NORMAL HISTOLOGY: SIXTH STAGE NYMPH
NORMAL HISTOLOGY OF THE MIDGUT OF SIXTH STAGE NYMPH OF
Poekilocerus pictus:

Internally the gut is lined by a stratum of enteric epithelium, the outer ends of its cells rest upon a basement membrane, the latter is followed by a inner layer of circular muscles and outer layer of longitudinal muscle. The outermost coat of the midgut is a thin peritoneal membrane. In the structure of the enteric epithelium two main types of cells are distinguished, these are the columnar cells, their boundaries are usually well defined, they almost invariably possess a striated border and these cells have well defined nucleus. The other type of cells are the regenerative nidi, their function is to renew other epithelial cells when they are destroyed (Fig. 117).

NORMAL HISTOLOGY OF THE ADIPOSE TISSUE OF SIXTH STAGE NYMPH OF
Poekilocerus pictus:

In the sixth stage nymph, the cell boundaries are not clearly visible, though it can be made out that the cells have increased in size. The cytoplasmic area is much elaborated and the central globules are less in number. Each nucleus has one or more nucleoli (Fig. 118).
NORMAL HISTOLOGY OF THE TESTIS OF SIXTH STAGE NYMPH OF
Poekilocerus pictus:

Each testicular tube is bound on the outside by a compact fibrous connective tissue layer forming the basement membrane which supports the germ tissues. The germ cells are grouped together in cysts. The earlier germ cells are in the form of a spermatogonia. (Fig. 119a). Each spermatogonia consists of a large nucleus surrounded by thin layer of cytoplasm. The spermatogonia divide mitotically and enlarge into primary spermatocytes which are incidentally the largest cells among the germ cell population. Each primary spermatocyte has a comparatively large nucleus showing chromatin threads (Fig. 119b). The secondary spermatocytes are obtained after the meiotic division of the primary spermatocytes. As sixth stage nymph becomes 15 to 18 days old the secondary spermatocytes divide mitotically to give rise to the spermatids. In the process of spermateleosis the spermatid nucleus starts condensing and elongating to form the sperm nucleus (Fig. 119c).

NORMAL HISTOLOGY OF THE OVARY OF SIXTH STAGE NYMPH OF
Poekilocerus pictus:

Each ovariole is lined with follicular epithelium which
is covered with two membranes the tunica propria and the external ovariole sheath. A short pedicle or ovariole neck connects each ovariole with the lateral duct and the genital chamber. The nucleus is oval situated at the terminal end of the oocyte, the chromatin material is amorphous and scattered irregularly. The cells of the follicular epithelium are cubical and rectangular with oval nuclei and the interfollicular tissue has small oval nucleus. The ooplasm is devoid of yolky material. (Fig. 120a and b).

CONTROL SERIES :

The control series of the present investigation did not show any variation from the normal histology in terms of the midgut, the adipose tissue and the gonads.
Fig. 117: Section of the midgut of normal sixth stage nymph of *P. pictus* showing well developed longitudinal muscles (arrow 1) circular muscle layer (arrow 2) regenerative nidi (arrow 3) columnar cells (arrow 4) and striated border (arrow 5) (X 120) Haematoxylin/Eosin.

Fig. 118: Section of the adipose tissue of normal sixth stage nymph of *P. pictus* showing elaborated cytoplasmic area, prominent nuclei and nucleoli (X 100) Haematoxylin/Eosin.

Fig. 119a: Section of the testis of normal sixth stage nymph of *P. pictus* showing compact arrangement of the germ cells (X 100) Haematoxylin/Eosin.

Fig. 119b: The same showing an enlarged view of the closely packed primary spermatocytes (X 450) Haematoxylin/Eosin.
Fig. 119c : The same showing normal structure of the spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and sperms (X 450) Haematoxylin/Eosin.

