CHAPTER FIVE

OBSERVATIONS ON DISTRIBUTION, HISTOCHEMICAL CHARACTERIZATION AND SEASONAL VARIATIONS IN THE MUCOSUBSTANCES IN THE CERVIX
1. *Reoematia leschenaulti* leschenaulti

(Seasonal breeder, two sex-cycles in a year, only one side of genitalia functional in each sex-cycle)

**A) Histological**

In this megachiropteran bat the uterine cornu were united only externally and each uterine horn opened into a separate cervical canal and hence initially two cervical canals were observed, they were completely separated by a septum and enclosed in a common sheath. Both the cervical canals then continued as a single canal after their union. The cervix column had characteristic histological appearance, as the uterine endometrial tissue formed the bulk of material going into the composition of the cervix. The myometrium continued down in the cervix and formed the central core for the pillar-like cervix. There were also few simple tubular glands in the mucosal fold. The mucosa was folded and the folds were short, lined by mucosa and contained connective tissue. The lumina of both cervical canals remained separate till the last quarter of the cervix where a single cervical canal was formed, which ultimately opened into the vaginal lumen. Histologically these two cervical canals resembled each other.

B) Cervix During the Anestras

(August-September)

Both the cervical canals presented identical histological
picture. The mucosa was less folded and the folds were short, lined by epithelium and contained connective tissue core. The cervical epithelium consisted of simple columnar epithelial cells. Very few cervical glands were observed at the base of the mucosal folds. The mast cells were evident in the connective tissue. The muscular layer was comparatively thick. During the anestrus there was overall reduction in the diameter of the cervical tube.

II) Cervix During the Proestrus
(October)

In the proestrus there was slight increase in the diameter of the cervix. Numerous mucosal folds in both the cervical canals increased and also there was increase in their height. The thickness of the mucosa was slightly increased. The muscularis became thicker. Cervical glands and secretory cells were seen in the mucosa. Mast cells were observed in the connective tissue.

III) Cervix During the First Estrus
(Mid-November)

During this period cervix attained its maximum diameter and the height of the mucosal folds also attained its maximum. The number of secretory cells in the epithelium was further increased. The glands also appeared to have increased in number. The muscularis was comparatively thick and the mast cells appeared to have increased in number.
IV) **Cervix During the First and Second Pregnancies**
(December to early July)

At the time of first pregnancy the diameter of cervix and the number of folds were further increased. The number of secretory cells and cervical glands numerically increased which exhibited intense mucification. There was no major change in the mast cells and muscle layer. Immediately after the first parturition and during postpartum pregnancy, practically there were no histological changes, hence the histological picture was found to be identical to that in the first pregnancy.

V) **Cervix During the Lactation After Second Pregnancy**
(July and August)

After the second parturition the cervical canal was dilated and the mucosal folds were reduced in number and height. The secretory cells and cervical glands were reduced in their number and also in their mucification. No significant changes were evident in the mast cells and muscular layer, later on cervix started resembling that of the anestrus.

b) **Histochemical**

The results obtained with various histochemical techniques for cervical mucous substances are recorded in Table No. 19 and the histochemical distributions are shown in photomicrographs (Plate No. 12, Figs. 07 to 20).
The cervical epithelial cells showed poor PAS reactivity which could be completely blocked by prior phenylhydrazine treatment and was eliminated by diastase digestion indicating the presence of glycogen. Moreover, these cells remained unstained with AB at pH 1.0 and 2.5, C.I., and AF and exhibited only blue orthochromasia with azure A at all the pH levels. With sequential staining procedures such as AB pH 1.0-PAS, AB pH 2.5-PAS (Plate No. 12, Fig. 87) and C.I.-PAS these cells exhibited only PAS reactivity, and they remained unstained in AB-AB pH 2.5 sequence. The histochemical reactivities remained unaltered even after pepsin digestion. Thus, these results showed occurrence of poor quantity of glycogen in cervical epithelium.

The cells of these glands exhibited weak to moderate PAS reactivity, which could be partly blocked by prior phenylhydrazine treatment, but was resistant to diastase digestion. These initial results indicated the absence of glycogen but presence of neutral mucosubstances rich in vic-glycols. Moreover, these glands remained unstained with AB pH 1.0 and exhibited only PAS reactivity in AB pH 1.0-PAS sequence which indicated the absence of sulfate groups in their mucosubstances. They also showed poor to weak alcianophilia at pH 2.5 and showed C.I. positivity of the
same intensity, indicating the presence of carboxymucins in them. The carboxymucins in them were further inferred from their purple-blue staining in AB pH 2.5-PAS (Plate No. 12, Fig. 67) and C.I.-PAS sequential staining procedures, blue staining in AB pH 2.5 sequence, metachromasia with azure A at and above pH 3.0, poor alcianophilia in GEC technique in presence of 0.1 M Mg++ but blockade of staining with 0.2 M and higher concentrations of Mg++ and reversible blockade of their alcianophilia in methylation-saponification techniques. The carboxymucins were further identified as sialic acid since their alcianophilia could be eliminated by acid hydrolysis and neuraminidase digestion. Hyaluronidase had no effect on their alcianophilia. These results showed the presence of neutral mucosubstances and sialomucins in the cervical gland cells during the anestrous.

III) Muscular Layer

It showed poor PAS reactivity, which could be blocked by phenylhydrazine pretreatment and was abolished by diastase digestion, indicating the presence of glycogen in it. Acidic mucosubstances were found to be absent in this layer since it reacted negatively towards AB pH 1.0 and 2.5, C.I., and AP even after pepsin digestion. The myometrium also exhibited only PAS reactivity in AB pH 1.0-PAS, AB pH 2.5-PAS and C.I.-PAS sequential staining techniques and showed only blue orthochromasia with azure A at all the pH levels. Thus, the muscular layer of cervix contained only poor amount of glycogen in this period.
iv) Mast Cells

The mast cells scattered in the connective tissue exhibited moderate PAS reactivity, which was partly blocked by phenylhydrazine pretreatment and was resistant to diastase digestion. Initial results indicated the absence of glycogen but presence of neutral mucosubstances rich in NAG-glycans. These cells exhibited poor alcianophilia at AB pH 1.0 and weak alcianophilia at AB pH 2.5, which indicated the presence of sulfate group-containing and carbonyl-group containing mucosubstances in them. The presence of sulfomucins in them was further inferred from their blue-purple staining in AB-AB pH 2.5 sequence, poor metachromasia with azure A at low pH (pH 1.5), persistent alcianophilia in CBB technique in the presence of 0.2 M Mg** and partial loss of alcianophilia following saponification of methylated sections. The presence of carboxymucins in the mast cells was further substantiated by their purple-blue staining in AB pH 2.5-PAS (Plate No. 12, Fig. 97) and CBB-PAS sequential staining procedures, blue-purple staining in AP-AB pH 2.5 sequence, enhancement in metachromasia with azure A at and above pH 1.5 and partial restoration of the alcianophilia following saponification of the methylated sections. The carboxymucins was further identified as hyaluronic acid, since hyaluronidase digestion partly removed their alcianophilia. Acid hydrolysis and neuraminidase digestion had no effect on their basophillia. These histochemical reactivities indicated the presence of neutral mucosubstances, sulfomucins and hyaluronic acid in the cervical mast cells.
Cervix During the Proestrus

(October)

1) Cervical Epithelium

The cells of the cervical epithelium exhibited poor to weak PAS reactivity. These cells contained increased amount of glycogen and their histochemical staining reactivities resembled those described for the anestrus, except that there was a slight enhancement in the staining intensities during this period (Plate No. 12, Fig. 68).

Some epithelial secretory cells (goblet cells) were observed within the cervical epithelium in this period. These cells exhibited moderate PAS reactivity, which could partly be blocked by prior phenylhydrazine treatment but was resistant to diastase digestion. These initial results indicated the absence of glycogen but presence of neutral mucosubstances rich in 

-glycals. Moreover, the secretory cells remained unstained with AB pH 1.0 and exhibited only PAS reactivity in AB pH 1.0-PAS sequence, which indicated the absence of sulfate groups in their mucosubstances. But these cells exhibited weak alcianophilia at pH 2.5 and showed C.I. positivity of the same intensity indicating the presence of carboxymucins in them. The presence of carboxymucins in them was further inferred from their purple-blue

staining in AB pH 2.5-PAS and C.I.-PAS sequential staining procedures, blue staining in AB-PAS pH 2.5 sequence, metachromasia with safran A above pH 1.5, poor alcianophilia in CIE technique in presence of 0.1 M Mg++ but blockade of staining
with 0.3 M and higher concentration of Mg$^{++}$ and reversible blockade of their alcianophilia in methylation-saponification techniques. The carboxymucins were further identified as sialomucins since their alcianophilia could be eliminated by acid hydrolysis and neuraminidase digestion. Hyaluronidase had no effect on their alcianophilia. These results showed the elaboration of neutral mucosubstances and sialomucins in those secretory cells.

ii) Cervical Glands

The cells of the cervical glands also exhibited moderate PAS reactivity and alcianophilia at pH 2.5. The remaining histochemical reactivities with various histochemical procedures were identical to those described for the cervical glands (Plate No.12, Fig. 89). Thus, it was concluded that the cervical glands contained neutral mucosubstances and sialomucins in slightly increased concentration during the proestrus.

iii) Muscular Layer

The muscular layer also exhibited poor to weak PAS reactivity. Only glycogen was identified in muscles of the cervix since the PAS reactivity could be completely blocked by prior phenylhydrazine treatment and diastase digestion effected in complete removal of their PAS reactivity. Moreover, acidic mucosubstances could not be visualized in them by any of the histochemical procedures.
iv) **Mast Cells**

The mast cells in the cervical connective tissue also exhibited moderate PAS reactivity and alcianophilia at AB pH 1.0 and at AB pH 2.5. The remaining histochemical staining reactivities were identical to those described for mast cells during the anoestrus (Plate No. 12, Fig. 89). Thus, it was concluded that the mast cells contained neutral mucosubstances, sulfomucins and hyaluronic acid. There was no significant difference seen in the staining intensities in the previous period and this preoestrus.

