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Professor

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October 20, 1988

Dr. Hector P. Garcia
Founder-American GI Forum
1315 Bright
Corpus Christi, Texas 78405
ATTN: GILBERT JASSO

Dear Dr. Hector and Mr. Jasso:

Enclosed please find the study which you requested on mosquitos and the viruses they carry.

We are still waiting for the study on hepititis conducted at Thomason Hospital in El Paso. It will be mailed to you as soon as we recieve it. If we can be of further assistance please do not hesitate to contact us.

Sincerely,

Karen

Karen Campbell
Legislative Assistant

/kc

P.O. Box 2910, Austin, Texas 78769 512-463-0462 District 34

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October 17, 1988

COLLEGE OF SCIENCES AND ENGINEERING
Division of Life Sciences

Mr. Jerry Trevino
P.O. Box 2910
Austin, Texas 78769

Dear Mr. Trevino:

Enclosed is a summary of the research my students and I are conducting with mosquitoes. We have been studying the sensitivity of chemical mutagens in mosquitoes; however, our focus at present is on the "Genetics of Filarial Development" in the mosquito. Our mosquito model is Aedes aegypti and the filarial nematode is the human pathogen, Brugia malayi. Filarial nematodes are the causative agents of the human disease called filariasis.

Our research is funded by the National Institutes of Health (NIH) through the Minority Biomedical Research Support (MBRS) Program. Current funding is $75,000 per year until 1991. The Program and my own project also provide funding and support for students, undergraduates as well as graduates.

Included also are some articles about our work which have appeared in newspapers and the University of Texas alummus magazine, *Sombrilla*. Perhaps this is what you were referring to during our phone conversation.

The reprints or scientific articles which deal with our work may also be helpful. Representative publications are included.

If you need any other materials, please let us know. I can be reached at (512) 691-5484 or our NIH/MBRS Office at (512) 691-4172.

Sincerely,

Paul H. Rodriguez

Paul H. Rodriguez, Ph.D.
Professor of Genetics
and MBRS/MARC Program
Director at UTSA

PHR:sb

SUMMARY OF RESEARCH INTERESTS

Genetic Control of Filarial Development

PAUL H. RODRIGUEZ, PH.D.
PROFESSOR OF GENETICS
DIVISION OF LIFE SCIENCES
THE UNIVERSITY OF TEXAS AT SAN ANTONIO

Studies will be conducted on the genetic control of filarial susceptibility and sensitivity of Brugia malayi to chemical mutagens. Specific susceptible and refractory genotypes of Aedes aegypti mosquitoes will be used, and various alkylating agents, including ethyl methanesulfonate (EMS), nitrosoguanidine, thiotepa, and triethylenemelamine (TEM) will be tested. The Jird, Meriones unguiculatus, will serve as the source of filarial infections for the mosquito susceptibility studies. Jirds will also be used to test the effects of chemical mutagens on infectivity.

Specific and major objectives will include (1) the heritable effects of chemical-mutagen-induced changes in the f^m gene for filarial susceptibility in known susceptible mosquito genotypes, (2) the molecular influences or changes associated with the presence or absence of the f^m susceptible gene, (3) the sensitivity of filarial nematodes to chemical mutagen stress in susceptible mosquito genotypes, and (4) biochemical and developmental effects of filarial infectivity subsequent to chemical mutagen exposure.

Mosquito pupae and adults will be exposed to various chemical mutagens. Viable adults derived from the different treated stages will be cross-mated with subsequent backcrosses and intercrosses. Genetic effects will be measured by biochemical assays for specific lysosomal enzymes and filarial infection experiments to test for chemical-induced changes in the f^m gene. Female susceptible mosquitoes will be exposed to chemical mutagens before and after filarial infections to determine the sensitivity of filariae to chemical mutagen stress. Specific comparative lysosomal enzyme assays and electrophoretic characterizations will be made to detect molecular changes associated with the f^m gene. Infected and non-infected susceptible and refractory genotypes will be tested at various time intervals. Infectivity changes in the definitive host will be measured by biochemical assays, the presence of circulating microfilariae, and post-mortem examinations of Jirds for adult nematodes.

