HISTOPATHOLOGICAL AND DEVELOPMENTAL EFFECTS OF THE CHEMOSTERILANT APHOLATE, ON THE MOSQUITO *AEDES AEGYPTI* [1]

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INTRODUCTION. Considerable work has been done in the last few years on the chemical sterilization of insects (see Borkovec 1966; LaBrecque and Smith 1968). As a result, important contributions have been made in recent years concerning the molecular, cytological and gross effects of some of the more commonly used chemosterilants on several insect species. However, most of this work has been based on studies of the reproductive tissues. Very little information on the histopathological effects of chemosterilants in other tissues has been reported.

In the case of *Aedes aegypti*, detailed studies dealing with apholate-induced aberrations in somatic chromosomes in the brain cells and the mechanism of apholate-induced changes in fecundity and fertility have been described earlier (Rai 1964a, b). The present paper reports the results of the effects of apholate on the midgut and the nerve fibers in the brain. The important role of both these organs in controlling normal ovarian development is well known (Larsen and Bodenstein 1959, and Roth and Porter 1964). Thus it is surprising indeed that scant attention has been given to the effects of chemosterilants on these organs. This paper also includes data on the effects of apholate on the development and metamorphosis of the growing larvae.

MATERIALS AND METHODS. Eggs of ROCK strain of *Aedes aegypti* were used in this study. They were hatched in deoxygenated water and the larvae were reared in enamel pans containing tap water. The growing larvae were fed on beef liver powder from time to time. The rearing of all the stages was done in an insectary maintained at 80° ±5° F. and 80±10 percent relative humidity. From the growing cultures, 2-day-old, second instar, larvae were hand picked and transferred to pint containers (50 per container) in 250 ml. solution of 15 p.p.m. apholate in tap water. Controls were run simultaneously in tap water without apholate. The midgut and the brain from treated and untreated adult female mosquitoes, 24 to 48 hours old were dissected in *Aedes aegypti* saline (Hayes, 1953). These organs were fixed in either Carnoy's or Susa fixative (see Galigher & Kozloff 1964). They were then dehydrated and embedded following the usual procedure of tissuemat embedding at 58° to 60° C. The sections were cut at 5 to 6 μ and stained in Delafield hematoxylin and eosin. For the study of nerve fibers, the brain tissue was fixed in Petrunkvitch paranitrophenol fluid containing 20 percent formalin (Rogoff, 1946) and then the usual procedure of tissuemat embedding was followed. The sections were cut at 10 μ and stained with Bodian's technique as modified by Rogoff (1946). Some observations were also made on the Golgi bodies of the midgut epithelium. For the demonstration of Goldi bodies the technique used by Baker (1957) was employed.

For the observations on the effect of apholate on the metamorphosis of *Aedes aegypti* larvae, second instar larvae were hand picked and transferred to pint con-

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Explanation of Figures on Page 11

FIG. 1. T. S. of brain showing nerve fibers in the corpus centrale region x 1,200.

FIG. 2. T. S. of brain in an apholate-treated female showing the degeneration and dissolution of the nerve fibers in the corpus centrale region x 1,200.

FIG. 3. T. S. of midgut showing normal histology x 700.

FIG. 4. T. S. of midgut in a chemosterilized female showing the pathological condition. Note the contraction and vacuolation of epithelium and dislocation of nuclei x 700.

FIG. 5. T. S. of midgut showing scattered Golgi bodies in the epithelium (untreated female) x 1575.

FIG. 6. T. S. of midgut showing fewer Golgi bodies in the epithelium of the treated females x 1575.

tainers. Various concentrations of apholate used in this experiment were 10, 50, 100, 200, and 500 p.p.m. (Table 1). In each case 50 second instar larvae were treated in 250 ml. of apholate solution. The data included in Table 1 are based on 8 replicates at each concentration. The controls were run simultaneously in tap water.

RESULTS. The nuclei in the cortical region of the brain tissues in treated mosquitoes showed considerable clumping of chromatin. Moreover, as shown in Fig. 2, the degeneration of nerve fibers was evident following apholate treatment. The normal nerve fibers in the untreated brain are shown in Fig. 1.

