Chapter IV.

Discussion
DISCUSSION

The term chemical carcinogens are generally defined to indicate the induction or enhancement of neoplasia by chemicals. It although in the strict etymologic sense, this term means the induction of carcinomas, it is widely used to indicate tumorigeiesis. In other words, it includes not only epithelial malignacies carcinomas, but also mesenchymal malignant tumours (sarcomas) and benign tumours.

Here, in the present investigation the effect of benzidine and 1-nitroso-2-napthol has been studied on the insect tissues in order to see if they have any carcinogenic effect in insect as well.

The insect selected for the present study is Periplaneta americana and various tissues taken are the foregut, the mid gut, the hind gut, the adipose tissue and ovary. The various chemicals tried are benzidine which is an aromatic amine and 1-nitroso-2-napthol which is a nitroso compound, these are known established carcinogens. The carcinogenic effect of benzidine has been shown in rats by Spitz et al. (1950), Vesselinovitch et al. (1975) and Norton et al. (1981). Maltoni and Grett (1964) have reported the formation of tumours in the upper urinary tract of worker in dyestuff factory, exposed to

Several authors have studied the effect of insecticides on mid gut epithelium of different insects Pilate (1935), Woke (1940), Salkeld (1950, 1951), Chadbarne and Rainwater (1953), Soliman and Soliman (1958), Lal et al. (1970) and Ahi (1985). But histopathology of the fore gut and hind gut of insects has been studied by a very few authors Sharma (1966), Lal et al., (1970), Mishra and Mukharjii (1973), Usha Chaturvedi (1991).

As far as the author is aware no literature is available on the histopathological effect on of carcinogens on the fore gut. In the present investigations the effect on the fore gut was: cuticular intima was ruptured and degenerated, epithelial cells showed dense chromatin material in their nuclei, longitudinal and circular muscles were degenerated and torn apart. The folds had become abnormal in shape and were filled with pycnotic nuclei in P. americana treated with benzidine. The epithelium folds were distorted and degenerated and formed clusters when treated with 1-nitroso-2-napthol. The maximum changes were observed from 5 to 40 days of treatment with benzidine and 5 to 20 days of treatment with 1-noitroso-2-napthol.
In confirmation with the present findings, Sharma (1966) reported that maximum changes were observed with dichloruus on the crop than any other part of alimentary canal, and the insecticide caused chromatolysis. Sharma (1966) reported in Poekilocerus pictus treated with nuvian, revealed shrinkage in the foregut of Dysdercus similis treated with DDT and severe poisoning. The muscular layer partially or fully disrupted showing degeneration and vacuolisation of epithelial cells whereas the muscular layer under dichloruus poisoning was swollen and epithelial cells also become enlarged and thickened, blocking partially the lumen of foregut Sharma (1968). In the present study the epithelial cells of the foregut increased in number and folds were filled with additional cells in insect treated with benzdine on the other hand the epithelial cells were degenerated, distorted and folds were contracted in insect treated with 1-nitroso-2-napthol. Lal et al. (1970) reported acute pathology caused by application of diazinon in the foregut of the larvae of Spodoptera litura F. Mishra and Mukherjee (1973) described the histology of foregut of I Cartaniun treated with DDT, diazinon, fenthion, dichloruus and carboryl and reported partial or total disrupted muscular layer around the crop. The cubiodal shape of epithelial cell became altogether straightened out with widening the crop lumen. The muscular layer under dichloruus poisoning became swollen and
the epithelial cell become enlarged, thick and partially blocked
the lumen of the crop. The extra foliation of epithelium either
from muscular layer or intima was also marked and
conspicious. Intima remains unaffected except with fenthion
poisoning when it loses its continuity and is dragged out
alongwith the parts of the cell contents at their breaking points
towards the lumen of the foregut. In the present observation
cuticular intima was ruptured in its continuity and epithelial
cells increased in number and were filled with pycnotic nuclei,
folds became abnormal at some places in benzidine treated
insects and on the other hand epithelium cells with contracted
folds and degenerated cells were filled with diffused chromatin
material in their nuclei. Circular and longitudinal muscles were
degenerated and distorted.