Significantly, more information will be developed on the effects of chemical mutagen stress on a host-parasite system. Likewise, additional and new information will be gained on the genetic control of filarial susceptibility in vector hosts as well as the development of such pathogens in vertebrate hosts. Characterization of specific and non-specific proteins, including lysosomal enzymes, associated with the presence or absence of the f^m gene could lead to good and reliable molecular genetic marker(s) for filarial susceptibility. Subsequently, our laboratory will use protein markers as probes to generate useful plasmid or phage vectors and mosquito DNA libraries. Eventually, studies such as these could even lead to the identification of mosquito DNA encoding for filarial susceptibility. Likewise, knowledge gained from the infectivity studies could be valuable by improving the treatment of filariasis or perhaps by gaining a better insight into host defense mechanisms. Also, as more is learned about chemical-altered changes in filarids and molecular infectivity-induced changes in the vertebrate host, better biochemical diagnostic procedures to detect filariasis in humans and other animals could be established.

SOMBRILLO

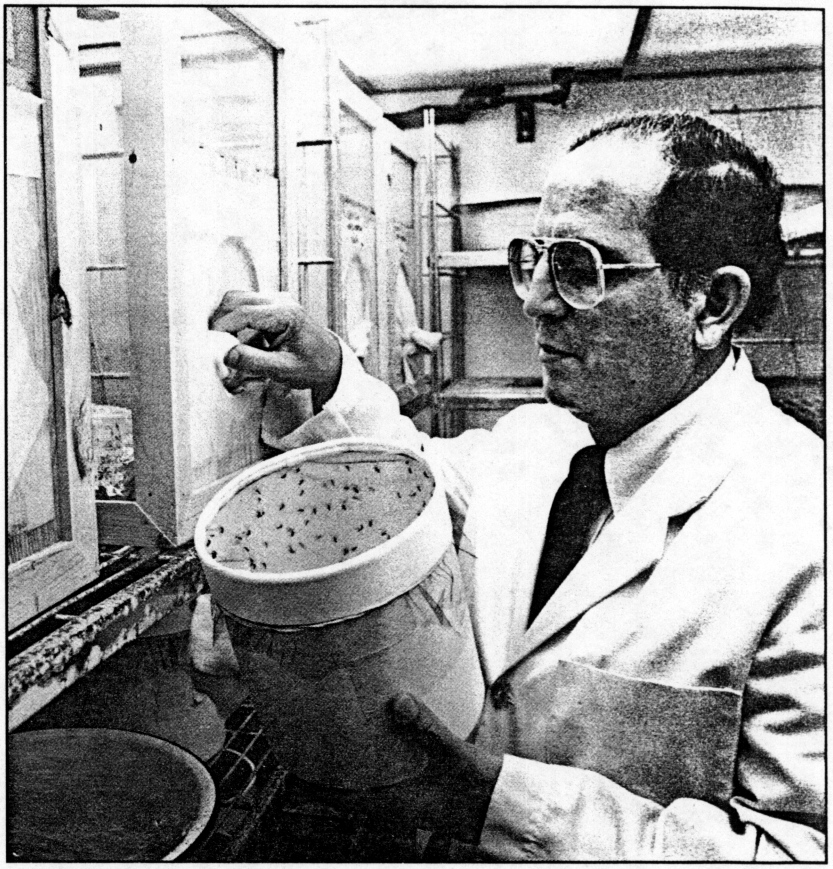
★ MAGAZINE OF THE UNIVERSITY OF TEXAS AT SAN ANTONIO

Summer '86

Investing in the Future

LAB NOTES

Mosquito Research Fights Disease



In the incubator, Dr. Paul Rodriguez exposes a female colony of mosquitoes to ethyl methane-sulfonate.

Mosquitoes, those pesky detractors from spring's arrival, are carriers of lethal diseases in many parts of the world. But promising research at UTSA might reduce the health risk of mosquito bites.

Dr. Paul H. Rodriguez, professor of genetics, and his students are breeding mosquitoes resistant to diseases and are studying the biochemical-molecular genetic mechanisms for the transmission of mosquito-borne diseases in the hope of developing better control measures.

As Rodriguez explained, only the female mosquitoes bite, but their potential damage is extensive. In humans, that bite can transmit elephantiasis, encephalitis, malaria and yellow fever. In dogs, mosquitoes are responsible for heartworms.

Rodriguez's work is focusing on the mosquito's transmission of elephantiasis which is caused by filarial nematodes. The insect picks up the tiny parasite by biting an infected host and transmitting it to another human or animal with the next bite.

Dr. Rodriguez and colleagues have recently experimented with filarial susceptible mosquitoes by treating them with chemicals to test for changes in the gene which permit development of the filarial parasite.