III) **Cervix During the First Oestrus**

(Mid-November)

1) **Cervical Epithelium**

The cells of the cervical epithelium exhibited weak PAS reactivity. Their PAS reactivity could completely be blocked by phenylhydrazine pretreatment and abolished by diastase digestion indicating the presence of glycogen in them. Moreover, acidic mucosubstances could not be visualized in them by any of the histochemical procedures even after pepsin digestion (Plate No. 12, Fig. 89).

The secretory cells in the cervical epithelium exhibited moderate to intense PAS reactivity, which could be partly blocked by phenylhydrazine pretreatment and was resistant to diastase digestion. These initial results indicated the absence of glycogen but presence of neutral mucosubstances rich in...
vial-glycals in them. Moreover, the goblet cells exhibited presence of poor to weak alcianophilia at pH 1.0 and weak to moderate alcianophilia at pH 2.5 which indicated the presence of both sulfomucins and carboxymucins in them. The presence of sulfomucins in these sites was further inferred from their blue-purple staining with AB pH 1.0-PAS sequence, blue-purple staining in AB-AB pH 2.5 sequence, weak metachromasia with azure A at lower pH (pH 1.5), persistent alcianophilia in CIC technique up to 0.4 M Mg** and partial loss of alcianophilia following saponification of the methylated sections. The presence of carboxymucins in them was further substantiated from their C.I. positive reactivity, purple-blue staining in AB pH 2.5-PAS and C.I.-PAS sequential staining techniques, blue-purple staining in AB-AB pH 2.5 sequence, enhanced metachromatic staining with azure A at pH 3.0 and above and partial restoration of their alcianophilia following saponification of the methylated sections. The carboxymucins in the secretory cells were further confirmed as sialomucins, since acid hydrolysis and neuraminidase digestion partly reduced their alcianophilia. These results remained unaltered following pepsin digestion. These histochemical results revealed the presence of neutral mucosubstances, sulfomucins and sialomucins in the secretory cells or goblet cells.

ii) Cervical Glands

The cervical glands exhibited moderate to intense PAS reactivity and showed weak to moderate alcianophilia at pH 2.5. The remaining histochemical reactivities with various techniques
were identical to those in goblet cells in the epithelium during estrus (Plate No. 12, Fig. 39). Thus, the cells in cervical glands contained neutral mucosubstances, sialomucins and sulfomucins.

iii) Muscular Layer

The muscular layer exhibited poor to weak PAS reactivity which could completely be blocked by phenylhydrazine pretreatment and diastase digestion effected in complete removal of their PAS reactivity. These initial reactions indicated the presence of only glycogen in muscles. Moreover, all the histochemical tests to visualize acidic mucosubstances in muscles were negative.

iv) Nest Cells

The nest cells during this period also indicated the presence of neutral mucosubstances, sulfomucins and hyaluronic acid. Their tinctorial affinities resembled those described for those cells during proestrus and there was no significant change in the staining intensities with various histochemical procedures.

IV) Cervix During the First and Second Pregnancies

(December to early July)

1) Cervical Epithelium

During the first and second pregnancies and in a short period in between those two pregnancies, the cells of the cervical epithelium showed weak PAS reactivity. These cells as in the estrus contained only glycogen, because histochemical staining
reactivities were identical to those described during estrus (Plate No. 12, Fig. 92).

Numerous secretory cells in cervical epithelium exhibited intense PAS reactivity, which could be partly blocked by prior phenylhydrazine treatment but diastase digestion had no effect on their PAS reactivity indicating absence of glycogen, but presence of neutral mucosubstances. Moreover, these cells exhibited weak alcianophilia at pH 1.0 and moderate alcianophilia at pH 2.5. These results indicated the presence of sulfomucins and carboxymucins. The remaining modifications in their staining reactivities and the results obtained were identical to those described for these cells during estrus except that there was a slight enhancement in the staining intensities (Plate No. 12, Fig. 92). Thus, it was concluded that these sites contained increased concentrations of neutral mucosubstances, sulfomucins and sialomucins.

11) Cervical Glands

The glandular cells also exhibited intense PAS reactivity and showed moderate alcianophilia at pH 2.5. The remaining histochemical reactivities with various histochemical procedures were similar to those described for goblet cells during this period (Plate No. 12, Fig. 92). Thus, the cells in the cervical glands contained neutral mucosubstances, sialomucins and sulfomucins during first and second pregnancies and a short time period between these two pregnancies.
iii) **Muscular Layer**

The muscular layer exhibited poor to weak PAS reactivity. The remaining histochemical reactivities of this layer were identical to those described during previous period. Thus, it was concluded that this layer contained glycogen.

iv) **Mast Cells**

No significant alterations were evident in the staining reactivities of the mast cells in this period and those described for the previous period. It was concluded that the mast cells contained neutral mucopolysaccharides, sulfomucins and hyaluronic acid during pregnancy.

v) **Cervix During the Lactation After the Second Pregnancy (July and August)**

The cells of the cervical epithelium exhibited poor PAS reactivity, which could be completely blocked by phenylhydrazine pretreatment and was abolished by diastase digestion indicating the presence of glycogen in them. Moreover, acidic mucopolysaccharides could not be visualized in these cells by any of the histochemical techniques even after pepsin digestion.

Very few secretory cells were observed in the cervical epithelium during this period, which exhibited weak PAS reactivity. These cells remained unstained with AB pH 1.0 but exhibited alcianophilia at pH 2.5. Except for a slight decrease in the staining intensities, the remaining histochemical
reactivities were identical to those described for these cells during the proestrus. Thus, these secretory cells contained neutral mucosubstances and sialomucins during lactation.

1) Cervical Glands

In general very few cervical glands were observed during this period. The granular cells exhibited weak PAS reactivity and remained unstained with AB at pH 1.0, but exhibited alcianophilia at pH 2.5. The remaining histochemical reactivities with various histochemical procedures were similar to those in goblet cells during this period. Thus, the cervical glands contained neutral mucosubstances and sialomucins during lactation.

iii) Muscular Layer

The muscular layer exhibited poor PAS reactivity which could be completely blocked by phenylhydratase pretreatment and diastase digestion affected in complete removal of their PAS reactivity. These initial staining reactivities showed the presence of only glycogen in muscles. Moreover all the histochemical tests to visualize acidic mucosubstances in muscular layer were negative.

iv) Neck Cells

These cells indicated the presence of neutral mucosubstances, sialomucins and hyaluronic acid. Their tinctorial affinities resembled those described for these cells during pregnancy. There was no significant change in the staining.
Plate No. 12 (Figs. 07 to 08)

Fig. 07a: Cervix of *H. leucophaeata* leucophaeata during anestrus in September, stained with AB pH 2.5-PAS. Note moderate staining in mast cells (C), weak to moderate staining in cervical glands (G) and poor staining in cervical epithelium (x 120).

Fig. 08a: Cervix of *H. leucophaeata* leucophaeata during proestrus in October, stained with AB pH 2.5-PAS. Note moderate staining in cervical glands (G) and mast cells (M) and poor to weak staining in cervical epithelium (x 120).

Fig. 08b: Cervix of *H. leucophaeata* leucophaeata during estrus in mid-November, stained with AB pH 1.0-PAS to show moderate to intense staining in cervical glands (G) and weak staining in cervical epithelium (x 200).

Fig. 09a: Cervix of *H. leucophaeata* leucophaeata during pregnancy in November, stained with PAS to show moderate staining in mast cells (M), weak to moderate staining in cervical glands (G) and poor staining in cervical epithelium (x 200).

Fig. 09b: Cervix of *P. australis* australis during anestrus in January, stained with AB pH 2.5-PAS. Note moderate staining in goblet cells (G0) and cervical glands (G) and weak staining in cervical epithelium (x 200).

Fig. 10a: Cervix of *P. australis* australis during proestrus in early June, stained with AB pH 2.5-PAS. Note moderate to intense staining in goblet cells (G0), weak staining in cervical epithelium (E) and poor to weak staining in muscle (M) x 200.

Fig. 10b: Cervix of *P. australis* australis during estrus in late June, showing cervical secretion in lumen, stained with AB pH 2.5-PAS. Note moderate to intense staining in goblet cells (G0), weak staining in cervical epithelium (E) and poor to weak staining in muscle (M) x 200.

Fig. 11a: Cervix of *P. australis* australis during pregnancy in mid-September showing cervical mucus secretion in lumen, stained with AB pH 2.5-PAS. Note intense staining in goblet cells (G0) x 200.
intensities with various histochemical procedures.

2. *Pipistrellus ceylonicus chrysophrrix*
   (Seasonal breeder, single sex-cycle in a year and both the sides of genitalia functional in each sex-cycle)

A) **Histological**

In this microchiroptera but both the uterine horns were united to form a cervix, while both the lumina of these uterine horns meet to open into a common cervical canal. The mucosal layer was folded and the folds were short and were lined by epithelium and connective tissue core. The mast cells were abundant in the connective tissue. The mucosal epithelium consisted of mainly stratified epithelial cells. Very few cervical glands were observed at the base of the mucosal folds. The muscular layer was comparatively thick.

I) **Cervix During the Anestrus**
   (Mid-October to mid-May)

During anestrus the mucosa was less folded and the folds were short, which projected into cervical lumen loosely. The cervical epithelium consisted of mainly stratified epithelial cells. Very few tubular cervical glands were observed at the base of the mucosal folds. The mast cells were evident in the connective tissue. The muscle layer was thick. During this period there was overall reduction in the diameter of the cervical tube.
II) Cervix During the Proestrus
(Late May and June)

During proestrus there was a slight increase in the
diameter of the cervix. The mucosal folds were increased in
their number and height. The most significant change observed was
the increase in the number of cervical glands and appearance of
secretory cells in the mucosa. The muscular layer was as in the
anestrus. The mast cells were observed in the connective tissue.

