Chapter - 4

AN EXPERIMENTAL APPROACH TO THE STUDY OF INNERVATION OF THE RAT PREPUTIAL GLAND

Rothman (1954) has reviewed the problem of innervation of sebaceous glands. He has reported that sebaceous glands in man are supplied by nerves going both to the gland proper and the surrounding connective tissue. He has described that a ramifying bundle of nerve fibers forms a coarse net work in the perigandular connective tissue and slender branches from the latter penetrate the interlobular septa breaking up into very fine fibers forming a plexus within the gland. Boecke (1934) has described a plexus of nerve fibres surrounding the sebaceous gland, which he thought to be sympathetic in origin. On the other hand, Montagna (1963), citing the work of Hurley et al.(1953), Hellmann (1955) and Thies and Galente (1957), favoured the general agreement that there are no nerves supplying the sebaceous glands. Winkelmann (1960) also failed to demonstrate any autonomous nerve supply to the sebaceous glands. Thus the literature on the subject is replete with discrepencies and also there is no report regarding the innervation of other modified sebaceous glands, which otherwise are being used as models to elucidate physiology of sebaceous glands. This report deals with pattern of

innervation in the preputial glands of rat. An attempt is also made to evaluate the significance of nervous system in the functional activity of this sebaceous analogue.

MATERIALS AND METHODS

Male albino rats weighing 120-140 gms were utilized for the study.

Anatomical Study : Rats were dissected from the ventral side so as to expose the preputial glands of either sides. The dorsal nerve supplying the penis was traced upto sacral plexus by following its course through pelvis. After confirming the origin of the dorsal penile nerve a branch was traceable from the pudendal nerve. This nerve after traversing the connective tissue between the penis and the preputial gland; was found to penetrate the capsule of the preputial gland.

Microscopy : Nerve fibers were demonstrable in paraffin sections (12 μ thick) employing silver impregnation techniques of Bloom et al. (1962). Acetyl Cholinesterase (AChE) and Butyryl Cholinesterase (BuChE) activities were histochemically demonstrated employing the method of Karnovsky and Roots (1964). Adrenergic innervation was studied by fluorescence microscopy using the technique of Falck and Owman (1965).

Monoamine oxidase (MAO) activity was studied following the method of Glenner et al. (1957).

Denervation : A group of six rats were subjected to unilateral surgical denervation. A piece of nerve (5 mm long) supplying one of the two preputial glands was cut out at its origin near the base of penis. The gland of the other side was treated as control. 15 days after unilateral surgical denervation, denervated as well as control preputial glands were removed and treated for histochemical study of AChE, BuChE and MAO.

Chemical denervation was performed by intraperitoneal administration of reserpine (1 mg/100 gms of body weight/day for 5 days) and 6-hydroxydopamine (2 mg/100 gms body weight/ 12 hours for 3 days) in two separate groups of four rats each. The glands from these rats were tested for AChE, BuChE and MAO activities and were compared with controls.

Adrenergic innervation was confirmed using the glands from reserpinized and 6-hydroxydopamine treated animals.

RESULTS AND DISCUSSION

Gross anatomical observations showed that the pudendal nerve supplying the penis, originated from the sacral plexus

EXPLANATIONS FOR FIGURES

Fig.1 Photograph showing innervation of the rat preputial gland *in situ*.

VC = Vertebral column

PN = Pudendal nerve

DP = Dorsal nerve of penis

P = Penis

PR = Preputial gland

1

and ran on the dorsal side of penis. These paired dorsal nerves on their way to the tip of the penis gave out a branch each on their respective sides. This branch, as shown in the Fig.1, ran laterally through the connective tissue capsule of the gland and penetrated the intralobular septa. In fact, histological study using silver impregnation technique clearly revealed the presence of nerve fibers in connective tissue capsule as well as that of a bundle of nerve fibers entering the glandular mass along an intralobular septum (Figs. 2, 3 and 4). Beaver (1960) also reported the presence of nerves in the trabaculae of preputial gland. As shown in Figs. 12, 13, 14 and 15 a network of cholinergic and adrenergic nerve fibers was found to surround the major secretory duct of the gland. However, during the course of the present study no direct connections between the bundle of nerve fibers entering the intralobular septa and nerve-network surrounding the chief excurrent duct could be observed at any stage. Thus the origin of nerves forming the network surrounding the main secretory duct could not be traced with certainty presently.