In confirmation present findings, Chaturvedi (1991)
reported the same observation in *P. pictus* in the case of
digestive system after Hempa and Apholate treatment.

Effect of the chemicals on the mid gut has been done by
few workers and the following deserve special attention.

Pilate (1935) reported proliferation of epithelial cells in the
lumen of the mid gut, as mitotically active epithelial cells were
produced as a result of some regenerative process. Soliman and
Sheheta (1963) observed the effect of some insecticides on the mid gut epithelium of *Musca domestica*. Woke (1940) reported chromatolysis in mid gut of southern armyworm when orally poisoned with arsenicals. Salkeld (1951) reported no changes in mid gut when honey bee *Apis mellifera* were treated with parathion. He also reported the distinct histological changes in the mid gut of DDT poisoned, honey bee *Apis mellifera* L. Chadbourn and Rainwater (1953) reported no histological change in the mid gut of larvae of *Haliotthis armigera* with DDT treatment. Sharma (1966) studied nuvian poisoning on *Poekilocerus pictus* F. and reported that the epithelium and their nuclei were badly shrunked and their cytoplasm became granular and cell structure and cell boundary was completely lost. In the present study the circular muscles and longitudinal muscles were distorted and ruptured the epithelial cells increased in number and become bigger in size. Their cells were filled with nuclei showing diffused chromatin material. Some additional growths were formed due to the proliferation of cells with benzidine as well as 1-nitroso-2-napthol treatment.

As the regard of the effect of certain carcinogen on the mid gut, the work of Cantwell *et al.* (1966), Sutherland (1969) and Baewald and Boush (1969) deserves special consideration, Cantwell *et al.* (1966) fed the larvae of housefly with 2-
fluorenamine derivatives and found that the area most affected was the mid gut. Both epithelial lining and regenerative nidi appear hypertrophic and hyper plastic. In the treated larvae the nidi apparently increased and the cells proliferated rapidly and increased size.

Southerland (1969) worked on the nymphs and adult of *Periplaneta americana* by treating them with MAC, FL and DPAA. He found that growth of abnormalities were not specific. Such growths were of wound healing type and growth of mid gut also consisted of exfoliation of the epithelium. Regeneration of the epithelium was controlled by the encapsulating haemocytes and the compounds were carcinogenic.

Here in *Periplaneta americana* similar observations have been made as mentioned by Cantwell *et al.* (1966), Southerland (1969), Bearwald and Boush (1969) and Ahi (1985). The cells of the regenerative nidi proliferated rapidly and increased in size in insects treated with benzidine. The gut epithelium showed hyper activity and additional layer of epithelium was seen with degenerated cytoplasm and nuclei are with diffused chromatin material. The circular muscles were swollen and distorted. The longitudinal muscles were degenerated. The striated border was also distorted after treatment with benzidine and 1-nitroso-2-napthol.
In confirmation with the present findings Ahi (1985), reported that the cells of the regenerative nidi proliferated rapidly and increased in size, numerous additional group of nidi were seen which were with pycnotic nuclei and degenerative cytoplasm. The circular muscles layer were much thicker distorted and swollen at some places. Gut epithelium was filled with numerous pycnotic nuclei. Many additional groups of cells were seen towards the gut lumen and the striated border peritoneal membrane was also distorted in benzidine treated P. pictus.