To prevent the mosquito from picking up the initial parasite Rodriguez treated the females with chemicals before they bit a diseased animal.

In a walk-in incubator, Rodriguez exposed a colony of females to a nontoxic chemical called ethyl methane-sulfonate. The females then mated with normal males and the first and second generation offspring were tested for susceptibility to the nematode. All mosquitoes were analyzed for particular enzymes and proteins which indicate the level of resistance the mosquitoes have developed to the microfilaria.

"We showed that with an increase of the concentration of the EMS chemicals we had a decrease in filarial susceptibility," said Rodriguez.

Selective breeding of the mosquitoes exposed to the EMS chemical has produced a second generation with 64 percent of the surviving offspring resistant to the elephantiasis-causing parasite.

The success of Rodriguez's experiment is important to the millions of victims of this disfiguring disease and could lead to ways of creating genetically resistant mosquitoes for other diseases.

"If we can determine the basic genetic mechanisms for the transmission of filariasis, maybe some of these basic principles could be applicable to malaria, viruses or other similar insect-borne diseases," said Rodriguez.

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REDUCED PRODUCTIVITY IN TRETAMINE-TREATED ADULT POPULATIONS OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE)[1]

Paul H. Rodriguez and Cynthia D. McCreless[2]

Abstract. Male and female *Aedes aegypti* mosquitoes of the feral strain PUGU were given orally 0.001, 0.025, 0.050, and 0.075% triethylenemelamine (tretamine or TEM). Productivity, or the average number of progeny surviving in a population over a 2-wk period, was used to assess mutagenic effects. The mean number of progeny decreased in all but 1 of the experimental populations. Populations in which the parental females were treated with TEM approximated a linear decrease in the total mean number of F_1 progeny as dosage of TEM increased. Progeny derived from TEM-treated parental males showed a slight decrease in number in the 0.001% group and a significant decrease in the 0.025 and 0.075% populations. Doses of 0.050% TEM induced complete sterility in males.

The mutagenic effects of triethylenemelamine [2,4,6-tris(1-aziridinyl)-*s*-triazine; tretamine or TEM] and other alkylating agents in various organisms have been reported by several investigators. Relatively few studies, however, have been conducted on mosquitoes. Also, few investigators have been concerned with the quantitative effects produced by such agents in mosquito populations.

TEM has been shown to induce sterility, developmental changes, and chromosomal damage in various mammals (Jackson et al. 1958, Lyon & Smith 1971, Cox & Lyon 1975). Several investigators have shown that TEM induces recessive lethals and chromosomal translocations in *Drosophila melanogaster* Meigen (Herskowitz 1955, 1956, Ratnayake et al. 1967). Also, TEM has been reported to induce dominant lethals in *Musca domestica* L. (North 1967) and to be an effective chemosterilant for the screwworm, *Cochliomyia hominivorax* (Coquerel) (Crystal & LaChance 1962, Crystal 1964). Specifically, dominant lethals were induced in 3-day-old male house flies by injection, and in 1-day-old screwworm adults by either topical application or by feeding.

Esparza & Rodriguez (1975) reported the mutagenic effects of thiotepa on the productivity of *Aedes aegypti* (L.) males in a heterozygous laboratory strain (ROCK). Oral treatments of 0.025, 0.050, and 0.075% thiotepa induced sterility in males. Also, the number of F_2 offspring derived from parental males treated with 0.001% of the chemomutagen was significantly reduced. Further investigations (Rodriguez & Torres, unpubl. data) have indicated that the number of F_2 offspring derived from male pupae treated directly with 0.0025, 0.005, and 0.025% thiotepa is affected significantly. Thompson & Rodriguez (1979) described the effects of ethyl methanesulfonate (EMS) and thiotepa on eggs, pupae, and adult populations of *Ae. aegypti.* Treatment with EMS did not significantly reduce egg production, percent hatch, or the total number of surviving F_1 adults when administered at any of the 3 stages. Thiotepa, however, induced sterility in adults and eggs treated with 0.050 and 0.075%. Also, significant reductions in total mean F_1 progeny were found when pupae were treated with 0.050, 0.075, and 0.10% thiotepa. That same year, Rodriguez & De La Peña (1979) published information on the quantitative effects of TEM on development of male pupae. A pupal dip method was used and significant reductions in total F_1 progeny were demonstrated for all concentrations of TEM.

