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University of Udaipur
College of Agriculture
Department of Entomology
Campus Jobner, Rajasthan (India)

V. P. SHARMA

Studies on the mode of action of insecticides

(Orthoptera: Blattidae)

With 37 textfigures

Histological effect of insecticides on the tissues of *Periplaneta americana* (LINNAEUS)

Out of the various branches of Organic insecticides, histopathology has received comparatively little attention. In fact, some work exists on the histopathological effect of insecticides, but most of it centres round the pyrethrum. However, there also exists some scattered work on the histopathological effect of some of the modern organic insecticides. In most of the previous works there is striking lack of information about the dose in relation to the body weight and doses in relation to the histopathology developed.

During the course of present study, author was confronted with the problem of development of identical pathology in various tissues of insects with the application of different insecticides. An effort has been made to study the possible cause and explain the phenomenon of the development of identical pathology; and also to find out some mode of action which may be common to all insecticides.

Material and Methods

Cockroaches were collected from the manholes and other dwelling places and reared in the laboratory. Adult cockroaches of equal weight and size were used for the treatments. Stock solutions of 10 gms/liter of aldrin, endrin, lindane and methoxychlor were prepared in acetone and required concentrations were obtained from this stock solution in the 50 c.c. volumetric flasks. These insecticide solutions were used for treatments and applied through the micro-applicator. The insecticides were administered topically on the pronotum and by feeding through the bread in sub-lethal and lethal doses. The lethal doses of male and female cockroaches were experimentally ascertained and sub-lethal doses were applied arbitrarily. Permanent preparations of tissues were made following the usual technique of paraffin embedding and sections were cut at 5 to 6 u. For staining nervous tissues, Bodian's gold chloride and silver albumose, toluidine blue, methylene blue and haemotoxylin eosin Y stains; and for other tissues haemotoxylin eosin Y, Man's methyl blue and Mallory's triple stains were used. Haemotoxylin eosin Y was found to be the most satisfactory general stain for both nervous and other tissues (HARTZELL 1945 & SHARMA 1965) and was widely

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used in these studies. In all the experiments, all procedures were kept identical and a control and normal experiment was also run for comparative study. The following insecticides were used for the study:

1. Aldrin, technical
   purity
   hexachlorohexahydro-end, exo-dimethanonaphtalene 77.9%
   insecticidally active, related compounds 4.1%
   other related compounds 18%
2. Endrin-active ingredients-endrin (hexachloro-epoxy Octahydro-ends 96% endo-dimethanonaphtalene)
3. Lindane
   purity
   1,2,3,4,5,6-hexachlorocyclohexane, gamma isomer 100%
4. Methoxychlor, technical
   purity
   1,1,1,-trichloro-2,2-bis (p-methoxyphenyl) ethane 89.5%
   Other related isomers of methoxychlor and related compounds 10.5%
   Total chlorine by analysis 30.18%

To study the water loss as a cause of histological degenerations, untreated cockroaches were left in an apparatus (Fig. 1) for 14 hours. Air dried by passing over the cockroaches left them almost dry. The tissues of the untreated air dried cockroaches were subjected to the histological investigations following the usual technique of paraffin embedding.

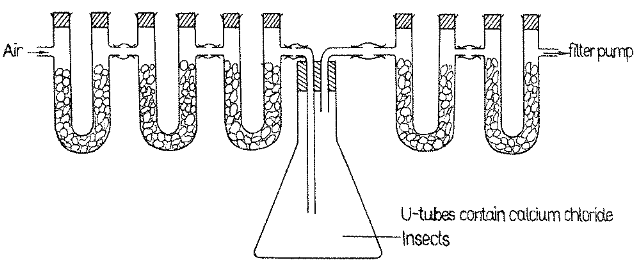

Fig. 1. Apparatus used for the dehydration of Cockroaches

Treatments: Cockroaches were applied with the following doses of insecticides in r/gm

Insecticides	Sub-lethal dose	Lethal dose	
	Female	Female	Male
Aldrin	9.74	24.26	3.63
Endrin	8.03	24.09	—
Lindane	6.30	18.90	2.19
Methoxychlor	16.46	28.08	—

Observations

Histological degenerations observed in various tissues of cockroaches are of the same type and vary only in the intensity.

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Alimentary canal

Oesophagus: The circular muscles of the Oesophagus are not much affected except, that the nuclei are slightly pressed from all sides and longitudinal muscles get detached from each other and lie scattered. The muscles appear vacuolated and the nuclei are thrown to one side by the vacuoles. The epithelial cells have been ruptured at various places, their cytoplasm become granular and a number of vacuoles of varying sizes lie scattered in the cells. The nuclei of the epithelial cells are either thrown to one side or lie extruded. Small air spaces are also observed in certain nuclei due to the dislocation of chromatin granules (Figs. 2 to 4).

Crop: Histological changes are acute in crop than any other part of the alimentary canal. The circular muscles appear contracted, their nuclei in most of the cells are surrounded with oblong air spaces of varying sizes. Longitudinal muscles also lie haphazardly detached from epithelial cells and big vacuoles are seen in the muscles. The nuclei of the muscles are pushed to one side and loose their shape and structure, they appear shrunk and their chromatin granules are thrown towards the periphery leaving the air space in the nuclei. The epithelial cells are remarkably reduced in size (Table 1) and major part of the cells have been occupied by vacuoles (Figs. 5 to 9) of varying size or else the cells appear crushed and their cytoplasm become granular and is not uniformly stained. In certain cells, the cell wall has been ruptured extending the cytoplasm or nuclei or both, and in others major part of the cells have been occupied by the vacuoles pushing the cytoplasm and the nuclei to one side. The nuclei are also pressed (Table 2) become pycnotic, acquire various shapes and loose uniformity in structure. The chromatin granules are pushed to one side leaving the air space. A remarkable feature of this region is the separation of intima from the epithelial cells by a layer which is uniformly stained with eosin (Figs. 5 to 9).

Hepatic Caeca: Vacuoles lie scattered in the epithelium of hepatic Caeca and the nuclei appear distorted and dislocated in the same way as that in the crop.

Midgut: The longitudinal muscles of the midgut develop air spaces which are either found enclosing the nuclei or otherwise lie scattered and the circular muscles also develop the same type of air spaces. The nuclei of the muscles are contracted, pushed to one side, their surface become uneven, the chromatin granules are thrown to one side leaving the air spaces and their shape appear distorted. Acute vacuolation completely destroys the epithelium, cytoplasm become granular and the cell walls are not clearly visible. The nuclei are also contracted, displaced and dislocated, extended, chromatin granules are pushed to one side leaving the air space and becomes distorted (Figs. 11 to 13).

Hindgut: Both the circular and longitudinal muscles of hindgut develop vacuoles and nuclear distortion. The epithelial cells develop vacuoles and nuclear

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distortion. The epithelial cells develop varying sizes of vacuoles, the cytoplasm and the nuclei of these cells are thrown to one side, air spaces are seen in the nuclei and at places major part of the cells have been occupied by the air spaces. There is slight reduction in the size of epithelial cells and the intensity of the pathology is very much reduced compared to the crop. Very slight exfoliation of epithelium takes place in this region (Figs. 15 and 16).