The midgut epithelium of untreated mosquitoes is uniform in size and nuclei usually lie in the center of the epithelial cells (Fig. 3). In the treated midguts on the other hand, the epithelial cells were greatly deformed, shrunken, uneven in size and the nuclei were displaced (Fig. 4). Furthermore, large vacuoles were observed in the midgut epithelium of the treated females.

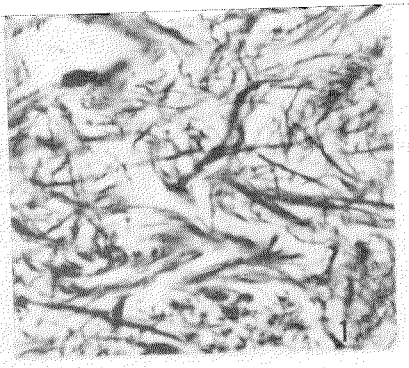
Normally, the Golgi bodies in the midgut epithelium are present dispersed in the cytoplasm. In the midgut of the untreated females, numerous Golgi bodies of various sizes of more or less round structure were observed (Fig. 5). In the midguts of the treated females, on the other hand, they were extremely few in number, (Fig. 6). Apparently, apholate caused dissolution of the majority of the Golgi bodies.

The results of the effect of apholate on the metamorphosis indicated that it interferes with the growth and development of *Aedes aegypti* larvae at relatively higher concentrations (Table 1). There was no significant difference in the rate of pupation between the treated and the untreated series at 10 p.p.m. apholate treatment. At 50 p.p.m. apholate treatment, the pupation was delayed and the percentage of pupation on day 1 was reduced to 23.5 from 80 in the controls. When

TABLE 1. Results of rearing second instar *Aedes aegypti* larvae in apholate solution.

Treatments	Percentage of pupation on day [a]						
	1	2	3	4	5	6	7
Control	80	20	..	..	..	..	..
10 p.p.m.	74	26	..	..	..	..	..
50 p.p.m.	23.5	45	9.5	22	..	..	..
100 p.p.m. [c]	0	14	9	21	13	4.5	0.5 [b]
200 p.p.m.	0	0.5	5	5.5	8	2.5	1 [b]
500 p.p.m. [d]	0	0	0	0	0	0	0
		(67.5)	(62)	(58.5)	(56.5)	(55)	(48)

[a] The day on which pupation began in controls is designated day 1.
[b] Remaining larvae continue to live up to 13th day.
[c] Adults are incapable of emergence, found dead attached to the pupal exuvium.
[d] Metamorphosis completely blocked, living larvae counted (percentage in parenthesis).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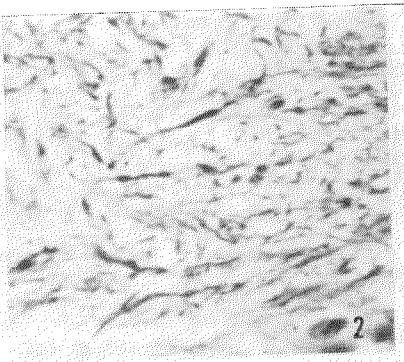
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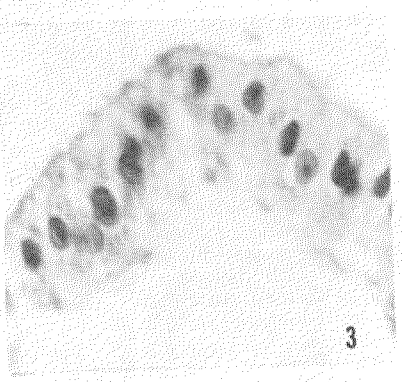
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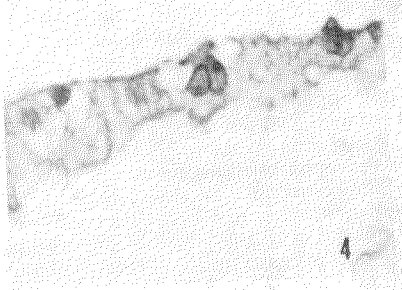
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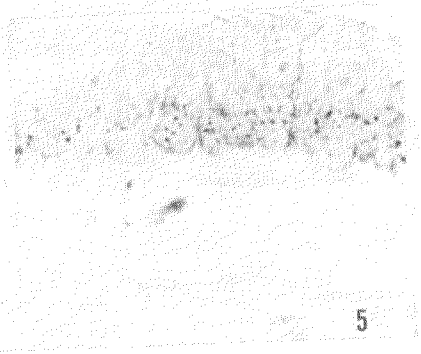
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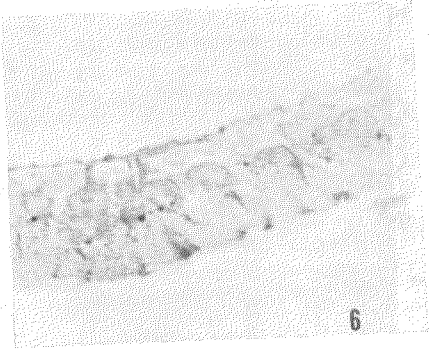
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exposed to a concentration of 100 p.p.m. apholate, there was no pupation on day 1 and the rate of pupation was low on the following days. Moreover, there were some larvae which did not pupate even up to day 13. The same effect was observed at 200 p.p.m. apholate treatment. At 500 p.p.m. treatment the metamorphosis was completely blocked.