Fig. 120a : Section of the ovary of normal sixth stage nymph of *P. pictus* showing normal oocytes which are connected by a pedicle to the genital chamber (arrow) (X 100) Haematoxylin/Eosin.

Fig. 120b : The same showing normal oocytes with terminally situated nucleus (X 100) Haematoxylin/Eosin.
EFFECT OF BENZIDINE
EFFECT OF BENZIDINE ON THE MIDGUT OF SIXTH STAGE NYMPH OF
Pseukilocerus pictus:

4 Days:

The columnar cells of the gut epithelium have lost their shape. The nuclei are unevenly scattered in these cells. The chromatin material of the nuclei of these cells is towards degeneration. The cytoplasm is also more or less degenerated. A few regenerative nidi cells can be seen which are with vacuolated cytoplasm and their nuclei are showing degenerated chromatin material. The circular muscle layer is thickened, while the longitudinal muscles are distorted and pycnotic. The peritoneal layer covering the gut wall is also thickened (Fig. 121).

8 Days:

Degeneration of the columnar cells of the gut epithelium is further evident. The nuclei of the gut epithelium as well as of the regenerative nidi are pycnotic. The cytoplasm is degenerated and vacuolated. The striated border of the gut epithelium is degenerated. The circular muscle layer is distorted and interrupted. The longitudinal muscles are
also distorted and degenerated. The peritoneal layer is distorted (Fig. 122).

12 Days:

The cells of the gut epithelium have lost their columnar shape and appear as masses of cells. Their nuclei as well as their cytoplasm are showing degeneration. A few regenerative nidi can be seen which are also showing degenerated nuclei and vacuolated cytoplasm. The striated border is also degenerated. The circular muscle layer is distorted. The longitudinal muscle layer is also distorted with degenerated cytoplasm and clumped nuclei. The peritoneal covering is also affected (Fig. 123).

16 Days:

The architecture of the gut epithelium is lost and irregular mass of cells can be seen which are seen migrating towards the gut lumen. The cells are with pycnotic nuclei and degenerated cytoplasm. Few regenerative nidi with degenerated cytoplasm and pycnotic nuclei can be seen. The striated border is degenerated and is not visible. The circular musculature is very much distorted and degenerated. The longitudinal muscles are also distorted and some of
them have become pycnotic (Fig. 124a and b).

18 Days:

Further degeneration of the gut epithelium is evident. The cells of the gut epithelium have increased in number and most of them are seen migrating towards the gut lumen. Cell exfoliation is also seen in the gut lumen (Fig. 125a). The nuclei of these cells with clumped chromatin material while the cytoplasm is degenerated and vacuolated. Many elongated cavities are also seen in between the epithelium cells. The striated border is degenerated. The circular muscle layer is very much distorted and degenerated. The longitudinal muscles are also with degenerated cytoplasm and vacuolated nuclei (Fig. 125b).
Fig. 121: Section of the midgut of sixth stage nymph of *P. pictus* showing thickened peritoneal covering (arrow 1) thickened circular muscle layer (arrow 2) and nuclei of the gut epithelium with degenerated chromatin material (arrow ) after 4 days treatment with benzidine (X 200) Haematoxylin/Eosin.

Fig. 122: Section of the midgut of sixth stage nymph of *P. pictus* showing distorted muscular- ture and pycnotic nuclei of the gut epithelium (arrows) after 8 days treatment with benzidine (X 50) Haematoxylin/Eosin.

Fig. 123: Section of the midgut of sixth stage nymph of *P. picta* showing degenerated longitudinal muscles and degenerated nuclei of the regenerative nidi and of the gut epithelium (arrows) after 12 days treatment with benzidine (X 60) Haematoxylin/Eosin.

Fig. 124a: Section of the midgut of sixth stage nymph of *P. pictus* showing pycnotic longitudinal muscles (arrow 1), distorted circular muscle (arrow 2) and inward migration of cells towards the gut lumen (arrow 3) after 16 days treatment with benzidine (X 100) Haematoxylin/Eosin.
Fig. 124b: The same showing degenerated gut epithelium and an additional mass of cells seen in the gut lumen (X 60) Haematoxylin/Eosin.