Malpighian tubules: Malpighian tubules are reduced in size (Table 2) and their outer surface become uneven (Figs. 17 and 19). The cells are pressed from all sides into the lumen and the cell walls are not visible. The nuclei become shrunk, pycnotic and at places they are found extruded.

Fat Bodies: The fat cells show varying stages of dissolution and thread like structures are left enclosing the nuclei. The nuclei become contracted, displaced and their surface become uneven (Figs. 18 and 19).

Central Nervous System

Brain: Neurilemma of the brain remains unaffected however, it has been separated at various regions with the endrin treatment. The cortex of the brain is intensely vacuolated causing acute degeneration of the cortical tissue and only thread like structures are left around the clear spaces. The cytoplasm of this region become granular and takes uneven stain. The I, II and III groups of globuli cells are damaged by the displaced and vacuolated nuclei, the chromatin granules are either thrown to one side or lie scattered and the cells collapse and the cell boundary is lost. The neurosecretory cells appear shrunk, unevenly stained, vacuolated, cytoplasm become granular and is pushed towards the nucleus. Their nuclei are also contracted, displaced and the chromatin granules are dislocated. The nuclei present in the cortex and neurospongium are deeply stained, shrunk and their surface becomes uneven. In neurospongium, vacuolation, tigrolysis, cellular degeneration, dissolution and destruction of fibre tracts and degeneration of the nerve tissue has been observed (Figs. 21 to 25). Lesions are found with the application of lindane and methoxychlor (Figs. 24, 25) in thoracic ganglia. Acute histological changes have been observed in thoracic ganglion than in the brain (Figs. 26 to 31). The neurilemma of the thoracic ganglia has been removed with aldrin, endrin and methoxychlor treatments, whereas it remains unaffected with lindane treatment. With aldrin and methoxychlor application, general tissue of the cortical region has been pushed to the peripheries along with their obliterated nuclei and the whole space has been occupied by air leaving only certain neurosecretory cells in the cortex (Figs. 28 and 29). With endrin and lindane treatment, the cortical cells show acute degeneration, intense vacuolation, lysis and the chromatin of the nuclei clump. The neurosecretory cells are damaged in the same way as that of the brain. Neurospongium show acute vacuolation, tigrolysis, cellular degeneration, dissolution of fibre tracts, other cell components and nerve tissue. Lesions have been observed with lindane and methoxychlor treatment (Figs. 30 and 31).

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Abdominal ganglia: Nature of the pathology remains same in abdominal ganglia but for the intensity of histological changes (Figs. 32 to 36). Neurilemma has not been removed with any of the insecticides. The cortex is vacuolated and cortical tissue show moderate to acute cellular degeneration. The nuclei and neurosecretory cells show similar degenerative changes as have been observed in the thoracic ganglia. The nuclei are deeply stained and the chromatins clump. The neurospongium develop acute vacuolation and the fibre tracts, other cell components and nerve tissue show moderate to acute dissolution. Lesions have been observed in patches with the methoxychlor treatment (Fig. 36).

Nerve cord: Neurilemma of the nerve cord becomes wavy and the nerve cells adhering to the neurilemma appears partially dissoluted.

The observations reveal minimum pathology with sub-lethal treatments, intense pathology with those roaches that survive the lethal dose and maximum pathology with the moribund insect's tissues. Further the histological changes are less acute in female cockroaches than in male cockroaches when given equal amount of insecticide. The oral dose of insecticides cause similar pathology, except the intensity which varies. The variation gave a picture of general intense pathology in the alimentary canal, Malpighian tubules and fat bodies and less intense pathology in the nervous tissues (with the same dose of treatment as that of topical application) when compared with topical application.

Pathology of dehydrated cockroaches

Alimentary canal

Foregut: The muscles of the foregut develop vacuoles of varying sizes around the nuclei. The nuclei become shrunk, distorted, the chromatins clump and lie displaced. The epithelial cells become shrunk, vacuolated, the cytoplasm become granular, unevenly stained and at places lie extruded. The nuclei appear shrunk, displaced, deeply stained, the chromatins clump and their surface becomes uneven. Exfoliation of epithelium is very conspicuous and marked. (Table 1 and Fig. 10). Epithelium of hepetic caeca also show vacuolation.

Table 1

Showing comparative shrinkage in the moribund tissues of crop with different treatments

Tissues	Control	Aldrin	Endrin	Lindane	Methoxychlor	Dehydration
Nucleus of muscles						
Length	11.9 ± .29	8.3 ± .59	8.4 ± .78	8.5 ± .39	8.0 ± .65	9.85 ± .47
Breadth	5.7 ± .31	3.0 ± .27	4.4 ± .25	3.0 ± .27	2.9 ± .43	4.5 ± .60
Epithelial cells						
Length	40.2 ±1.77	12.7 ±1.05	13.0 ± .98	14.5 ± .48	10.9 ±1.63	18.0 ± .65
Breadth	16.1 ±1.38	9.7 ±0.67	10.0 ± .93	11.5 ± .39	8.2 ±1.3	12.7 ± .79
Nucleus of epithelium	9.7 ± .41	5.0 ±0.35	5.0 ± .31	5.2 ± .37	4.7 ± .63	5.6 ± .36
Exfoliation	–	3.4 ±0.24	2.4 ± .25	2.62 ± .17	2.9 ± .68	5.78 ± .42

All sizes are in u

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Midgut: The muscles of the midgut do not show apparent degenerations. The epithelial cells are vacuolated, cytoplasm become granular, the nuclei are displaced and the chromatins show breakdown (Fig. 14).
Hindgut: There are no appreciable histological changes in the muscles of the hindgut. The epithelial cells are slightly shrunk, the nuclei are displaced and become pycnotic.
Malpighian 'ubules: The Malpighian tubules are shrunk, the lumen is pressed from all sides, surface become uneven, and the cell walls are not visible. The nuciei are deeply stained, at places lie extruded and become pycnotic (Table 2 Fig. 20).
Fat bodies: Fat cells show moderate to acute dissolution (Fig. 20).

