CHAPTER THREE

OBSERVATIONS

The Indian leaf-nosed bat, H. fulvus fulvus under present investigation is a seasonal breeder and shows a single annual breeding cycle. Annual breeding activities in this species of the bat have been reported by Patil (1968) and Vibhute (1981). The annual breeding cycle of the bat, H. fulvus fulvus is described in the chapter two on Material and Methods. The testes undergo morphological and histological changes throught the year and accordingly the breeding or sex cycle is distinguished into four phases viz. the inactive or sexually quiescent period (March to July) when spermatogenesis is not seen, the preparatory or prebreeding period (August to September) when initiation of spermatogenesis occur, the active breeding period (October to December) when the spermatozoa meture and are liberated and postbreeding period (January to Febuary) of testicular regression. The seasonal morphological and histological observations on testes of H. fulvus fulvus are presented hereafter which confirm the earlier reports on sex cycle of this species of bat, followed by observations on the frontal sac gland.

OBSERVATIONS

TESTES

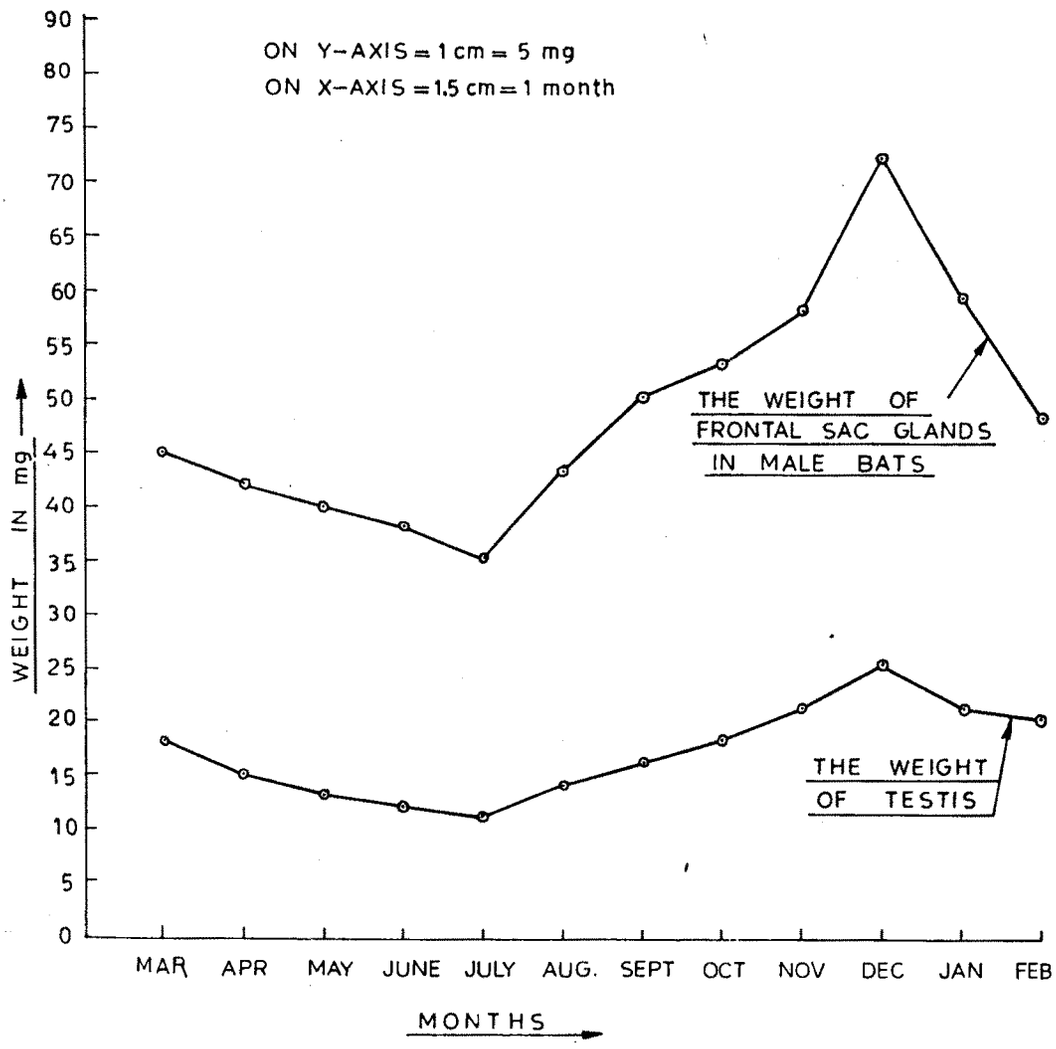
I) Seasonal Variations in the Testicular Weight of the Bat :

The testes showed seasonal variations in morphology and in weight in accordance to the annual breeding cycle.

Table No. 1 : Seasonal variations in the weight of the testis and the frontal sac glands of the male bats.

Months	Weight of the testis in mg.	Weight of the frontal sac gland in mg.
March	18 ± 0.9	45 ± 1.5
April	15 ± 0.8	42 ± 3.0
May	13 ± 0.9	40 ± 3.5
June	12 ± 0.5	38 ± 4.0
July	11 ± 0.7	35 ± 3.7
August	14 ± 1.1	43 ± 2.5
September	16 ± 1.4	50 ± 2.0
October	18 ± 1.0	53 ± 4.0
November	21 ± 0.9	58 ± 5.5
December	25 ± 1.2	72 ± 4.8
January	21 ± 1.4	59 ± 3.0
February	20 ± 1.0	48 ± 2.5

SEASONAL VARIATIONS IN THE WEIGHT OF TESTIS AND FRONTAL SAC GLANDS IN MALE BATS



The changes occuring in the weight of each testis are recorded in Table No. 1 and illustrated in Graph. During sexually guiescent period the testes appear small elongated and their minimal weight was 11 mg in the month of July. With the onset of pre-breeding period there was gradual increase in size and in weight of the testes. The testicular weight increased gradually from 14 mg in August to 16 mg in September. The testis showed further increase in size and in weight during the sexually active period reaching the peak of 25 mg in December. During the post breeding period testis showed a gradual decline in the weight from 21 mg in January to 20 mg in early February. This decline in weight continues in sexually quiescent period reaching 11 mg in the following July. The testis shows highest weight just prior to and during active breeding period whereas in the sexually quiescent period the testicular weight is low. Thus, the changes in the weight of the testis run closely parallel to the sexual activity of the bat.

II) Seasonal Histological Variations in the Testes :

Histological variations are evident in the different components of the testes during various reproductive seasons which are described hereafter.

(a) Testes During the Sexually Quiescent Period :

Histologically, the seminiferous tubules were reduced in their overall diameter with an apparent increase in the outer intertubular connective tissue.

