a photoperiod of 12 h light/12 h darkness at 25 °C and received tap water and food *ad libitum*. Animals were divided into two groups, each containing 6 rats. One group was subjected to adrenalectomy, the other was sham-operated under ether anaesthesia. One month after the operation, the rats were decapitated and harderian glands were dissected from the orbits. The glands were cut in small pieces with a razor blade washed and fixed 10% neutral formaldehyde for 24 h. The tissues were rinsed in water.

The glands were subsequently dehydrated in increasing concentrations of alcohols, cleared in xylol and embedded in paraffine. Sections (5 μm) for light microscopy were obtained with microtome. Tissues were stained with hematoxylin and eosin, toluidin blue, cresyl each violet. Mast cell numbers in the Per unit were counted under a light microscope. (magnification: ×40).

Results

The harderian glands was located at the medioposterior pole of the orbit, surrounded by a collagenous capsule. They were lobulated and appeared homogeneous in color and structure. The lobules were divised by connective tissue. The structure of the glands was tubuloalveolar. The gland consisted of branching tubules with wide lumina, was

lined by a single layer of cylindiric epithelial cells (Fig. 1). The nuclei were situated basally. Epithelial cells were occasionally binucleated. The glandular epithelium presented two cell types. The type A cell was more frequently encountered. Its cytoplasm was acidophilic and filled with large lipid vacuoles. The type B cell was characterised by basophilic cytoplasm with smaller lipid vacuoles. Lipid vacuoles were more numerous in type A than in type B cells (Fig. 2). The interstitium of the gland contained blood vessels, fibroblasts, mast cells (Fig. 3), nerve fibres, plasma cells and macrophages.

Myoepithelial cells were observed at the base of the secretory epithelium. The long fusiform nuclei of the myoepithelial cells lay parallel to the basal lamina. They were characterized by an elongated nucleus (Fig. 4). The lumina of the tubules frequently contained an accretion of reddish, brown porphyrin pigment (Figs 1, 4). No ducts were observed within the gland. The tubules were directly connected with each other.

Following adrenalectomy, the morphology of both male and female harderian gland differed considerably from those of sham-operated animals. They showed histological alterations that included thinning of the tubule walls, widening of the lumina and the accurance of porphyrin (Fig. 5). Most of the acini were completely disorganised. The acinar lumina were filled with debris. Numerous glandular cells showed pyknotic nuclei and an empty cytoplasm (Resim 6,a ve b). In some A cells few large vacuolised droplets could be seen (Fig. 7).

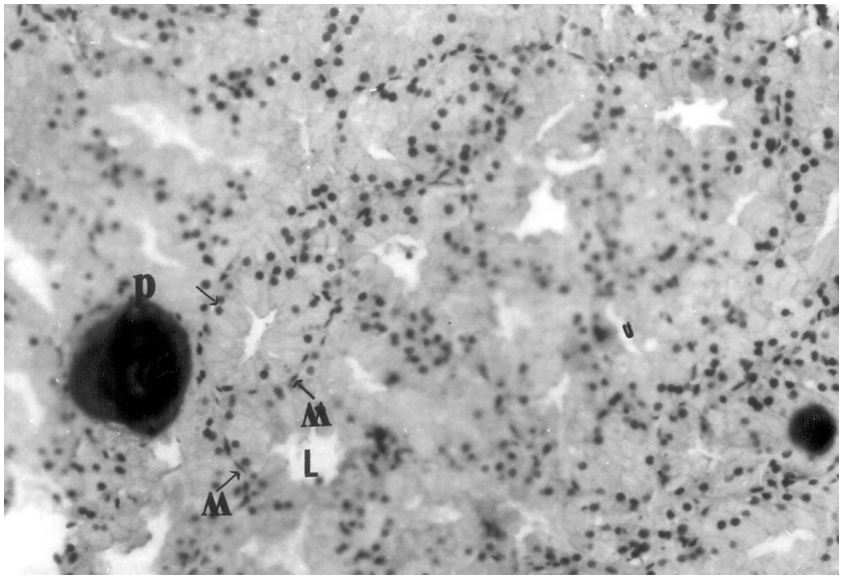


Fig. 1. Control group. Light micrograph from a 5 μm section stained with H.E. The tubules of the gland with wide lumina is lined by a single layer of typical tubule cells. The nuclei (arrow) are situated basally. p: porphyrin pigment, L: lumen, m: myoepithelial cell. ×10