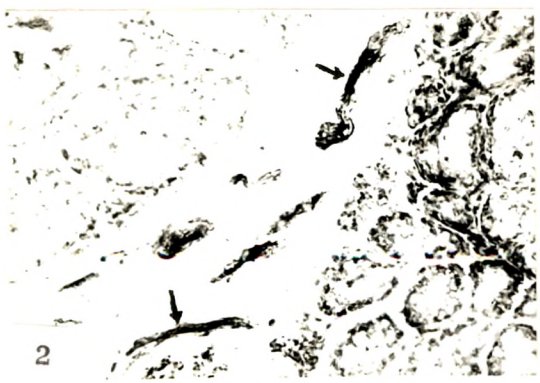
Additionally, histological preparations subjected to silver impregation treatment also revealed that a part of connective tissue surrounding the gland showed presence of some nerve fibers, which follow an undulating course, and

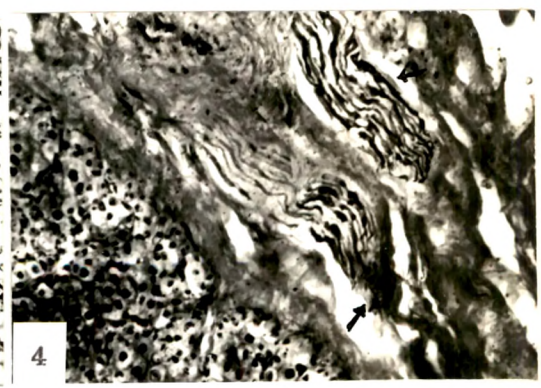
EXPLANATIONS FOR FIGURES

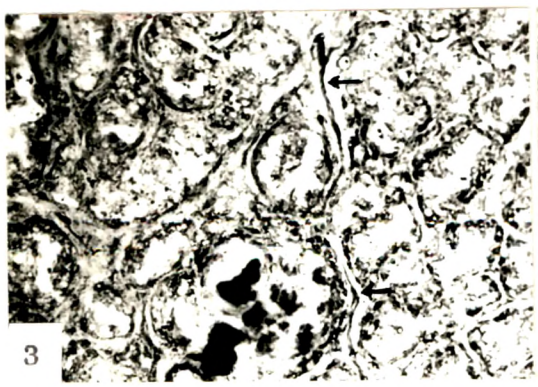
Fig.2 Photomicrograph of the section of rat preputial gland showing bundle of nerve fibgres (arrows) in interlobular septum. Silver impregnation technique. 125X

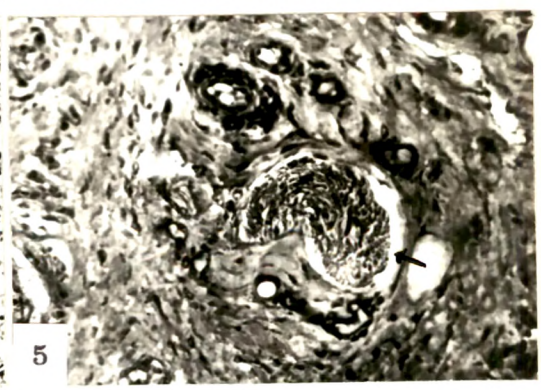
Fig.3 Photomicrograph of the section of rat preputial gland showing nerve fibres (arrows) in between acini and surrounding duct (DT). Silver impregnation technique. 125X

Fig.4-5 Photomicrographs of the Sections of rat preputial gland showing encapsulated end organs (arrows) in the connective tissue surrounding the gland. Silver impregnation technique. 125X