The present investigation was undertaken to determine the effects of TEM given to male and female adults derived from heterozygous populations of *Ae. aegypti.* Quantitative estimates, often used to measure genetic fitness and similar to those of Crenshaw (1965), Esparza & Rodriguez (1975), and Thompson & Rodriguez (1979), were employed. Specifically, productivity, or the average number of adult F_1 progeny surviving in a population per unit time, was used to assess the mutagenic effects of the trifunctional alkylating agent.

MATERIALS AND METHODS

All test populations originated from the same PUGU-strain colony of *Aedes aegypti.* The strain was obtained from Professor George B. Craig, Jr, Director of the W.H.O. International Reference Centre for *Aedes,* University of Notre Dame. As indicated by Rodriguez & Craig (1973), this strain was initially collected as larvae from tree holes in

[1] This study was supported in part by grant number RR-08194 from the General Research Support Branch, Division of Research Resources, National Institutes of Health, USA.

[2] Division of Life Sciences, The University of Texas at San Antonio, San Antonio, Texas 78285, USA.

TABLE 1. Total number of F_1 progeny produced over a 14-day test period after adult ♂ *Aedes aegypti* were fed TEM and mated to untreated virgin ♀♀.

% CONCEN-TRATION	SEX OF PROGENY	TOTAL NO. PROGENY	TOTAL MEAN PRODUCTIVITY ± SE
0	♂	568	94.67 ± 43.85
	♀	607	101.17 ± 46.28
0.001	♂	598	99.67 ± 4.81
	♀	561	93.50 ± 3.90
0.025	♂	10	1.67 ± 0.42
	♀	13	2.17 ± 0.87
0.050	♂	0	0.00 ± 0.00
	♀	0	0.00 ± 0.00
0.075	♂	37	6.17 ± 3.71
	♀	29	4.83 ± 3.12

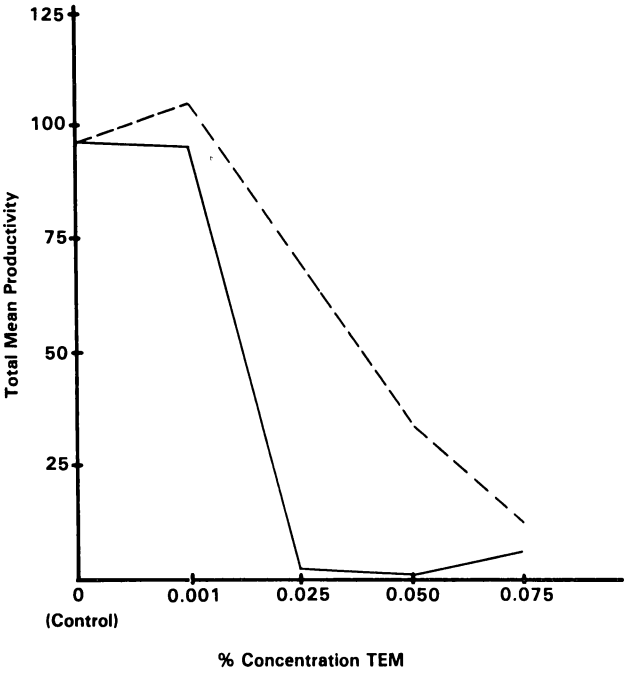

FIG. 1. Relationship between percent concentration of TEM and total mean productivity of F_1 *Aedes aegypti* (PUGU), ♂ and ♀ progeny inclusive. Solid lines indicate groups in which ♂ parents were treated orally with TEM; broken lines indicate populations in which ♀ parents were exposed to the chemomutagen.

0.001% concentration of TEM showed a slight increase in total number of F_1 progeny produced over 14 days (Table 1 and 2). Specifically, the populations in which the parental females were treated with the chemomutagen approximated a linear decrease in the total mean number of F_1 male and female progeny as the concentration of TEM was increased (Fig. 1). The F_1 progeny derived from TEM-treated parental males showed a slight decrease in the 0.001% group and a significant decrease in the 0.025% populations. At 0.050%, however, complete sterility was induced. Similarly, few F_1 progeny were produced in the 0.075% group (Fig. 1).

A factorial analysis of variance test indicated that the effect on productivity owing to the concentrations of TEM was statistically significant ($F = 9.339$, $P < 0.05$). However, neither the observed differences between the male and female progeny ($F = 0.003$, $P > 0.05$) nor the interaction of the sexes × treatments proved to be significant ($F = 0.007$, $P > 0.05$).