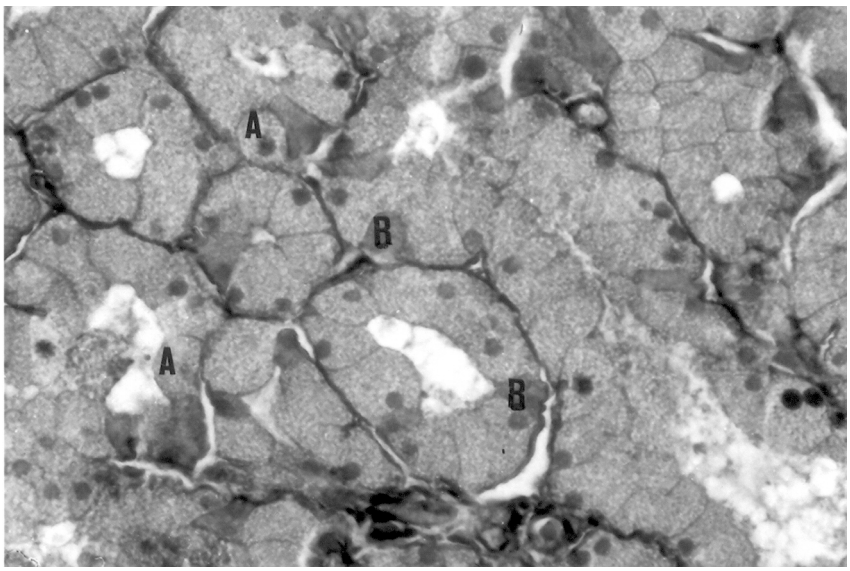


Fig. 2. The two types of cells in the control harderian gland epithelium. A cells (A) are acidophilic and filled large lipid vacuoles. B cells (B) are characterised by basophilic cytoplasm with smaller lipid vacuoles. H.E. ×40

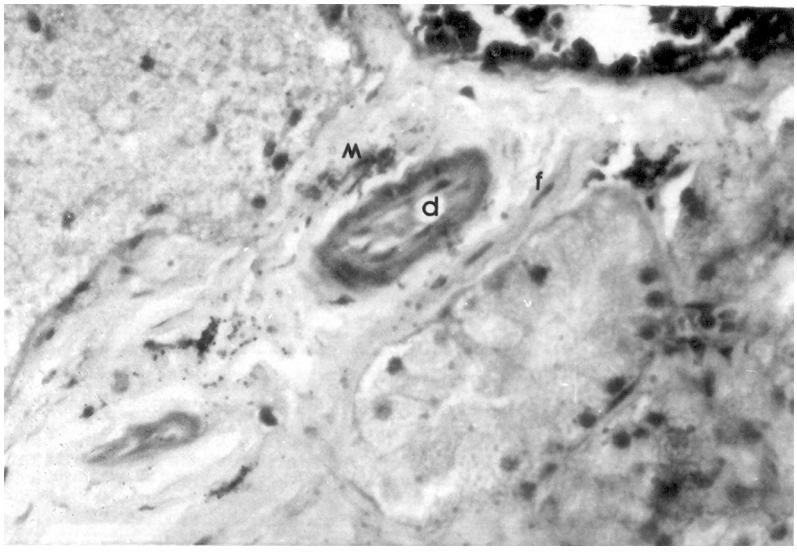


Fig. 3. Light micrograph section stained with Toluidine blue, showing mast cells (m), fibroblasts (f), blood vessels (d) in the connective tissue. ×40

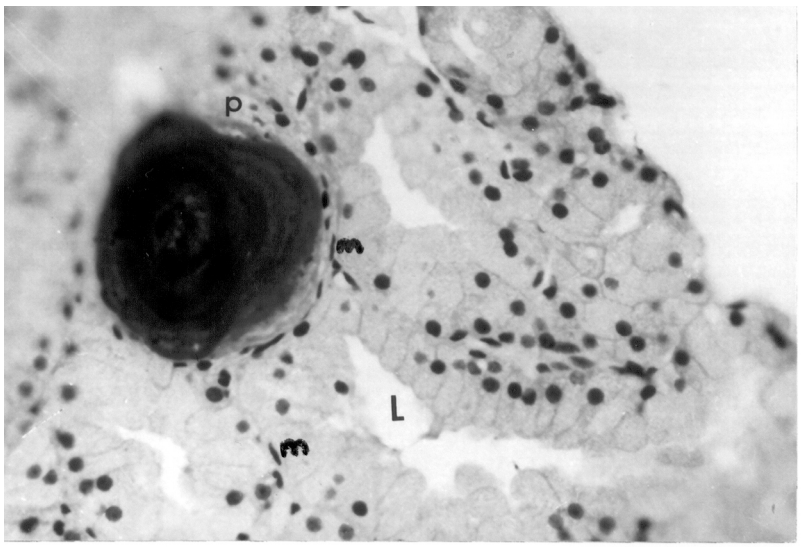


Fig. 4. Light micrograph from a 5 μm section stained with H.E. Lumen (L) of the tubulles is filled with reddish-brown porphyrin pigment (p). The nuclei of the myoepithelial cells (m) lie parallel to the basal lamina. ×40