III) Cervix During the Estrus
(Late June to early July)

In the estrus maximum diameter of the cervix was evident.
The number of mucosal folds was increased and their height
attained maximum size. The secretory cells and the cervical
glands increased in their number and also appeared to be modified.
The mast cells were evident in the connective tissue, and muscular
layer was comparatively thick.

IV) Cervix During the Pregnancy
(July to mid-September)

The number of folds was further increased in early and
midpregnancy, but the folds were decreased numerically during
late pregnancy. One of the prominent features was intense
modulation of the surface epithelium. There was no major
change in the mast cells and muscle layer.
v) Cervix During the Lactation
(September to mid-October)

Soon after the parturition the cervical canal was dilated.
The mucosal folds were reduced in number and height. The thickness of epithelium and the number of goblet cells and cervical glands gradually reduced. No significant changes were observed in the mast cells and muscular layer.

b) Histochemical

The histochemical reactivities of various mucosubstances present in the cervix in this variety of bats during different periods are shown in Table No. 20, in which the histochemical staining intensities have been shown comparatively with +++ as the strongest activity as determined by the visual comparison. The histochemical distribution and variations in the mucosubstances in the different sites of the cervix of this bat are similar to those described for megachiropteran bats, P. lageothrix lageothrix. Hence the details of the histochemical reactivities of various cellular sites in the cervix of P. cephalicus chrysocercus are not described and only the conclusions derived from these reactivities have been described in brief. The histochemical distribution and alterations in the cervix have been shown in Plate No. 12, Figs. 91 to 94.

i) Cervix During the Anastrus
(Mid-October to mid-May)

Cervical Epithelium

The cells of the stratified epithelium of cervix exhibited
poor PAS reactivity (Plate No. 12, Fig. 91) which could completely be blocked by prior phenylhydroxazone treatment and was eliminated by diastase digestion indicating the presence of glycogen in it. These cells remained unstained with ABC pH 1.0 and 2.5, C.I. and 2F and exhibited only orthochromasia with safranin A at all the pH levels. Moreover, this layer exhibited only PAS reactivity in ABC pH 1.0, PAS, ABC pH 2.5, PAS and C.I. - PAS sequential staining procedures. These histochemical reactivities could not be altered by pepsin digestion. Thus, the cells of stratified cervical epithelium contained only poor quantity of glycogen.

ii) Cervical Glands

Very few cervical glands were observed during this period, which showed staining reactivities similar to those exhibited by the cervical glands in the cervix of R. lechrchenaulti lechrchenaulti during their anestrus (Plate No. 12, Fig. 91). Thus, cervical glands elaborated presence of neutral mucosubstances and sialomucins only.

iii) Muscular Layer

During anestrus muscular layer was found to be thick, in which the histochemical reactivities resembled those exhibited by the cervical muscular layer of R. lechrchenaulti lechrchenaulti during their anestrus. Thus, the muscular layer exhibited only poor quantity of glycogen.
iv) **Mast Cells**

These cells showed the histochemical reactivities very much identical to those exhibited by the mast cells in the cervical connective tissue of *R. leachonauli* *leachonauli* during their anestras (Plate No. 12, Fig. 91). Thus, the mast cells revealed the presence of neutral mucosubstances, sulfomucins and hyaluronic acid.

II) **Cervix During the Proestrus**

(Late May-June)

1) **Cervical Epithelium**

The epithelial cells of cervix contained only glycogen, but during this period the intensity of staining was enhanced over that of the previous period and hence the concentration of glycogen content was increased during this period (Plate No. 12, Fig. 92).

A few secretory cells were observed in the cervical epithelium, which exhibited moderate PAS reactivity. These cells remained unstained with AB pH 1.0 but exhibited alcianophilia at AB pH 2.5. The remaining histochemical reactivities were similar to those described for these cells in the *R. leachonauli* *leachonauli* during their proestrus. Thus, the neutral mucosubstances and sulfomucins were revealed by these goblet cells in proestrus.
ii) Cervical Glands

Cervical glands in this bat exhibited moderate PAS reactivity and showed alcianophilia at pH 2.5. The remaining staining reactivities with various histochemical techniques were similar to those described for cervical glands in *H. leucocephalus leucocephalus* during their proestrus (Plate No. 12, Fig. 39). Thus, the cervical glands in this bat elaborated neutral mucous substances and sialomucins during proestrus. At this time slight enhancement could be observed in staining intensities in these cells over the anoestrus; thus, the concentration of mucous substances increased during proestrus.

iii) Muscular Layer

The muscular layer showed staining reactivities very much similar to those exhibited by the muscular layer of cervix of the *H. leucocephalus leucocephalus* during their proestrus, but now the staining intensity was enhanced over that of the anoestrus of this bat. Thus, muscular layer showed higher concentration of glycogen during proestrus.

iv) Nest Cells

These cells contained neutral mucous substances, sialomucins and hyaluronic acid.

III) Cervix During the Estrus
(near June and early July)

1) Cervical Epithelium

At this time slight enhancement was evident in the staining
intensities over that of the previous period (Plate No. 12, Fig. 93). Thus, epithelial cells showed higher concentration of glycogen during estrus.

The number of the secretory or goblet cells was increased in the cervical epithelium which exhibited moderate to intense PAS reactivity, poor to weak alcianophilia at AB pH 1 and weak to moderate alcianophilia with AB pH 2.5. The remaining histochemical reactivities were very much identical to those described for these cells in the *B. leachianula* leachianula during their estrus (Plate No. 12, Fig. 93). Thus, it was concluded that the secretory cells contained neutral mucosubstances, sialomucins and sulfomucins. During this period the elaboration of sulfomucins was actively started.

11) **Cervical Glands**

The glandular cells of the cervical glands resembled in their staining reactivities with the staining reactivities of the cervical glands in the *B. leachianula* leachianula during their estrus. Thus, these cells exhibited presence of neutral mucosubstances, sialomucins and sulfomucins during estrus.

111) **Muscular Layer**

The muscular layer showed presence of only glycogen during this period (Plate No. 12, Fig. 93).

iv) **Muscle Cells**

Muscle cells contained neutral mucosubstances, sulfomucins
and hyaluronic acid.

IV) Cervix During the Pregnancy
(July to mid-September)

i) Cervical Epithelium

The cells of the cervical epithelium exhibited very much similar staining reactivities to those exhibited in estrus. Thus, the epithelial cells of the cervix contained glycogen only.

The goblet cells showed presence of neutral mucosubstances, sialomucins and sulfomucins. At this period the enhancement was observed in the staining intensities (Plate No. 12, Fig. 94).

ii) Cervical Glands

The glandular cells of the cervical glands showed enhanced staining intensities over the estrus and contained increased amount of neutral mucosubstances, sialomucins and sulfomucins.

iii) Muscular Layer

The muscular layer showed presence of glycogen only.

iv) Mast Cells

These cells showed presence of neutral mucosubstances, sulfomucins and hyaluronic acid.

v) Cervix During the Lactation
(September to mid-October)

i) Cervical Epithelium

The cervical epithelial cells exhibited poor PAS reactivity.
and the remaining staining reactivities of this layer were identical to those described for this site during anestrus. Thus, the cervical epithelial cells contained poor quantity of glycogen only.

Very few secretory cells were evident during lactation, which showed weak PAS reactivity and remained unstained with AB pH 1.0 but exhibited alcianophilia at pH 2.5. Except for a slight decrease in the staining intensities the remaining histochemical reactivities were identical to those described for these cells during the proestrus. Thus, these secretory cells showed presence of neutral mucosubstances and sialomucins during lactation.

11) Cervical Glands

The number of cervical glands was reduced during this period, which exhibited weak PAS reactivity and remained unstained with AB pH 1.0 but showed alcianophilia at AB pH 2.5. The remaining staining reactivities with various histochemical procedures were identical to those in goblet cells during this period. Thus, the cervical glands showed presence of neutral mucosubstances and sialomucins during lactation.

111) Muscular Layer

This site showed presence of glycogen only.

iv) Rept Cells

There was no significant change in the staining intensities
with various histochemical procedures and hence their tinctorial affinities resembled those described for these cells during the pregnancy period. Thus, these cells contained neutral mucosubstances, sulfomucins and hyaluronic acid during lactation.

3. Hippopotamus species
   - Seasonal breeders, single sex-cycle in a year and only one side of genitalia functional in each sex-cycle
   - *Hippopotamus amphibius* fulvus fulvus
   - *Hippopotamus amphibius* larvadiva
   - *Tadarida aegyptiaca* opaci
   - *Taphoosous kachhoensis*

**Histological**

In the above bats excepting *T.kachhoensis* both the uterine cornua were united to form a common cervix, as their lumina met to open into a common cervical canal. But in *T.kachhoensis* both the uterine horns opened into a separate cervical canal, hence initially two cervical canals were observed. Both the cervical canals then continued as a single cervical canal after joining. In all these bats the mucosa was folded and lined by epithelium and contained connective tissue core. The mucosal epithelium consisted of mainly stratified epithelial cells. The mast cells were evident in the connective tissue. The cervical glands were observed at the base of the mucosal folds. The muscularis was comparatively thick.

**1) Cervix During the Anestrous**

- *H.sororis*: August to October
- *H.sulvus fulvus*: July to September
H. Lankadiva: July to September
T. aepyptiana gossi: December to March
T. kachhensis: November to January

In this period, mucosa was less folded, folds were short and projected into cervical lumen. The cervical epithelium consisted mainly of stratified epithelial cells in H. speoritis and H. fulvus fulvus. But the same in H. Lankadiva, T. aepyptiana gossi and T. kachhensis consisted of a single layer of columnar epithelial cells. The outermost stratified epithelial cells were keratinised in H. speoritis and H. fulvus fulvus. The mast cells were evident in the connective tissue. The muscularis was comparatively thick. There was overall reduction in the diameter of the cervical tube in all the bats during anoestrus.