2


4


3

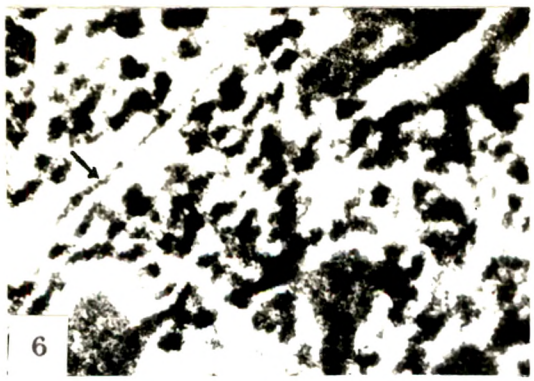

5

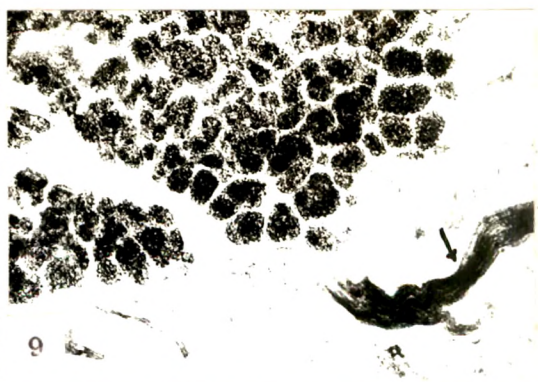
EXPLANATIONS FOR FIGURES

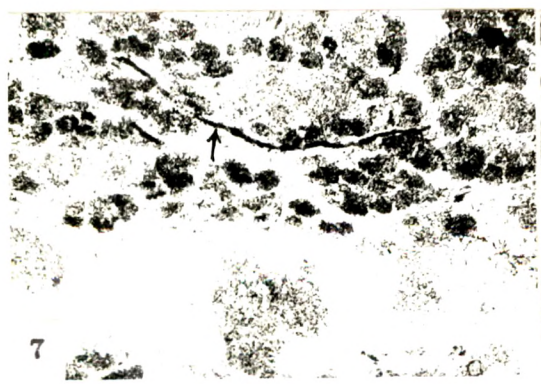
Figs. 6 and 7 Photomicrographs of the sections of the preputial gland of rat showing AChE activity. Also depicts presence of AChE positive nerve fibres (arrow) in between the acini. 65X

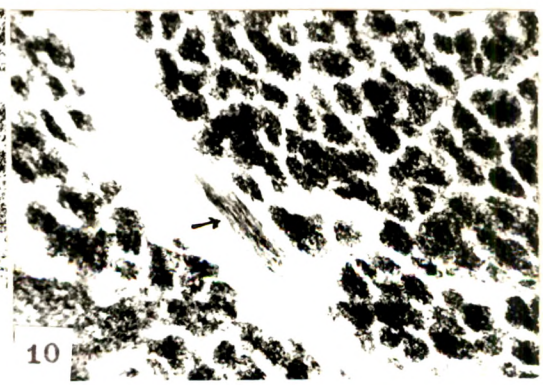
Fig.8 Photomicrograph of the section of the preputial gland of rat showing BuChE activity. 125X

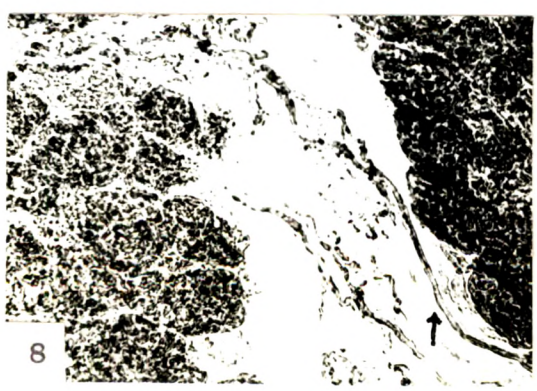
Figs. 9 to 11 Photomicrographs of the sections of rat preputial gland showing MAO activity. Note the enzyme activity in the acini as well as in nerve fibres (arrows). 65X