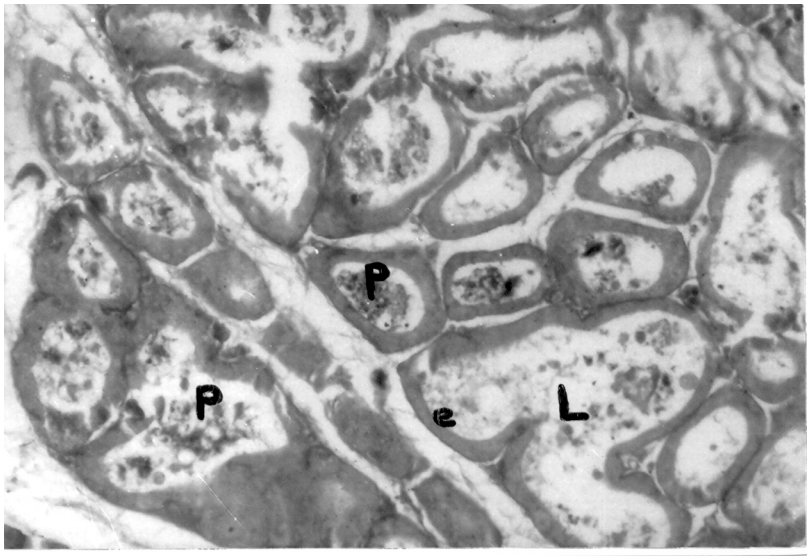
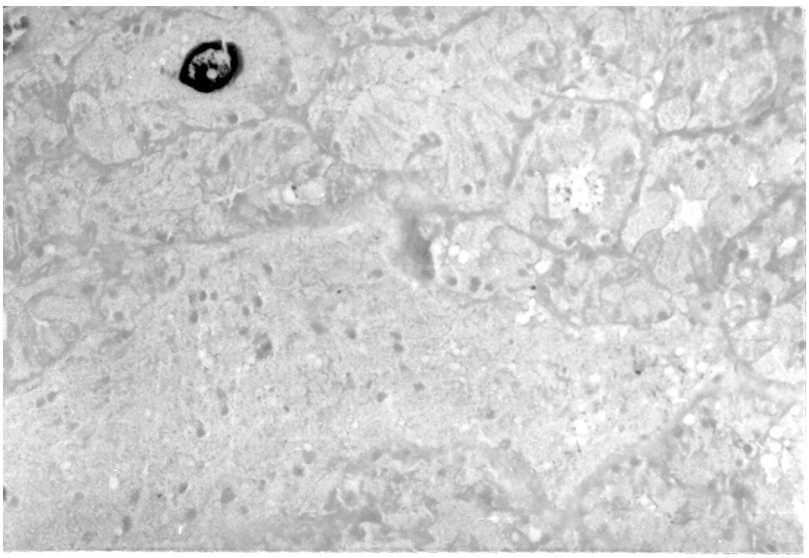


Fig. 5. Degenerative changes which occur in the harderian gland of the Wistar rats after adrenalectomy, including epithelial thinning (e), widening of the lumina (l) and the occurrence of porphyrin (p). H.E. ×20

a



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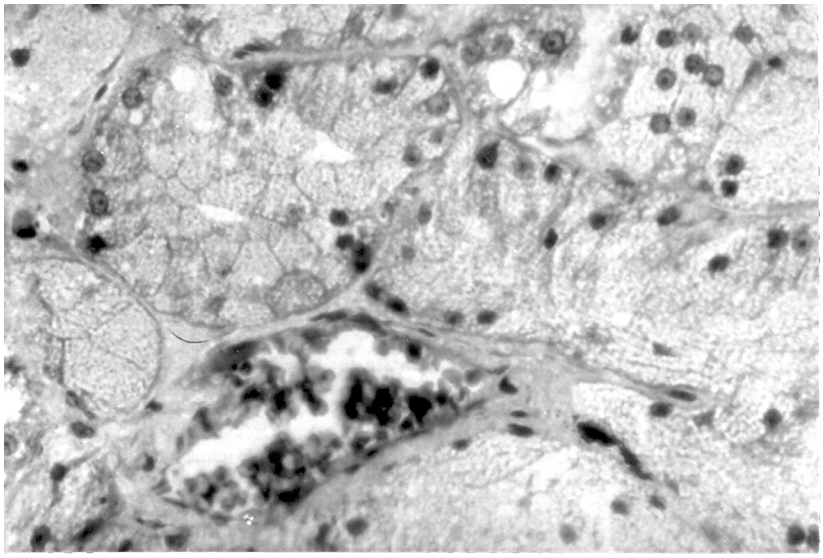