Table 2

Showing comparative shrinkage in moribund Malpighian tubules with different treatments

Tissues	Control	Aldrin	Endrin	Lindane	Methoxychlor	Dehydration
Size of nucleus	9.32 ± .91	8.15 ± .74	7.96 ± .36	8.2 ± .89	5.82 ± .77	4.8 ± .59
Size of lumen	23.39 ± 1.1	7.78 ± .83	6.13 ± .54	6.9 ± .5	5.61 ± .69	10.2 ± 1.35
Size of cells	8.15 ± .73	6.91 ± .18	7.1 ± .47	6.17 ± .4	5.72 ± .65	7.6 ± .95
Size of tubules in T.S.	46.97 ± 1.98	33.26 ± 1.2	24.5 ± 1.9	23.0 ± 1.35	20.79 × 1.27	35.98 ± 1.79

All sizes are in u

Central Nervous System

Nature of the histological changes are of the same type in brain, throracic and abdominal ganglia. Intensity of pathology is less in the brain and it is maximum in the thoracic and abdominal ganglia. There is no effect on the neurilemma. The cortical cells are vacuolated, show acute cellular degeneration and presence of clear spaces. The nuclei are shrunk, their chromatins clump and migrate to the periphery leaving the vacuoles. The neurosecretory cells are also shrunk, vacuolated, the cytoplasm become ragged, disintegrated and vacuolated. Neurospongium show vacuolation, uneven staining, partial degeneration of the nerve fibre, fibre tracts, nerve tissue and the nuclei. Lesions are absent (Fig. 37).

Discussion

KRÜGER (1931) observed with pyrethrum treated living transparent *Corethra* larvae, the appearance of vacuoles in the ganglia and connectives of the nerve cord within 10 to 20 minutes, after the onset of convulsions. HARTZELL & WILCOXON (1932a) found that in case of pyrethrum intoxication, death may ensue from the external application of pyrethrum concentrates, under conditions

Abbreviation

CM – Circular muscles; *COR* – Cortex; *CS* – Clear spaces; *DIS* – Dissolution; *EP* – Epithelium; *EX* – Exfoliation; *INT* – Intima; *LU* – Lumen; *LM* – Longitudinal muscles; *LES* – Lesions; *M* – Muscles; *NU* – Nucleus; *NC* – Neurosecretory cells; *NEL* – Neurilemma; *NER* – Neurospongium; *VAC* – Vacuoles.

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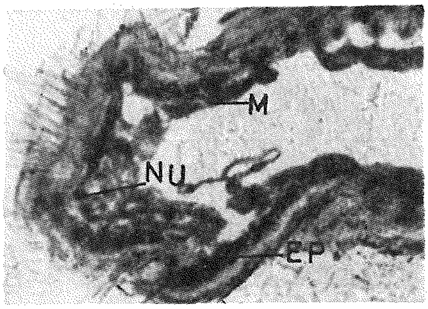

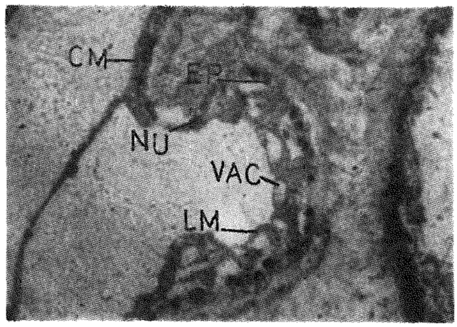

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Fig. 2. Control. Oesophagus showing muscles, epithelium and their nuclei

Fig. 3. Aldrin treatment. Oesophagus showing vacuolation in muscles and epithelium, degeneration of epithelium and distortion of nuclei

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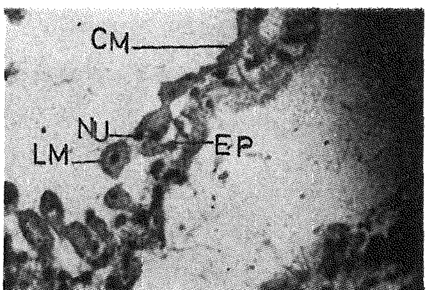

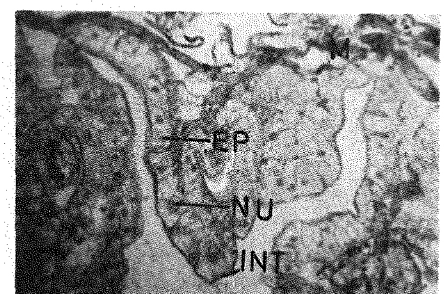

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Fig. 4. Lindane treatment. Oesophagus showing vacuolation in muscles, degeneration of epithelium and distortion of nuclei

Fig. 5. Control. Crop showing muscles, epithelium and their nuclei; and intima (Intima not exfoliated)

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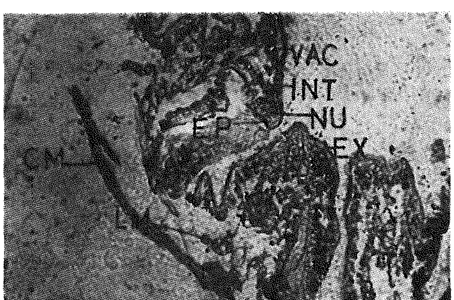

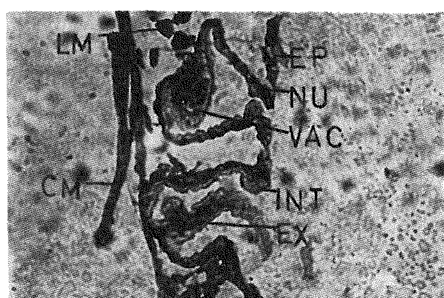

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Fig. 6. Aldrin treatment. Crop showing vacuolation in muscles and epithelium, degeneration of nuclei, rupture and contration of epithelium, degeneration of cytoplasm and exfoliation of epithelium

Fig. 7. Endrin treatment. Crop showing vacuolation in muscles; acute shrinkage, vacuolation and rupture of epithelial cells, extrusion of cytoplasm and nuclei, distortion of nuclei and exfoliation of epithelium

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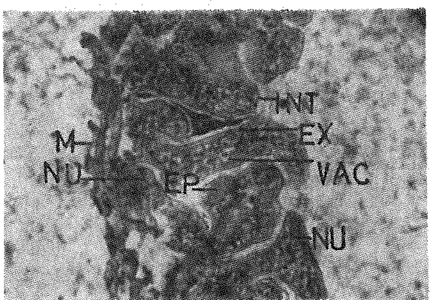

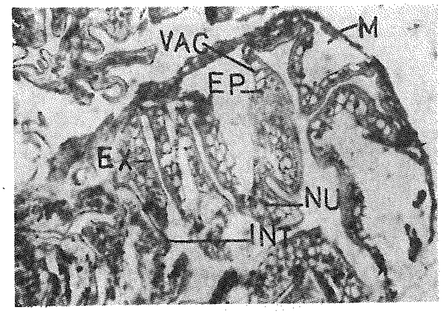

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Fig. 8. Lindane treatment. Crop treated with lethal dose (moribund) showing vacuolation in muscles and epithelium, disintegration of cytoplasm, distortion of nuclei and contraction and exfoliation of epithelium

Fig. 9. Lindane treatment. Crop treated with lethal dose (Survived insects) showing less acute histological changes than Fig. 8

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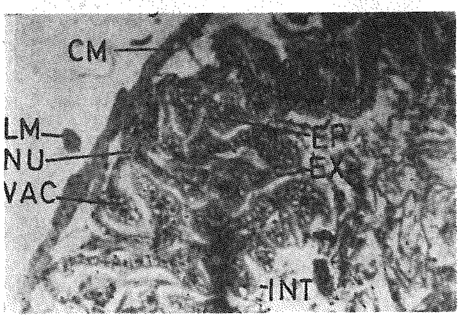