IX) Cervix During the Proestrus

H. speoritis: November
H. fulvus fulvus: October
H. Lankadiva: October
T. aepyptiana gossi: April
T. kachhensis: February

During proestrus there was slight increase in the diameter of the cervix in all these bats. The mucosa folds were increased and projected into cervical lumen. The most significant change observed was the increase in number of cervical glands and secretory cells in the mucosa. The nature of the mast cells and
the muscular layer was as in the anestrus.

III) Cervix During the Estrus

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<tr>
<td>T. egypthaca coasi</td>
<td>Late May to early June</td>
</tr>
<tr>
<td>T. kochensis</td>
<td>Late March</td>
</tr>
</tbody>
</table>

At this time in these bats the maximum diameter of cervix was observed. The number of mucosal folds was increased and their height attained maximum size. The secretory cells were abundant in the epithelium. The cervical glands also appeared to be multiplied. The mast cells also appeared to have increased in number and the muscularis was comparatively thick.

IV) Cervix During the Pregnancy

<table>
<thead>
<tr>
<th>Species</th>
<th>Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. aequora</td>
<td>Mid-December to early May</td>
</tr>
<tr>
<td>H. fulvus fulvus</td>
<td>November to end of April</td>
</tr>
<tr>
<td>H. lankadiva</td>
<td>December to May</td>
</tr>
<tr>
<td>T. egypthaca coasi</td>
<td>June to September</td>
</tr>
<tr>
<td>T. kochensis</td>
<td>April to July</td>
</tr>
</tbody>
</table>

The number of mucosal folds was further increased during early and mid-pregnancy, but the folds were decreased numerically in late pregnancy. The secretory cells were present in the epithelium and the intense mucusification of the surface epithelium was observed. The cervical glands were observed in the mucosa.
which were found to be loaded with mucins. There was apparently no change in the mast cells and muscularis.

V) Cervix During the Lactation

<table>
<thead>
<tr>
<th>Species</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. aurita</td>
<td>May to July</td>
</tr>
<tr>
<td>H. fulvus fulvus</td>
<td>May and June</td>
</tr>
<tr>
<td>H. jamaicensis</td>
<td>May and June</td>
</tr>
<tr>
<td>A. aegyptiaca coqui</td>
<td>September to November</td>
</tr>
<tr>
<td>P. kachensis</td>
<td>August to early October</td>
</tr>
</tbody>
</table>

After the parturition the cervical tube was dilated. The height and number of the folds were reduced. The thickness of epithelium and the number of goblet cells and cervical glands gradually reduced. The mast cells and muscular layer were observed as in the previous periods.

b) Histochemical

The histochemical reactivities of various mucous substances present in the cervix in these five varieties of bats during their different periods are shown in Table No. 21 in which the histochemical staining intensities have been shown comparatively with ++++ as the strongest activity as determined by the visual comparison. The histochemical distribution and variations in the mucous substances in the different sites of the cervical tubes of these bats are mostly identical to those described for the megachiropteran bat, P. kachensis. Hence the details of some of the histochemical reactivities of various cellular sites in the cervical tubes of these bats are not
described and only the conclusions derived from these reactivities are described in brief. The histochemical distribution and alterations in the cervical tubes are shown in Plates No. 13 to 15.

I) Cervix During the Anestrus

<table>
<thead>
<tr>
<th>Species</th>
<th>Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. angoria</td>
<td>August to October</td>
</tr>
<tr>
<td>H. fulvus fulvus</td>
<td>July to September</td>
</tr>
<tr>
<td>H. leucogaster</td>
<td>July to September</td>
</tr>
<tr>
<td>T. angystica comis</td>
<td>December to March</td>
</tr>
<tr>
<td>T. kochhoesta</td>
<td>November to January</td>
</tr>
</tbody>
</table>

1) Cervical Epithelium

Keratinised Layer: The outermost stratified epithelial cells were keratinised which exhibited poor to weak PAS reactivity in H. angoria (Plate No. 13, Fig. 95) and H. fulvus fulvus (Plate No. 13, Fig. 95). The PAS reactivity in this layer could be completely blocked by prior phenylhydrasine treatment and was eliminated by diastase digestion indicating the presence of glycogen in it. This layer remained unstained with AB pH 1.0 and 2.5, C.I. and AF, and exhibited only blue orthochromatic staining with saure A at all the pH levels. Moreover, this layer exhibited only PAS reactivity in AB pH 1.0-PAS, AB pH 2.5-PAS and C.I.-PAS sequential staining procedures. These histochemical reactivities could not be altered by pepsin digestion. Thus, the keratinized layer in H. angoria and H. fulvus fulvus contained only poor quantity of glycogen.
The epithelial cells exhibited poor PAS reactivity in *H. aspersa* (Plate No. 13, Fig. 95), *H. fulva* fulva* (Plate No. 13, Fig. 96), *H. lankadelia* (Plate No. 13, Fig. 97), *Z. neoplasticum costasi* (Plate No. 13, Fig. 98) and *Z. kochensea* (Plate No. 13, Fig. 99). These cells also contained poor quantity of glycogen as their histochemical reactivities resembled those described above for keratinised layer.

11) **Muscular Layer**

The muscular layer of the cervix in these bats exhibited poor PAS reactivity (Plate No. 13, Figs. 95 to 99) which could be completely blocked by phenylhydrazine pretreatment and was abolished by diastase digestion. The efforts to visualise acidic mucosubstances had failed. These results indicated the presence of glycogen in the muscles.

111) **Mast Cells**

The mast cells in the cervical connective tissues of all these bats exhibited moderate PAS reactivity, alcianophilia at AB pH 1.0 and at AB pH 2.5. The remaining histochemical staining reactivities were identical to those described for mast cells in cervix of *A. leuchowauldii leuchowauldl* during their anestrus (Plate No. 13, Figs. 96 to 99). Thus, it was concluded that the mast cells contained neutral mucosubstances, sulfomucins and hyaluronic acid.
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II) Cervix During the Proestrus

- *H. speciosa* : November
- *H. fulvus fulvus* : October
- *H. lankadiva* : October
- *T. serpentina gossi* : April
- *T. kachhornia* : February

1) Cervical Epithelium

The cervical epithelial cells exhibited poor to weak PAS reactivity in *H. speciosa* (Plate No. 13, Fig. 100), *H. fulvus fulvus* (Plate No. 13, Fig. 101), *H. lankadiva* (Plate No. 13, Fig. 102), *T. serpentina gossi* (Plate No. 13, Fig. 103) and *T. kachhornia* (Plate No. 14, Fig. 104). These epithelial cells contained only glycogen.

The goblet cells in the cervical epithelium of these bats exhibited moderate PAS reactivity in *H. speciosa* (Plate No. 13, Fig. 100), *H. lankadiva* (Plate No. 13, Fig. 102) and *T. kachhornia* (Plate No. 14, Fig. 104). These cells remained unstained with AB pH 1.0 but exhibited alcianophilia at AB pH 2.5. The remaining histochemical reactivities were identical to those exhibited by these cells in the cervix of *R. leschenaulti leschenaulti* during their proestrus. Thus, these secretory cells showed presence of neutral mucopolysaccharides and sialomucins.

II) Cervical Glands

The cervical glands in these bats also exhibited moderate
PLATE No. 13 (Figs. 95 to 103)

Fig. 95: Cervix of *H. angolensis* during anestrus in September, stained with PAS. Note poor to weak staining in keratinized layer (K) and poor staining in cervical epithelium (E) and muscles (M) x 180.

Fig. 96: Cervix of *H. fulvus fulvus* during anestrus in August, stained with PAS to show moderate staining in mast cells (MC), poor to weak staining in keratinized layer (K) and poor staining in cervical epithelium (E) and muscles (M) x 180.

Fig. 97: Cervix of *H. lankadiva* during anestrus in August, stained with PAS to show moderate staining in mast cells (MC) and poor staining in cervical epithelium (E) and muscles (M) x 180.

Fig. 98: Cervix of *H. mehelyi* during anestrus in December, stained with PAS. Note moderate staining in keratinized layer (K) and poor staining in cervical epithelium (E) and muscles (M) x 180.

Fig. 99: Cervix of *H. mehelyi* during anestrus in December, stained with PAS. Note moderate staining in cervical epithelium (E) and muscles (M) x 180.

Fig. 100: Cervix of *H. angolensis* during proestrus in November, stained with PAS to show moderate staining in goblet cells (G) and cervical glands (C) and poor to weak staining in cervical epithelium (E) and muscles (M) x 200.

Fig. 101: Cervix of *H. fulvus fulvus* during proestrus in October, stained with PAS. Note poor to weak staining in cervical epithelium (E) x 180.

Fig. 102: Cervix of *H. lankadiva* during proestrus in October, stained with PAS. Note moderate staining in goblet cells (G) and poor to weak staining in cervical epithelium (E) x 180.

Fig. 103: Cervix of *H. mehelyi* during proestrus in April, stained with PAS to show poor to weak staining in cervical epithelium (E) x 180.
PAS reactivity (Plate No. 13, Fig. 100; Plate No. 14, Fig. 104). These glandular cells remained unstained with AB pH 1.0 but exhibited alcianophilia at AB pH 2.5. The remaining histochemical reactivities with various histochemical procedures were identical to those in secretory cells in the epithelium of those bats during this period. Thus, the cells in cervical glands in the bats elaborated neutral mucosubstances and sialomucins.

iii) Muscular Layer

The muscular layer showed similar staining reactivities to those exhibited by the cervical muscular layer of the U. leschenaulti leschenaulti during their proestrus (Plate No. 13, Fig. 100). Thus, muscular layer of cervical tubes of these bats exhibited higher concentration of glycogen during their proestrus, as the staining intensities were enhanced compared to that of the anestrus.

iv) Nest Cells

There was no significant change in the staining intensities during anestrus and proestrus of these bats, as similar staining reactivities were evident in both the periods. Thus, these cells contained neutral mucosubstances, sialomucins and hyaluronic acid during proestrus.

