دراسة هستوكيميائية الغدة الهاردرين
فى الجمل ذو السنم

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ملخص .

وصفت فى هذا البحث دراسة هستوكيميائية لتركيز وتوزيع أنزيمات السكسينك هيدروجنيز . وانزيم الغوسفاتيز القاعدى والحامضى وانزيم الكولين استرايز كذلك حامض الربيونيوكليك والديزوكس نيوكليك . والسكريات المركبة المخاطية كذلك الجليكوجين والدهون فى غدة الهاردرين .

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The HARDERIAN GLAND OF THE ONE HUMPED CAMEL (CAMELUS DROMEDARIUS)

II. Histochemistry

(with 7 figures)

By

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SUMMARY

A histochemical study of the concentration and localization of succinic dehydrogenase, acid and alkaline phosphatases, cholinesterase, RNA, DNA, mucopolysaccharides, glycogen and lipid in the harderian gland in Camel were Fully described

INTRODUCTION

During the course of histological investigation on harderian gland in the camel, it became necessary to have more detailed knowledge of the distribution of the various enzymes and chemical components. Only a few studies on the histochemistry of the harderian gland have been published. PAULE AND HAYES (1958) found that the composition of harderian gland secretions varies in the different classes of vertebrates ; in mammals it is usually lipoidal, in reptiles serous or sermucoid and in birds mucoid. WIGHT, MACKENZIE, ROTHWELL AND BURNS (1971) described the harderian gland of the domestic fowl histochemically to identify certain enzymes and chemical components.

The present study deals with the histochemical aspects of the harderian gland of the one humped camel.

MATERIALS AND METHODS

Harderian glands were taken from 32 healthy camels of both sexes of 2-7 years old, slaughtered at Cairo and Giza abattoires. The glands were quickly removed and subjected to the appropriate histochemical technique. Unless

a special reference is cited for the methods, the technical recommendations of PEARSE (1968) were followed.

Material were either fixed in 10% neutral formalin, calcium fromol Carnoy's fluid and Bouin's fluid and processed for paraffin sections at a thickness of 5-7 microns, or frozen in dry ice and cut in a freezing microtome at a th ickness of 10-20 microns.

The following chemical compounds and enzyme activities were investigated by various methods.

ENZYMES :

1 — *Succinic dehydrogenase* : Sections of fresh-frozen tissue were examined. Control sections were incubated in solutions without substrates.

2 — *Alkaline and acid phosphatases* were detected by of the calcium cobalt method and the lead nitrate method respectively (GOMORI).

3 — *Cholinesterase* : Tissues were fresh-frozen and sections of 20 microns thickness were examined by the method of GOMORI (1952). Actylthiocholine (ATHCH) iodide was used as substrates. Cholinesterase inhibitor namely 2.5 × 10 — 6 M — physostigmine salycilate (eserine) in 0.25 M— sodium acetate (PH 5.4) was used empirically to identify the true cholinesterase enzyme.

Chemical compounds :

1 — *Glycogen, glycoprotein and mucopolysaccharides* : The presence of glycogen and glycoprotein was investigated in paraffin sections treated with celloidin to prevent any possible diffusion of glycogen. Control sections were treated with diastase or saliva. The periodic acid Schiff (PAS) and Best's carmine methods were used to test for the presence of these compounds. PAS also was used to identify the presence of neutral mucopolysaccharides. The alcian blue was used for acid mucopolysacchaides.

2 — *Lipids :* The identify the presence of lipids, frozen sections were cut at 10 microns thickness from blocks of tissue fixed in10% formol calcium and stained in a saturated solution of Sudan black B in 70% alcohol for 30 minutes at room temperature.

Pieces of freshly removed glands were fixed in solutions of osmium tetraoxide ($Os O_4$) and embedded in paraffin to identify unsaturated lipid.

3.— *Nucleic Acid* : Methyl green — pyronin and ribonuclease method, Feulgen reaction and the gallocyanin — chromalum were the methods used on sections previously fixed in Carnoy's fluid and embedded in paraffin for identification of nucleic acids.