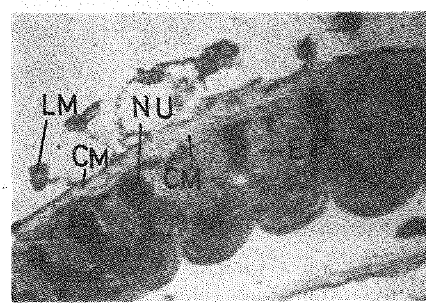

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Fig. 10. Dehydration. Crop showing vacuolation contraction and exfoliation of epithelium; and nuclei become pycnotic
Fig. 11. Control. Midgut showing muscles, epithelium and their nuclei

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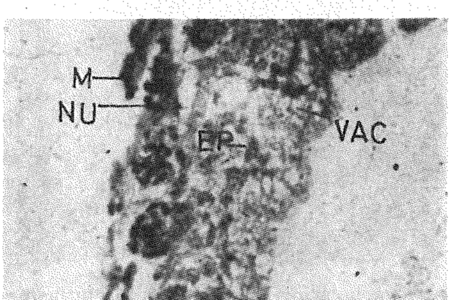

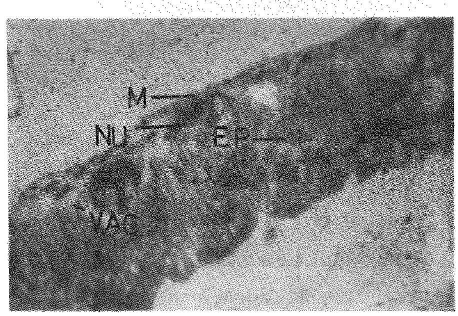

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Fig. 12. Aldrin treatment. Midgut showing vacuolation in muscles and epithelium, dislocation and distortion of nuclei and disintegration of cytoplasm

Fig. 13. Endrin treatment. Midgut showing vacuolation in epithelium and dislocation and distortion of nuclei

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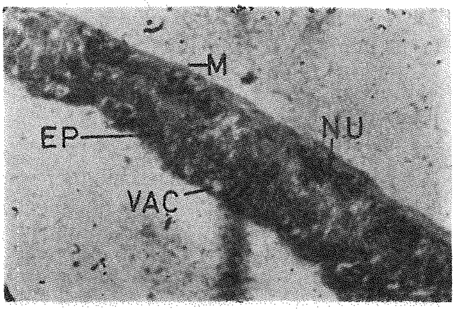
14 

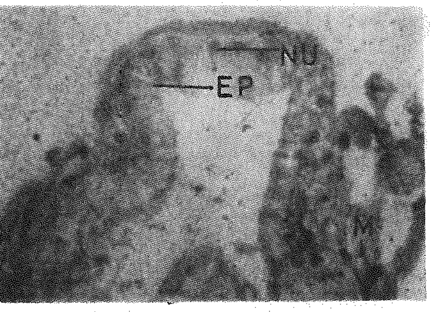
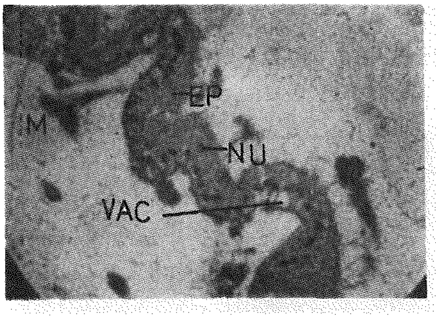
 15

Fig. 14. Dehydration. Midgut showing vacuolation, contraction and cytoplasmic degeneration in epithelium; and distortion of nuclei

Fig. 15. Control. Hindgut showing muscles, epithelium and their nuclei; and intima (Intima not exfoliated)

16 

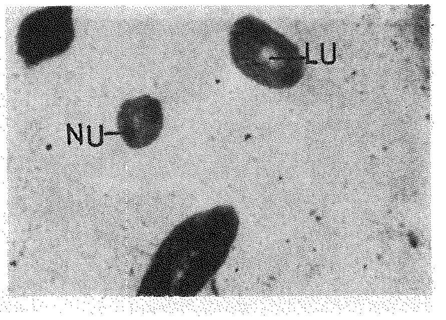
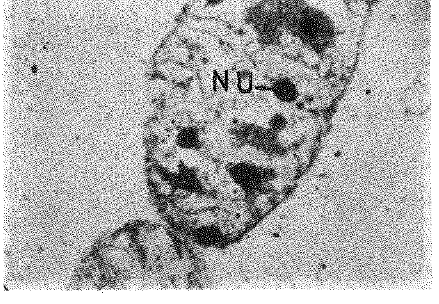
 17

Fig. 16. Endrin treatment. Hindgut showing vacuolation in epithelium and dislocation and distortion of nuclei. (Exfoliated intima)

Fig. 17. Control. Malpighian tubules showing lumen and nuclei

18 

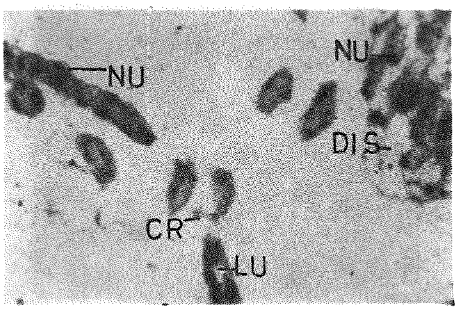
 19

Fig. 18. Control. Fat bodies showing fat cells and their nuclei

Fig. 19. Aldrin treatment. Malpighian tubules showing contraction of lumen. The surface of tubules become uneven. Section of fat bodies showing dissolution of fat cells and deep staining of nuclei

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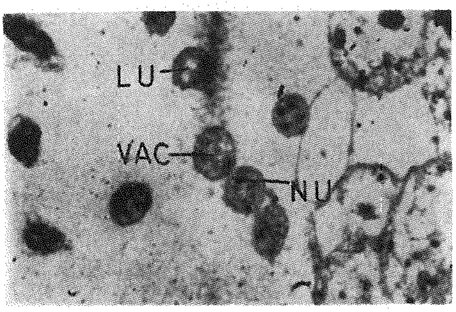

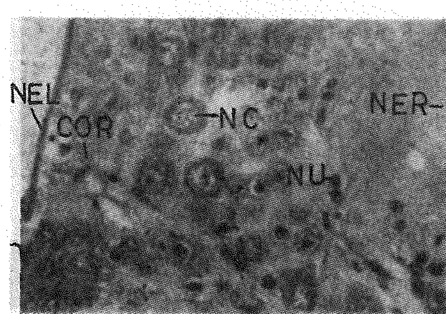

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Fig. 20. Dehydration. Malpighian tubules showing reduced lumen and their nuclei. Fat bodies showing dissolution of fat cells and nuclei are enclosed by clear spaces