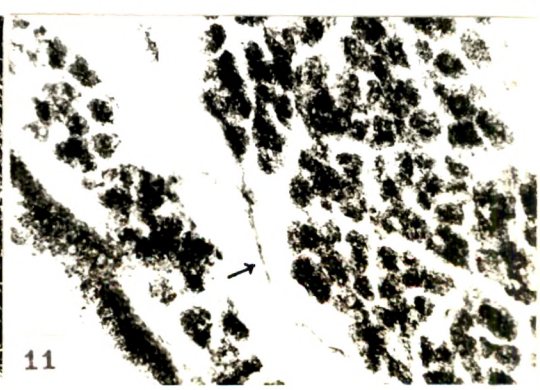

6


9


7


10

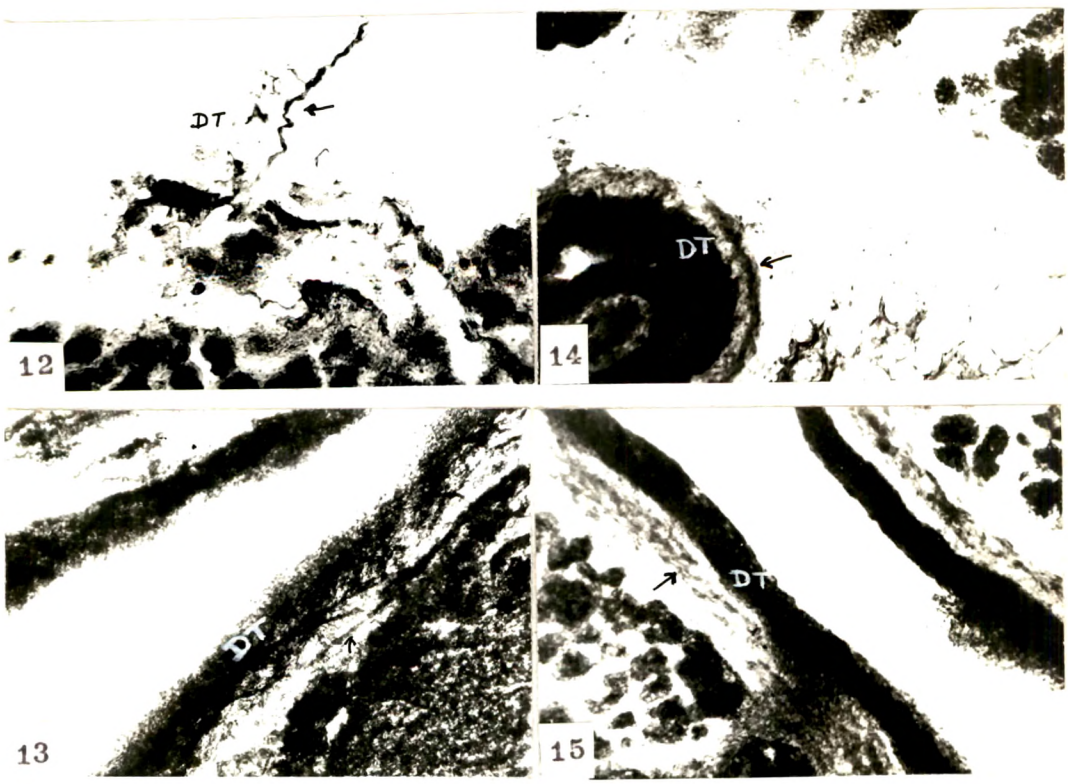

8


11

EXPLANATIONS FOR FIGURES

Figs. 12 and 13 Photomicrographs of the section of the rat preputial gland showing BuChE (Fig.12) and AChE (Fig.13) positive nerve fibres (arrows) surrounding the duct (DT) respectively. 65X

Figs. 14 and 15 Photomicrographs of the sections of rat preputial gland showing MAO activity. Note MAO activity surrounding the duct (DT). 65X


DT
12
DT
14
DT
13
DT
15

have terminal encapsulations (Figs. 4 and 5) in the connective tissue. Such encapsulated nerve endings in the connective tissue capsule of the gland represent, in all probability sensory end-organs.