The seminiferous tubules were composed of a single layer of germinal epithelium and one or two layers of resting spermatogonia. Very few Sertoli cells could be distinguished amidst the spermatogonia. The seminiferous tubules appeared to be collapsed but with unobliterated lumina. Hypotrophied Leydig cells appeared in the loose connective tissue of the intertubular areas. Thus the testes were in the regressed condition during sexually quiescent period.

(b) Testes During the Pre-breeding Period :

Each testes during the pre-breeding period became some what larger and showed preparatory changes for spermatogenesis. The testis during this period histologically, consisted of larger seminiferous tubules and reduced intertubular connective tissue. Each of the seminiferous tubule consisted of the germinal epithelium, three or four layers of spermatogonia and spermatocytes and the spermatids in the late prebreeding period. The Sertoli cells could be well distinguished near the basement membrane. The lumina of the seminiferous tubules gradually became reduced due to addition of spermatogenic element. There appeared increase in size and number of Leydig cells in this period.

(c) Testes During the Active Breeding Period :

The size and the weight of testes showed a gradual increase during breeding period reaching its peak activity in December. Histologically, the seminiferous tubules consisted of the germinal epithelium, spermatogonia,

Spermatocytes, spermatids and mature sperms. The Sertoli cells could be well distinguished. The sperm bundles were observed in the lumina and clear lumina were not evident. The intertubular areas were greatly reduced and contained only thin streaks of connective tissue. Either isolated or groups of hypertrophied Leydig cells occured in the connective tissue. The testes showed intense spermatogenesis during this period. The seminiferous tubules attained their maximum diameters.

(d) Testes During the Post-breeding Period :

Soon after the breeding activities the testes showed progressive decrease in the size and weight during the post breeding period. This decrease in size correspond to the histological changes occuring in testes. The rate of spermatogenesis was declined and the seminiferous tubules were reduced in their diameter. The spermatocytes and spermatids which did not reach spermatogenesis started degenerating and resulted in the formation of cell-debris in the lumina of the seminiferous tubules. The seminiferous tubules mainly contained germinal epithelium, few resting spermatogonia and Sertoli cells. The intertubular areas contained well developed connective tissue with ill defined and hypotrophied Leydig cells.

FRONTAL SAC GLAND:

A) Morphology :

Position : The frontal sac gland in H. fulvus fulvus appeared as a saccular invagination of the median frontal skin in the head region. The gland was observed well developed in sexually matured males where as females

possessed inconspicuous papilla. The young immature bats showed a small buldge on innerface of the skin. This organ appeared as a thickened gland with small orifice without any secretory material. When the bat attains sexual maturity the gland attains full size with secretory material in the sac.

Frontal sac gland in male bat : The frontal sac gland in the male bats occupied the deeper part of the dermis, presented an ovoid outline and lay behind the nose leaf. The prominent frontal sac gland of the male has a transversely oriented slitlike orifice or opening. The sac contained yellow-brown, odouriferous secretory material in it. Furtermore, the sac was adorned with a special type of white hair growing centrally and with skin lappets on its lips.

Frontal gland in female bat : The frontal sac gland in female bats was inconspicuous and appeared in the form of a rudimentary papilla on innersurface of median frontal skin. The shallower and less developed female sac possessed only a tuft of black hairs. The female glandular area exhibited no changes in its size and activity throughout the year.

B) Seasonal Variations in Size and Weight of the Frontal Sac Gland in Male Bats. :

The size and the weight of the frontal sac gland in the male bats showed seasonal variations in accordance to the testicular cycle. These variations were parallel to the sex cycle. The variations occuring in wet weight of the frontal sac gland of the male bats are recorded

in Table No. 1 and illustrated in Graph.

The frontal sac gland during the sexually quiescent period was small ovoid structure. The diameter was about 3-4 mm and weight was minimal which was 35 ± 3.7 mg in the month of July. The size and weight showed progressive increase in August and September i.e. during the pre-breeding period. The average size of the gland was 4-5.5 mm in diameter and was 8-9 mm long. The weight of the gland was 43 ± 2.5 mg in August and 50 ± 2.0 mg in September. The sac of the gland contained yellowish secretion. The active breeding period witnessed continued increase in size and weight of the gland reaching the peak in December. The size of the gland reached 14 to 16 mm long and 6-7 mm in diameter in December when the breeding activities of the bat were at its maximum. The average weight of the gland reached the peak of 72 ± 4.8 mg in early December. The secretary material in the sac of gland appeared yellowish brown and waxy in nature. The sac contained maximum secretory material which covered orifice of the gland and spread over the skin hair of leafnose area. There appeared a gradual decline in the size and weight of the gland in late December which progressively continued in the post-breeding period. After cessation of breeding activities during post breeding period the size of the gland gradually reduced together with fall in the weight of the gland. The size of the gland was 10 to 12 mm long and 6-7 mm in diameter. The weight of the gland was 59 ± 3.00 mg in January and 48 ± 2.5 mg in February. The decrease in the quantity of the secretary material in sac was also evident

during post breeding period. In the early sexual quiescence, there was further reduction in weight of the gland which was 45 ± 1.5 mg and 38 ± 4.00 mg in March and June respectively. The secretory material present in the sac of the gland showed further decrease during the sexually quiescent period and sac contained a little secretory material.

C) Blood Supply :

The blood supply varied in the frontal sac gland of the male bat in accordance to the testicular cycle. The mature male bats had a very little blood supply to the frontal sac gland during the sexually quiescent period which showed progressive increase during prebreeding period and attained the maximum vascularization during active breeding period. The network of the blood vessel of the gland was complex and blood vessels were large during active breeding period. This supply regressed considerably during the post breeding period which was closely similar to that in the sexually quiescent period.

Thus the variations in the frontal sac gland in male bats paralleled to that of testicular cycle and invalved seasonal changes in size, weight, vascularization and functional state of the gland.

D) Histology of the Frontal Sac Gland :

The histology of the frontal sac gland of the male and female bats in H-E stained preparation is shown in figs. 1 to 8 and figs. 9-12 respectively.