Fig. 21. Control. Brain showing neurilemma, cortex, neurosecretory cells, nuclei and neurospongium

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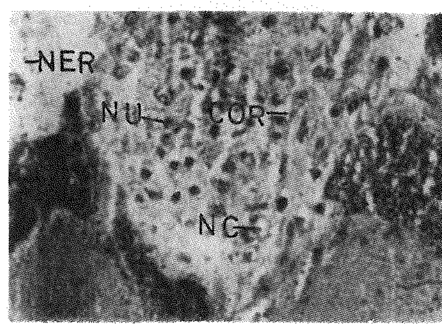

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Fig. 22. Control. Brain showing neurospongium and certain nuclei

Fig. 23. Aldrin treatment. Brain showing vacuolation of cortex, contraction of neurosecretory cells, the nuclei appear shrunk and deeply stained

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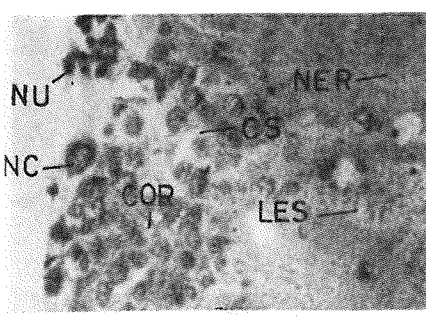

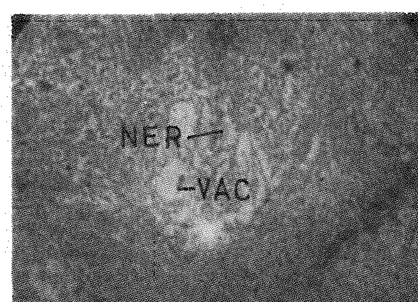

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Fig. 24. Lindane treatment. Brain showing vacuolation of Cortex, contraction of neurosecretory cells and nuclei, presence of clear spaces, acute vacuolation in cortex and lesions

Fig. 25. Methoxychlor treatment. Brain showing acute vacuolation and lysis of neurospongium

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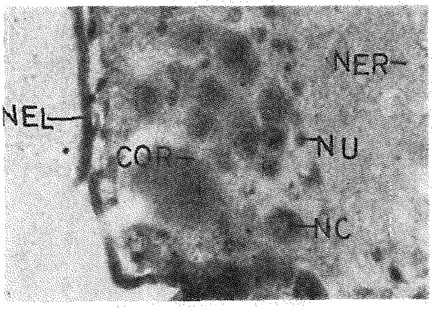

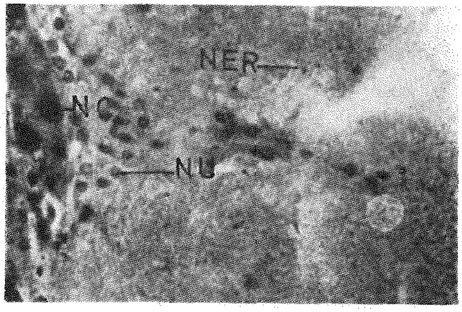

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Fig. 26. Control. Thoracic ganglion showing neurilemma, cortex, neurosecretory cells, nuclei and neurospongium

Fig. 27. Control. Thoracic ganglion showing neurosecretory cells, nuclei and neurospongium

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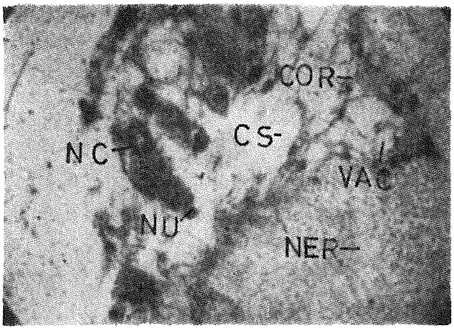

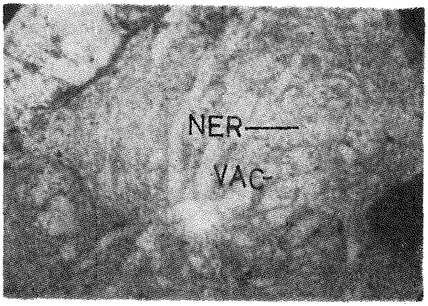

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Fig. 28. Aldrin treatment. Thoracic ganglion showing disappearance of neurilemma, presence of clear spaces, acute degeneration and lysis in cortex, contraction and vacuolation of neurosecretory cells, deep staining and dislocation of nuclei, and vacuolation in neurospongium

Fig. 29. Aldrin treatment. Thoracic ganglion showing degeneration and acute vacuolation of neurospongium

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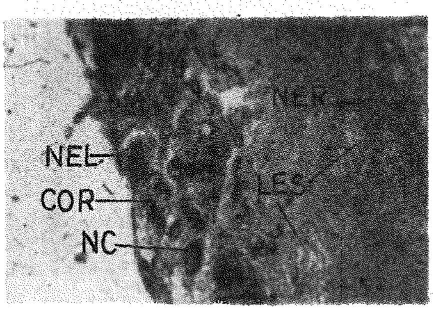

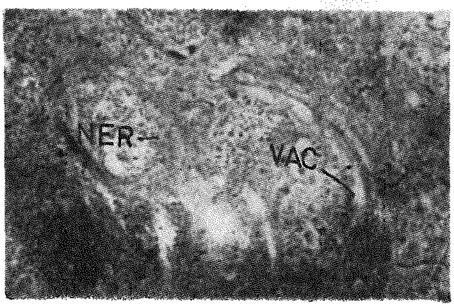

31

Fig. 30. Lindane treatment. Thoracic ganglion showing contraction of neurosecretory cells and nuclei; vacuolation in neurospongium and presence of lesions

Fig. 31. Lindane treatment. Thoracic ganglion showing acute vacuolation in neurospongium

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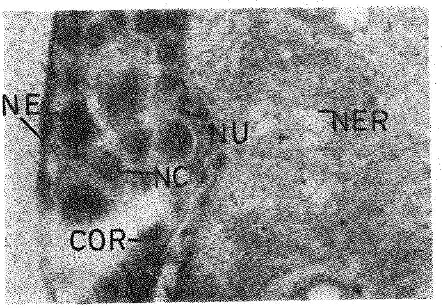
32 

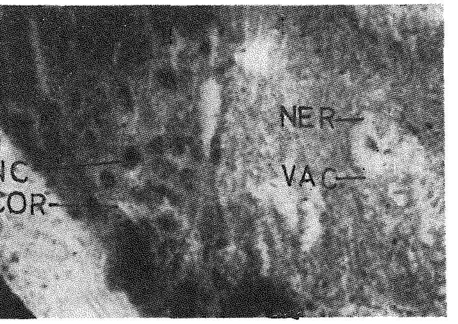
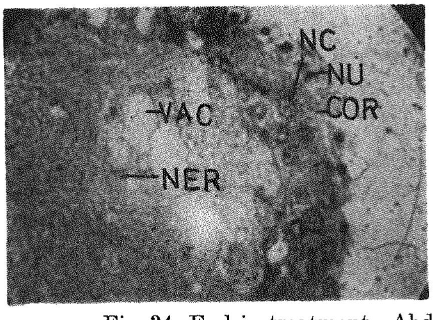
 33

