CYTOGENETIC EFFECTS OF CHEMOSTERILANTS IN MOSQUITOES. III. DEVELOPMENT OF TRANSPLANTED OVARIES IN NORMAL AND CHEMOSTERILIZED FEMALES OF *AEDES AEGYPTI*

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Introduction

Considerable work has been done during recent years on the mode of action of chemosterilants and the detailed mechanisms by which they induce sexual sterility in various insect species. Two recent books (Borkovec, 1966 and LaBrecque and Smith, 1968) summarize pertinent developments in this field.

In the case of female insects, chemical treatment may either inhibit oogenesis and egg maturation (infecundity) or may result in the induction of dominant lethal mutations in the eggs that are produced. Whereas the mechanism of the induction and expression of dominant lethality is fairly well understood (Fahmy and Fahmy, 1954, Rai, 1964a and LaChance, 1967) much less is known about the detailed pathways or causal events that lead to female infecundity. It may be mentioned that normal oogenesis in insects depends upon:

(1) Continued mitotic divisions of the oogonial cells which ultimately produce the nurse cells and oocytes.
(2) Endomitotic replications of the nurse cell chromosomes and related metabolic processes.
(3) Synthesis of the specific yolk proteins and the associated enzyme systems either in all parts of the insect body (Telfer, 1965) or as in *Aedes aegypti* principally in the midgut (Roth and Porter, 1964).
(4) Proper functioning of the endocrine system or more specifically the availability of the gonadotrophic hormone essential for egg maturation.

Depending upon several factors, various chemicals may induce female infecundity by interfering with any one or more of these essential requirements. To cite a few examples, interference with step 1 has been concluded by Rai (1964b), with step 2 by LaChance and Leverich (1968) and step 3 by Akov (1965) among others. With regard to interference with step 3, it may be mentioned that whereas 5-fluorouracil inhibits protease activity in the midgut of *A. aegypti* (Akov, 1965), apholate does not (Akov, 1966). Nevertheless, an important aspect which has been neglected so far is the effect, if any, of chemosterilants on the endocrine system. The experiments reported in this paper were designed to obtain information in this field and to ascertain if female infe-

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cundity induced by a commonly used chemosterilant, apholate, in *Aedes aegypti* might be due wholly or partially to one or more of the following causes:

(*a*) Apholate might interfere with the availability of the gonadotrophic hormone to the developing oocytes either by combining with the hormone molecules thus inactivating them or by inhibiting the release of the hormone due to possible chemosterilant-induced changes in the endocrine organs. Molecules of proteins, nucleic acids and different vitamins etc. have many sites to which alkylating agents can and do attach (Alexander, 1960).

(*b*) Apholate might be causing the inhibition of yolk protein synthesis in the midgut.

(*c*) Apholate might be causing degenerative changes in the cells and tissues of the ovary itself thus preventing the oocytes from completing their development.

It may be pointed out that our earlier studies with *Aedes aegypti* have demonstrated that rearing of larvae in 15 ppm apholate solution from second instar until pupation induces drastic chromosomal aberrations in brain cells (Rai, 1964a), almost complete female infecundity (Rai, 1964b) and various histopathological abnormalities in nerve fibres and the midgut epithelium (Sharma and Rai, 1968). The usefulness of the ovarian transplantation technique for several types of developmental studies has been emphasized elsewhere (Rai, 1967, 1968). In particular, this technique makes it possible to determine the role of intrinsic and genetic factors in the development of tissues which are implanted in alien environments and thereby to resolve problems concerning interactions of genotypes and environment.

Material and Methods

Eggs of ROCK strain of *Aedes aegypti* were hatched in deoxygenated water. The larvae were reared in enamel pans and fed with liver powder from time to time. The rearing was done in a room maintained at $80 \pm 5°F$ and $80 \pm 10\%$ relative humidity. Two days after hatching, when most of the larvae were second instars, they were hand-picked and transferred to pint containers (50 per container). Each of these contained 250 ml. solution of 25 ppm apholate in tap water. The second instar larvae were reared in this solution till pupation. Control larvae were reared simultaneously in tap water. The adult mosquitoes were fed on canned apple slices or sugar cubes.

One ovary from each of the 4 to 6 day old, unfed donor females, was removed in *Aedes aegypti* saline (Hayes, 1953) with the help of watchmaker's forceps under a stereoscopic microscope. The dissections were made from both the treated and control females. In the case of the recipient (host) females, a small slit was made in the intersegmental membrane of the fifth or sixth abdominal segment. The ovary from each of the donor females was inserted into the abdomen of the host females through this opening. Usually the latter females were approximately of the same age as the donor females (4-6 days old) at the time of implantation. After 24 to 48 hours of these transplantations, the females were given a blood meal on anaesthetized mice. Seventytwo hours after the blood meal, the implanted ovary and host ovaries were dissected and the process of oogenesis studied. By this time, the ovaries in the untreated host were fully mature.