Sharma (1966) reported the effect of DDT poisoning in the alimentary canal of P. pictus (Fabr.), showed shrinkage and vacuolisation of the muscles and the nuclei of the epithelium. The vacoules were usually big and uniform in size and become unevenly stained and disintegrated. Toppozade et al. (1968) reported contents or oral uptake of carboxyl parathion, DDT and aldrin causing considerable cytopathological changes in the mid gut epithelia of Spodoptera litura, such that there was continuous elongation of epithelial cells having vacuolisation, folding of epithelial boundary, chromatin clumping or lysis shedding of cytoplasm and degeneration of epithelial cells. Gupta and Southerland (1968) reported that with chlordan poisoning in the mid gut epithelium of Periplaneta americana
there was disruption in the formation of the peritrophic membrane and increased vacuolisation and accelerated rate of mitotic and secretary activities in the epithelial cells. Lal et al. (1970) reported that when tobacco caterpillar *Spodoptera litura* was treated with three insecticides, viz. endosulfan, diazinone and dichloruos the mid gut become highly vacuolated and degenerated with endosulphan and dichloruos treatment. Most of the cells of epithelial layer were clumped together and had taken deep stain. The histological appearance of the mid gut of this larvae poisoned with diazonon showed insignificant change in the nuclei. In present study the epithelial cells increased in number due to rapid mitotic activity, nuclei of epithelial cells were pycnotic in benzidine treated *P. americana* while in 1-nitroso-2-napthol treated insects the nuclei were with diffused chromatin material and additional groups of regenerative nidi were found.

Sharma (1966) reported maximum effects of insecticides on the hind gut in comparison to the fore gut and mid gut of *P. pictus*. Lal et al. (1976) reported histopathological degeneration in all the region of hindgut (Pylorus anterior and posterior intestine) poisoned with dichloruos, endosulfan and diazinon. The intensity of damage caused in the hind gut was likely to the fore gut.
In the present study in *Periplaneta americana* the epithelial folds become abnormal in shape and cells were filled with pycnotic and enlarged nuclei, columnar cells as well as cuboidal cells increased in size and formed additional fold of cells. Connective tissue was also degenerated after treatment with benzidine while 1-nitroso-2-napthol treatment showed that epithelial folds were shrunken and degenerated and cells were filled with nuclei showing diffused chromatin material.

In confirmation with the present findings, Chaturvedi (1991) reported in *P. pictus* that the epithelium folds were reduced in size, columnar cells and cuboidal cells were degenerated when treated with Hempa and Apholate.

With reference to toxicants, the adipose tissue has been studied by Chadbourn and Rainwater (1953) they studied the effect of DDT, calcium arsenate and dialdrin on the larval tissue of bollworm. They showed that nuclei of the adipose tissue were clumped into dense masses. Soliman and Soliman (1958) studied that effect of DDT, parathion, tapoxophena on *Proderia litura* and Soliman *et al.* (1971) studied the effect of DDT, malathione and sevin on the larvae of *Drosophila melanogaster* and found shrinkage of the cytoplasm of the fat cells and distortion of the fat body structure.
Here, in the present findings similar observation were made in adipose tissue of *Periplaneta americana* when treated with benzidine and 1-nitroso-2-napthol. The basement membrane of fat cell was obliterated and degenerated. The central globules as well as peripheral globules have lost their normal configuration and are degenerated. The adipose cells show pycnotic nuclei. The cytoplasm is severely vacuolated after treatment with 1-nitroso-2-napthol.

In contrast due to the present finding Ahi (1985) reported the formation of 'brown coloured body' and their subsequent melanization in the adipose tissue of HCH, aldrin and benzidine treated *P. pictus* but in confirmation with the present observations Ahi (1985), also showed pycnotic and fragmented nuclei and vacuolisation of the fat cells by Aldrin, HCH, and endosulfan treatment. However, in the present finding 'brown colour body' and their encapsulation by haemocytes was not observed.