Fig. 125a: Section of the midgut of sixth stage nymph of *P. pictus* showing degenerated and thickened circular muscle layer (arrow 1) and cell exfoliation (arrow 2) after 18 days treatment with benzidine (X 100) Haematoxylin/Eosin.

Fig. 125b: The same showing degenerated longitudinal muscles (arrow 1), degenerated circular muscle layer (arrow 2), vacuolated gut epithelium (arrow 3) and along cavity in the epithelium (arrow 4) (X 100) Haematoxylin/Eosin.
EFFECT OF BENZIDINE ON THE ADIPOSE TISSUE OF SIXTH STAGE NYMPH OF *Poekilocerus pictus*:

4 Days:

The cell boundaries of few fat cells are visible while in rest of the cells, the boundaries are obliterated. The cytoplasm shows vacuolization. The chromatin material of the nuclei are clumped and the nucleoli are not visible (Fig. 126).

8 Days:

The cell boundaries of the fat cells are obliterated. The cytoplasm shows degeneration and vacuolization. The nuclei are also disintegrated and the nucleoli are not visible (Fig. 127).

12 Days:

In this stage too, degeneration of the adipose tissue is evident. The cell boundaries are further obliterated and the cytoplasm shows much degeneration with prominent vacuoles. The nuclei of the fat cells have come close together and are showing degenerated and vacuolated chromatin material. A few nuclei are with pycnotic chromatin material.
The nucleoli were not visible (Fig. 128).

16 Days:

Further degeneration of the adipose tissue is evident. The adipose tissue is accumulated together and the cell boundaries are not visible. The cytoplasm is much degenerated with more prominent vacuoles. Most of the nuclei which have come close together are showing pycnotic chromatin material (Fig. 129a and b).

18 Days:

The entire adipose tissue is disintegrated and obliterated. The cytoplasm is also disintegrated. The nuclei have come close together and are irregular in shape with degenerated chromatin material. Though the adipose tissue is not melanized but a few spindle shaped cells can be seen (Fig. 130).
Fig. 126: Section of the adipose tissue of sixth stage nymph of *P. pictus* showing nuclei with clumped chromatin material (arrow 1) and vacuolated cytoplasm (arrow 2) after 4 days treatment with benzidine (X 250) Haematoxylin/Eosin.

Fig. 127: Section of the adipose tissue of sixth stage nymph of *P. pictus* showing nuclei with degenerated chromatin material (arrows) and degenerated cytoplasm after 8 days treatment with benzidine (X 250) Haematoxylin/Eosin.

Fig. 128: Section of the adipose tissue of sixth stage nymph of *P. pictus* showing degenerated adipose tissue with degenerated nuclei (arrows) after 12 days treatment with benzidine (X 250) Haematoxylin/Eosin.
Fig. 129a : Section of the adipose tissue of sixth stage nymph of P. pictus showing pycnotic nuclei (arrows) and degenerated cytoplasm after 16 days treatment with benzidine (X 250) Haematoxylin/Eosin.

Fig. 129b : The same showing degenerated adipose tissue with pycnotic nuclei (X 250) Haematoxylin/Eosin.

Fig. 130 : Section of the adipose tissue of sixth stage nymph of P. pictus showing obliterated cells with pycnotic nuclei (arrow 1) and some spindle shaped cells (arrow 2) after 18 days treatment with benzidine.
EFFECT OF BENZIDINE ON THE TESTIS OF SIXTH STAGE NYMPH OF
Poekilocerus pictus:

4 Days:

Loosening of the germ cells is evident and some of the germ cells in turn have become pycnotic. The cytoplasm of the testis follicle shows somewhat degeneration and much vacuolization (Fig. 131a and b).