The histological observations revealed a glandular organization in male bat. The glandular area consisted

of the sebaceous and sudoriferous apocrine glands. The sac wall was lined by haired skin consisting of the thin epidermis, frequently 1-3 cell thick (Figs. 1,3,5,7) and fairly thick dermis (figs. 1,3,5,7). The dermis contained interwoven collagen fibres. The sac contains were mainly crenate, non-medullated white hair and fragments of stratum corneum. Embeded in the superficial zone of dermis were sebaceous glands (Figs.1,3,5,7) associated with hair follices. The deeper zone of dermis contained a simple saccular type of apocrine glands (Figs.3,4,5). Each apocrine gland has a proximal secretory tubule and a distal non-secretory duct connected to the neck or near the orifice of a hair follicle. The epithelium of the secretory tubules was lined by columner cells with basal nuclei and that of ducts consisted of cuboidal cells with centrally placed nuclei. The lumen of apocrine tubules contained secretion of the gland (Figs. 5,6) which was eosinophilic. The apocrine gland cells exhibited blebbing (Figs.3,5,6). The peripheral part of the gland consisted of clumped apocrine glands without an apparent lumen (Figs. 2,3,5,8). This part stained darker with haematoxylin and had little blood supply indicating passive secretory function. Beneath these apocrine glands was a dense layer of collagen fibres. Next to this collagenous layer was a muscle layer, fascicles of which run parallel to the antero-posterior axis of the sac. This arrangement of muscles in relation to collagen fibres

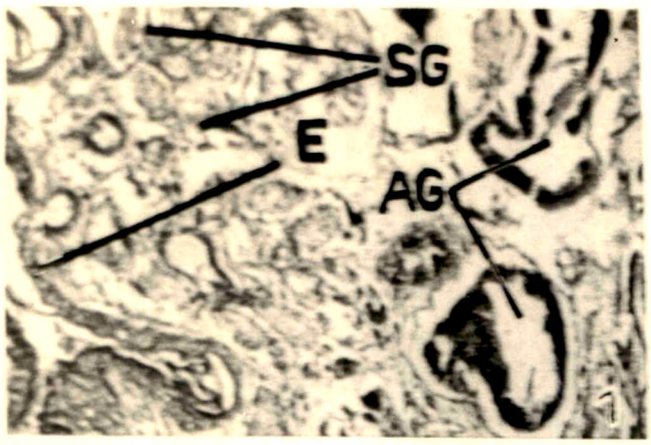
provide a machanism for everting the sac due to which secretion from sac could be expelled out on fore head of the male bat.

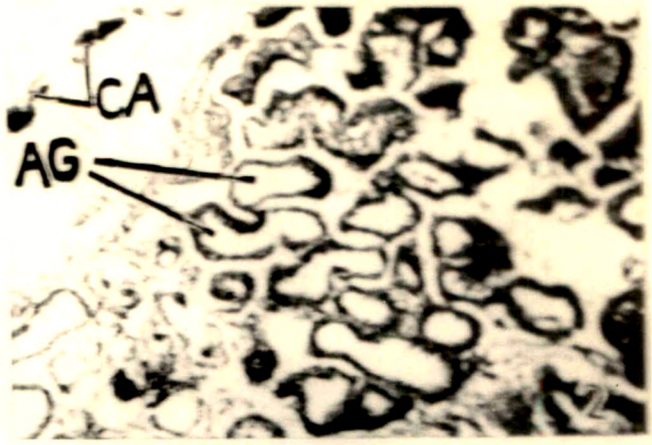
The histological observations on the glandular area in female bat showed that gland consisted of few tubules (Figs. 9 to 12). These tubules were scattered in adipose tissue layer of dermis. These tubules were reduced to clusteres of nuclei in adipose tissue. The tubules had narrow lumina and no secretory material throughout the annual breeding cycle. The rudimentary glandular area was incapable of eversion due to lack of collagen fibres and muscle layer. The glandular area in female bat showed no histological variations throughout the annual breeding cycle.

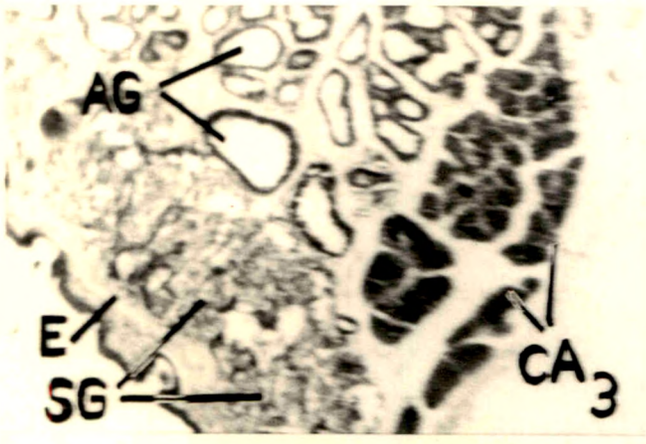
E) Seasonal Histological Observations in Male Bat :

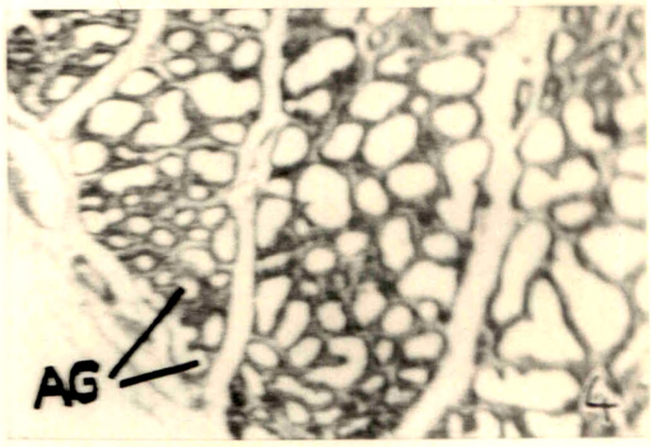
(a) Frontal Sac Gland in the Sexually Quiescent Period :

During the sexual quiescence, from March to July, the frontal sac gland in male bats was in regressed condition. The sebaceous gland were in regressed condition. with small and compactly arranged sebaceous cells (Fig.1). The sebum was very poorly seen in these glands. Thus the superficial zone of dermis containing sebaceous glands was collapsed. The apocrine gland tubules were with larger lumina and contained residual secretion (Figs.1,2). The intertubular region was occupied by large amount of connective tissue with few blood vessels. The