TABLE 2. Total number of F_1 progeny produced over a 14-day test period after adult ♀ *Aedes aegypti* were fed TEM and mated to untreated ♂♂.

% CONCEN-TRATION	SEX OF PROGENY	TOTAL NO. PROGENY	TOTAL MEAN PRODUCTIVITY ± SE
0	♂	568	94.67 ± 43.85
	♀	607	101.17 ± 46.28
0.001	♂	676	112.67 ± 36.81
	♀	591	98.50 ± 27.00
0.025	♂	400	66.67 ± 23.68
	♀	439	73.17 ± 26.37
0.050	♂	201	33.50 ± 12.89
	♀	202	33.67 ± 13.34
0.075	♂	61	10.71 ± 3.98
	♀	81	13.50 ± 5.74

A Student-Newman-Keuls' multiple range test (Steel & Torrie 1960) was employed to analyze the specific differences between the means of F_1 progeny for each test group. Accordingly, several significant differences were shown. The 0.001% treatment group in which the parental females had been exposed demonstrated the greatest mean value of 105.58 F_1 progeny per culture. This mean was significantly different from the means of the 0.025, 0.050, and 0.075% groups in which the parental males were treated, and from the means of the 0.025 and 0.050% groups in which the parental females were treated. The control group, which showed the 2nd highest mean value per culture (97.92), and the 0.001% population, in which the parental males were exposed to TEM (96.59), showed significant differences when compared to the 0.001% group in which the female parents were treated (105.58). The 0.025, 0.050, and 0.075% treatment groups in which the parental females were exposed showed a significant decrease in productivity when compared to the 0.001% treatment group of parental females.

the Pugu forest, 32 km west of Dar Es Salaam, Tanzania, East Africa, in December 1968.

Mosquitoes were reared according to methods outlined by Craig & VandeHey (1962) and Esparza & Rodriguez (1975) in a walk-in environmental chamber (Environmentor, Calumet Scientific, Inc., Elk Grove Village, IL) at a temperature of 27 ± 1 °C and 85 ± 10% RH. All eggs were conditioned in the incubator 24 h before hatching in 475-ml (1-pt) cardboard containers with deoxygenated water (ca. 375 ml of distilled water plus 5 ml of a solution of liver powder, Nutritional Biochemical Corp., Cleveland, OH).

Two days after hatching, the larvae were transferred to round, white enamel pans (General Housewares Corp., Terre Haute, IN) containing 2 litres of tap water and 15 ml of liver powder solution. Special care was taken to prevent overcrowding by allowing no more than 200 larvae per pan. Pans were covered with a clear polyethylene plastic (Glad Wrap) and secured to prevent contamination of the cultures. Pupation generally occurred 5–6 days after hatching. Pupae were sexed according to size (females are larger than males) and transferred to cages constructed from 3.8-litre (1-gal) paper cartons. Adults were fed on sugar cubes and water.

Three-day-old male and female mosquitoes were treated orally with tretamine (TEM) by allowing them to feed freely for 5 days on sugar cubes containing a given amount of the chemomutagen. The nitrogenous chemical was supplied by Dr E.R. Soares, North Carolina University Memorial Hospital, Chapel Hill, North Carolina. Four different concentrations of TEM were used: 0.001, 0.025, 0.050, and 0.075%. The highest concentration (0.075%) was initially prepared by dissolving 37.5 mg of TEM in 50 ml of deionized water. The remaining 3 concentrations (0.001, 0.025, and 0.050%) were made by dilutions of the 0.075% solution using the concentration vs. volume relationship ($C_1V_1 = C_2V_2$).

Approximately 0.25 cm^3 of the chemical solution was applied to each of 4 sugar cubes for a total of 1 cm^3 per experimental cage. One group of 60 males and another group of 60 females were exposed to 0.001% TEM. Two other cultures of the same number of males and females were exposed to 0.025%. Four other identical cultures were treated with concentrations of 0.050 and 0.075%, respectively. One group of 60 male and 60 female mosquitoes (controls) received no exposure to TEM.

After treatment with TEM, 9 groups of 20 pair matings each (including a control) were established in 3.8-litre (1-gal) mosquito cages. All treated males were mated to untreated virgin females. Conversely, all treated virgin females were mated to untreated males. All matings were replicated once.

