CHAPTER IV

HISTOPATHOLOGICAL STUDIES ON THE CUTICLE OF P. PICTUS
HISTOLOGY OF CUTICLE OF P. PICTUS

A. OBSERVATIONS

The cuticle is a secretion of the epidermis and forms the outer side covering of the body of an insect as well as lining of the ectodermal invagenations such as the stomodaenum, proctodaenum and the trachae. The cuticle can be differentiated into two main regions. 1. An inner region, which contains chitin and forms the bulk of the cuticle, and 2. The thin outer epicuticle.

The chitinous cuticle as it is first secreted is known as procuticle, but subsequently the outer part often becomes tanned or sclerotised to form exocuticle while the inner undifferentiated part is called endocuticle. Between these two layers there may be a region of hardened, but not fully darkened cuticle which is fully sclerotised cuticle, does not stain readily and such a layer is called mesocuticle.

The cuticle of P. pictus is like that of any insect as seen in the section of control insect (Fig. 1).
HISTOPATHOLOGICAL OBSERVATIONS OF CUTICLE OF P. pictus.

The present Chapter deals with the histopathological observations of the cuticle induced by the different doses of alkylating chemosterilants apholate and non alkylating chemosterilant hempa. The observations were made in both chemosterilants with different dose for the period of 50 days. The stages selected are 3, 7, 14, 21, 28, 35, 42 and 50 days after the treatment of the chemosterilants and the doses administered were .065 ml. and .125 ml. of apholate and hempa respectively given to different group of insects to study the necrotic effects. The necrosis induced is given as follows:

EFFECT OF .065 ml. OF APHOLATE ON THE CUTICLE OF Poekilocerus pictus

3 days: Epicuticle, exocuticle and mesocuticle became thin and endocuticle became thick and discontinuous and it was broken at number of places. (Fig. 2).

7 days: Exocuticle and mesocuticle were fused together and epicuticle became thick. Endocuticle became more thick discontinuous and was ruptured at places (Fig. 3).

14 days: Epicuticle and mesocuticle became thin and exocuticle became thick. At some places exocuticle gets separated from mesocuticle. Endocuticle became thick and was ruptured. (Fig. 4).

21 days: Epicuticle became thin and mesocuticle and exocuticle gets fused together and showed splitting. Endocuticle became thick, ruptured and was degenerated. (Fig. 5).
28 days: Entire cuticle became thick at this stage. Epicuticle and endocuticle became thin and exocuticle and mesocuticle became more thick. (Fig. 6).

35 days: Epicuticle and mesocuticle became thin and exocuticle and endocuticle became thick. (Fig. 7).
EFFECT OF .125 ml. OF APHOLATE ON THE CUTICLE OF *Poekilocerus pictus*:

3 days: Epicuticle and mesocuticle became thin and was ruptured. Exocuticle also became thick than mesocuticle. Endocuticle became thick and discontinuous. (Fig. 8).

7 days: Epicuticle became thick and mesocuticle and exocuticle became thick and were fused together. Endocuticle became thin and was irregular in arrangement. (Fig. 9).

14 days: Epicuticle, exocuticle and mesocuticle became thin and endocuticle became thick. (Fig. 10).

21 days: Entire cuticle became thin and mesocuticle and exocuticle were fused together. (Fig. 11).

28 days: Entire cuticle became thin. Endocuticle showed splitting. (Fig. 12).

35 days: Entire cuticle became thin at this stage. (Fig. 13).
EFFECT OF .065 ml. OF HEMPA ON THE
CUTICLE OF *Poskillocerus pictus*:

3 days: Epicuticle and mesocuticle became thin and exo-
cuticle became thick and endocuticle became disconti-
nuous or irregular in arrangement. (Fig. 14).

7 days: Epicuticle was ruptured and exocuticle and mesocuticle
became thin. Endocuticle became thick and irregular
in arrangement. (Fig. 15).