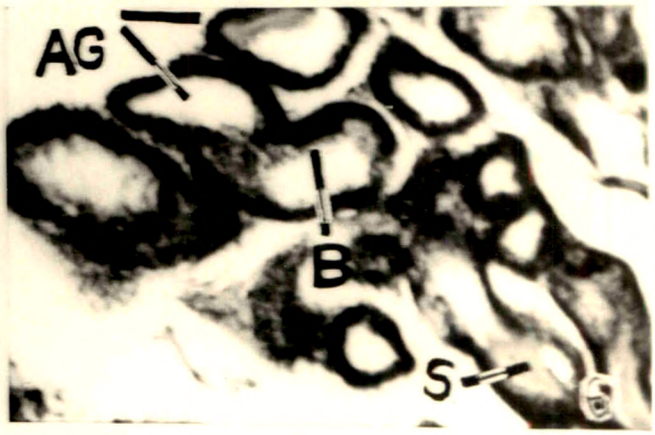

SG
E
AG
1

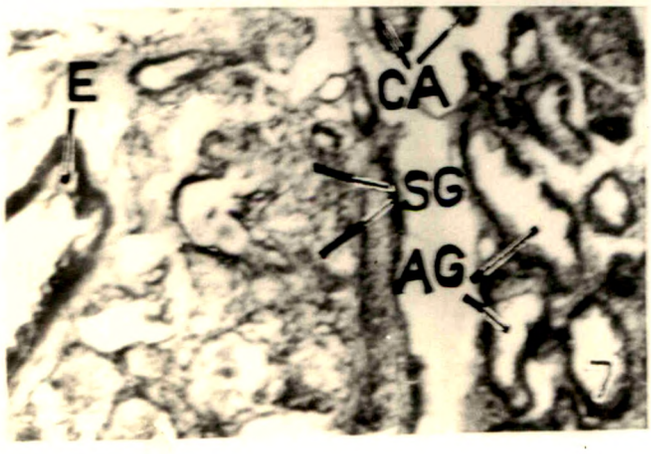

CA
AG
2

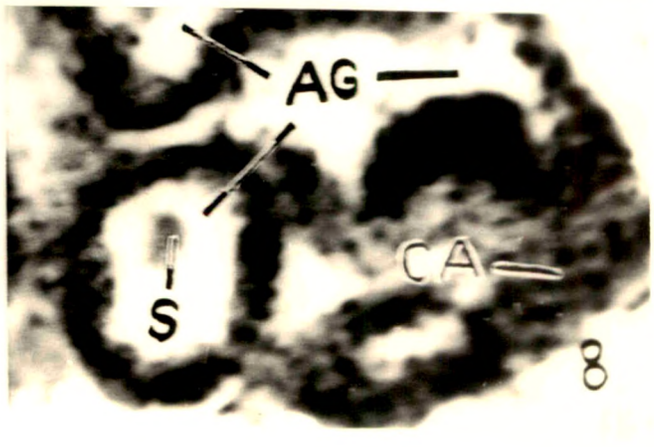

AG
E
SG
CA
3


AG
4


E
S
SG
CA
AG
5


AG
B
S
6


E
CA
SG
AG
7


AG
CA
S
8

CAPTIONS TO FIGURES

Fig. 1 Section passing through the frontal sac gland of the male bat during sexual quiescence stained with H-E to show epidermis (E), sebaceous glands (SG), apocrine gland tubules (AG) x 240.

Fig. 2 Section passing through the frontal sac gland of the male bat during sexual quiescence stained with H-E to show apocrine gland tubules (AG) and peripheral clumped apocrine glands (CA) x 240.

Fig. 3 Section passing through the frontal sac gland of the male bat during prebreeding period stained with H-E to show epidermis (E), sebaceous glands (SG), apocrine gland tubules (AG) and clumped apocrine glands (CA) x 320.

Fig. 4 Section passing through the frontal sac gland of the male bat during prebreeding period stained with H-E to show apocrine gland tubules (Ag) x 320.

Fig. 5 Section of the frontal sac gland of the male bat during active breeding period stained with H-E to show epidermis (E), sebaceous glands (SG) and apocrine glands (AG) and peripheral clumped apocrine glands (CA). Note secretion in the apocrine gland tubules x 300.

Fig. 6 A magnified view of apocrine gland tubules during the active breeding period to show blebbing (B) and secretion (S) in apocrine gland (AG) in H-E stained section x 450.

Fig. 7 Section of the frontal sac gland of the male bat during the postbreeding period stained with H-E to show epidermis (E), sebaceous glands (SG), reduced apocrine glands (AG) and clumped apocrine glands (CA) x 240.

Fig. 8 A magnified view of apocrine gland tubules during the postbreeding period to show regressing apocrine tubules (AG) and clumped apocrine glands (CA). Note scanty secretion (S) in the apocrine gland tubules x 900.

appcrine tubules were lined by single layer of cuboidal cells without blebs. The peripheral clumped apocrine glands were small and compact without lumina.

(B) Frontal Sac Gland in the Prebreeding Period :

Histological structure of the frontal sac gland in male bat, during the prebreeding period (August and September) showed slight changes than during the sexual quiescence. There was increase in size of the sebaceous glands in the dermis (Fig. 3). The sebaceous gland lobes consisted of enlarged sebaceous cells with central large nucleus in each cell. Very little sebum was observed in the glands and the sebaceous duct was not distinct. The dermis appeared thicker due to increase in size of the sebaceous gland. The deeper sudoriferous part of the frontal sac gland showed reduced connective tissue in the intertubular region and larger appcrine tubules. The connective tissue contained larger blood vessels in this period. The apocrine tubules showed increase in diameter (Fig. 4). The cells lining the apocrine gland tubules became gradually columner. The columner cells showed moderate amount of blebbeing which increased gradually at the end of the prebreeding period. The blebs formed at the apical surface of columner cells were released free in the lumina of the tubules. There was very little eosinophilous secretion in the tubular lumina. The tubules contained less to moderate amount of secretion indicating that the apocrine gland tubules were in different stages of the secretary activity. The clumped apocrine gland did not show any significant changes except slight enlargement in size.

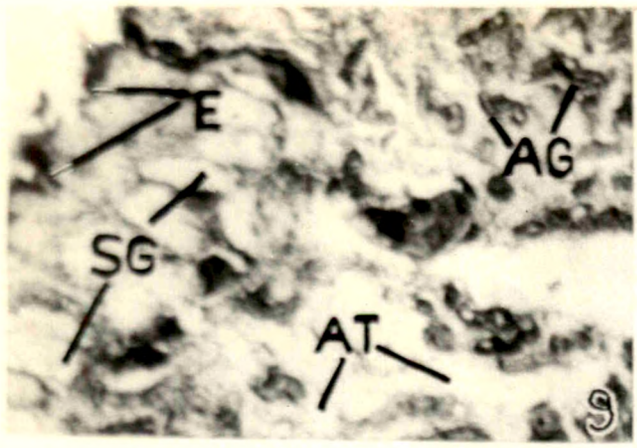
(c) Frontal Sac Gland in the Active Breeding Period :