Fig. 32. Control. Abdominal ganglion showing neurilemma, cortex, neurosecretory cells, nuclei and neurospongium

Fig. 33. Aldrin treatment. Abdominal ganglion showing disappearance of neurilemma, contraction of neurosecretory cells, and vacuolation of cortex and neurospongium

34 

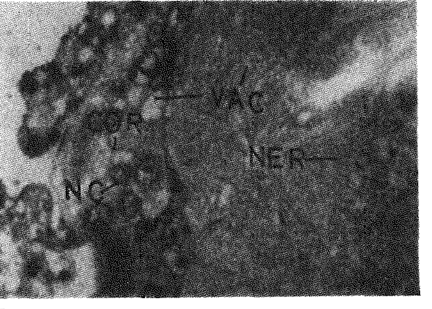
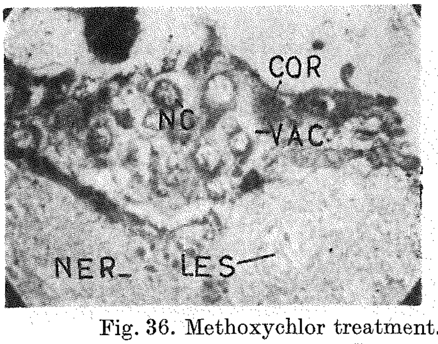
 35

Fig. 34. Endrin treatment. Abdominal ganglion showing acute vacuolation and lysis in cortex; contraction and vacuolation of nucleiand the neurosecretory cells, and vacuolation in neurospongium

Fig. 35. Lindane treatment. Abdominal ganglion showing vacuolation in cortex and neurospongium, contraction of neurosecretory cells and distortion of nuclei

36 

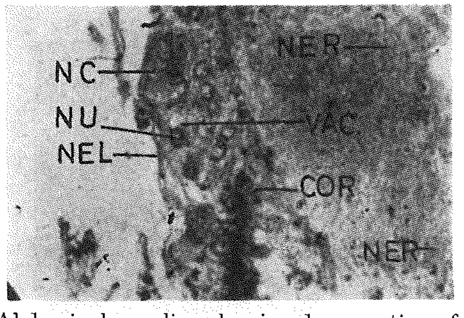
 37

Fig. 36. Methoxychlor treatment. Abdominal ganglion showing degeneration of cortex, neurosecretory cells, acute vacuolation of neurospongium and presence of lesions

Fig. 37. Dehydration. Abdominal ganglion showing vacuolation in cortex and neurospongium

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where not tracheal penetration takes place. Wilcoxon & Hartzell (1933) found that pyrethrum extracts entered the body of the insect through the trichogen or hair cells in the integument and thence reached the nerve endings. Hartzell et. al. (1934a) has observed that three organic thiocyno containing insecticides are now known to produce nerve lesions, namely Lethane 384, Thanite, and gamma thiocyanopropylphenyl ether. Hartzell et. al. (1934b) reported nerve lesions throughout the main part of the central nervous system of the *Tenebrio molitor* larvae and adult grasshopper *Melanoplus femur — rubrum* that had been killed by pyrethrum extracts applied externally. Klinger (1935) made a histological study of the nerve lesions of the *Porthetria dispar* larvae after application of 24 hours of 15% pyrethrum extract. He reported that nerve appeared to be isolated and surrounded by spaces as contrasted with the check where the tissue was not dislocated. A similar histological change was reported from moribund insects. Richards & Cutkomp (1945) using a variety of insecticides found that nerves were paralyzed and presumably dead prior to appearance of any abnormalities or lesions, except the chromatin clumping. They also observed that in dead nerve tissue the neurones become granular internally. Srivastava (1951) concluded that the application of the gamma isomer on the meso-sternum of German cockroach caused the changes in hypodermal cells, fat cells and metathoracic ganglion. Chadbourne & Rainwater (1953) were unable to detect any histopathological change in the nervous system of *Heliothis armigera* under the effect of several insecticides, though changes in other tissues were observed (midgut epithelium) muscles, fat and Malpighian tubules) Luers, Kopf & Luers (1955) reported with BHC poisoning lack of stainability of the cell fibres in a number of greatest somatic cells and strong staining with appearance of folds in a number of small somatic cells and several karyochromatic cells. Lower (1961) observed the pathology of starvation in larvae of Army Worm (*Persectania ewingii*). He concluded that the larvae of Army Worm are ill adapted to withstand starvation. Without food, young larvae soon died, older larvae may either die or pupate and produce defective moths after a greatly prolonged period of development. Certain structural and chemical changes are common to all larvae undergoing starvation. The midgut epithelium degenerates, the hypodermis shrinks and retracts irregularly from both the cuticle and the basement membrane, while uric acid crystal accumulate in it; secondary pigments are deposited in the cuticle and in other internal tissues, the proportion of the stored fat rapidly diminishes, the nitrogen content significantly rises. A relatively constant proportion of water is maintained, which differs little from that of the feeding larvae. Even a short period of starvation may bring about a permanent derangement of the metabolism, so that if larvae subsequently resume feeding they are either capable of limited or abnormal growth only, or, if they complete their development, the resultant moths are sexually defective.

Although a good amount of work has been done on the induced histopathology, both in invertebrates and vertebrates, yet the exact mode of action of

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certain organic insecticides at the disposal of man remains obscure. During the course of present study, author was confronted with the problem of development of identical pathology in various tissues of insects with the application of different groups of insecticides (SHARMA 1965, 1966). An effort has been made to study the possible cause and explain the phenomenon of the development of identical pathology; and also to find out some mode of action which may be common to all the insecticides.

There was no difficulty in making comparison with sub-lethal treatments where the insect could tolerate the toxic dose. However, care was taken to study the histological degenerations of moribund than dead insects, and in all cases moribund insects tissues were examined in order to avoid any post-mortem changes owing to death. It may be recalled that the term moribund has been used to indicate a condition of complete inability of insects to move and this condition is preceded by death.

Pathological picture produced by the action of insecticides on the tissues of insects has been reported by a number of workers and an exhaustive work on the induced histological changes has been contributed by HARTZELL (1945). Appreciable histological changes caused by lindane on insects have been reported by SRIVASTAVA (1951), LUERS, KOPF & LUERS (1955) and others and there are no accounts available on the histological effect of aldrin, endrin and methoxychlor.