8 Days:

Many testis follicle have come close together. Loosening of the germ cells is further evident in some of the follicles (Fig. 132a). The germ cells and the differentiating germ cells show arrested growth. The spermatogonia are with pycnotic nucleus and degenerated cytoplasm. The primary spermatocytes are also with diffused chromatin material. The cytoplasm of the testis follicle is degenerated. The basement membrane covering the germ cells is also seen to be very weak at some places (Fig. 132b).

12 Days:

Further degeneration of the germ cells and the differentiating germ cells is evident. Loosening of the germ
cells is seen in this stage too (Fig. 133a). The basement membrane is also affected. The spermatogonia are with pycnotic nucleus. The spermatocytes are also showing degeneration of the nucleus and the cytoplasm. A few pycnotic spermatids can be seen (Fig. 133b). A cluster of clumped pycnotic sperm heads can be seen in the lumen.

16 Days:

The entire testis is in a much degenerated state. Loosening of the germ cells is evident in some of the testis follicle. The cytoplasm of the follicles is very much degenerated. The spermatogonia, the spermatocytes and the spermatids are with pycnotic nucleus and degenerated cytoplasm (Fig. 134a). Hypertrophied spermatozoans are seen scattered in the lumen (Fig. 134b). A small melanized scab is seen for the first time in this stage, which is formed inbetween the interfollicular space of the testis in the connective tissue.

18 Days:

The testis is in a deformative and degenerative state. Loosening of the germ cells is further evident. The cytoplasm of the testis follicle is much degenerated.
The basement membrane covering the germ cells and differentiating germ cells is also distorted and interrupted. The spermatogonia, the spermatocytes and even the spermatids are with pycnotic nucleus. Some hypertrophied spermatozoans can be seen scattered in the lumen (Fig. 135a), while in some cases pycnotic sperm heads are seen accumulated (Fig. 135b).
Fig. 131a: Section of the testis of sixth stage nymph of *P. pictus* showing pycnotic germ cells (arrows) after 4 days treatment with benzidine (X 60) Haematoxylin/Eosin.

Fig. 131b: The same showing vacuolated cytoplasm of the testis follicle (arrows) (X 100) Haematoxylin/Eosin.

Fig. 132a: Section of the testis of sixth stage nymph of *P. pictus* showing distorted shape of the follicles, loose arrangement of the germ cells which have become pycnotic (arrows) after 8 days treatment with benzidine (X 50) Haematoxylin/Eosin.

Fig. 132b: The same showing loose arrangement of spermatogonia which are with pycnotic nucleus (arrow 1) degenerated primary spermatocytes (arrow 2) and loosely scattered secondary spermatocytes which are with pycnotic nucleus (arrow 3) (X200) Haematoxylin/Eosin.
Fig. 133a: Section of the testis of sixth stage nymph of *P. pictus* showing degenerated follicles and pycnotic germ cells (arrows) after 12 days treatment with benzidine (X 100) Haematoxylin/Eosin.

Fig. 133b: The same showing degenerated chromatin threads of primary spermatocytes (arrow 1), pycnotic nucleus of secondary spermatocytes (arrow 2) and some pycnotic spermatids (arrow 3) (X 200) Haematoxylin/Eosin.

Fig. 134a: Section of the testis of sixth stage nymph of *P. pictus* showing the formation of 'brown coloured body' after 16 days treatment with benzidine (X 80) Haematoxylin/Eosin.
Fig. 134b: The same showing hypertrophied scattered sperms in the lumen (X 225) Haematoxylin/Eosin.

Fig. 135a: Section of the testis of sixth stage nymph of *P. pictus* showing loose arrangement of the pycnatic germ cells and cavities are also seen in the follicles (arrows) after 18 days treatment with benzidine (X 120) Haematoxylin/Eosin.