RESULTS

Succinic dehydrogenase (SDH) activity were manifested by deposition of black granules (formazan) in the cytoplasm of the cells. The ducts of the gland showed maximal evidence of dehydrogenase activity in cells and moderate activity was seen in the acinar epithelium (Fig. 1).

Alkaline phosphatase activity was confined to the blood capillaries surrounding the acinar epithelium and ducts (Fig. 2).

The reaction of acid phosphatase was variable in the epithelial cells of the acini. This appears in the form of granules specially concentrated in the apical part of the acinar epithelium. The nuclei show activity to the acid phosphatase (Fig. 3). Moderate activity have been observed in ducts.

Sites of cholinesterase activity were manifested by the dark brown precipitate of copper sulfide. Numerous numbers of sensory fibers and their endings containing active acetylcholinesterase in harderian gland have been observed. These fibers were of fine calibre forming plexiform receptors about the acinar epthelium and their ducts in the gland (Figs. 4 and 5). Under high magnifications, the enzyme appears distributed non uniformly along the axons in receptor terminals. These structures resemble varicosities or vesicles that have been detached from the main fibre, forming pictures suggestive of secretion (Fig. 4). Furthermore, positive reaction was seen in many of the nerve cells surrounding the ducts (Fig. 5) and the blood vessels.

Neither osmiphilic lipid nor Sudan black material was demonstrated by the methods used in the acinar epithelium and ducts.. Different sizes of unsaturated lipid stainable by osmium tetraoxide were seen scattered between the acini and ducts. Furthermore, numerous amount of lipid droplets were seen in capsula adiposa (Fig. 6).

The lumen of the ducts has a unique distribution of glycogen which gave us the indirect evidence we were seeking. The total amount varies in the various portions of the ducts, as well as from individual to individual and from gland to gland, but the relative distribution remains the same.

The cells of the tertiary duct which is closely associated with the secretory end piece have no glycogen. There is a fine granular, perinuclear distribution of glycogen in the secondary duct cells and also in its lumen. The glycogen in the secretory cells of the gland can be completely absent. Thus glycogen degradation is clearly linked to the functional activity of the secretory cells. Furthermore, the chondrocytes of the traversing hyaline cartilage through the gland show marked positive reaction with Best's carmine for glycogen.

According to the functional activity of the gland, PAS — positive material was present in the epithelium and lumina of the ducts but only in frequent acinar epithelial cells. The epithelial cells of the tertiary and secondary ducts contained positive material which appeared to accumulate towards the apical border (Fig. 7). Positive material was seen in goblet cells which were scattered in the pseudostratified columnar epithelium and also in stratified columnar epithelium of the large central collecting duct.

Epithelial cytoplasm and intraluminal material were orthochromatic after treatment with alcian blue. Positive material was observed in the apical border of the cytoplasm of the ducts and some acini. Furthermore, high activity was observed in the cytoplasm of the chondrocytes.

RNA was distributed as minute granules in subnuclear part of the acinar epithelium and their ducts. The nucleolus shows ANA activity. The chromatin is located chiefly peripherally adjacent to the nuclear membrane. The cells of the ducts show strong positive Feulgen and also gallocyonin — chrumalum reactions.

DISCUSSION

Succinic dehydogenase plays a vital role in the respiratory processes of most living cells and forms a link in the chain of reactions concerned with the oxidation of lipids, carbohydrates and proteins (SELIGMAN AND RUTENBURG, 1951). PADYKULA (1952) found succinic dehydrogenase in greatest concenrations within those portions of tissues and organs which show high metabolic activity, such a muscle, or are engaged in absorptive or secretory activities. In our observations, succinic dehydrogenase activity is much more marked in the cells of the secondary and tertiary ducts than in those of the secretory pieces. This also is in agreement with the results obtained by LOBITZ, HOLYOKE AND BROPHY (1955) on the histochemical evidence for human eccrine sweat duct activity.