III) Cervix During the Estrus

<table>
<thead>
<tr>
<th>Species</th>
<th>Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. aequalis</td>
<td>Mid. December</td>
</tr>
<tr>
<td>U. salvensis salvensis</td>
<td>Mid. November</td>
</tr>
</tbody>
</table>
1) **Cervical Epithelium**

The cells in the epithelium contained only glycogen during estrus (Plate No. 14, Figs. 105 to 109).

The secretory epithelial cells in the epithelium in cervix exhibited moderate to intense PAS reactivity. The remaining histochemical reactivities were identical to those described for these cells in *E. moomoutedi* during their estrus (Plate No. 14, Figs. 105, 107, 109). Thus, the secretory epithelial cells contained neutral mucosubstances, sulfomucins, and sialomucins during estrus.

ii) **Cervical Glands**

The cervical glands contained neutral mucosubstances, sialomucins, and sulfomucins during estrus (Plate No. 14, Figs. 105, 107, 108, 109).

iii) **Muscular Layer**

There was no significant difference observed in this site over that of the previous period (Plate No. 14, Figs. 106, 107, 109). Thus, the muscular layer exhibited only glycogen during estrus.
iv) Mast Cells

The mast cells in the cervix of these bats contained neutral mucopolysaccharides and hyaluronic acid (Plate No. 14, Figs. 105, 106, 109).

IV) Cervix During the Pregnancy

H. nasalis
H. fulvus fulvus
H. langsdorfi
T. dermestes punch
T. bachmanni

: Mid-December to early May
: November to end of April
: December to May
: June to September
: April to July

1) Cervical Epithelium

These cells contained only glycogen during this period (Plate No. 14, Figs. 110 to 112; Plate No. 15, Figs. 113, 114).

The secretory epithelial cells also exhibited intense PAS reactivity in these bats, which could partly be blocked by prior phenylhydrazine treatment but diastase digestion had no effect on their PAS reactivity indicating absence of glycogen but presence of neutral mucopolysaccharides. Moreover, these cells showed weak alcianophilia at AB pH 1.0 and moderate alcianophilia at AB pH 2.5. These results indicated the presence of sulfomucins and carboxymucins. The remaining modifications in their staining reactivities and the results obtained were identical to those described for cats (Plate No. 14, Figs. 110 to 112; Plate No. 15, Fig. 113). Thus, it was concluded that these cells contained
Fig. 104: Cervix of *E. kohbohuni* during preovulation in February, stained with PAS to show moderate staining in goblet cells (G) and cervical glands (C) and poor to weak staining in cervical epithelium (E) x 150.

Fig. 105: Cervix of *H. sparsa* during estrus in mid-November, stained with AB pH 2.5-PAS to show moderate to intense staining in goblet cells (G) and cervical glands (C), moderate staining in mast cells (MC) and weak staining in cervical epithelium (E) x 150.

Fig. 106: Cervix of *H. fulvus fulvus* during estrus in mid-November, stained with AB pH 1.4-PAS to show moderate to intense staining in goblet cells (G) and cervical glands (C), weak staining in cervical epithelium (E) and poor to weak staining in muscles (M) x 150.

Fig. 107: Cervix of *H. senegalensis* during estrus in December, stained with PAS. Note moderate to intense staining in goblet cells (G) and cervical glands (C), weak staining in cervical epithelium (E) and poor to weak staining in muscles (M) x 150.

Fig. 108: Cervix of *H. senegalensis* during estrus in early June, stained with AB pH 2.5-PAS. Note moderate to intense staining in goblet cells (G) and cervical glands (C), weak staining in cervical epithelium (E) and poor to weak staining in muscles (M) x 150.

Fig. 109: Cervix of *E. kohbohuni* during estrus in late March, stained with AB pH 1.4-PAS to show moderate to intense staining/cervical glands (C), moderate staining in mast cells (MC) and weak staining in cervical epithelium (E) x 150.

Fig. 110: Cervix of *H. sparsa* during pregnancy in January, stained with PAS, note intense staining in goblet cells (G), weak staining in cervical epithelium (E) and poor to weak staining in muscles (M) x 200.

Fig. 111: Cervix of *H. fulvus fulvus* during pregnancy in December, stained with PAS. Note intense staining in goblet cells (G) and weak staining in cervical epithelium (E) x 200.

Fig. 112: Cervix of *H. senegalensis* during pregnancy in February, stained with PAS to show intense staining in goblet cells (G) and cervical glands (C), weak staining in cervical epithelium (E) and poor to weak staining in muscles (M) x 200.
increased concentration of neutral mucosubstances, sulfomucins and sialomucins.

ii) Cervical Glands

These glands showed presence of neutral mucosubstances, sialomucins and sulfomucins during pregnancy of these bats, but there was increase in the staining intensity indicating an increase in the concentration of these mucosubstances (Plate No. 14, Figs. 110, 112; Plate No. 15, Figs. 113, 114).

iii) Muscular Layer

The muscular layer of cervix in these bats contained only glycogen during pregnancy (Plate No. 14, Figs. 110, 112).

iv) Mast Cells

The mast cells also showed identical staining reactivities as they exhibited in the uterus of these bats (Plate No. 15, Fig. 114). Thus, mast cells showed the presence of neutral mucosubstances, sulfomucins and hyaluronic acid during pregnancy.

v) Cervix During the Lactation

- *H. scapuligera* : May to July
- *H. fulvus fulvus* : May and June
- *H. lankadiva* : May and June
- *T. sequoiae sequoiae* : September to November
- *T. kochhensis* : August to early October
1) **Cervical Epithelium**

The epithelial cells contained only poor quantity of glycogen.

Very few secretory cells were observed during this period, which resembled in their staining reactivities those described for these cells during the proestrus of these bats. Thus, these secretory cells showed presence of neutral mucosubstances and sialomucins during the lactation.

ii) **Cervical glands**

The cervical glands exhibited weak PAS reactivity and remained unstained with AB pH 1.0 but showed alcianophilia at AB pH 2.5. The remaining staining reactivities with various histochemical procedures were identical to those in the goblet cells during this period. Thus, the cervical glands showed presence of neutral mucosubstances and sialomucins during lactation.

iii) **Muscular Layer**

The muscular layer contained poor quantity of glycogen during lactation in these bats.

iv) **Mast Cells**

The mast cells showed no significant difference in their staining reactivities during lactation. These cells contained neutral mucosubstances, sialomucins and hyaluronic acid during
Plate No. 15 (Figs. 113 to 119)

Fig. 113: Cervix of *Microtus oeconomus* during pregnancy in September, showing cervical mucus secretion in lumen stained with PAS. Note intense staining in goblet cells (a) and cervical glands (c) and weak staining in cervical epithelium (b) x 100.

Fig. 114: Cervix of *Microtus oeconomus* during late pregnancy in June, stained with PAS. Note intense staining in cervical glands (c), moderate staining in mast cells (b) and weak staining in cervical epithelium (a) x 100.

Fig. 115: Cervix of *Peromyscus maniculatus* during oestrus, stained with PAS to show moderate staining in mast cells (b), weak to moderate staining in goblet cells (a) and poor staining in cervical epithelium (c) and muscles (d) x 100.

Fig. 116: Cervix of *Peromyscus maniculatus* during proestrus, stained with AB pH 2.8-PAS. Note moderate staining in goblet cells (a) and cervical glands (c) and poor to weak staining in cervical epithelium (b) x 100.

Fig. 117: Cervix of *Peromyscus maniculatus* during oestrus, stained with AB pH 2.8-PAS. Note moderate to intense staining in cervical glands (c), weak staining in cervical epithelium (b) and poor to weak staining in muscles (d) x 200.

Fig. 118: Cervix of *Peromyscus maniculatus* during oestrus, showing numerous goblet cells in epithelium, stained with PAS. Note moderate to intense staining in goblet cells (c) and weak staining in cervical epithelium (a) x 300.

Fig. 119: Cervix of *Peromyscus maniculatus* during pregnancy, stained with PAS. Note intense staining in goblet cells (a), moderate staining in mast cells (b) and weak staining in cervical epithelium (c) x 200.
A) Histological

In this continuous breeder microchiropteran bat both the uterine horns posteriorly meet to form common cervix, hence both the lumen meet to continue as a single common cervical canal. The mucosa layer was folded which consisted of epithelium and connective tissue core. The mast cells were evident throughout the different phases of reproductive cycle in the connective tissue. The cervical glands were observed in the mucosal folds. The muscularis was thick.

I) Cervix During the Anestrus

In this period the mucosal folds were short and loosely projected into cervical lumen. The cervical epithelium consisted of columnar epithelial cells. The goblet cells were observed in the epithelium. Very few cervical glands were evident in the mucosal folds. Mast cells were found to be present in the connective tissue. The muscularis was thick. In general during anestrus there was overall reduction in the diameter of the cervix.

II) Cervix During the Proestrus

At this time the cervical diameter was increased due to
increase in thickness and height of mucosal folds. The secretory cells increased numerically. Cervical glands were observed at the base of folds. The mast cells and muscle layer were as in the anoestrus.

III) Cervix During the Estrus

During estrus the diameter of the cervix increased to its maximum. The mucosal folds were increased and their height attained maximum level. The number of cervical glands and the secretory cells was increased and they appeared to be mucified. The mast cells were increased in their number and muscularis was comparatively thick.

IV) Cervix During the Pregnancy

All the cellular sites showed similar histological structure up to the mid-pregnancy, but during late pregnancy the number and height of mucosal folds were decreased. The goblet cells and cervical glands were evident and were found to be loaded by mucus. There was no significant difference in the mast cells and muscularis compared to that of the estrus.

V) Cervix During the Lactation

After parturition the number of mucosal folds, goblet cells and cervical glands reduced. The height of mucosal folds and the thickness of epithelium decreased. The mast cells and muscular layer were significantly evident as in the previous period.
The histochemical reactivities of various mucosubstances present in the cervix of *H. mus mus* during the breeding cycle are shown in Table No. 22 in which the histochemical staining intensities have been shown comparatively with ++++ as the strongest activity as determined by the visual comparison. The histochemical distribution and variations in the mucosubstances in the different sites of the cervix of this bat are mostly similar to those described for the megachiropteran bat, *R. leschenaultii leschenaultii*. Hence the details of the histochemical reactivities of various regions of cervix of *H. mus mus* are not described and only the conclusions drawn from these reactivities have been described in brief. The histochemical distribution and alterations in the cervix are shown in Plate No. 15, Figs. 115 to 119.