To ensure maximum egg production in all matings, female mosquitoes were given 2 blood meals prior to mating and at 3-day intervals after mating using anesthetized mice (Nembutal, Abbott Laboratories, Chicago, IL, diluted to a 10% solution). Females were allowed to deposit eggs in 148-ml (5-oz) oviposition cups, which were lined with a double thickness of paper toweling and had a central moistened cotton plug to prevent desiccation. Eggs were collected at 4- and 3-day intervals. Also, a new oviposition cup was provided after each egg collection and dead adults were counted and removed. Numbers of live F_1 adult males and females produced during a 14-day period were recorded and analyzed statistically.

RESULTS

Mortality for parental males and females that were treated orally with TEM was variable. About 13% of the control males survived the 14-day test period and the survival of all of the test groups was approximately the same as that of the control groups. Mortality in the parental females, however, was significantly different from that of the parental males. Survival of the control female population was approximately 75%, while 89% of the females exposed to the 0.001% concentration of TEM survived. Females exposed to 0.025% TEM showed little difference from the control groups, as indicated by 73% survival. Viability of females treated with the 0.050 and 0.075% TEM differed greatly from the control groups, as they showed 98 and 93% survival, respectively.

All parental males and females in this study were about 22 days old by the end of the 14-day test period. The poor survival of control males is explained by the longevity of *Ae. aegypti* males, since under normal circumstances the PUGU-strain males have a mean life expectancy of 8.11 days. The mean life expectancy of females of the same strain was 24.18 days (Crovello and Hacker 1972).

Based on total number of progeny surviving over a 14-day test period (total productivity), the mean number of F_1 progeny (♂ + ♀) decreased in all but 1 of the experimental populations. When compared to the control populations, the group in which the female parents had been treated orally with a

DISCUSSION

Dominant lethal mutations believed to be associated mainly with with chromosomal breakage have been used for several years to measure the mutagenic effects of various chemical agents and ionizing radiation (Bateman 1958, Barety & Freese 1960, Partington & Bateman 1964). Soares (1972) and Soares & Crenshaw (1974) have estimated the frequency of induced dominant lethal mutations in mice by uterine dissection. With insect systems, several investigators have reported the induction of sterilization (Fahmy & Fahmy 1955, 1961, Bertram 1963, Amirkhanian 1972, Grover et al. 1972). The results obtained in the present study substantiated those of previous investigations and indicate that dominant lethal mutations induced by TEM reduced reproductive potential in *Aedes aegypti.* Simultaneously, administration of TEM might have induced lethal physiological or morphological abnormalities during oogenesis.

Exposure to various concentrations of TEM produced a general decrease in numbers of F_1 progeny in all but 1 of the experimental groups. However, the survival rates of the parents (male and female) that were treated orally with the alkylating agent were equal to or greater than those of the controls. This observation agrees with the experimental data obtained by Weidhaas et al. (1961), which showed that when mosquitoes of both sexes were exposed to different concentrations of the alkylating agent apholate, survival was normal for concentrations of 0.01 to 0.5%.

Numbers of F_1 progeny derived from males that had been treated orally with TEM showed significant decreases from the control at the 0.025, 0.050, and 0.075% concentrations. Complete sterility was induced at the 0.050% concentration, and very few progeny were produced by the 0.025 and 0.075% groups. Esparza & Rodriguez (1975) obtained similar results when male mosquitoes were treated orally with 0.025, 0.050, and 0.075% thiotepa. However, the 0.001% group produced almost the same number of F_1 progeny as did the control population. Auerbach (1967) reported that various alkylating agents may have a delayed mutagenic effect.

Numbers of F_1 progeny derived from females treated orally with TEM approximated a linear decrease compared to control population progeny as the concentration of TEM increased (Fig. 1). The 0.050 and 0.075% treatment groups showed a significant decrease from the controls, while the 0.001% group showed a slight but nonsignificant increase over the controls. The 0.025% group also showed a decrease in productivity, which proved to be not significant. Studies with apholate on female mosquitoes have shown that this chemosterilant acts on the ovaries alone, and that the early formation of follicles is particularly susceptible to the action of the alkylating agent. Moreover, a follicle beyond this stage may possibly proceed with uninterrupted development (Rai 1964). Perhaps, as with the male experimental groups, the lower concentrations may not have had enough time for any dominant lethal mutations to show up in the eggs. The higher concentrations, however, were probably strong enough to affect even the mature gonadal cells.

