Cytogenetic Effects of Chemosterilants in Mosquitoes

I. Apholate-induced aberrations in the somatic chromosomes of *Aedes aegypti* L.¹

K. S. Rai

Department of Biology, University of Notre Dame
Notre Dame, Indiana, U.S.A.

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Introduction

Considerable work has been done in the last few years on chemical sterilization of insects (LaBrecque 1961, Chamberlain 1962, Crystal 1963 and others). As a result, the possibility of using certain chemicals, commonly referred to as chemosterilants, for eradication of noxious insect species has attracted great attention (Knipling 1962). Several chemical compounds have been tested for their effectiveness against such species as the housefly (*Musca domestica*), Mexican fruit fly (*Anastrepha ludens*), the stable fly (*Stomoxys calcitrans*), Screw-worm fly (*Cochliomyia hominivorax*) and others. Weidhaas *et al.* (1961) have extended these studies to mosquitoes. They have shown that three alkylating agents—tepa, aphalate and aphamide induced sterility in both sexes of *Anopheles quadrimaculatus* and *Aedes aegypti*. Murray (1963) has studied the effects of apholate on *Culex pipiens quinquefasciatus*.

The interest in the study of the cytogenetic effects of chemical compounds dates back to 1947 when Auerbach and Robson showed that mustard gas produces mutations and chromosomal breakages in *Drosophila*. Subsequently a number of studies of the mode of action of “nitrogen mustards” and other alkylating agents have been made in *Drosophila melanogaster* (Fahmy and Bird 1953, Fahmy and Fahmy 1954, 1958) and in other organisms “ranging from bacterial viruses to mammals” (Alexander 1960). However, the emphasis, by and large, especially in entomological literature has been on screening a variety of chemical compounds as effective chemosterilants. Consequently, a large body of data is available concerning the potentialities of some of these chemicals as chemosterilants (Knipling 1962). However, considerably less is known about some of their biological effects and the mechanism by which they induce sexual sterility in different insects. This is especially true in the case of mosquitoes. With the possible exception of

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Murray (1963), no basic studies in this field with any mosquito species have yet been reported.

The present studies were initiated with a view to analyzing the cytogenetic basis of chemosterilant-induced sterility in *Aedes aegypti*, the yellow fever mosquito. Effects of a commonly used chemosterilant, apholate (2, 2, 4, 4, 6, 6-hexahydro — 2, 2, 4, 4, 6, 6-hexakis [1-aziridinyl] — 1, 3, 5, 2, 4, 6-triazatriphosphorine) on the somatic chromosomes and on the development of the reproductive tissues were studied. The present paper will deal with the former aspect of this study. To my knowledge, this is the first detailed report of the effects of a chemosterilant on the mitotic chromosomes of any mosquito species.

The normal karyotype of *Aedes aegypti* is characterized by the presence of 3 pairs of rather large chromosomes which are individually recognizable. The 3 pairs have been designated I, II and III based on their length and the position of the centromere (Rai 1963 and Fig. 1). In the brain tissue they measure approximately 5.4, 6.9 and 7.6 micra respectively. Some of the advantage of the use of this species in basic cytogenetic studies have been emphasized elsewhere (Rai 1963a).

Materials and methods

The Rock strain of *Aedes aegypti* was used in this study. Relatively fresh eggs of this strain were hatched in deoxygenated water. The growing larvae were reared in white enamel pans containing tap water. For the first 2 to 4 days, they were maintained in a room with a temperature of 80°F ($\pm 1^\circ$ F) and relative humidity of 80% ($\pm 10\%$). Two days after hatching, second instar larvae were hand picked and transferred to pint containers (50 per container). Each of these contained 250 ml solution of 10 ppm apholate in tap water. Fifty larvae per cup were kept as controls in 250 ml untreated tap water. Lids were put on individual containers. Both the treated and control larvae were then transferred to an incubator maintained at 84.2°F. Some batches of third instar larvae were similarly treated four days after hatching. Appropriate controls were kept in this case also. The larvae were fed with commercial dog-food pellets (Gaines) from time to time.

Twenty-four - 96 hours after putting the larvae in apholate solution cytological fixations of both treated and untreated fourth instar larvae were made. The fixative used was a solution of methanol 6 parts; chloroform 3 parts; propionic acid 2 parts (Pienaar 1955). This solution acts as both fixative and preservative.

Mitotic chromosomes were studied from squashed preparations of larval brains stained with acetolactic orcein. Detailed methods for making the squashes have been described by Breland (1961) and Rai (1963).

The temporary slides were ringed with ordinary nail polish and photomicrographs of normal and aberrant mitoses were taken with a 35 mm Zeiss/Ikon camera. Panatomic-X, black and white film was used.