DISCUSSION. The above observations revealed that apholate caused degeneration of the midgut epithelium and the nerve fibers in the brain. These degenerative changes are in keeping with the known effects of apholate on other organelles and tissues (Rai 1964a, b). The Golgi bodies also in the treated midgut epithelia either underwent degeneration and dissolution or were not produced in the same numbers as in the controls. Although the biochemical properties and function of Golgi bodies have long been debated and are still not exactly clear, it appears that they are secretory in function. They are also implicated in the absorption of vitamin C, iron, copper compounds etc. (De Robertis *et al.*, 1960). Furthermore, alkaline phosphatases have been reported to be concentrated in the Golgi bodies of the intestinal epithelium (Emmel 1945). Similarly, acid and alkaline phosphatases may be present in the Golgi bodies of several kinds of epithelial cells (Dean and Dempsey 1945). These observations indicate a participation of the Golgi bodies in the metabolic processes. Obviously the fewer number of Golgi bodies in the treated midguts along with other histological degenerations may be expected to lead to reduced metabolic activity. The available evidence indicates that this is indeed what happens (Rai and Sharma 1968). Whereas Akov (1966) has shown that apholate treatment given to adult females does not interfere with proteolytic activity, female infecundity induced by 5-fluorouracil, a pyramidine antagonist, results from an inhibition of proteolytic activity in the midgut (Akov 1965).

Following a blood meal, it is generally believed that the neurosecretory cells in the brains of adult female mosquitoes activate the corpora allata. These in turn release the gonadotrophic hormone which is essential for egg maturation (Larsen and Bodenstein 1959). Furthermore, according to Roth and Porter (1964), the synthesis of yolk proteins necessary for follicular development takes place principally in the midgut. Although, because of technical difficulties, it was not possible to study the effects of apholate on neurosecretory cells in *A. aegypti,* the implications of the observed histopathological effects on the nerve fibers in general and the midguts are worth emphasizing. From these results one might assume that following apholate treatment the synthesis of both yolk proteins and the gonadotrophic hormone may be blocked. However, this does not happen. By making transplants of ovaries from apholate-treated to normal females and vice versa Rai and Sharma (1968) have suggested that both the above mentioned processes remain functional in chemically treated females. Normal ovaries transplanted in the abdomens of infecund females, develop more or less normally although they do not reach the same stage of maturation as in controls. Thus, either there is a considerable repair and histolysis of the brain tissue and the midgut epithelium from the chemosterilant-induced damage induced during immature stages or these tissues continue to function, though probably at a suboptimal level in spite of their structural abnormalities reported here. In contrast, radiation-induced damage to the midguts of boll weevils at sterilizing doses results in high mortality (Riemann and Flint 1967). Furthermore, in the case of reproductive tissues, Rai (1964b) showed that chemosterilant-induced infecundity was irreversible i.e., there was no repair for at least three gonadotrophic cycles.