14 days: Epicuticle and endocuticle became thin, exocuticle
and mesocuticle became thick. (Fig. 16).

21 days: Entire cuticle became thick and thick epicuticle
becomes dense in structure. Mesocuticle and exo-
cuticle were fused together. Endocuticle became
thick and showed splitting. (Fig. 17).

28 days: Entire cuticle became thick. Epicuticle became
somewhat thick but was thin than 21 days stage.
Mesocuticle and exocuticle were fused together.
Endocuticle became thick and showed splitting.
(Fig. 18).

35 days: Epicuticle, exocuticle and mesocuticle became thin
but some parts of endocuticle became thick. (Fig. 19).

42 days: Epicuticle became thick and dense in structure.
Mesocuticle and exocuticle were somewhat normal in
thickness. Endocuticle became thin. (Fig. 20).

50 days: Epicuticle, exocuticle and mesocuticle became some-
what normal in thickness. Endocuticle was ruptured
at places. (Fig. 21).
EFFECT OF .125 ml. OF HEMPA ON THE CUTICLE OF Poekilocerus pictus:

3 days: Entire cuticle showed splitting and epicuticle became thick. Exocuticle and mesocuticle were fused together and endocuticle was ruptured. (Fig. 22).

7 days: Epicuticle became thin having dense layer and exocuticle and mesocuticle showed splitting. At places endocuticle became more thick. (Fig. 23).

14 days: Entire cuticle became very much thick and epicuticle became thin. Mesocuticle and exocuticle became thick and endocuticle became thin. (Fig. 24).

21 days: Epicuticle became discontinuous and irregular in appearance. Exocuticle became thin and mesocuticle was thick. Endocuticle became thin and irregular in appearance. (Fig. 25).

28 days: Epicuticle became thin, exocuticle became thick and mesocuticle became thin. Endocuticle became thick and was ruptured at places. (Fig. 26).

35 days: Epicuticle, exocuticle, mesocuticle and endocuticle became thin at places, endocuticle gets separated from mesocuticle. (Fig. 27).

42 days: Epicuticle became thin and discontinuous. Exocuticle and mesocuticle became thin. Endocuticle became thick and was discontinuous and ruptured. (Fig. 28).

50 days: Epicuticle was thin and exocuticle and mesocuticle became fused together, endocuticle became thick and discontinuous. (Fig. 29).
EXPLANATION TO FIGURE

Fig. 1 : Section of cuticle of control *Poekilocerus pictus*. Periodic acid Schiff's reaction (PAS). X 200

Fig. 2 : Section of cuticle of *Poekilocerus pictus* after 3 days of .065 ml apholate treatment. Periodic acid Schiff's reaction (PAS). X 200

Fig. 3 : Section of cuticle of *Poekilocerus pictus* after 7 days of .065 ml apholate treatment. Periodic acid Schiff's reaction (PAS). X 200

Fig. 4 : Section of cuticle of *Poekilocerus pictus* after 14 days of .065 ml apholate treatment. Periodic acid Schiff's reaction (PAS). X 200
EXPLANATION TO FIGURE

Fig. 5: Section of cuticle of *Poekilocerus pictus* after 21 days of .065 ml apholate treatment. Periodic acid schiff's reaction (PAS). X 200

Fig. 6: Section of cuticle of *Poekilocerus pictus* after 28 days of .065 ml apholate treatment. Periodic acid schiff's reaction (PAS). X 200

Fig. 7: Section of cuticle of *Poekilocerus pictus* after 35 days of .065 ml apholate treatment. Periodic acid schiff's reaction (PAS). X 200

Fig. 8: Section of cuticle of *Poekilocerus pictus* after 3 days of .125 ml apholate treatment. Periodic acid schiff's reaction (PAS). X 200
Fig. 9: Section of cuticle of *Poekilocerus pictus* after 7 days of .125 ml apholate treatment. Periodic acid schiff's reaction (PAS). X 200