Although non — specific acid and alkaline phosphatases have been detected ; the first enzyme shows the granular localization typical of lysosomal enzymes, and the second is confined to blood vessels. Both enzymes hydrolyse β —glycerophate more readily than other substrates. WIGHT, MACKENZIE, ROTHcELL AND BURNS (1971) found that acid phosphatase is important in energy transfer through high energy phosphate compounds, so the occurrence of a strongly positive reaction for this enzyme would be likely in an actively secreting gland. Our findings in regard to these materials are essentially the same as previously reported.

It is of interest to note that the results in the present studies concerning the localization of specific cholindesterase activity in harderian gland of camel are entirely in agreement with the results which have been made upon endocardium (KROKHINA AND PLECHKOVA, 1964) AND (PLECHKOVA, 1966), the urinary bladder mucosa (PLECHKOVA, 1962) and the uterine mucosa (GURVICH, 1960). As stated that the fine sensory fiber and their plexiform, receptors, which are widely distributed throughout the body do take part in acetylcholine metawhenever required an enzyme to inactivate excess tissue acetylcholine, thus bolism. Moreover, this system of afferent fibres is capable of releasing; whenever required an enzyme to inactivate excess tissue acetylcholine, thus participating in tissue trophism (PLECHKOVA, 1970). In reference to the harderian gland, it should be pointed out that cholinergic fibers and nerve cells were seen about the portions of the glands. This was of significance, since some observer have felt that the duct plays an active role in the process if secretion, and that this dudtal function under the control nerve fibers.

Specific cholinesterase activity, which is present in nerve fibers distributed as a network over the whole secretory pieces. The nerve fibers presumably innervate the myoepithelium. These observations are in agreement with the results obtained by LOEWENTHAL AND HINS, (1963) on the eccrine sweat gland.

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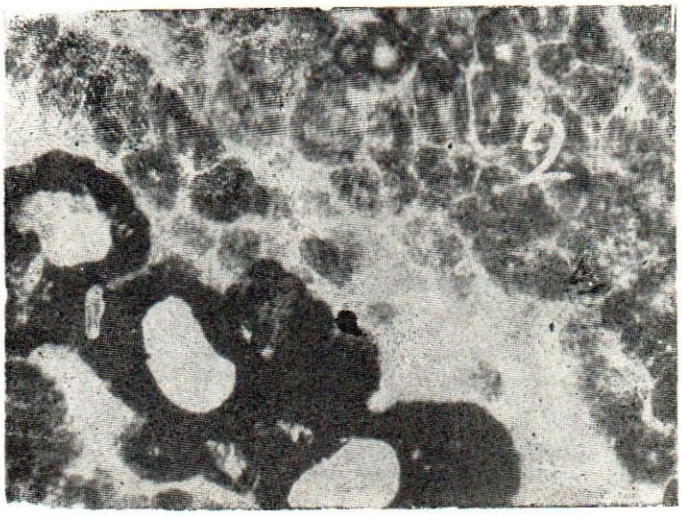

Fig. 1.—High succinic dehydrogenase activity were manifested in the ducts (1) and mode-rate in acini (2). × 250.

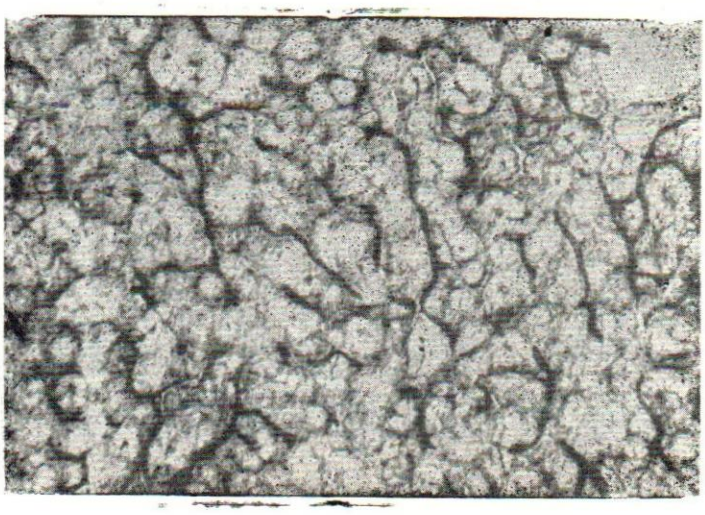

Fig. 2.—Distribution of alkaline phosphatase in blood vessels surrounding the acini. × 100