Histochemical observations on the distribution patterns of AChE are of great value in the study of cholinergic innervation at microscopic level. Both acetyl and butyryl cholinesterases were studied histochemically. Figs. 6, 7 and 8 indicate presence of nerve fibers showing positive reaction with acetylcholine (Figs. 6 and 7) as well as butyrylcholine (Fig.8). Network of cholinergic fibers was also observed surrounding the duct. Comparison of the distribution pattern of cholinesterase positive fibers with results of silver staining technique confirms that the nerve fibers running into the intralobular septa and surrounding the main duct are cholinergic in nature (Figs. 12 and 13). Strong cholinesterase activity was observed in non-neuronal components of the preputial gland of rat. The acinar cells and the cells of the duct system in the rat preputial gland depicted strong acetyl and butyrylcholinesterase activities (Figs. 6 and 7). However, cholinesterase positive staining is not a property of all sebaceous glands. Montagna (1963) reported that human skin sebaceous glands are negative to cholinesterase while those

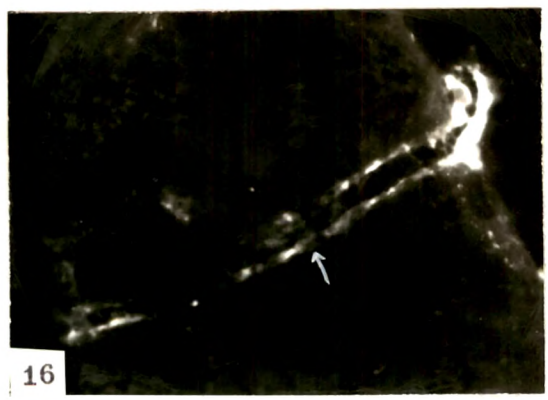
EXPLANATIONS FOR FIGURES

Figs.16 to 18 Photomicrographs of the sections of the preputial gland of rat showing fluorescence (arrows) indicating the presence of adrenergic fibres. 125X

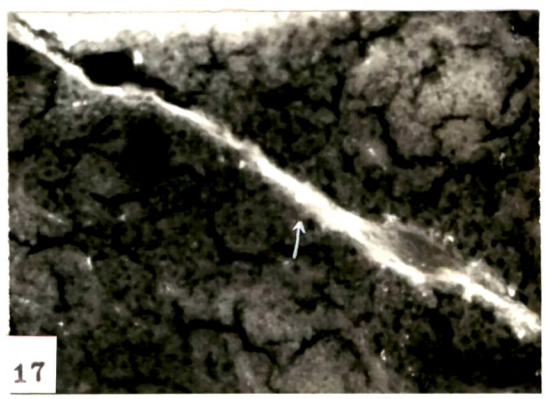
Fig.16 Adrenergic fluorescence along the blood vessel.

Fig.17 Adrenergic fluorescence in the connective tissue septum running between lobules.

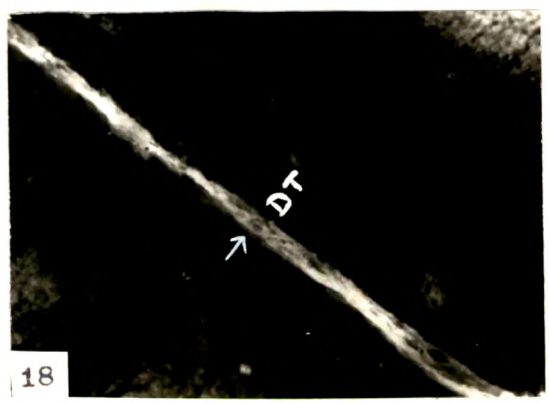
Fig.18 Adrenergic fluorescence surrounding the duct (DT).



16



17



18

in nipple of the female breast contain cholinesterase (Giacomelti and Montagna, 1962). Ballantyne and Bunch (1967) reported the presence of non-neuronal esterase in the sebaceous glands of mammary skin. It has been reported that the Meibomian glands are surrounded by many nerves which contains specific cholinesterase activity (Montagna and Ellis, 1959; Montagna, 1962).