There is striking lack of literature on the histological effect of the Organochlorine compounds on the alimentary canal, Malpighian tubules and fat bodies of insects. PILAT (1935) has reported exfoliation of epithelium, vacuolation, deeper staining, disintegration of cytoplasm and many other degenerations with intestinal poisoning. Pilat held the view that owing probably to a change in osmotic conditions the epithelium begins to exfoliate from the subjacent connective membrane in large sheets, but retains at least in beginning its typical morphological character. Histological degenerations are of the same magnitude as have been observed during the present investigations and reported earlier by SHARMA 1965, 1966. WOKE (1940) carried out studies in the larvae of Southern Army Worm *Prodenia eridania* (CRAMER) after the administration of stomach poisons principally arsenicals or arsenic derivatives. He observed with calcium arsenate poisoning, desorganization of the midgut, with the epithelial layer appearing as a rather solid mass, occasionally presenting fragments recognized as cell structures. He further observed that the different insecticides varied widely in type of degree of action, ranging from no apparent histological effect to the well marked damage to the midgut walls.

SALKELD (1951) carried out histological studies of DDT on the midguts of honey bees, *Apis mellifera* LINNAEUS. He observed that the epithelial cells are vacuolated, elongated, straited borders are disrupted, the cytoplasm become granular, vacuolated and unevenly stained. CHADBOURNE & RAINWATER (1953) reported histological changes in the midgut epithelium, fat cells and Malpighian tubules of *Heliothis armigera* larvae poisoned with DDT and dieldrin. The present

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findings reveal acute histological degenerations in the alimentary canal, Malpighian tubules and fat bodies of cockroaches treated with aldrin, endrin and methoxychlor.

Chadbourne & Rainwater (1953) reported the degeneration of cell nuclei of the Malpighian tubules of *Heliothis armigera* larvae with dieldrin poisoning. Soliman & Soliman (1958) and Sharma (1965 and 1966) reported degenerative changes in the Malpighian tubules poisoned with a number of insecticides. Author observed the effect of these insecticides on the Malpighian tubules and found varying degrees of degenerations in Malpighian tubules (Figs. 17 and 19). A statistical analysis on Table II gave a picture of the intensity of pathology.

Dissolution of fat bodies under the effect of dieldrin has been reported by Chadbourne & Rainwater (1953) and Sharma (1965) with a number of Organochlorine compounds. Such dissolution of fat cells has been observed in Cockroaches during the course of present studies, however, the intensity of dissolution varies greatly (Figs. 18 to 20).

The observations show that the variations in the intensity of histological changes depends on the sex, mode of application, dose of insecticides and other factors. The histological changes reported in the text are of the same generalized nature and are in agreement with the earlier investigations on the histopathology of alimentary canal.

There is a striking lack of literature on the neuropathological effect of cyclodiene insecticides on insects. Chadbourne & Rainwater (1953) were unable to detect any neuropathological change under the effect of aldrin in nervous system of *Heliothis armigera.* Acute histological degenerations throughout the central nervous system have been reported by Sharma (1965) with dieldrin poisoning in *Poecilocerus pictus* Fabricius. During the present findings, author found that aldrin and endrin have been causing acute vacuolation throughout the central nervous system along with other histological degenerations (Figs. 23, 28, 29, 33, 34) however, lesions were not observed with the aldrin and endrin treatment which were reported by Sharma (1965) with dieldrin treatment.

A good amount of work has been done on the histological effect of lindane. Author's findings are in agreement with the earlier workers however, it is interesting to note that lesions were present in brain and thoracic ganglia which were not reported with the lindane treatment (Figs. 24 and 30).

There are conflicting reports on the histological effect of DDT and Sharma (1966) reported definite histological degenerations with DDT poisoning in Red Cotton bugs. There are no accounts available on the neuropathological effect of methoxychlor. It was found that methoxychlor has been causing acute neuropathological degenerations in the brain, thoracic ganglia and abdominal ganglia as have been reported by Sharma (1966) with DDT poisoning in Red Cotton bugs. However, lesions have been observed in brain and thoracic ganglia which were absent with DDT poisoning as reported by Sharma (1966) (Fig. 36).

The observations reveal that all the insecticides tested in the present findings and reported by Sharma (1965, 1966) gave almost identical pathology leaving

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only the effect on chromatins and the formation of lesions. The variation in histological degenerations have been observed only in the intensity of the induced pathology in all the tested tissues and it is difficult to say, what particular poison has effected the tissue. The question naturally arises as to what can be the possible cause of identical pathology in all the tissues (SHARMA 1965, 1966, and present findings) when such a varied nature of insecticides were selected for the study?

BERNHEIM (1948) held the view that the establishment of a single basic mode of action of certain poisons may be impossible, since life processes are so numerous and interdependent; for the pathologist precision is attained with visible changes in certain cells and tissues; for the physiologist, the impairment of function in certain life processes; and for the bio-chemist with the inhibition of certain life processes and individual enzymes.

Consequently, studies more advanced to know of some common effect of insecticides, since the pathology observed with various groups of insecticides was identical. It was found that water loss takes place in insects treated with insecticides (CHATTORAJ & SHARMA 1964, SHARMA & CHATTORAJ 1964). Earlier such studies were carried out by WIGGLESWORTH (1941) and INGRAM (1955).

Histophysical mode of action of insecticides

The water loss due to the insecticidal action is common to a variety of insecticides and this led the present investigator to think, that this loss of water might be causing histological degenerations observed during the present studies and reported by HARTZELL (1945), SHARMA (1965, 1966) and other investigators.

In order to find out the histological evidence to support the above idea, untreated cockroaches were run in dry air for fourteen hours in an apparatus (Fig. 1) and then the tissues were examined histologically. It was found that the loss of water from the body caused histological degenerations which are similar to that caused by the insecticides (Figs. 10, 14, 20 and 37). This suggests that somehow or other, insecticides by their action might be allowing the withdrawal of water from the tissues and this act of loosing the water caused histological changes. LOWER (1961) carried out such studies with starved Army Worm larvae *Prodenia eridania* and observed that midgut epithelium shrinks and retracts irregularly from both cuticle and basement membrane and the proportion of body fat rapidly diminishes. During the present studies, effect of starvation has been eliminated since death occurs in acute cases of poisonings within a short time and yet the pathology develops. The work of LOWER (1961) suggests that due to the starvation a vacuum is developed inside the body; cells start loosing turgidity and in turn tissues starts collapsing and the pathology develops.

It is well recognized that insects loose water with the change in their morphological and, or physiological state (MELLANBY 1935) and this change is also attained with the insecticide treatment (WIGGLESWORTH 1941), INGRAM 1955,

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Chattoraj & Sharma 1964, Sharma & Chattoraj 1964). During the loss of water from the body, there is a continuous outflow of water from within of the insects i.e., from all the effected tissues. Naturally, this outflow of water is likely to set the internal tissues of insects at a different morphological, physiological and bio-chemical state thereby changing their structural appearance.