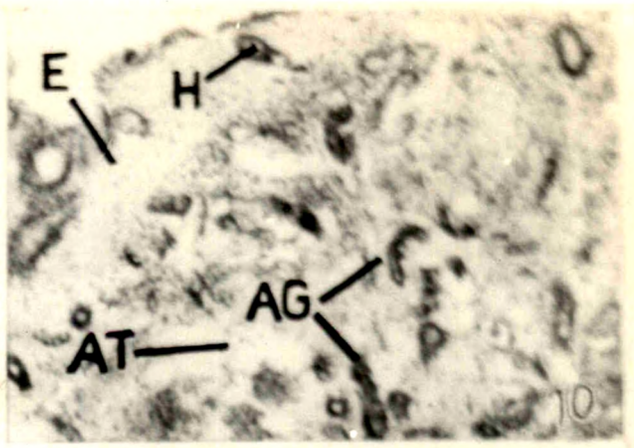
Histologically, the frontal sac gland during the active breeding period (October to December) showed maximum secretory activities in the apocrine gland tubules (Figs. 5,6). The sebaceous zone of the dermis showed gradual increase in dimensions in October and November and attaining maximum peak in December. The sebaceous glands (Fig. 5) were large, lobulated and extend inside the dermis, reaching to the deeper apocrine glands. The ducts of the sebaceous gland opened at the base of hair follicles. The cells of the gland were large with centrally located nucleus in each cell. The sebaceous glands contained sebum. The maximum size and the secretory activity were observed in late November and early December. The apocrine tubules showed maximum diameter during active breeding period due to [illegible] which the intertubular areas were greatly reduced. The streaks of connective tissue in the intertubular areas, contained large blood vessels. The epithelial cells lining the apocrine tubules were tall columnar and showed maximum blebbing (Fig. 6). The blebbing was maximum in late November and early December. The lumens of the apocrine gland tubules were full of eosinophilic secretion (Fig. 5). In late December the cellular activities, blebbing and secretion in apocrine tubules and size of sebaceous gland showed slight depletion. The peripheral apocrine glands were slightly larger than the prebreeding period and no other significant changes were observed in this part.

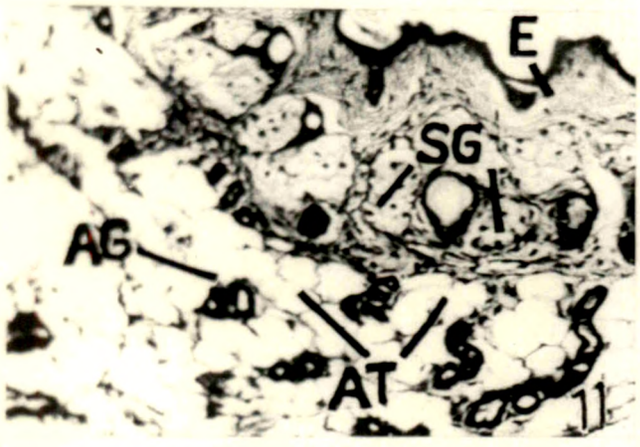
(d) Frontal Sac Gland in the Postbreeding Period :

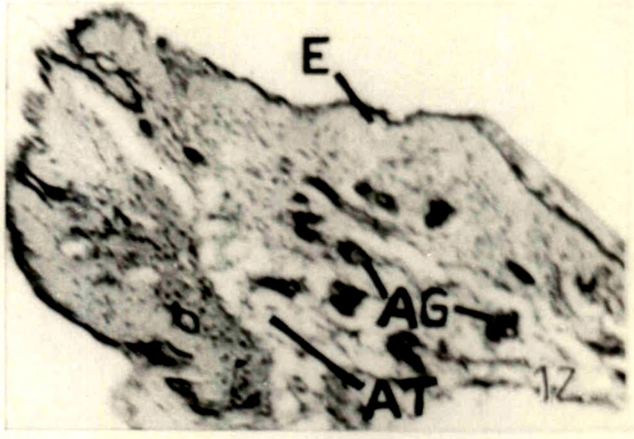
The regressive changes that initiated in the late December continued further in January and February in the postbreeding period. With the complete cessation of the breeding activities there was gradual decline in the histological structure of the gland. The sebaceous glandular region showed considerable decrese in the size of the glands and secretion of sebum (Fig.7). The sebaceous gland were with small sebaceous cells which contained very little of sebum. The glands appeared as compact mass of cells with illdefined ducts. There was sharp decrease in the diameter of the apocrine tubules with the increase of intertubular connective tissue (Fig. 7). The intertubular connective tissue contained few blood vessels. The cells lining the apocrine tubules appeared cuboidal in shape and showed very little blebbing (Fig. 8). The change in size of the cells, secretory activity and degenerating blebs resulted in the formation of cell -debris which occupied the lumina of the apocrine tubules. The peripheral part of clumped apocrine tubules showed decrease in size. The cells were reduced in size. This gradual decline in the histological structure continued in the sexually quiescent period and reached to the minimum in following July.

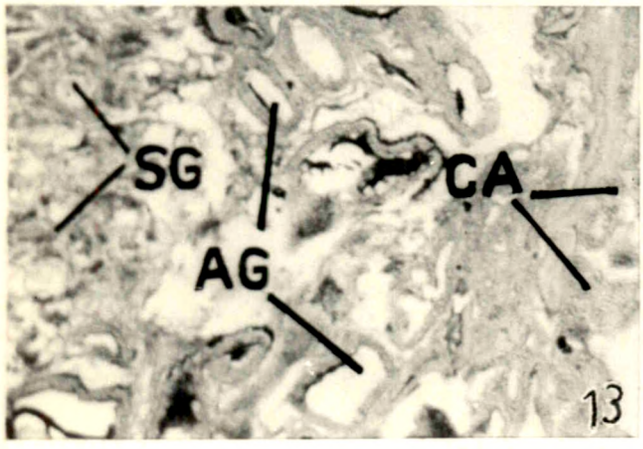
The histological observations on the frontal sac gland of the male bats exhibit seasonal variations in the histology and the amount of secretion in the sebaceous and apocrine glands. These changes correspond