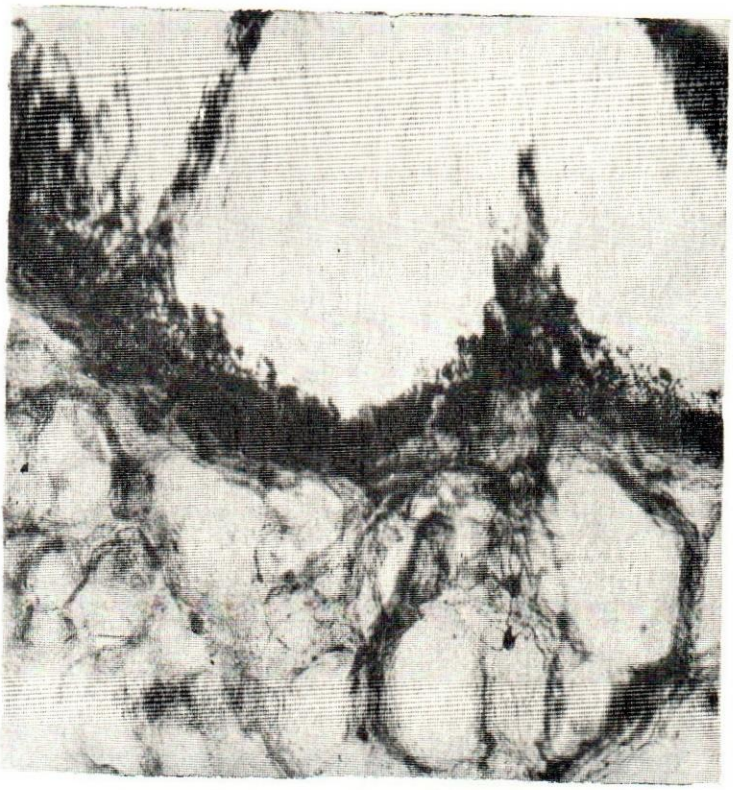

Fig. 5.—Cholinesterase activity insensory fibers and nerve cells in the duct of harderian glands. × 250.

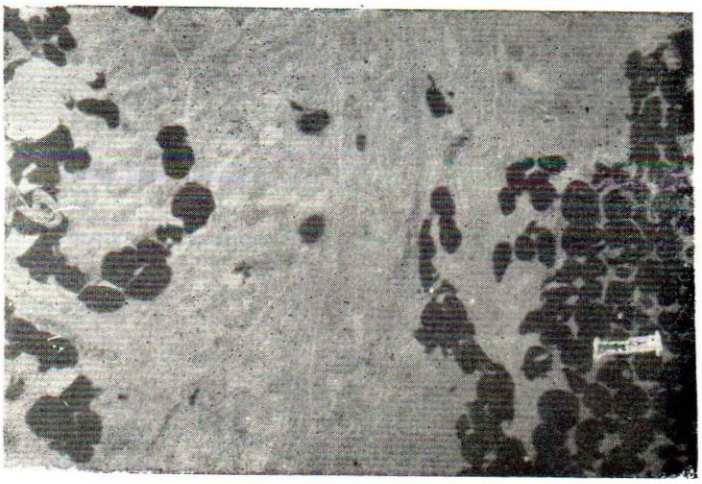

Fig. 6.—Unsaturated lipid demonstrated in the capsula adiposa (1) and between the acini ducts (2) × 100