The ovaries were transplanted as soon as they were removed from the donor female. The delay in implantation often retards the development of the implanted ovary. Besides, if the age of the implanted ovary is less than the host ovary, many implanted ovaries do not develop (Larson and Bodenstein, 1959). Care was taken to avoid all the above-mentioned artifacts. The stages of ovarian development described here are based on the categorization of Clements (1963).

Results

The condition of the ovary before and after a blood meal in apholate-treated and control females is shown in Figs. 1, 2 and 3, 4 (Plate 11) respectively. The details of oogenesis in untreated and apholate-treated females of *A. aegypti* have been described by Rai (1964b) and will, therefore, not be included here. As also shown by Rai (1964b), a few, often 1 or 2 follicles may develop in treated ovaries (Fig. 2). The ovaries from untreated donors were at the resting stage (Stage IIb, Fig. 3) at the time of implantations. When fully developed, the primary follicles in these ovaries assumed the shape of mature eggs (Stage IVb) as shown in Figures 4 and 5. It may be pointed out that on the whole resting stage ovaries of treated females were smaller than those of the untreated ones of the same age. Furthermore, whereas the latter ovaries contained a large number of uniformly developing follicles, in treated ovaries the follicles varied greatly in size and stage of development and showed considerable degeneration. Thus a categorization of a treated ovary with regard to the exact stage of follicular development is almost impossible.

Table 1. *Results of ovarian transplantation in apholate-treated (infecund) and normal females of* Aedes aegypti

Transplantations			Development of the transplanted Ovary in the Host
Donor		Host	
Untreated	(53)*	Untreated	(45)**
Untreated	(49)	Treated	(47)
Treated	(53)	Untreated	(0)
Treated	(56)	Treated	(0)

*Figures in parenthesis indicate number of females utilized (one ovary dissected from each donor ♀ and implanted in a host).
**No. of females in which implanted ovaries underwent positive development.

Results of transplantation experiments are included in Table 1 and Figs. 5 to 8. When an ovary at resting stage of development from an untreated donor was implanted into an untreated, control female, the follicles of both the host and the implanted ovary developed to maturity (Stage IVb) following a blood meal (Fig. 5: implanted ovary on the left and host ovary on right). Alternatively, when the resting stage ovaries from untreated donors were implanted in apholate-treated females, the development of several follicles was initiated and progressed to Stage IIIb-IVa (Fig. 6, right). However,

the follicles of such implanted ovaries never developed to Stage IVb as they normally did in the untreated host. The under-developed host ovary is shown in Fig. 6, left. In another series of experiments when the resting stage ovaries from apholate-treated hosts were implanted in either the untreated hosts or the treated hosts they did not develop beyond Stage IIb (Fig. 7, left and Fig. 8, right respectively). However, the follicles of the recipient untreated host ovaries reached maturity as expected (Fig. 7, right). Nevertheless, the ovaries of recipient, treated host never proceeded beyond the resting stage i.e. IIb (Fig. 8, left).

Discussion

Following a blood meal, the neurosecretory cells in the brain of an adult female mosquito activate the corpora allata. These in turn release the gonadotrophic hormone which is essential for egg maturation (Larson and Bodenstein, 1959). In the absence of this hormone the ovary remains underdeveloped. Furthermore, in *A. aegypti*, the synthesis of yolk proteins necessary for follicular development takes place principally in the midgut (Roth and Porter, 1964). The yolk proteins are taken up from the haemolymph by developing follicles by micropinocytosis. Thus, according to the currently held views, the involvement of the brain and the midgut for proper follicular development cannot be overemphasized.

Earlier studies on the biology of chemosterilization in *A. aegypti* emanating from this laboratory had shown that these same organs (besides the reproductive tissues) undergo extensive cytological aberrations (Rai, 1964a) and histological degeneration (Sharma and Rai, 1969) following chemosterilant treatment in the immature stages. Thus, it was of considerable interest to investigate if the structural abnormalities induced by apholate in the brain and midgut affect their functional capabilities also.