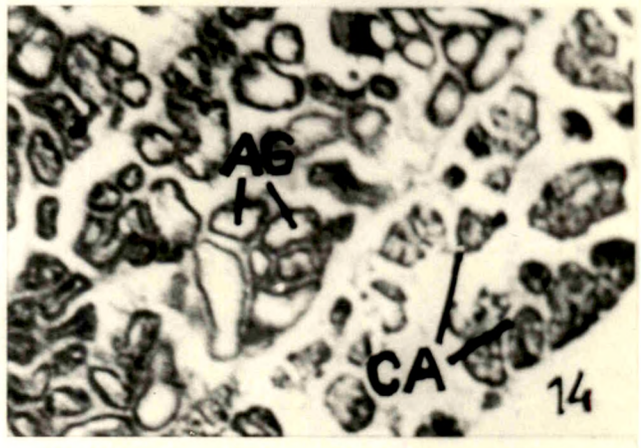

E
AG
SG
AT
9

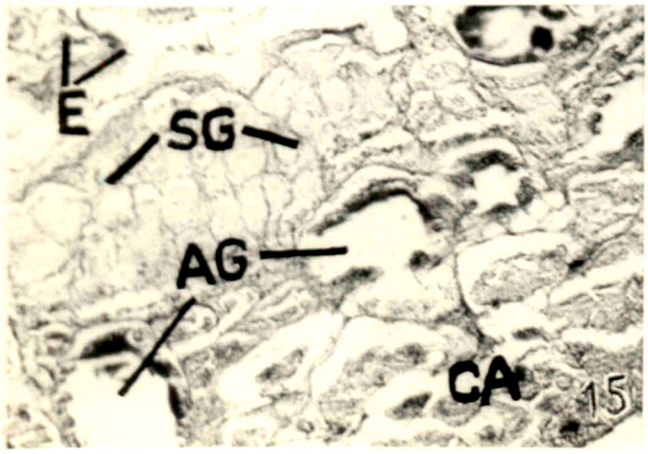

E
H
AG
AT
10

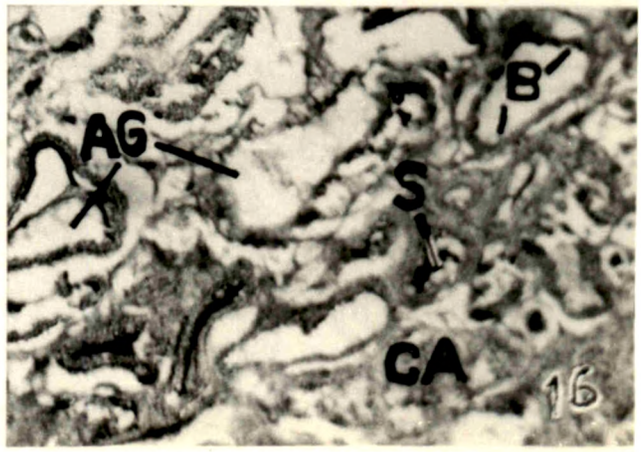

E
SG
AG
AT
11


E
AG
AT
12


SG
CA
AG
13


AG
CA
14


E
SG
AG
CA
15


B
AG
S
CA
16

CAPTIONS TO FIGURES

Fig. 9 Section of the frontal sac gland papilla of the female bat during sexually quiescent period stained with H-E to show epidermis (E), sebaceous glands (SG) and very few and small apocrine gland tubules (AG) and adipose tissue (AT) x 240.

Fig.10 Section of the frontal gland papilla of the female bat during prebreeding period stained with H-E to show epidermis (E), hair (H), apocrine glands (AG) and adipose tissue (AT) x 240.

Fig.11 Section of the frontal gland papilla of the female bat during the active breeding period stained with H-E to show epidermis (E), apocrine glands (AG) and adipose tissue (AT) x 240.

Fig.12 Section of the frontal gland papilla of the female bat during the postbreeding period stained with H-E to show epidermis (E), apocrine glands (AG) and adipose tissue (AT) x 300.

Fig.13 Section of the frontal sac gland of the male during sexual quiescence stained with PAS showing trace reactivity in sebaceous glands (SG), clumped apocrine glands (CA) and weak to moderate staining in apocrine glands (AG) and residual secretion (S) x 240.

Fig.14 Same section as in fig. 13, showing weak to moderate staining in apocrine glands (AG) and trace reactivity in clumped apocrine glands (CA) x 300.

Fig.15 Section of the frontal sac gland during prebreeding period stained with PAS showing very poor staining in epidermis (E), sebaceous glands (SG) and clumped apocrine glands (CA) and moderate staining in apocrine glands (AG) x 240.

Fig.16 Same section as in fig. 15, showing moderate PAS staining in apocrine glands (AG), intense staining in blebs (B) and secretion (S), very poor staining in the clumped apocrine glands (CA) x 240.

to the seasonal changes in the testicular activity of the bat.

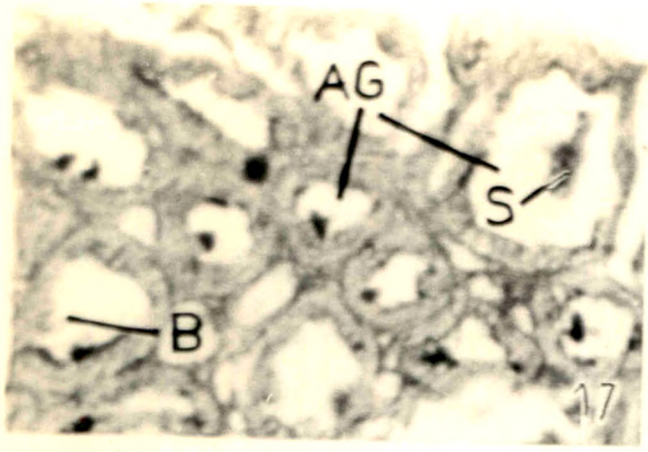
F) Seasonal Histological Observations in Female Bat :

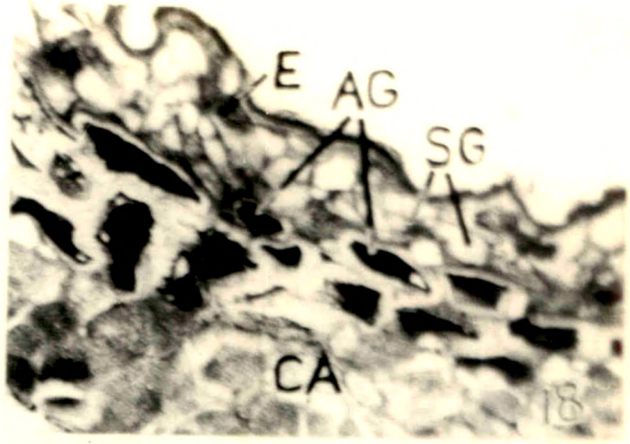
Histologically, the frontal gland papilla consisted of few apocrine gland tubules. These tubules were narrow and lined by flat cuboidal epithelium. The lumina of the tubules were narrow and did not contain any secretary material. The tubules were scattered singly or in groups in the adipose tissue. The glandular area had very poor blood supply. The tubules appeared to be coiled but without duct. The skin covering the gland was comparatively thin and contained projecting hairs. The small sebaceous glands were located at the base of hairs. This structure of the female frontal gland papilla (Fig. 9) in sexually quiscent period showed no significant change in the prebreeding (Fig. 10), the active breeding (Fig. 11) and the postbreeding period (Fig. 12) of the annual breeding cycle. Thus the frontal gland papilla of the female bats exhibited no histological variations, changes in cells size and activity throughout the sex cycle.