1) **Cervix During the Anestrus**

1) **Cervical Epithelium**

The epithelial cells contained poor quantity of glycogen only (Plate No. 15, Fig. 115).

Some secretory cells or goblet cells in the cervical epithelium exhibited weak to moderate PAS reactivity (Plate No. 15, Fig. 115) which could partly be blocked by prior phenylhydrazine treatment but was resistant to diastase digestion. These initial results indicated the absence of glycogen but presence of neutral mucosubstances rich in xylose-glycols. Moreover, these
glands remained unstained with AB pH 1.0 and exhibited only PAS reactivity in AB pH 1.0-PAS sequence which indicated the absence of sulfate groups in their mucosubstances. They showed weak alcianophilia at pH 2.5 and C.I. positivity of the same intensity, indicating the presence of carboxymucins in them. The carboxymucins in them were further inferred from their purple-blue staining in AB pH 2.5 PAS and C.I.-PAS sequential staining procedures, blue staining in AB-AB pH 2.5 sequence, metachromasia with azure A at and above pH 3.0, poor alcianophilia in C.I.C technique in the presence of 0.1 M Mg\(^{++}\) but blockade of staining with 0.2 M and higher concentrations of Mg\(^{++}\) and reversible blockade of their alcianophilia in methylation-saponification techniques. The presence of carboxymucins was further proved in them as their alcianophilia could be eliminated by acid hydrolysis and neuraminidase digestion. Hyaluronidase had no effect on their alcianophilia. These results showed the presence of neutral mucosubstances and sialomucins in the secretory cells.

iii) Cervical Glands

Very few cervical glands were evident during estrus which exhibited similar staining reactivities to those described for the cervical glands in the cervix of *H. leucophaea* during their anestrus. Thus, cervical glands contained glycogen and sialomucins.
iii) Muscular Layer

This site of the cervix showed staining reactivities similar to those exhibited by the cervical muscularis of *R. loschenaulti loschenaulti* during their anestrus (Plate No. 15, Fig. 115).

iv) Mast Cells

These cells showed presence of neutral mucosubstances, sulfomucins and hyaluronic acid (Plate No. 15, Fig. 115).

II) Cervix During the Proestrus

1) Cervical Epithelium

The epithelial cells showed identical staining reactivities to those showed by the cervical epithelial cells of *R. loschenaulti loschenaulti* during their proestrus. But now the staining intensity was enhanced compared to the anestrus (Plate No. 15, Fig. 116). Thus, these epithelial cells contained higher concentration of glycogen during proestrus.

The secretory cells in the epithelium exhibited moderate PAS reactivity, which remained unaltered with AB pH 1.0 but exhibited alcianophilia at AB pH 2.5. The remaining histochemical reactivities were similar to those showed in these cells of the *R. loschenaulti loschenaulti* during their proestrus (Plate No. 15, Fig. 116). Thus, the secretory cells in the cervical epithelium contained higher concentration of neutral mucosubstances and
sialomucins.

ii) Cervical Glands

The cervical glands contained higher concentration of neutral mucosubstances and sialomucins during proestrus as compared to the anestrus, as the staining intensity was increased (Plate No. 15, Fig. 110).

iii) Muscular Layer

The muscular layer showed increased amount of glycogen during proestrus.

iv) Mast Cells

The cervical mast cells during proestrus exhibited very much identical histochemical staining reactivities to those showed by the mast cells of this bat during anestrus. There was no significant difference in the staining intensities of anestrus and proestrus. Thus, mast cells contained neutral mucosubstances, sulfomucins and hyaluronic acid during proestrus.

XII) Cervix During the Estrus

1) Cervical Epithelium

The epithelial cells showed weak PAS reactivity (Plate No. 15, Fig. 110). At this time staining intensity was slightly increased over the previous period. Thus, epithelial cells showed higher concentration of glycogen during estrus.
The secretory cells exhibited moderate to intense PAS reactivity (Plate No. 15, Fig. 112), poor to weak alcianophilia at AB pH 1.0 and weak to moderate alcianophilia with AB pH 2.5. The remaining histochemical reactivities were similar to those described for these cells in the earlier period. Thus, these secretory cells contained neutral mucosubstances, sialomucins and sulfomucins. During this period the elaboration of sulfomucins actively started.

11) Cervical Glands

The cells of cervical glands resembled in their staining reactivities the histochemical staining reactivities of the cervical glands in the *H. leucogaster* during their estrus (Plate No. 15, Fig. 117). Thus, the glandular cells of the cervical glands exhibited presence of neutral mucosubstances, sialomucins and sulfomucins during estrus.

111) Muscular Layer

The cervical muscular layer showed presence of glycogen during estrus of this bat (Plate No. 15, Fig. 117).

14) Mucos Cells

The mucos cells in the cervix of this bat showed identical staining reactivities to those exhibited by them in earlier phase. Thus, mucos cells contained neutral mucosubstances, sulfomucins and hyaluronic acid during estrus.
IV) Cervix During the Pregnancy

1) Cervical Epithelium

The epithelial cells of the cervix showed presence of glycogen only (Plate No. 15, Fig. 119).

The goblet or secretory cells showed identical staining reactivities to those exhibited by these cells in the *L. leucogenulti* during their pregnancy. At this period the staining intensity was enhanced over that of the oestrus (Plate No. 15, Fig. 119). Thus, the goblet cells contained higher concentration of neutral mucosubstances, sulfomucins and hyaluronic acid during pregnancy.

2) Cervical Glands

The cervical glands of this bat exhibited enhanced staining intensities over the oestrus which contained increased amount of neutral mucosubstances, sulfomucins and sulfates.

3) Muscular Layer

The cervical muscular layer showed presence of glycogen only.

4) Mast Cells

The mast cells showed presence of neutral mucosubstances, sulfomucins and hyaluronic acid during pregnancy (Plate No. 15, Fig. 119).
v) Cervix During the Lactation

1) Cervical Epithelium

The cells of the cervical epithelium showed poor PAS reactivity and the remaining reactivities were identical to those described for this site during anestrus of this bat. Thus, it was concluded that the cervical epithelial cells contained poor quantity of glycogen only.

During lactation period the number of goblet cells was reduced over that in the pregnancy, which showed weak PAS reactivity and the remaining histochemical reactivities were similar to those described for the goblet cells in anestrus. Thus, the goblet cells contained neutral mucosubstances and sialomucine in small amounts during the lactation.

ii) Cervical Glands

Very few cervical glands were observed during this period and they showed presence of neutral mucosubstances and sialomucine during lactation.

iii) Muscular Layer

This layer gave histochemical reactivities for glycogen only.

iv) Neck Cells

As in earlier period these cells indicated the presence of neutral mucosubstances, sialomucine and hyaluronic acid.
DISCUSSION

As it is brought to the notice earlier in the introductory chapter, the mammalian cervix has mostly been studied to find out the role of cervical mucus, whether it acts as a barrier or aids in conception and also to augment the hormone-mucous substance relationship. The present chapter deals with the nature and cyclic alterations in cervical mucous substances in eight varieties of bats with different breeding habits such as continuous breeding, seasonal breeding with a single sex-cycle and seasonal breeding with two sex-cycles, in a year.

A) Histochemical Reactivities of Cervical Mucous Substances in Bats

The histochemical reactivities of the mucous substances in various cellular sites of the cervix in these varieties of bats resemble those of the identical mucous substances in other organ-systems. There is no indication of the presence of any atypical mucous substances in cervical sites of the bats.

B) Distribution of Mucous Substances in the Cervix of Bats

This study was also undertaken with a view to find out whether the mucous substances in various cervical sites in different varieties of bats are similar or whether there exists any heterogeneity in one and the same tissue and identical type of the cells. The histochemical results obtained in the present investigation show the presence of glycogen, neutral mucous substances, sialomucins, sulfomucins and hyaluronic acid in the
cervix at a very general level. But such different components of
mucous substances are not present in all the cellular elements of the
cervix; instead they show a very selective distribution. There are
minor differences in the elaboration of the mucous substances in
anestrus, but other phases of reproduction show wide differences.
Thus, the glycogen is found in the keratinized layer only in
*H. caporia* and *H. salvus fulva* during anestrus, but the keratinized
layer is not observed in the cervix of these two varieties of
bats during the rest of the phases of their reproductive cycles.
The keratinized layer is not observed in the cervix of the
remaining bats in the present investigation.

The glycogen is also found in cervical epithelium and
muscular layer in these bats, in all the stages of reproductive
cycle. The neutral mucous substances containing *N-glycine* and
acidic mucous substances are found to be absent in the epithelium
and muscular layer of cervix in all the reproductive phases. The
goblet or secretory epithelial cells, which are observed in
cervix of *P. minus minus* only during the anestrus, do exhibit
presence of neutral mucous substances. The goblet cells are not
observed in cervical epithelium of the remaining bats during
their anestrus. The neutral mucous substances also reveal their
presence in the cervical glands of *G. leucogena multis, leucogena*,
*P. pilonidens chrysostrich* and *P. minus minus* during anestrus, but
the cervical glands are not found during this period in the
remaining bats. The neutral mucous substances are found in goblet
cells, cervical glands and mast cells in all these bats during
their proestrus, estrus, pregnancy and lactation. The cholinesterase
showed their absence in the cervical epithelium, muscles and mast cells during the entire reproductive cycle, but during anestrus only the goblet cells of *P. major* and the cervical glands of *A. leucogaster*, *A. leucomystax*, *P. cyclonius*, and *P. major* exhibit presence of sialomucins. The goblet cells and the cervical glands of all these bats reveal presence of considerable amount of sialomucins during proestrus, estrus, pregnancy and lactation. The goblet cells and the cervical glands during estrus and pregnancy only and the mast cells during all the phases of reproductive cycle exhibit presence of sialomucins, but the rest of the cervical sites during respective periods of reproductive cycle do not exhibit presence of sialomucins. The hyaluronic acid is selectively found in the mast cells during all the phases of sex-cycles of these bats, but it is not found in any other site during different phases of sex-cycles.