Results

Apholate-induced aberrations in the somatic chromosomes of *Aedes aegypti* can be grouped as follows:

Physiological aberrations: One of the observable effects was the induc-

Figs. 1-15. Apholate induced chromosomal aberrations in the brain cells of *Aedes aegypti*. Figs. 1-7, 9, 12-14 $\times 2000$; Fig. 10 $\times 1560$ and Figs. 8, 11 and 15 $\times 1250$. The time in hours, between the start of the chemical treatment and fixation of the larva for cytological examination is indicated in () following each figure. 1, shows the normal karyotype in an

tion of chromosome stickiness. Often this stickiness was most pronounced at the chromosome ends (Fig. 2). In extreme cases, the chromosomes lost their individuality completely and appeared clumped and vacuolated (Fig. 3). Although physiological changes are mostly regarded to be reversible, it is doubtful whether a cell as badly damaged as shown in Fig. 3 will recover completely from such effects.

Structural aberrations: The small number (3 pairs) and relatively large size of the somatic chromosomes make them well suited for studies of this type.

A major effect of apholate observed in this study was its ability to break chromosomes. Fig. 4 shows an early prophase chromosome complement with one of the homologues of what appears to be the largest pair (Chromosome III) broken approximately in the middle. The stump of the broken chromosome is clearly visible although it was not possible to account for the broken piece.

Rather frequently the chromosomes appeared constricted (Fig. 5). A fusion of two chromosome arms also was observed in this case. This may have resulted from either the ends sticking to each other or a fusion at two broken ends. Fig. 6 shows a metaphase chromosome broken at or near the region of its centromere. Such a breakage was not observed after X-irradiation (Rai 1963a). One of the larger chromosome pairs showed a relatively frequent breakage towards the terminal portion of one arm (Fig. 7).

Ring chromosomes were observed during late prophase (Fig. 8) and anaphase (Fig. 9). In the former case, the somatic pairing which characterizes the chromosomes at this stage was disrupted. In the latter, the ring chromosome was left in the equator of the spindle, probably because of its acentric nature. If apholate were to break a chromosome at two loci, and if the two broken ends of the interstitial piece joined, a ring chromosome would result. The size of the ring will depend on the length of the broken piece. However, if the two ends healed, an interstitial deletion would result.

Most of the fragments were acentric and were observed at all stages during the mitotic cycle. Because of their inability to move on the mitotic spindle, these fragments usually lagged behind the centric chromosomes. This

untreated fourth instar larva. Chromosomes I, II and III have been marked. In Fig. 2 (48) chromosomes appear to stick to each other. In Fig. 3 (72) all three pairs have lost their discreteness completely. 4 (48), shows prophase chromosome breakage near the center of one of the homologues of the middle pair. Note the constricted appearance of the chromosomes in Fig. 5 (24). Two chromosome arms from two pairs are also seen fused in this figure. 6 (48), shows a metaphase chromosome broken at or near its centromere. 7 (24), shows breakage at or near what appears to be the region of the secondary constriction in one of the larger pairs. 8 (96) and 9 (24), show ring chromosomes at prophase and anaphase respectively. A number of acentric fragments not included in the daughter telophase nuclei are seen in Fig. 10 (48). 11 (24), shows what appears to be a dicentric bridge with a DNA droplet lying near the equator of the spindle. 12 (48), shows a similar telophase bridge but without any accompanying fragment. 13 and 14 (48), show tetraploid cells. One of the chromosome pairs in Fig. 15 (48) is a duplicate entity while the other two are not.

resulted in their exclusion from the two daughter nuclei (Fig. 10).

Chromosomal bridges during anaphase and telophase were also observed. Some of them (Fig. 11) were accompanied by a small fragment while the others (Fig. 12) were not. The fragment in Fig. 11 appears more like a droplet of DNA. Probably because of anaphasic pull, the bridge shown in Fig. 12 broke towards one end partly snapping back on itself.

Miscellaneous effects:

Induction of somatic polyploidy: Rarely apholate induced polyploidy in some cells of the brain tissue. Tetraploid cells were observed at early prophase (Fig. 13), late prophase or early metaphase (Fig. 14). Chromosomes in some of the polyploid cells were clumped, presumably due to physiological changes in their surface properties.

Probable interference with the normal replication mechanism: Fig. 15 shows a dividing cell in which one of the chromosome pairs has clearly replicated. The other two pairs for the most part appeared as unsplit entities.

It may be emphasized that none of the above-mentioned aberrations were observed in untreated material. The induction of all the above-mentioned chromosomal aberrations in the brain cells of this species did not result in any significant mortality of the treated individuals.