Fig. 135b: The same showing degenerated germ cells (arrow 1) and scattered sperms in the lumen (arrow 2) (X 120) Haematoxylin/Eosin.
EFFECT OF BENZIDINE ON THE OVARY OF SIXTH STAGE NYMPH OF
Poekilocerus pictus:

4 Days:

The oocytes are distorted in shape. The ooplasm is also degenerated, showing vacuoles in some oocytes. The nuclei show scattered chromatin material. The follicular epithelium cells are pycnotic. The genital epithelium also shows degeneration (Fig. 136).

8 Days:

The oocytes are distorted in shape in this stage too. The ooplasm is much degenerated showing severe vacuolization. The follicular epithelium cells are pycnotic and distorted. The nuclei show degenerated chromatin material. The cytoplasm of the genital epithelium is vacuolated and the nuclei are pycnotic (Fig. 137a and b).

12 Days:

Further degeneration of the oocytes is visible. The ooplasm is much degenerated. In some oocytes contraction of the ooplasm is seen, while in some oocytes degenerating remnants of the ooplasm is seen. The nuclei are also
showing degenerated chromatin material. The follicular epithelium cells are pycnotic in some oocytes while in some oocytes the follicular epithelium cells are vacuolated. In some oocytes it is also seen that the follicular epithelium cells have lost contact among themselves. The nuclei of the genital epithelium are pycnotic (Fig. 138a and b).

16 Days:

Degeneration of the ovary is further evident due to the distorted shape of the oocytes. The ooplasm is very much degenerated and vacuolated. Contraction of the ooplasm is also evident in some oocytes. The follicular epithelium cells are distorted, most of them have lost contact among themselves and most of the follicular epithelium cells have also become pycnotic. The nuclei are also degenerated. The genital epithelium is contracted and pycnotic (Fig. 139a and b).

18 Days:

The entire ovary is in a very degenerated state. The oocytes are distorted with degenerated ooplasm. Contraction of the ooplasm is also evident and in some oocytes degenerated remanants of the ooplasm are also seen. The follicular epithelium layer is also distorted, most of the follicular
epithelial cells have lost contact among themselves and are showing degenerated chromatin material. The oocyte nucleus is also with degenerated chromatin material. The genital epithelium is pycnotic and degenerated (Fig. 140a and b).
Fig. 136 : Section of the ovary of sixth stage nymph of *P. pictus* showing distorted shape of the oocytes after 4 days treatment with benzidine (X 60) Haematoxylin/Eosin.

Fig. 137a : Section of the ovary of sixth stage nymph of *P. pictus* showing degenerated ooplasm and genital epithelium after 3 days treatment with benzidine (X 30) Haematoxylin/Eosin.

Fig. 137b : The same showing distorted shape of the oocytes and degenerated ooplasm (arrows) (X 80) Haematoxylin/Eosin.
Fig. 138a: Section of the ovary of sixth stage nymph of *P. pictus* showing degenerating oocytes (arrows) after 12 days treatment with benzidine (X 50) Haematoxylin/Eosin.

Fig. 138b: The same showing an enlarged view of the oocyte with degenerated ooplasm (arrows) and pycnotic follicular epithelium layer (X 200) Haematoxylin/Eosin.

Fig. 139a: Section of the ovary of sixth stage nymph of *P. pictus* showing almost degenerated and distorted oocytes (arrows) and pycnotic genital epithelium after 16 days treatment with benzidine (X 50) Haematoxylin/Eosin.
Fig. 139b: The same showing distorted oocytes with degenerated ooplasm (arrows) (X 50) Haematoxylin/Eosin.

Fig. 140a: Section of the ovary of sixth stage nymph of *P. pictus* showing degenerated oocytes and degenerated genital epithelium (arrows) after 18 days treatment with benzidine (X 50) Haematoxylin/Eosin.

Fig. 140b: The same showing an enlarged view of the degenerated oocytes with contracted ooplasm (arrows) and pycnotic follicular epithelium cells (X 200) Haematoxylin/Eosin.