The adrenergic innervation of the gland was investigated by treating the tissue with paraformaldehyde vapour to observe fluorescence due to catecholamines (Falck and Owman, 1965). A few adrenergic fibers, showing a characteristic yellow green fluorescence, with a distribution pattern similar to that of AChE containing fibers were seen in the sections of the gland (Figs. 17 and 18). Such fluorescence was not observable in the glands of reserpinized and 6-hydroxydopamine treated rats, thus confirming the demonstration of adrenergic fibers using the technique employed. Some of the fibers were seen to be present in the wall of the larger blood vessels (Fig.16). Since the extrusion of preformed secretion in this gland is mediated through α-receptors and can be induced by adrenaline treatment (Chapter-5), it is obvious that the observed fluorescence surrounding the excretory ducts of the glands would be present indicating the adrenergic fibers there.

In addition to the fluorescence technique, histochemical study of MAO also indicated the presence of adrenergic fibers. However, the intensity of MAO was found to be comparatively high in acinar cells (Figs. 9, 10 and 11). It has been suggested that MAO may participate in the enzymatic degradation of amines and the formation of biologically active metabolites (Gorkin, 1973). Presence of MAO is an indication of turnover of catecholamines at the site of its localization. On the basis of this knowledge of the functional significance of MAO, it could be suggested that high MAO activity in acinar cells is an index of turnover of catecholamines in the cells of the rat preputial gland. It has been reported that deamination reaction catalysed by MAO has a regulatory influence on the SDH activity of mitochondria in liver (Gorkin and Krivchenkova, 1967) and heart muscle (Gorkin and Krivchenkova, 1971). It could be implied that high MAO activity observed in the acinar cells of the preputial gland may play such a role in the regulation of metabolic processes of the gland; besides its role im degradation of biogenic amines. It is worth mentioning here that sebaceous glands of all mammals studied, including sebaceous glands of man show significant levels of MAO activity (Montagna, 1963).

The present investigation provides clear evidence that the rat preputial gland is supplied by both adremergic and

cholinergic nerve fibers; though other sebaceous glands may lack distinct innervation (Winkelmann, 1960).

Cholinesterase and MAO activities were studied histochemically following surgical denervation, reserpinization and 6-hydroxydopamine treatment to evaluate functional importance of nerve supply. It is known that both reserpinization and 6-hydroxydopamine treatment deplete catecholamines at the adrenergic nerve terminals (Kostrzewa and Jacobowitz, 1974). However, when activities of AChE, BuChe and MAO in the preputial glands of surgically denervated (15 days after denervation), reserpinized and 6-hydroxydopamine treated rats were compared with that of normal, no detectable change was observed. This raised doubts against direct involvement of nervous system in the physiology of rat preputial gland. Doupe and Sharp (1943) also found that denervation of a skin area had no effect whatsoever on sebaceous excretion. Melczer and Deme (1942) claimed that pilocarpine stimulates sebaceous gland activity but others could not corroborate (Rothman and Hermann, 1953). In the light of these observations it could be suggested that, like in sebaceous gland of skin, nervous system is not directly involved in the physiology of the rat preputial glands except, for its role in excretory mechanism as reported in Chapter-5.

Administration of reserpine and 6-hydroxydopamine is known to increase adrenal catecholamine secretion (Mueller et al., 1969). It was observed that MAO activity of the rat preputial glands did not undergo any alteration following reserpine and 6-hydroxydopamine treatments. It could, therefore, safely be presumed that MAO activity, which remains unaltered after experimental treatments, is due to the availability of catecholamines from adrenal medulla via blood circulation. From the present investigation it appears that the neurohormones of adrenal origin might play an important role in the physiology of the preputial glands since even surgical denervation did not affect the enzyme activity in the gland.

In Chapter-6 it is shown that administration of isoproterenol, a β-adrenergic agonist, reduces the acid phosphatase activity of the gland and that it also influences glandular steroid metabolism (Chapter-8). These findings strongly favour the view that circulating catecholamines play an important role in the physiology of this sebaceous analogue. However, the nervous component of the gland may be of minor significance as evident from its involvement only in excretory activity of the glamd (Chapter-5).