Discussion

The mechanics of various X-ray-induced chromosomal aberrations in the brain tissues of fourth instar larvae of *Aedes aegypti* has been described in considerable detail (Rai 1963a). Based on the present study it is evident that most of the effects of apholate on the somatic chromosomes of this species are strikingly similar to those of X-rays, with some differences. The precise distribution of either X-ray or apholate-induced breaks along the different chromosomes of this species has not yet been studied in detail. Apparently, no departures from a random distribution of breaks after X-irradiation were observed (Rai 1963a). However, it appears that after apholate treatment one of the two larger pairs showed a rather frequent breakage towards the end of one of its arms. Akstein (1962) has reported the presence of a secondary constriction in an arm of one of the larger pairs in this species. It may be that the satellited chromosome breaks more frequently at the region of the secondary constriction than in other regions. It is generally agreed that the distribution of breaks in the case of at least some chemical compounds is non-random. Kihlman (1963) showed that after treatment with 8-ethoxycaffeine (EOC), the so-called isolocus breaks in the chromosomes of the root tips of *Vicia faba* were localized in the nucleolar constriction. Furthermore, "the degree of localization increased with increasing time between treatment and fixation". Root-tips treated in early

interphase (fixed 16 hours after treatment) showed 95.2% aberrations originating in the nucleolar constriction. Kihlman (1963) assumed that the non-random distribution of EOC induced breaks resulted from a non-random distribution of EOC within the interphase nucleus. The fact that the nucleolus is in contact with the secondary constriction during interphase meant, according to Kihlman, that the latter would be "exposed to higher concentrations of EOC than would other chromosome regions". The same phenomenon may be involved in the case of *Aedes aegypti*. Studies using lower concentrations of apholate and with cytological fixations made at more frequent intervals would be more definitive in this regard.

Furthermore the differential appearance of the three chromosome pairs seen in Fig. 15 may be explained on the basis of unequal distribution of apholate within the dividing nucleus. A particular concentration may interfere with the normal replication in some pairs. Structurally, the chromosome pair which was clearly duplicated appeared "normal" i.e. without any apparent aberrations. This was not so for the remaining two pairs which were not visibly duplicated in the same nucleus. Also, apholate-induced cross linking of the two chromatids or the DNA chains in the chromosomes (Alexander 1960) might not only result in breaks but possibly in interference with normal replication as well.

Another effect of apholate, not observed in the case of X-ray-induced chromosomal aberrations in this species, was the induction of somatic polyploidy in the brain cells. Apholate in this case may have acted in a manner similar to that of colchicine (Eigsti and Dustin 1955). Interference with the spindle apparatus of the dividing cells would not permit anaphasic separation of the duplicated chromosomes. Naturally occurring polyploidy in the larval epidermine of *A. aegypti* (Rissler 1959) and other mosquitoes is a common phenomenon. However, it has never been observed in the brain tissue.

The constricted appearance of the chromosomes (Fig. 5) observed frequently may be due to incomplete fusions of the broken ends. Alternatively, these may represent partial or potential breaks. Apholate may be regarded to break the DNA core without, in some cases, completely breaking the matrix that envelops the chromosomes.

Dicentric chromosome bridges may result from a number of causes. An isochromatid break, or a chromosomal break before replication, can, after proper fusion of the broken ends, result in anaphase bridges and acentric fragments (Lea 1947). Induction of heterozygous paracentric inversions in interphase chromosomes, followed by odd-numbered exchanges while the prophase chromosomes are somatically paired, will also result in similar configurations. A proportion of the bridges unaccompanied by chromosomal fragments may result from chromosomal stickiness particularly at their ends.

Depending on the time of their application, chemosterilants may inhibit and completely prevent the production of gametes in insects. However, when

the gametes are produced after chemical treatment, "sexual sterility" usually results from induction of dominant lethality in sperms and ova. Results obtained by various workers fit with this explanation. It is generally believed that dominant lethals are predominantly drastic chromosomal structural aberrations. If some of the major apholate induced chromosomal changes observed in the brain cells of *A. aegypti* occur during gametogenesis also (and there is no reason why they would not), gross genetic disturbances would be expected to ensue. Mitotic anomalies and the resulting genetic imbalance which would result from the presence of, for example, large deletions, acentric or dicentric chromosomes or polyploid complements in a developing embryo would result in its death before embryogenesis is completed. Such fertilized eggs would not hatch. Cytological examination of unhatched eggs in *Drosophila melanogaster* fertilized by chemically treated sperm has revealed "disturbed cleavage due to non-eucentric chromosome configurations" (Fahmy and Fahmy 1954). There seems little doubt that similar mechanism are responsible for chemosterilant-induced dominant lethality in the sex cells of *Aedes aegypti*, too.

Summary

Some cytological effects of an alkylating agent, apholate, a commonly used chemosterilant, on the Rock strain of *Aedes aegypti* were studied. Two to four days old larvae were reared until pupation in 10 ppm apholate. Mitotic chromosomes were studied from squash preparations of larval brains stained with acetolactic orcein.

Apholate induced numerous chromosomal aberrations by virtue of its ability to break and physiologically modify chromosomes. Among the chromosomal aberrations observed were stickiness, deletions, ring chromosomes, dicentric chromosomes and anaphase bridges. Chromosome breakage appeared to be frequently induced in the region of the secondary constriction in one of the larger chromosomes. Also, treatment with apholate resulted in the induction of somatic tetraploidy in some brain cells. It may also interfere with normal replication of chromosomes.

Explanations for the induction of these aberrations have been discussed.

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