C) **Species-specific Differences in Cervical Mucous Substances of Bats**

For the present investigation eight species of bats were selected. Such a selection was done with a view to find out the species difference, if any, in cervical mucous substances. The observations at hand show that there is no major species-specific difference. Some minor differences observed in the present investigation are as follows:

1) Keratinization of a higher degree is evident in the cervical epithelium in *P. major* and *H. salvus*. Keratinization is
not evident in other bats during anestrus.

2) Keratinization is not evident in the cervix of *H. leucogaster* because of two successive cycles and the cervical mucosa remains unmodified, although the degree of mucification varies in different phases of the sex-cycles.

3) During anestrus the secretory epithelial cells or goblet cells, although numerically few are evident in *P. micus micus*. From proestrus onwards the secretory cells gradually increase in number in all the bats.

4) During anestrus very few cervical glands are evident in *H. leucogaster*, *P. evelonimus chrysophlebix* and *P. micus micus*. From proestrus onwards the glands gradually increase in number in all the bats. The glands elaborate different mucous substances according to the stage of the sex-cycle.

5) Higher degree of cervical mucification is evident in *H. leucogaster*, *H. hastalis*, *H. cryptice goasi*, *P. evelonimus chrysophlebix* and *P. micus micus* than in *H. aceros*, *H. fulves fulves* and *H. jactilina*.

D) Comparative Distribution of Mucous Substances in Chiropteran Cervix

The next interesting aspect is to view comparatively the mucous substances in various cervical sites of the bats under the present study and the mucous substances reported in the cervix of other studied bats to find out similarities or differences, and
if possible to draw a common chiropteran pattern of the cervical mucousubstance content. The histochemical observations obtained from eight varieties of bats in the present project are in good agreement with most of the reports on cervical mucousubstances in other bats.

The keratinized layer in the cervix of *J. speoris* and *S. fulves* contains only glycogen. There are no histochemical reports on the mucousubstances of this layer in other chiroptera.

The cervical epithelium in the bats under investigation contains only glycogen, and secretory epithelial cells contain neutral mucousubstances, sialomucins and sulfomucins. In similar histochemical studies glycogen has been reported in bat, *E. theobaldi* (Partado, 1981). The neutral mucousubstances, sialomucins and sulfomucins have been demonstrated histochemically in the secretory epithelial cells of *G. sphinx sphinx* (Pawar, 1976), *P. giganteus giganteus* and *P. longipes* (Gadegone, 1977) and *E. theobaldi* (Partado, 1981), Gadegone (1977) reported presence of only neutral mucousubstances in the cytoplasm of secretory epithelium of cervix in *E. lyra lyra*, but he also observed neutral mucousubstances, sialomucins and sulfomucins in the surface secretion of the epithelium in the same bat. In the present investigation the cervical epithelium contains glycogen, while the secretory epithelial cells contain neutral mucousubstances, sialomucins and sulfomucins particularly during the estrus and pregnancy.

The neutral mucousubstances, sialomucins and sulfomucins are
histologically identified in the glandular cells of the cervical glands of these bats in the present investigation. In the similar studies Pawar (1976) demonstrated the neutral mucosubstances, sialomucins and sulfomucins in the cervical glands of G. niger.

The cervical muscular layer in the bats under investigation contains glycogen only. The glycogen has been identified histologically in the bat, P. theobaldi (Vartodo, 1981). Thus, the results obtained for the presence of glycogen in the muscles of eight varieties of bats in the present investigation are identical to those reported in other bats.

The present investigation also reveals the presence of neutral mucosubstances, sulfomucins and hyaluronic acid in the cervical mast cells in all bats under present project. There are no comparable histochecmical reports on the mucosubstances for the cervical mast cells in other chiroptera.

Thus, from this discussion on the histologically demonstrated mucosubstances in the cervix of eight varieties of bats and existing literature on cervix of other bats it can be noted that the chiropteron cervix exhibits very selective distribution. The keratinized layer, if present, contains glycogen. The cervical epithelium shows presence of glycogen, neutral mucosubstances, sialomucins and sulfomucins are found in the secretory epithelial cells and cervical glands. The muscles contain only glycogen. The mast cells show presence of neutral mucosubstances, sulfomucins and hyaluronic acid.
The next interesting aspect is to view comparatively the
mucosubstances in various cervical sites of the bats and the
mucosubstances reported in cervix of other mammals to find out
similarities and differences, if any. The mucosubstances demontrated-
ed in the present investigation in cervix of eight variety of
bats are practically identical to those reported in other mammals
except for the presence of glycogen in the keratinised layer of
some bats.

The glycogen is found in the cervical epithelium of the bats
under investigation and the presence of glycogen has also been
reported in cervical epithelial cells of guinea pig (Gurgo and
Sialocki, 1956), rabbit (Gregoire and Hafez, 1971), hamster
(Gregoire and Guimera, 1968; Gregoire and Richardson, 1970) and
human (Scheeler and Danziger, 1955; Masslow, 1961; Gregoire, 1963),
bats like E. theobaldi (Partado, 1931). Colborn et al. (1967)
described numerous glycogen granules in the stratified squamous
epithelial cells in cervix of monkeys. Earlier Henry and Latour
(1957) reported that the glycogen in human cervix-uteri parallel-
ed the cell differentiation, but was inversely proportional to the
cell activity.

In the cervical secretory epithelial cells neutral mucosub-
stances, sialomucins and sulfomucins are found in the bats investi-
gated for the present study. While studying some aspects of
mammalian cervical mucous, Gibbons and Waffner (1966) identified
glycoproteins in the epithelial cells. The presence of saliva and diastase resistant PAS positive material has been reported in the cervical epithelium of rat (Persberg, 1962) and monkey (Colborn et al., 1967). Presence of neutral mucopolysaccharides has been reported in cervix-uteri of cows (Grabholz and Kühnel, 1957) and human (Graumann et al. 1966; Tock and Shilkin, 1970) and cervical epithelium in rabbit and cow (Yamashita et al., 1971) and secretory cervical epithelial cells in several bats such as C.aphrines aphrines (Pawar, 1976), P. gigantea gigantea, P. lyra lyra and P. longimanus (Andegowa, 1977) and P. theobaldi (Fartado, 1981).

Acidic mucopolysaccharides were reported in variable amounts in human cervix-uteri (Graumann et al., 1966), Tock and Shilkin (1970) after studying the mucopolysaccharides in the cervix of postmenopausal human cervix-uteri, reported the presence of mucins in the surface epithelium which are mostly acidic and a lesser portion was neutral. Kondo (1972) also demonstrated acidic mucopolysaccharides in the surface epithelium of cervix-uteri of nonpregnant women. The presence of acidic mucopolysaccharides has also been reported in bovine cervix-uteri by Grabholz and Kühnel (1957). Yasuda (1960) also observed histochemically acidic mucopolysaccharides in cervix of rabbit. According to him at the tubal epithelium there is mesenchymal substance which is independent of the pH and hormones. It contained hyaluronic acid, mucosin sulfate, chondroitin sulfate and chondroitin. He also observed uptake of 35S in cervical epithelium and stroma. By employing viscometric measurements and paper electrophoresis, Iversen (1950) showed the presence of hyaluronic acid and chondroitin sulfate in human endocervix.
Fractionation and isolation studies by Taakaya (1973) on human cervix-uteri revealed the presence of chondroitin-4-sulfate, chondroitin-6-sulfate, dermatan sulfate and hyaluronic acid. The changes in sialic acid in human cervix during menstrual cycle were studied by Vernik and Iruela (1971). Marinov and Lovell (1967) observed two types of cells in cervix of cow during all the phases of estrous cycle. The mucus secreting cells were predominant over the nonsecreting ciliated cells in the epithelium. Vorderer et al. (1973) also identified ciliated and nonciliated columnar epithelial cells in cervix of cow. They further reported that 4th day proestrus the nonciliated cells contained a large amount of stored mucin in the supra-nuclear area. They also identified sulfated and nonsulfated acidic carboxylic mucopolysaccharides in the stored mucin and secretion in the cervical human. Yoshinata et al. (1971) demonstrated neutral, acidic and sulfated mucopolysaccharides in epithelial cells of rabbit and cattle cervix. They identified eight cell types, but found absence of glycogen and hyaluronic acid in all of them. The presence of sialic acid and sulfomucins has also been reported in cervical epithelium (secretory epithelial cells) of the bats such as C.ephring ephring (Pawar, 1976), A.egyptius egypius and T. longipes longipes (Ladegene, 1977) and T. ca Activity (Partade, 1981).

Ladegene (1977) also reported neutral mucosubstances, sialomucins and sulfomucins in the surface secretion of cervical epithelium in S. lyra lyra. In the present investigations also neutral mucosubstances, sulfomucins and sialomucins were identified in the cervical secretory epithelial cells of the bats particularly
during the estrus and pregnancy.

The neutral mucosubstances, sialomucins and sulfomucins were found in the cervical glands in eight varieties of bats in the present investigation. The mucosubstances in the cervical glands have not been studied in detail, although it is reported that cervical glands in some mammals develop enormously during pregnancy and secrete large masses of mucus (Stieve, 1927; Hamilton, 1949; Nesbitt and Hallman, 1952). Kondo (1972) by employing alcian blue and histoplanimetric procedures demonstrated acidic mucopolysaccharides in human cervix-uteri. In cervical glands of one megachiropteran bat, Cynopterus sphinx Pater (1976) demonstrated neutral mucosubstances, sulfomucins and sialomucins. In this regard the cervical glands in all the varieties of bats investigated resembled Cynopterus sphinx.