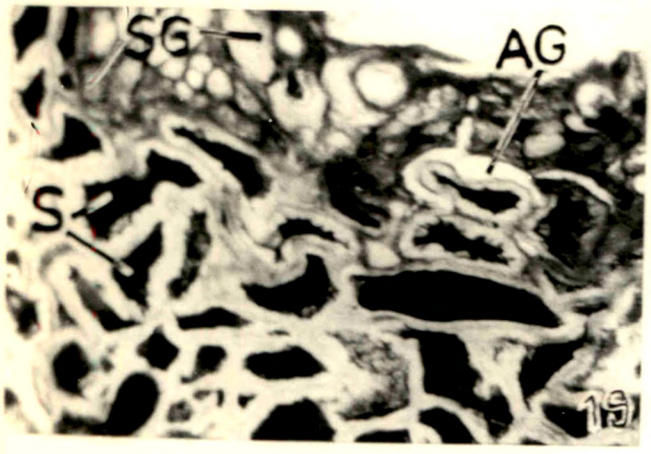
G) Histochemical Observations on Mucosubstances :

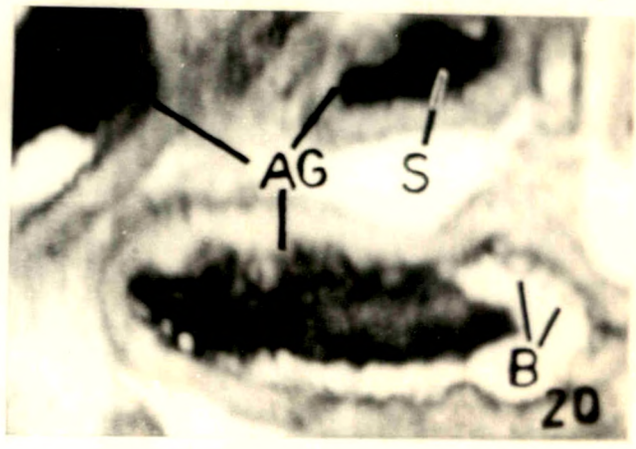
The frontal sac glands were observed functional only in male bats, therefore, histochemical studies on mucosubstances have been carried out only for the male frontal sac glands.

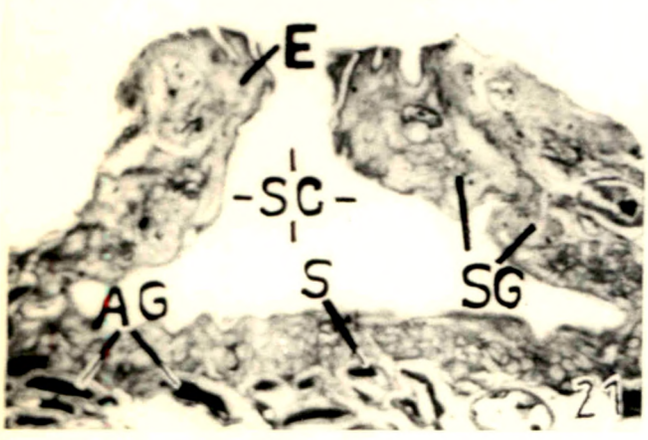
In the present investigation on the frontal sac glands in the male bats mucosubstances were investigated

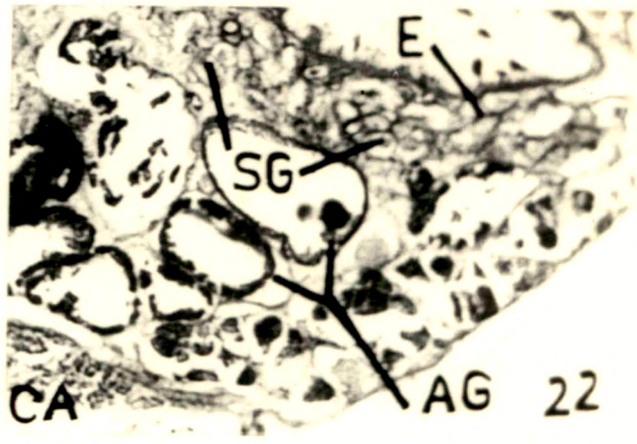

AG
S
B
17

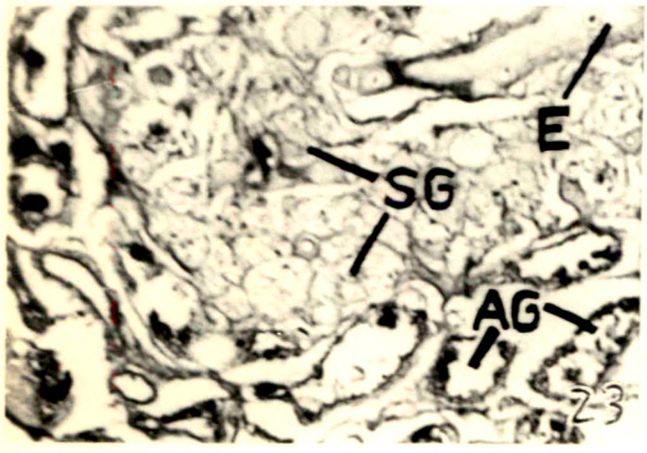

E
AG
SG
CA
18

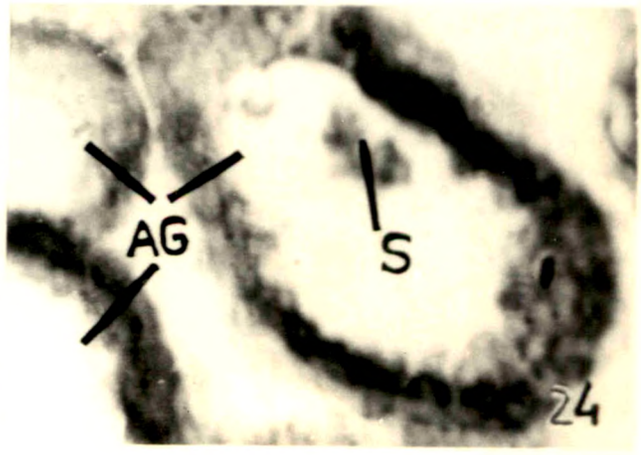

SG
AG
S
19


AG
S
B
20


E
SC
S
SG
AG
21


E
SG
CA
AG
22


E
SG
AG
23


AG
S
24

CAPTIONS TO FIGURES

Fig.17 A magnified view of apocrine gland tubules during the prebreeding period to show moderate staining in apocrine glands (AG) and intense staining in blebs (B) and secretion (S) in PAS stained section x 450.

Fig.18 Section of the frontal sac gland of the male bat during active breeding period stained with PAS to show weak to moderate staining in epidermis (E), very poor staining in sebaceous glands (SG) and clumped apocrine glands (CA) and intense staining in apocrine glands (AG) x 240.

Fig.19 Same section as in fig. 18, but middle region of the gland showing very poor staining in sebaceous gland (SG), very intense staining in apocrine glands (AG) and secretion (S) x 200.

Fig.20 A magnified view of apocrine gland tubules during the active breeding period to show intense staining in blebs (B) and secretion (S) in apocrine glands (AG) x 600.