In confirmation with the present finding, Mishra (2005) also reported that in *D. similis* the adipose tissue when treated with dyflubenzuron has lost its architecture, central and peripheral globules had lost their normal configuration and degenerated cytoplasm was severely vacuolated and fragmented chromatin material seen in the nuclei.
As regard the female gonads (ovary) the visible damage to the oocytes due to the effect of benzidine and 1-nitroso-2-napthol treated insects, showed that follicular epithelium in immature oocytes were distorted and degenerated. The immature oocytes were with contracted and degenerated ooplasm and at some places the follicular epithelium migrated inside the ooplasm. In mature oocytes yolk platelet present, prior to the treatment become disintegrated and distorted and corpus luteum was also distorted. In confirmation with present finding Kaur et al. (1986, 87) reported defects concerning differentiation of oocytes in ovary alongwith reduced germarium and previlellarium with few or no oocytes. In benzidine treated insect the follicular epithelium cells of mature oocytes become multi layered and showed pycnotic nuclei while in 1-nitroso-2-napthol treated insects in immature oocytes the follicular epithelial cell were with pycnotic nuclei and some nuclei were with diffused chromatin material, lacking yolky material. Follicular epithelium layer in immature oocytes was obliterated and distorted. In confirmation with the present finding Ahi (1987, 88) also reported that the follicular epithelium migrated inside the ooplasm which appeared as lecitholytic cells and which helps in the resorption of yolk.
Ahi (1992) reported pycnotic follicular epithelium cells which had lost contact among themselves in endosulfan treated *P. pictus* while according to Saxena *et al.* (1998) follicular epithelium cells of Plumbagin treated *Musca domestica* changed their shape from cuboid to columnar in a usual way and yolk was also disputed through in marked vacuolisation cytoplasm was seen in the cell. Thus according to these authors presence of yolk droplets under the follicular lining is a clear indication that yolk depleted deposition continues to such cells.

In confirmation with the present study Mishra (2005) reported in *D. similis* that in the germarium the trophocytes were with pycnotic nuclei in cypermethrin treated insect while in diflubenzuron treated insect the germarium was obliterated and necrotic, yolk platelet become degenerated and distorted. The contracted ooplasm interferes with yolk deposition, probably leads to dysfunction of the ovaries.

As regards the histochemical studies on the mid gut, adipose tissue and ovary by 1-nitroso-2-naphthol treatment in *P. americana*, as far as the author is aware such studies have not been done so far.
Protein

As regard the effect of 1-nitroso-2-napthol, on the mid gut the circular as well as the longitudinal muscles were distorted and less protein positive. The regenerative nidi were found in groups and less protein positive. The columnar cells had increased in number their nuclei were with diffused and pycnotic chromatin material and less protein positive. The striated border was distorted and less protein positive in nature.

The histochemical work on adipose tissue of insect is very scanty. Barlese (1999) has reported the presence of protein granules in the fat body of normal and treated insect.

Wigglesworth (1942, 49) reported Aedes and Drosophila cell, protein granules as protein vacuoles. As regard the adipose tissue of Rhodnius, Wigglesworth (1941) has described that protein was found in a condensed zone around the nucleus with filaments radiating outwards between the fat droplets.

Odhibambo (1947) has not found protein granules in the fat body of the Schistocerca, however, Banerjee (1971) in P. pictus and Bhakthen and Gilbert (1972) in H. cecropia and H. gloveri have not found discard granules of protein in the cytoplasm as well as in the peripheral globules. Aggrawal (1976)
reported protein positive substances are observed in cytoplasm and also in peripheral globules.

As regard the histochemical studies due to the effects of 1-nitroso-2-naphthol on the adipose tissue, the basement membrane of the adipose tissue was obliterated and less protein positive. The cell membrane was obliterated and was less protein positive. The central and peripheral globules lost their configuration and were less protein positive. The architecture of the adipose tissue was lost and nuclei were few in number, pycnotic and less protein positive during 1 to 20 days of treatment.

In confirmation with the present findings Mishra (2005) also reported in *D. similis* after treatment with diflubenzuron that adipose tissue had lost its architecture and very weak protein positive in nature.

In the present investigation, the ovary in 1-nitroso-2-naphthol treated insects showed that the follicular epithelium in immature oocytes were obliterated and distorted and was less protein positive. The immature oocytes were degenerated and distorted in shape. Their ooplasm was contracted and vacuolated which was less protein positive.
In mature oocytes the follicular epithelium and tunica propria was pycnotic and less protein positive. In mature oocytes the ooplasm was highly vacuolated, yolk bodies were contracted and degenerated and weak protein positive. The follicular epithelium cells of mature and immature oocytes were weak in construction and are with diffused pycnotic chromatin material and are very less protein positive in nature.