Fig. 6. Section of the harderian gland of an adrenalectomized rat. Most of the acini are completely disorganised. The nuclei of the acinar cells appear pyknotic. a: Cresyl each violet. ×20, b: Toluidine blue. ×20

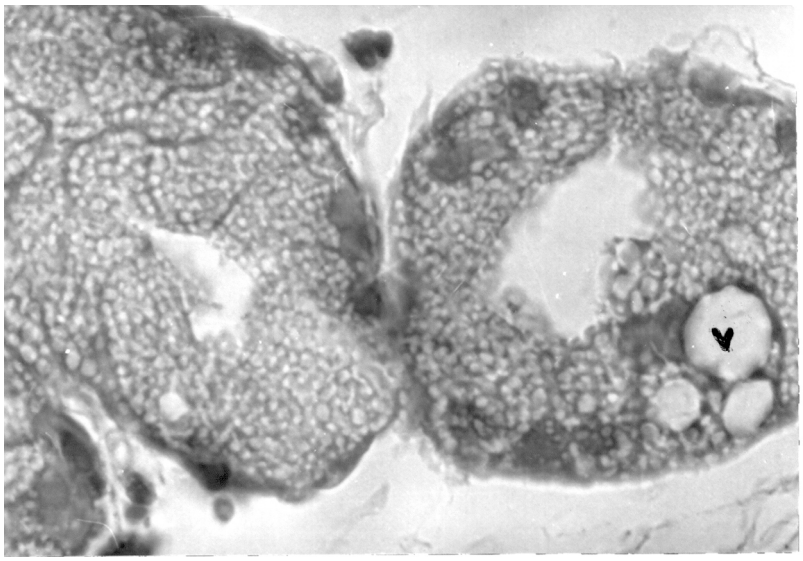

Fig. 7. Section of the harderian gland of an adrenalectomized rat. In some cells a few large vacuolised droplets can be observed (v). H.E. ×100

The morphometric studies revealed that the number of mast cells in the per unit area showed sex differences and altered with hormonal status. The mast cell number was 30 in the female rats while this number rose with adrenalectomy ($p<0.05$). The mast cell number in the adrenalectomised rats proved to be 60 in the female and 30 in the male rats ($p<0.005$).

Discussion

It is now well known that the harderian gland is part of a retinal pineal axis. The gland is surrounded by a collagenous capsule. Fibroblasts, macrophages, plasma cells, mast cells are frequently observed in the connective tissue. As in the hamster two distinct cell types can be distinguished in the glandular epithelium of the Wistar rat. These two cell types are designated as type A and B cells. The type A cells are constituted of approximately 75% epithelial cells. Their cytoplasm is acidophilic and filled with large lipid vacuoles. The type B cell is characterised by basophilic cytoplasm with smaller lipid vacuoles (9, 14, 18).

The lumina contain brown pigment accretion seen by light microscopy. The luminal material is identical with the dense component noted in the vacuoles of the type A cell, suggesting that porphyrins may be selectively secreted by this cell type. The content of the vacuoles of the type A cell is released primarily by exocytosis (14). This mechanism has been reported in all rodent species studied however so far, holocrine

secretion also occurs in the Wistar rat. Carriere (19) noted that the harderian free porphyrins are released through cell death and suggested that this degeneration may be due to the fact that free porphyrins can be toxic to cells.

The hormone melatonin involved in the photoperiodic regulation of gonadal activity, has been demostrated in harderian glands of mammals (20).

Feria Welasco et al. (21) concluded that secretory activity of the harderian gland is modified by enviromental lighting conditions and that secretions produced by this gland appear to be directly related to the control mechanism of melatonin production by the pineal gland in neonatal rats.

Harderian gland type II 5'-deiodinase activity exhibits a night-time increase with maximal values during the daytime (22). The nocturnal rise of the rat pineal type deiodinating activity is prevented by bilateral superior cervical ganglionectomy (23).

The rat harderian gland selectively accumulates the precursor of a particular pheromone steroid (24).

According to Lopez and Alvarez (15), the hamster harderian gland is influenced directly or indirectly by ovariectomy and ageing.

The androgen control of the harderian gland was first described in male golden hamster (25). Castration may be correlated with different levels of circulating androgens (26). We found that also by way of the lack of such adrenal androgen may be correlated similar case in the structure of the harderian gland. Harderian glands of the adrenalectomised rats had effected with alterations similar as that of castration.

In the male mice subjected to combinations of adrenalectomy castration alone caused a significant increase in the count resulting in about three times the mast cells number found in intact males. Casration plus adrenalectomy increased the count over 6 fold (17). Our results have shown that adrenalectomy increased the mast cell count over 3-fold in the female and male rats. Finally, adrenalectomy effected the structure of the gland by changes in adrenal androgens.

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