The development of follicles from untreated females following implantation in the abdomens of apholate-treated infecund females suggests that the synthesis of yolk proteins in the midgut is not blocked. This eliminates the possibility of the inhibition of yolk protein synthesis in the midgut as a consequence of chemosterilant treatment. Besides, this also eliminates the possibility that apholate may be interfering with the availability of the gonadotrophic hormone to the ovaries. The positive development of the normal ovaries following implantation in the chemosterilant-treated, infecund host indicates that the yolk proteins and the gonadotrophic hormone continue to be available in the haemolymph of such females. Nevertheless, it must be emphasized that when an ovary from an untreated female is implanted in the abdomen of a treated female, fewer follicles develop. Moreover, they do not reach the same stage of development as they do in an untreated environment. This may be due to several factors. The general metabolism in the treated host may be taking place at a reduced rate. As already mentioned, Sharma and Rai (1969) have demonstrated that the nerve fibres and the midgut in the treated females undergo histological degenerations. Furthermore, the number of Golgi bodies was greatly reduced in the epithelial cells of the midguts of apholate-treated adults. It is conceivable, therefore, that the cytological and/or patho-

logical condition of the brain and the midgut in the treated females may be affecting the quantity of neurosecretion and/or yolk protein synthesis and thus limiting the development of the follicles in the treated environment. "Histological alterations and changes in quantity of neurosecretion" have been reported following intense long-term irradiation of *Gryllus domesticus* (see Hrdy and Hrdlickova, 1968).

Although a chemosterilant, 6-azauridine has been reported to "completely inactivate" the corpora allata in *Pyrrhocoris apterus* (see Hrdy and Hrdlickova, 1968), on the whole the endocrine system is rather resistant to the damaging effects of mutagens, e.g. to irradiation (Casarett, 1968). Similarly, based on their cytological study of oogenesis in screw-worm fly treated topically with an alkylating agent, 2, 5-bis (1-aziridinyl)-3-6,-bis (methoxyethoxy)—p-benzoquinone, LaChance and Leverich (1968) have concluded "a direct effect of chemosterilants on the developing follicles rather than through hormonal interaction." They have shown that if chemical treatment is applied to young, 0 to 4 hours old females, endomitotic replication of the nurse cell chromosomes is interfered with. These chromosomes do not attain proper ploidy level and stay in bundles rather than unravel or "fall apart". The latter condition is essential for normal vitellogenesis and egg maturation (LaChance and Leverich, 1968).

From the available evidence, it may be concluded that in *A. aegypti* the primary cause of ovarian inhibition following apholate treatment at immature stages lies in the treated ovary itself and results from degenerative changes in the oogonial cells. This may be brought about by inhibition of and aberrant mitoses in the primary follicles and the germarium resulting in gonial death. The detailed events leading to such a degeneration have been reported earlier (Rai, 1964b).

Summary

1. Two-day old larvae of *Aedes aegypti* were reared until pupation in 25 ppm of a commonly used mosquito chemosterilant, apholate. This concentration induced complete female infecundity.

2. Transplantation experiments involving ovaries from normal adults and adults arising from the above chemical treatment were undertaken. The object was to investigate whether apholate-induced female infecundity results from direct effects on the ovary or from indirect effects on the hormonal and/or yolk protein synthesis or supply.

3. The fact that the ovary from an untreated female when implanted in the abdomen of an infecund female develops more or less normally, at least in a qualitative sense, following a blood meal indicates that the yolk proteins and the gonadotrophic hormone are available for ovarian development in chemically treated females. Thus, the humoral factors are not involved in female infecundity and the primary site of action of apholate must be the oocyte. The inhibition of the ovaries in apholate-treated females, must be brought about by degenerative changes in the ovary itself.

4. Quantitatively, fewer untreated follicles developed following implantation in the abdomen of a treated female. Furthermore, they did not reach the same stage of development as they did in untreated environment. Possible mechanisms for this retarded development are discussed.

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LEGENDS TO PLATE 11

Fig. 1. Ovary of the treated female at the resting stage before blood meal. ×44.

Fig. 2. Ovary of the treated female at the resting stage 3 days after blood meal. ×44.

Fig. 3. Ovary of the untreated female at the resting stage IIb, before blood meal. ×44.

Fig. 4. Ovary of the untreated female, showing mature, Stage IVb, follicles 3 days after blood meal. ×44.

Fig. 5. Condition of the implanted untreated ovary, Stage IVb, left and the untreated host vary, also Stage IVb, right.

Fig. 6. Condition of the implanted untreated ovary, Stage IIIb-IVa, right and the treated host ovary, left. ×44.

Fig. 7. Condition of the implanted treated ovary, left, and the untreated host ovary, Stage IVb, right. ×44.

Fig. 8. Condition of the implanted treated ovary, right and the treated host ovary, left. ×44.

PLATE 11