Although the cervical mucus has not been studied separately in the present investigation, the secretions from the secretory epithelial cells and glands appear to contain neutral mucosubstances, sulfomucins and sialomucins. Shuttles (1951) and Shuttles et al. (1981) have reported that in human cervical mucus during the mid-cycle about 75-80% of the mucopolysaccharides consist of a neutral mucopolysaccharide containing methylpentose, galactose and hexosamine. Reggie and Petella (1955) found glycogen and reducing sugars in human cervical mucus. Batka et al. (1969) also noted an increase in cervical glycogen in contracept during the secretory phase.

Presence of glycoprotein or mucoid in human cervical mucus
has been reported by Neubaus and Moghissi (1962). Agrawal et al. (1977) also studied glycoproteins from cervical mucus of cows, buffaloes and hybrid cows, and reported that the glycoprotein concentration in the cervico-vaginal mucus may influence fertility. The presence of sialic acid also has been reported in the cervical mucus of humans (Ö inhabit, 1955; Moghissi and Syner, 1976) and bovines (Igumnov, 1967; Iacobelli et al., 1971; Humana et al., 1971).

Recently, Hatcher et al. (1977) have purified a sialic acid fraction from cervical mucus of bonnet monkeys. The presence of sulfomucins has been demonstrated in bovine cervical mucus (Igumnov, 1967). Takamine et al. (1951) identified chondroitin sulfate in vaginal lumen which was believed to be synthesized in cervix-uteri. In a later study Takamine (1951) identified keratosulfate in the bovine cervical mucus. In addition to sulfomucins and sialic acid, neutral mucosubstances were demonstrated in bovine cervical mucus (Igumnov, 1967). Cadogan (1977) has reported the presence of neutral mucosubstances, sialic acid and sulfomucins in cervical secretion of a bat Lycon. lyca.

The present investigation on cervix of eight varieties of bats showed the presence of glycogen in the muscles. Fortade (1921) also detected glycogen in cervical muscles of a bat, T. theobaldi.

In the present project presence of neutral mucosubstances, sulfomucins and hyaluronic acid is seen in the mast cells. Practically there is no comparable histochemical report on the mucosubstances in these mast cells in other animals.
Therefore, at a general level the cervical epithelium in different mammals seems to contain glycogen. Also the cervical epithelial secretory cells and glandular cells in the cervix of bats and other mammals secrete a complex mixture of neutral and acidic mucosubstances. Glycogen is found in the muscle layer of cervix in most of the mammals. The keratinized layer contained glycogen in some bats whereas the cervical mast cells contain a complex mixture of neutral and acidic mucosubstances.

F) Mucosubstances and the Functional States of the Cervix in Bats

Though the eight varieties of bats selected for the present investigation differ in the breeding habits, the variations occurring in the mucosubstances at various cervical sites of these bats in seasonal breeding cycle appear practically identical. These alterations are shown in Tables No. 23 to 26.

In all the phases of the reproductive cycles of these bats the nonglandular cervical epithelium and the muscular layers show presence of glycogen only, this content being at its poorest in anestrous but increasing through proestrus reaches its maximum in estrus and pregnancy. The mast cells also show presence of a complex mixture of mucosubstances containing neutral mucosubstances, sulfomucins and hyaluronic acid. The mast cells undergo mucosal changes, being minimum in anestrus and maximum in estrus. Their staining intensities are poor in anestrus but high in estrus. The glandular cervical epithelial cells in all the bats are not seen in anestrus except Nyctimene rhyncha, but in proestrus some
epithelial cells seem to undergo a transformation and become glandular, when they elaborate neutral mucosubstances and sialomucins. The number of such glandular cells and also their typical mucin elaboration attain their maximum in estrus and pregnancy. In lactation they undergo a depletion and in the successive anestrus these cells again disappear. The cervical glands in some bats contain poor quantities of neutral mucosubstances and sialomucins in anestrus, but their concentrations increase in all the bats through proestrus reaching maximum levels in estrus when in addition they also elaborate sulfomucins, the condition remaining practically the same in pregnancy also with the difference that the sulfomucin elaboration stops following parturition. These glands undergo structural and functional hypotrophy during lactation.

The above discussion, thus, shows the dependence of the elaboration of mucosubstances in the cervix on its functional state and it may have a far-reaching significance in the functional activities of the cervix of these bats. Some observations in the available literature also support the above described alterations in the cervical mucosubstances in the cervix. Maximum quantity of cervical epithelial mucin was observed in the late proliferative and early secretory phases of the human menstrual cycle by Shoemaker and Danzliger (1956). Forchberg (1962) reported glycogen in stratified epithelium of the rat cervix epithelium during proestrus and estrus, Fedorov (1966) observed very little glycogen in cervical epithelial cells of sheep. Their glycogen content was slightly increased in the preovulatory period but
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*Example Table*
decreased during pregnancy. Yakovleva (1967) and Botnar (1968) demonstrated that in women during menstrual cycle epithelial glycogen content increased during the proliferative phase and decreases during the secretory phase, when in the latter phase acidic mucopolysaccharides increased. Moghissi and Synar (1976) observed that in women the cervical mucus increases 10 to 20 fold at ovulation with the peak coinciding the LH surge. Sialic acid was found to decrease at mid-cycle and increase after ovulation. Cyclic variations in sialic acid in cervix have also been reported in cow (Hamans et al., 1971; Iacobelli et al., 1971) and monkey (Batcher et al., 1977). Cyclic alterations in neutral mucosubstances, sulfomucins and sialic acid have been observed in bats like C. sphinx sphinx (Fawcett, 1973), T. giganteus giganteus, H. lyra lyra and P. lactemplana (Gadegasse, 1977) and T. theobaldi (Partida, 1981).

3) Probable Hormone Control of Cervical Mucosubstances

The aforementioned cyclic alterations in cervical mucosubstances of bats appear to be under the control of ovarian hormones. The results obtained in other mammals involving hormone administration support this conclusion. Gregoire and Hafe (1971) showed that cervical glycogen synthesis responded to estrogen but not to progesterone administration in rabbits. Earlier Gregoire et al. (1967) demonstrated that in spayed rats, estrogen administration caused accumulation of glycogen in cervix but progesterone was found to have glycogenolytic effect. Colborn et al. (1957) described diastase-resistant PAS material in cervical epithelium
and glandular lumina in monkeys. This material appeared more abundant with synergistic action of estrogen and progesterone. Sundaravadhan (1974) found that mucin (I) and PAS-positive material in bovine cervical mucus was dependent on estrogen levels. Kakaya (1973) also observed an increase in human cervical mucopolysaccharides by estradiol and estradiol treatments, but progesterone or HCG were without any effect.

Koescru and Westphal (1973) found highest levels of proteins and sialic acid in cervical mucus of normal women during ovulatory phase. Women treated with estradiol on days 5-14 of the cycle showed depletion in them over the ovulatory level, but nonestrous administration during ovulatory phase increases the levels of both. Raynud (1973) found that an injection of synthetic progesterone, fluraxestorone lowered the sialic acid content in cervical mucus of mice. Agrawal and Datee (1976) reported that incorporation of sialic acid in mucoid fraction may be regulated by estrogen:progesterone titer. Hishino and Heymann (1976) also reported that the sialic acid content in cervix of ovariectomized mice was not significantly affected by estrogen or progesterone alone, but was affected by their combination. It was observed that when progesterone was dominant sialic acid was increased, whereas under estrogenic dominance the effect was reversed.

Sacharlas (1980) reported that estrogen causes cervix to secrete acid mucopolysaccharides, one of them being keratosulfate. Vasuda (1980) also observed an accumulation of large amounts of acid mucopolysaccharides in cervix of rabbit by estradiol
treatment. He further reported that the uptake of $^{35}$S was evenly distributed in struma and cervical epithelium. Guthrie et al. (1966) also showed that intra-cardiac injection of Na$_2^{35}$O$_4$ in guinea pigs, radioactivity in cylindrical cells in cervical epithelium increased in first 10 min. following estradiol benzoate treatment, which was not observed in castrated animals. However, when estradiol treatment was followed by progestrone, the radioactivity was also found in the secretion. These studies indicate that cervical mucous substances are governed by ovarian steroid hormones. This may be true in rats also. This is only a suggestion and further studies are required to establish this hormone-mucous substance relationship by involving castration and hormone replacement therapy.

With respect to the cervical mucous substances, there are some suggestions as to their functional significance in female physiology of reproduction in the mammals. Regales and Botella (1955) suggested that the cervical mucoproteins are an essential product in the process of fertilization. Högstedt and Synner (1970) studied the human cervical mucus, mucoid and their relation to sperm penetration, and showed that sperm migration was accelerated in hydrolysed cervical mucus. Kondo (1972) studied the human cervix-uteri, where he observed ample amount of acid mucopolysaccharides, and suggested that these mucous substances might be playing an important role in the ripening and dilation of the cervix-uteri. Carlberg et al. (1969) have done some considerable work on the cervical sialic acid and its relation to sperm receptivity. They have noted that with increase in the estrogen excretion, there is a decrease in the sialic acid, while the
spora receptivity increases. They also suggested that in the
normally menstruating woman the cervical mucus decreased during
proliferative phase but increased during the secretory phase of
menstrual cycle. These cyclic changes in the sialic acid were
related to sperm receptivity. Linford (1974) has recently reviewed
the cervical mucus and has come to the conclusion that this
cervical secretion in some cases can, in itself, form a barrier
to sperm receptivity and as such can be used as a contraceptive,
whereas in other cases it aids the conception favouring the sperm
receptivity. In the present investigation cervix showed some
elaboration of acidic mucosubstances, nacrocucins and sulfocucins
in addition to neutral mucosubstances during anestrus, whereas
such elaboration of mucosubstances increased during proestrus,
estrus, and at pregnancy it was maximum. Hence in the bats it
appears that the cervical mucosubstances may be favouring the
sperm receptivity and thus aiding conception, since in these
wild breeding animals copulation invariably ends in conception.