Results in Table 1 indicate that apholate interrupted the growth and development of *Aedes aegypti* larvae at certain concentrations. Results indicating that apholate interfered with the growth of *Anatis mali*

Auct. and prevented it from reaching the adult stage have been similarly reported by Smith and Berube (1966). Such results may be expected if high chemical concentrations inhibit cell division (Kihlman 1966). Rai (1964b) suggested that the unhatchability of the few eggs laid by apholate-treated females inseminated by sperm from untreated males may have resulted from an interruption of the embryonic development due to the presence of induced dominant lethals in ova.

Recently, Slama and Williams have observed the ability of synthetic juvenile hormone to block the embryonic development of three insect species. Spielman and Williams (1966) have reported the same for *Aedes aegypti*. The synthetic juvenile hormone also interferes with the growth and metamorphosis of *Aedes aegypti* larvae. Thus, there are similarities between the action of apholate and the synthetic juvenile hormone. It may be emphasized, however, that similar end results do not imply a similar mode of action or a similar type of pathway.

SUMMARY. Second instar larvae of *Aedes aegypti* were reared in 15 p.p.m. apholate solution in water until pupation. This treatment resulted in almost complete female infecundity. The midgut epithelium of these sterilized females had fewer Golgi bodies and showed histological degenerations. In brains of such infecund females, the nerve fibers appeared degenerated. Extensive chromosomal damage in brain cells resulting from similar apholate treatment was demonstrated earlier. Based on these and ovarian transplantation experiments, which will appear elsewhere, it may be concluded that in spite of chemosterilant-induced structural abnormalities, the brain tissue and the midgut continue to function, though at a reduced rate in controlling ovarian development.

Apholate at relatively high concentrations inhibited the growth and metamorphosis of *Aedes aegypti* larvae. This may result from inhibited cell division.

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MONOFACTORIAL INHERITANCE OF AUTOGENY IN *AEDES ATROPALPUS*[1]

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INTRODUCTION. Ovarian development in mosquitoes can be classified into two types. Females which require a blood meal for the production of mature eggs are called anautogenous, whereas those lacking this requirement at least for the first egg batch are called autogenous.

Although biochemical and physiological aspects of autogeny have been extensively investigated in several species (Beckel, 1954; Chen, 1967; Kardos, 1959; Lea, 1964), the mode of inheritance of this character has been carefully analyzed only in the *Culex pipiens* (L.) complex. Multifactorial modes of inheritance of autogeny have been postulated for this mosquito group (Spielman, 1957; Laven, 1967). In an effort to ascertain if a similar, complex genetic control mechanism was present in another mosquito species, the present study was undertaken. Two rather closely interrelated biological activities were examined in the rockpool mosquito, *Aedes atropalpus* (Coq.). First, both types of egg development were analyzed and the mode of inheritance of this character was determined with crossing experiments. Second, blood-feeding activities of autogenous and anautogenous strains were characterized and the genetic basis of blood-feeding was investigated.

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MATERIALS AND METHODS. Laboratory colonies were initiated from field collections made at 12 sites in 7 states (Table 1). In all cases except one, only larvae and pupae were collected. The exception involved eggs collected from an oviposition trap. Large bulbed pipettes were used in extracting the aquatic stages from rockpools.

Aquatic stages were reared in enamel pans with a water temperature range of $26 \pm 3°$ C. Larvae were fed on liver powder (Nutritional Biochemicals Co.). Adults were maintained at $24 \pm 1°$ C. and a relative humidity of 70–80 percent. Sugar cubes and slices of canned apples were provided as food for adults.

Initial observations involved measurement of autogeny by counting eggs that had been deposited by females that had been denied access to blood. However, some females developed eggs but retained them in the abdomen. Therefore, the criterion for autogeny used in the present work was based on the degree of follicular development, as measured in dissected females.

Ovaries were dissected from anesthetized females which had the abdomen in a drop of saline. The abdomen was grasped with two forceps, one holding the first segment and the other holding the seventh. The latter segment was slowly pulled until the intact ovaries appeared.