It has been hypothesized that, probably due to the action of insecticides there is developed a physical force inside the body which allows the water to withdraw from the tissues. Further this withdrawal of water can be the direct withdrawal of water from the tissues or it can reverse or check the usual process of osmosis and absorption. Consequently, this withdrawal of water creates a pressure or pull on the tissues and the cells start contraction. The shrinkage dislocates and disturbs the cell contents and allows the air to occupy the place and cause nuclear distortions and cytoplasmic degenerations.

If ones the shrinkage has been effected, recovery is not possible as long as the process is not reversed. Bursting of cells causing extrusion also suggests some force on the cells which allows them to rupture. Due to the loss of water in tissues homogeneous distribution and even staining of cytoplasm is lost. Further, the more is pressure on cells, more is the pressure on nucleus and the nuclei give distorted and pycnotic appearance.

The main function of crop is storage and the epithelial cells of crop are provided with a layer of intima which is thick and chitinous in nature. When the cells starts contraction and loose water, the thick chitin fails to adhere with the contracting cells and, therefore, it must separate. This causes exfoliation of epithelium, cell contents of the epithelium come upto cover up the space created by the separating intima and lie in between the intima and the epithelium (the cell contents probably do not pass through the chitin as the nature of chitin is hard). Further the nature of newly formed layer is uniform and structureless suggesting some fluid deposits. There is no exfoliation of epithelium in midgut because of the absence of chitinous intima. In hind gut the intima is comparatively thin and allows exchange of some fluids and show slight exfoliation, possibly because it is least affected with the insecticides.

When fat cells loose water, air spaces are formed enclosing the nuclei and the cytoplasm appear degenerated and lie as thread like structures. Such dissolution of fat bodies has been observed by Lower (1961) with starvation, Chadbourne & Rainwater (1953) with dieldrin and Sharma (1965) with a number of organio chlorine compounds and in present findings with dehydration and insecticidal poisoning. Since water loss is common in the insecticide treated insects and dehydrated insects, it is plausible to assume that water loss might be causing degenerations of the fat cells.

Nervous tissues of all the affected insects (Sharma 1965, 1966 and present findings) also show varying degrees of vacuolation. This can also be possibly explained on the basis that due to the loss of water, air comes to cover up the space and in turn cause vacuolation. Further the formation of air space or vacuoles should also cause some pathology.

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Moreover, with the application of insecticides hyperactivity sets in, in the body, its appendages and internal tissues. To cope with this developed hyperactivity, insects need more energy and as such they appear exhausted within a short time. Further the body hyperactivity can also cause or enhance certain damages in the tissues.

However, the above explanation regarding the identical pathology does not rule out the possibility of direct chemical actions and reactions going on inside the body which also disrupts the normal physiology and behaviour; and thereby are also responsible for bringing about pathology and death. The above hypothetical explanation of histophysical force is one possible way of identical pathology observed with various insecticides.

Conclusions

1. Histological changes have been observed throughout the alimentary canal of the cockroaches with the effect of aldrin, endrin, lindane and methoxychlor. However, maximum pathology has been observed in crop than in any other region of the alimentary canal.

2. Malpighian tubules and fat bodies also show varying stages of degenerations and dissolutions with the effect of these organochlorine compounds.

3. Neuropathological changes have been observed throughout the central nervous system and the variations have been observed in the intensity of the induced pathology. Neurilemma has been separated from the thoracic and abdominal ganglia with the aldrin treatment and it has been separated from the cortex in thoracic ganglia with endrin and methoxychlor treatment. Neurilemma remains unaffected with lindane treatment. Nuclei, neurosecretory cells and globuli cells are damaged in the same way with all the insecticides. No lesions were observed with aldrin and endrin treatment, instead acute vacuolation and lysis has been observed in cortex and neurospongium. Lindane causes lesions in the brain, thoracic ganglia with other histological degenerations. Lesions are observed in brain, thoracic ganglia and abdominal ganglia with the treatment of methoxychlor along with sub-acute to moderate vacuolation and other degenerations.

4. Minimum pathology has been deserved with sub-lethal treatments, intense pathology in individuals that survive the lethal dose and maximum pathology with moribund insect's tissues. Further the observations reveal that, histological changes are less acute in female insect's than the male insect's tissues when given equal amount of insecticides.

5. Tissues examined after the oral application of insecticides of male and female cockroaches show the same nature of pathology however, there is variation in the intensity of the pathology. This variation gave a picture of general intense pathology in the alimentary canal and fat bodies and less intense pathology in the nervous tissues, (with the equal dose of insecticide) when compared with the topical application.

6. The present findings reveals that practically all the insecticides tested produced identical pathology in insects leaving only the minor variations. Histological changes reported in these findings are of the same pattern as reported by earlier workers with various insecticides and author is in agreement with the views of PILAT (1935) that the histological picture of the intestinal tube of the insects is characterized by identical features and does not give itself any identification as to what insecticide has been used for poisoning; further the final effect of the usually employed insecticides shows itself in the destruction and disintegration of epithelium.

7. It has been hypothesized that probably, due to the action of insecticides there is developed a physical force inside the body which allows the water to withdraw from the tissues.

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Consequently, this withdrawal of water causes shrinkage, nuclear distortions and cytoplasmic degenerations in the tissues which are observed under the microscope as histological degenerations.

Summary

Application of aldrin, endrin, lindane and methoxychlor induces pathology in the alimentary canal, Malpighian tubules, fat bodies and central nervous system of the cockroach *Periplaneta americana* LINNAEUS. The pathology is characterized by the development of identical features and does not indicate which insecticide has been used. In view of the development of identical pathology caused by the action of insecticides and the loss of water from the body of the cockroaches, it is supposed that the insecticides by their action cause the withdrawal of water from the tissues and this withdrawal of water in turn induces pathology.

Zusammenfassung

Die Anwendung von Aldrin, Endrin, Lindan und Methoxychlor ruft bei der Schabe *Periplaneta americana* LINNAEUS Krankheitserscheinungen im Magen-Darm-Kanal, den Malpighischen Gefäßen, den Fettkörpern und dem Zentralnervensystem hervor. Die Krankheitserscheinungen sind dadurch gekennzeichnet, daß sie gleichartig verlaufen und nicht erkennen lassen, welches Insektizid benutzt wurde. Angesichts der Gleichartigkeit der durch die Insektizide bewirkten Krankheitserscheinungen und des Wasserverlustes im Körper der Schaben wird angenommen, daß durch die Wirkung der Insektizide den Geweben Wasser entzogen wird und dieser Wasserentzug wiederum die Krankheitserscheinungen hervorruft.

Резюме

Применение Алдрина, Ендрина, Линдана и Метоксихлора вызивает у *Periplaneta americana* LINNAEUS болезненные явления в пищеварительной системе, в малпигиевых сосудах и в центральной нервной системе. Явления характеризированны тем, что они вместе возникают и не позволяют узнать, какой инсектицид был применён. В виду одинаковости этих болезненных явлений и потерии воды в теле тараканов устанавливается, что под влиянием инсектицидов из тканей удаляется вода и поэтому возникают эти болезненные явления.

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