Fig. 10: Section of cuticle of *Poekilocerus pictus* after 14 days of .125 ml apholate treatment. Periodic acid schiff's reaction (PAS). X 200

Fig. 11: Section of cuticle of *Poekilocerus pictus* after 21 days of .125 ml apholate treatment. Periodic acid schiff's reaction (PAS). X 200

Fig. 12: Section of cuticle of *Poekilocerus pictus* after 28 days of .125 ml apholate treatment. Periodic acid schiff's reaction (PAS). X 200
EXPLANATION TO FIGURE

Fig. 13 : Section of cuticle of *Poekilocerus pictus* after 35 days of .125 ml apholate treatment. Periodic acid schiff's reaction (PAS). X 200

Fig. 14 : Section of cuticle of *Poekilocerus pictus* after 3 days of .065 ml hema treatment. Periodic acid schiff's reaction (PAS). X 200

Fig. 15 : Section of cuticle of *Poekilocerus pictus* after 7 days of .065 ml hema treatment. Periodic acid schiff's reaction (PAS). X 200

Fig. 16 : Section of cuticle of *Poekilocerus pictus* after 14 days of .065 ml hema treatment. Periodic acid schiff's reaction (PAS). X 200
**EXPLANATION TO FIGURE**

**Fig. 17** : Section of cuticle of *Poekilocerus pictus* after 21 days of .065 ml hema treatment.
Periodic acid schiff's reaction (PAS). X 200

**Fig. 18** : Section of cuticle of *Poekilocerus pictus* after 28 days of .065 ml hema treatment.
Periodic acid schiff's reaction (PAS). X 200

**Fig. 19** : Section of cuticle of *Poekilocerus pictus* after 35 days of .065 ml hema treatment.
Periodic acid schiff's reaction (PAS). X 200

**Fig. 20** : Section of cuticle of *Poekilocerus pictus* after 42 days of .065 ml hema treatment.
Periodic acid reaction (PAS). X 200
EXPLANATION TO FIGURE

Fig. 21 : Section of cuticle of *Poekilocerus pictus*
after 50 days of .065 ml hempa treatment.
Periodic acid schiff’s reaction (PAS). X 200

Fig. 22 : Section of cuticle of *Poekilocerus pictus*
after 3 days of .125 ml hempa treatment.
Periodic acid schiff’s reaction (PAS). X 200

Fig. 23 : Section of cuticle of *Poekilocerus pictus*
after 7 days of .125 ml hempa treatment.
Periodic acid schiff’s reaction (PAS). X 200

Fig. 24 : Section of cuticle of *Poekilocerus pictus*
after 14 days of .125 ml hempa treatment.
Periodic acid schiff’s reaction (PAS). X 200
EXPLANATION TO FIGURE

Fig. 25 : Section of cuticle of *Poekilocerus pictus*
after 21 days of .125 ml hempo treatment.
Periodic acid schiff's reaction (PAS). X 200

Fig. 26 : Section of cuticle of *Poekilocerus pictus*
after 28 days of .125 ml hempo treatment.
Periodic acid schiff's reaction (PAS). X 200

Fig. 27 : Section of cuticle of *Poekilocerus pictus*
after 35 days of .125 ml hempo treatment.
Periodic acid schiff's reaction (PAS). X 200
Fig. 28: Section of cuticle of *Poekilocerus pictus* after 42 days of .125 ml hempo treatment. Periodic acid schiff's reaction (PAS). X 200

Fig. 29: Section of cuticle of *Poekilocerus pictus* after 50 days of .125 ml hempo treatment. Periodic acid Schiff's reaction (PAS). X 200
B. DISCUSSION

The use of insecticides in the field of public health has been largely confined to the restricted habitat of the human dwelling and has had comparatively little impact on non-target organisms. Insecticides have contributed to environmental pollution though more from their use in agriculture than in the control of insect borne diseases. Rachel Carson (1962) in writing her book "Silent Spring" brought the seriousness of the situation to the general public in the exaggerated and emotional way of presenting the spectre of a world without birds, bees and butterflies. However, the readiness with which those concerned took to the use of insecticides after the Second World War was in itself an indication of the relative inefficiency of existing methods while control operators will undoubtedly take another look at existing and tried methods.