Morya (1995) and Kharya (2003) reported that the pycnotic nuclei of follicular epithelium and cytoplasmic syncytium showed gradual decrease in protein positive nature whereas in later stage of the corpus luteum formation, the same observation were noticed by Bhide (1978) in *P. pictus*, *P. americana, D. similes, S. rusticum and S. lineapocollis*, Jain (1991) observed gradual decrease in protein positive intensity in corpus luteum of normal as well as short term to long treated insects of *P. pictus* and all stages of corpus luteum formation form newly laid to later stage were observed in *P. pictus, D. similes* by Sahai (1971).

Mishra (2005) reported the typhocytes, in conformation with the present findings oogonia and follicular cells in the germarium were necrotic and were less protein positive in nature in diflubenzuron treated *D. similis*. Mature oocytes were degenerated and distorted their ooplasm was contracted which
was less protein positive. The mature oocytes were with degenerated yolk bodies which were less protein positive. The follicular epithelium in mature and immature oocytes were with diffused chromatin material and less protein positive.

**Carbohydrate**

As regards the study on the mid gut, the circular muscles as well as longitudinal muscles are degenerated and distorted at some places. The circular muscles were swollen and were less PAS positive. The regenerative nidi were in groups and degenerated and were weak PAS positive. The cytoplasm was degenerated and vacuolated and PAS negative. The columnar cells of gut epithelium were bigger in size and less PAS positive. The striated border was distorted and less PAS positive.

As regards the study on adipose tissue the basement membrane of the adipose tissue was distorted at many places and less PAS positive. The cell membrane was degenerated and very weak PAS positive. The cells were accumulated together. The peripheral and central globules were not differentiated and were PAS negative in nature. The cytoplasm was distorted, vacuolated and PAS negative in nature. The nuclei are very few in number and very weak PAS positive nature.
Glycogen has been described in the vacuoles by Wigglesworth (1942) in *Aedes* and in cytoplasm in the fat cells of *Drosophila* by Butterworth *et al.* (1965). Coupland (1967) has described the distribution of glycogen in diffused and granular form in adipose tissue of *Schistocereca* while Odhiambo (1967) had found only granular deposition in the cytoplasm.

Nair and George (1964) have mentioned the presence of glycogen in the peripheral globules and over the cytoplasmic stand around them.

In confirmation with the present finding, Mishra (2005) reported in *D. similis* after dilubenzuron treatment that the basement membrane was distorted and less PAS positive. The central and peripheral globules came close together and were less PAS positive. The nuclei were with fragmented chromatin materials which were less PAS positive. The cytoplasm was obliterated and degenerated and was very less PAS positive.

In the female gonads the immature oocytes were with degenerated, contracted and vacuolated ooplasm and was less PAS positive. The follicular epithelium in immature oocytes was degenerated and less PAS positive. The ooplasm of mature oocytes was highly vacuolated, contracted and yolk bodies showed less PAS positive nature. The follicular epithelium was
pycnotic and very less PAS positive. In confirmation with the present finding Jain (1991) reported in *P. pitus* and *P. americana* that the PAS positive intensity gradually increased in short term to long term treated insect.

In confirmation with the present finding Mishra (2005) reported in *D. similis* by cypermatherin and diflubezuron treatment that the vacuolated ooplasm in mature oocytes was PAS negative. The degenerated yolk bodies were less PAS positive. The follicular epithelium cells of the oocytes in cypermethrine treated were pycnotic and less PAS positive whereas follicular epithelium cells in diflubenzuron treated insect were also pycnotic but PAS positive.