Fig.21 Section of the frontal sac gland of the male bat during active breeding period showing central lumen (SC) in PAS stained section x 120.

Fig.22 Section of the frontal sac gland of the male bat during post breeding period stained with PAS to show weak to moderate staining in epidermis (E), weak staining in sebaceous glands (SG) moderate staining in apocrine glands (AG) and very poor reactivity in clumped apocrine glands (CA) x 300.

Fig.23 Section of the frontal sac gland of the male bat during post breeding period stained with PAS to show weak staining in sebaceous glands (SG) and moderate staining in apocrine glands (AG) x 200.

Fig.24 A magnified view of apocrine gland tubules during post breeding period to show intense staining in secretion in apocrine glands (AG) in PAS stained section x 900.

for their cellular localization and seasonal alterations in them. The mucosubstances were studied by histochemical techniques, details of which are given in chapter two on material and methods. The localization and seasonal alterations in the mucosubstances are recorded in the tabulated from according to the visually estimated intensity of staining and shade. A very intense or strong reaction is designated as ++++, an intense staining reaction as +++, moderately positive reaction as ++, poor or weak reaction as +, trace reaction as ± and negative reaction as -. The results obtained with various histochemical techniques for mucosubstances in the frontal sac gland on the male bats are recorded in Table No.2. The histochemical localization and seasonal variations in mucosubstances in frontal sac gland of the male bats are shown in photomicrographs (figs. 13-24).

(a) Sexually Quiesent Period :

The frontal sac gland in males were with minimal size and weight during the sexually inactive period. Comparatively the staining intensities towards various histochemical techniques for mucosubstances were low during this period. The various histological sites exhibited varied PAS reactivity during this period (figs. 13, 14) viz. the epidermis and apocrine gland cells exhibited weak to moderate PAS reactivity, the blebs and residual secretion in apocrine gland tubules exhibited moderate staining. The cells in the peripheral

clumped apocrine glands could be differentiated in to two types according to the staining intensity. The cells which showed very poor staining were referred to as light cells and the remaining which exhibited poor staining activity were referred to as dark cells. Both the cell types were uniformly scattered in the clumped apocrine gland. The light and dark cells in clumped apocrine glands so also the sebaceous gland cells showed trace reaction and the secretion in sebaceous gland remained unstained. The PAS reactivity in the respective cells remained unaltered following diastase digestion but could completely be blocked by phenelhydrazine pretreatment. These initial staining reactivities indicated the presence of only neutral mucosubstances but absence of glycogen in the aforemetioned sites. Moreover all these sites remained unstained with AB at pH 1.0, pH 2.5, C.I. and AF even after pepsin digestion and exhibited only blue orthochromatic staining with azure A at high pH levels (pH 3.0 to 5.0).

These histochemical results indicated the absence of any acidic mucosubstances. The presence of only neutral mucosubstances in the aforementioned sites was further substantiatied by their only PAS reactivity in sequential staining procedures such as AB pH 1.0 - PAS, AB pH 2.5 - PAS and C.I. - PAS. Thus, the various histological sites which were PAS reactive contained only non-glycogen neutral mucosubstances during the sexually inactive period.

(b) Prebreeding Period :

During the prebreeding period the size and weight of the frontal sac gland in male bats gradually increased together with enhancement in the staining in some of the histological sites. The various histological sites exhibited varied PAS reactivity. The epidermis lining the sac exhibited weak to moderate PAS reactivity. The PAS reactivity in sebaceous gland cells (fig. 15) and dark and light cells in the peripheral clumped apocrine glands (figs. 15, 16) was very poor. Sebaceous gland secretion remained unstained with PAS. On the other hand the cells in the apocrine gland tubules exhibited moderate PAS reactivity in these aforementioned sites could completely be blocked by phenylhydrazine pretreatment but diastase digestion had no effect on their PAS reactivity. These staining reactivities indicated the presence of neutral mucosubstances in them but absence of glycogen. This conclusion was further substantiated from their only PAS reactivity in sequential staining procedures such as AB pH 1.0 - PAS, AB pH 2.5 - PAS and C.I. - PAS. Absence of acidic mucosubstances in all the histological sites in the frontal sac gland was inferred from the absence of staining with AB pH 1.0, AB pH 2.5, C.I. and AF even after pepsin digestion and from only the orthochromatic staining with azure A at all the pH levels. These histochemical results indicated slight increase in the

neutral mucosubstances in apocrine gland cells, blebs and the luminal secreation than the sexually quiescent period.

(c) Active Breeding Period :

The frontal sac gland in male bats attained maximal size and weight during this period. The various histological sites exhibited varied PAS reactivity during this period. No significant changes in intensity of staining occurred in the epidermis, sebaceous glands and clumped apocrine glands (figs.18, 19, 21). Their PAS reacitivity was practically identical to that observed during the previous periods. The PAS reactivity was intense in the apocrine gland cells and very intense in the blebs and luminal secretion (figs.18, 19, 20).

The remaining histochemical results were more or less identical to that described for prebreeding period which indicated the presence of only neutral mucosubstances but absences of glycogen and acidic mucosubstances in various PAS reactive histological sites.

(d) Postbreeding Period :

The regressing frontal sac gland in males showed reduction in size and weight. The intensity of PAS reaction was different in various histological sites in this period. The epidermis showed weak to moderate PAS staining (fig.23). The sebaceous gland cells and sebaceous secretion exhibited weak PAS reactivity (fig.23). The cells in the clumped apocrine glands reacted very poorly. Blebbing was not seen in the

apocrine gland tubules and the tubules appeared collapsed. The cells in apocrine glands and residual secretion in superficial zone exhibited moderate PAS reactivity (figs. 22, 24), whereas in deeper zones the cells showed moderate PAS reactivity but the residual secretion reacted very intensely towards PAS (fig.22). Some of the apocrine tubules were free of secretion during this period (figs. 22, 23). The remaining tinctorial affinities were practically identical to those described for previous period except slight change in staining intensity. These results indicated the presence of only neutral mucosubstances in PAS reactive sites of the frontal sac gland.

Thus, in general the histochemical observations on mucosubstances revealed the presence of only neutral mucosubstances and absence of glycogen and acid mucosubstances. Histochemical observations further show that very little changes in staining reactivity occur in epidermis, sebaceous gland cells and peripheral clumped apocrine glands. The apocrine gland tubules, blebs and the secretion in the tubules undergo cyclic variations in the neutral mucosubstances according to the sexual status of the males. The neutral mucosubstances in the apocrine gland tubules, cells, blebs and secretion in the lumen, were low during the sexual quiescence which progressively increased during the prebreeding period and active breeding period and showed slight decrease in post breeding period reaching the minimal activity again during the approach of the sexual quiescence.