Use of chemosterilants or genetic control methods are species specific and non-polluting where they lead to population elimination there may be an "upset in the balance of nature" though so far as is known the successful eradication of insect pests in the past by other means has not to any major catastrophe in this direction. Genetic control methods have the advantage over most other methods of being most efficient when the target insect is in low density as the released insects have the capacity, if they are competitive to search out the wild populations. However, they are least efficient against those insects with a high reproductive potential. Against such
Populations they are best used in season of low population numbers or in combination with other methods designed to reduce population numbers (Davidson, 1974).

Present study of the effect of apholate and hempa on the cuticle of *P. pictus* has been done to investigate its structure after the treatment with chemosterilants. So far as the author is aware of, no work has been done on the effect of chemosterilants on the cuticle and very little work has been done on the effect of insecticides on the cuticle and chitin synthesis.

Polyoxin D and diflubenzuron are known to inhibit chitin synthesis in insects including *L. cuprina* and seems to have different biochemical modes of action (Sowa and Marks, 1975; Vardanis, 1976; Deul et al., 1978; Turnbull and Howells, 1982). Polyoxin D is known to inhibit the enzyme chitin synthetase (Hori et al., 1974), but diflubenzuron is inactive (Cohen and Casida, 1980; Mayer et al., 1981) or only weakly inhibitory against cell free insect chitin synthetase (Turnbull and Howells, 1983). Diflubenzuron is inactive also against fungal chitin synthetase (Leighton et al., 1981) and alternative ways in which might interfere with chitin synthetase have been suggested (Leighton et al., 1981; Cohen and Casida, 1980; Turnbull and Howells, 1983). Diflubenzuron may block chitin synthesis at some site involved in chitin polymerization (Cohen and Casida, 1980) or may inhibit the formation of glycolipid or glycoprotein intermediates necessary for chitin (Turnbull and Howells, 1983). A further possibility is that diflubenzuron
may act against a protease which activates a zymogen form of chitin synthetase (Leighton et al., 1981). In view of the apparent differences in the mode of action of polyoxin D and diflubenzuron, it is not surprising that there are differences in their effects on ultrastructure. Thus in L. cuprina both chemicals cause the secretion of an unstructured procuticle but diflubenzuron produced globules of material, perhaps unstabilized protein (Ker, 1978) and Polyoxin D has a more drastic effect on the epicuticle. Polyoxin D and diflubenzuron interfere with the normal deposition of epicuticle, a part of the integument, generally thought to be devoid of chitin (Filshie, 1982).

In the present study the cuticle of Poekilocerus pictus treated with apholate shows that epicuticle becomes discontinuous and thin and similar to those reported by Binnington (1985). The cuticle of larvae L. cuprina treated with polyoxin D, epicuticle are discontinuous and contain a thinner than usual dense layer but distorted and same results were found when treated with aminopterin. The effect of the diflubenzuron on the cuticle of L. cuprina are that, epicuticle is more subtle, the dense layer does not form a regular interface with the procuticle and there are numerous infoldings of the outer epicuticular layers. The cuticle affected larvae contain globular bodies.

Here in the present study the cuticle of Poekilocerus pictus treated with .065 ml. of apholate, the epicuticle and
mesocuticle became thin and endocuticle became thick and when treated with .125 ml. of apholate the entire cuticle became thin in most of the stages.

The treatment of .065 ml. of hempa causes thickness to the cuticle but after 50 days of treatment cuticle became somewhat normal. Treatment of .125 ml. of hempa, the entire cuticle became thick from 3 to 14 days and after 21 to 50 days, the entire cuticle became thin but endocuticle remain thick.

As such it is concluded that the chemosterilants apholate and hempa causes necrosis to the cuticle in *P. pictus*. 