**Lipid**

As regards the study on mid gut, the epithelial layer was distorted in shape. The circular muscles as well as the longitudinal muscles were distorted and ruptured and showed less lipoid nature. The columnar cells increased in number and lost their architecture and showed very less lipoid nature. The cytoplasm is degenerated and vacuolated and showed weak lipoid nature. The nuclei are pycnotic and showed very less lipoid nature.
Lipids in the adipose tissue were observed in form of drops and vacuoles by different authors, Wigglesworth (1942) has shown that in *Aedes* they were minute droplet of the fat chiefly around the nuclei. Later they pass outward in large numbers becoming the most obvious inclusions of the adipose cells. In *Rhodhius* fat droplet of different size were observed throughout the adipose tissue. Wigglesworth (1947) and Coupland (1957) have noted big vacuoles of fat in the cytoplasm of the fat body of *Schistocera*. However, he has also shown patterns of phospholipids in the fat body cell by staining materials. In the present observation same droplets of lipid were seen around the nucleus of fat cells.

Nair and George (1964) were the first to point out that the central globules have neutral lipids and around them are the peripheral globules which perhaps contain certain acidic lipid. They have also noticed diffused staining of fat around the peripheral globules.

Butterworth *et al.* (1965) has not mentioned that peripheral globules in *Drosophila* though he has shown various size of small and big lipid globules.

In the ovary the immature oocytes were with contracted ooplasm and were less lipoid in nature. The mature oocyte were
vacuolated and have three types of lipid bodies L₁, L₂ and L₃ whereas the corpus luteum also showed three type of lipid bodies L₁, L₂ and L₃. Nucleus in corpus luteum showed very less lipoid nature. The follicular epithelial cell was distorted and degenerated and were very weak lipoid in nature. In mature oocytes the tunica propria and follicular epithelium were also distorted and seen without nucleus and were very weak lipoid in nature.

Mishra (2005) reported in *D. similis* after treatment with cypermethrin that the typhocytes and oogonia and pre-follicular cells were in very deformed state which were very less lipoid in nature, contracted ooplasm was very less lipoid in nature. The follicular epithelial cells were less lipoid in nature. In mature oocytes L₁ lipid were more in number than the L₂ lipid bodies. These were lipoid positive. The L₃ lipid bodies could not be seen. This is in conformation with the present findings.

**Enzyme : Alkaline phosphotase**

Appreciable alkaline phosphotase activity is to be found in variety of neoplasm. This activity was initially found to be reflected, in certain cases, in the vera of individuals with neoplastic disease, Franseen and Mclean (1935) and Fishman (1959) reported the first significant study dealing with the
histochemical distribution of enzymes in tumours was reported by Kabat and Furth (1941) who studied number of human tumours including hypernephroma, fibrocircoma, osteogenic cercome and carcinoma of the gastro intestinal tract.

Alkaline phosphatase activity in tumours of central nervous system has been studied by Buttuguer et al. (1957) and Feigin and Wolf (1959). It appeared that an increasing degree of malignancy in meningiouras could be correlated with a decrease in phosphatase activity.

Foraker (1956) found decreased phosphatase activity in breast tumours as compared with normal controls.

Fanger and Barker (1959, 1960) noted a characteristic staining of capillaries and hypoepithelial cells in normal breast tissue as well as in adinosis. In carcinomas the enzyme was absent or else exhibited a source what diffused and patchy distribution of staining. Though no mitotic and melanotic tumours were observed by 1-nitroso-2-napthol treatment with Periplaneta americana, but interestingly the decrease in phosphatase activity in mid gut and adipose tissue of Periplaneta americana could be correlated with the neoplastic studies in mammals.
However, it can be concluded that aromatic amine and nitroso compound, no doubt, cause cellular deformation, irritation and stress and this lead to abnormalities and mitotic division in the regenerative cells of the mid gut.

The present findings in *P. americana* suggested that benzidine and 1-nitroso-2-napthol causes disruption of the adipose tissue which leads to the dysfunction of the intermediary metabolism. As regard the female gonads there is inhibition in the maturation of the oocytes, yolk platelet wherever present are disintegrated. The damage to the follicular epithelium cells interrupted the passage of nutrients, thus affecting the maturating of the oocytes which leads to their resorption. The toxication by these chemicals in *P. americana* cause not only for the degeneration, disintegration and resorption of germ cells but also the decline of protein positive, PAS positive and lipoid positive contents and alkaline phosphatase activity in their intensity in gut, adipose tissue and ovarian tissue.