Productivity as used in this study was a compounded measure of adult fecundity, egg fertility, and progeny viability (Crenshaw 1965, Esparza & Rodriguez 1975, Thompson & Rodriguez 1979). However, this did not permit a direct analysis of TEM effects on fecundity. Recent experiments in our laboratory with low concentrations of thiotepa (*N,N',N''*-triethylene-thiophosphoramide) on *Aedes aegypti* specifically showed that this trifunctional alkylating agent affects egg production. Fewer eggs were produced and both percent hatchability and productivity were significantly decreased in F_1 progeny derived from surviving females treated orally and exposed directly during the pupal stage to 0.001, 0.0025, 0.005, and 0.01% thiotepa (Larson & Rodriguez, unpubl. data). By contrast, egg production in female mosquitoes was not reduced when they were mated with males exposed to similar concentrations of thiotepa during the same developmental stages (Rodriguez, Marotta & Rodriguez, unpubl. data). Accordingly, TEM probably also had a direct effect on fecundity when administered to *Ae. aegypti* female adults in the present study.

Acknowledgments. We are grateful to Prof. George B. Craig, Jr, Director, W.H.O. International Reference Centre for *Aedes,* University of Notre Dame, for supplying the PUGU strain of *Ae. aegypti.* We also wish to thank Dr Eugene R. Soares, North Carolina University Memorial Hospital, Chapel Hill, North Carolina, for supplying the TEM, and Mrs Karen P. Hoskins for typing the manuscript.

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Comparative Development of *Brugia malayi* in Susceptible and Refractory Genotypes of *Aedes aegypti*

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Relatively few papers have been published on the comparative development of filarids in specific genotypes of a vector host. Esslinger (1962, Am. J. trop. Med. Hyg. **11:** 749–758) reported on the behavior of microfilariae during the first 60 min of *Brugia pahangi* infections in *Anopheles quadrimaculatus.* In 1962 Schacher (J. Parasit. **48:** 679–692) also published detailed information on the distinctive morphology of *B. pahangi* microfilariae and other larval stages in both *An. quadrimaculatus* and *Amerigeres obturbans.* Comparative migration of microfilariae and subsequent behavior and development of *B. patei* in *Mansonia uniformis* (Laurence and Pester, 1961, J. Helminth. **35:** 285–300) and *B. pahangi* in various mosquitoes (Ewert, 1965, Am. J. trop. Med. Hyg. **14:** 254–259) have also been demonstrated.

The present paper reports basic quantitative information on the comparative development of *B. malayi* larvae in susceptible (BLACK EYE) and refractory (ROCK) genotypes of *Aedes aegypti.* Both genotypes were previously tested with *B. pahangi.* The BLACK EYE strain was shown as 95% susceptible (Rodriguez, 1973, J. Med. Ent. **10:** 194–197); whereas, ROCK was refractory to development of third-stage or L_3 larvae (Rodriguez and Craig, 1973, Am. J. trop. Med. Hyg. **22:** 53–61). Accordingly, 200 3-day-old female mosquitoes of either strain were fed simultaneously on 2 jirds, *Meriones unguiculatus,* having a mean microfilarial density of 243 per 20 mm^3 of blood. Fully engorged mosquitoes were then placed in an incubator maintained at 27 C with a RH of 80 ± 10%. Twenty-five mosquitoes from each strain were dissected 2 hr after infection. Also, 6 other comparable samples of 25 mosquitoes each were dissected at 24-hr intervals over a period of 5 days and at 8 days post-infection. Mosquitoes were dissected initially under a stereoscopic microscope in *Aedes* physiological saline (Hayes, 1953, J. Econ. Ent. **46:** 624–627) and 1–2 drops of dilute Giemsa, then terminated and analyzed under a binocular compound microscope. During dissection each female was anesthetized with chloroform and specifically separated into head, thorax and abdomen using dissecting needles. Individual parts were then carefully teased and analyzed. The number of microfilariae (mf) and subsequently active developing larvae (days 1–8) per female mosquito and strain were recorded.

The susceptible genotype (BLACK EYE) maintained an overall infection of 88% throughout the 8-day period. The refractory genotype (ROCK) was 44% over the same period, decreased by 36% on the third day after infection (2 hr = 88%; 3 days = 52%), and gave 0% susceptibility at 8 days. Both genotypes showed a decrease in mean number of larvae per female. However, the refractory genotype had a significantly greater or 23-fold decrease by day 3, with continuing decreases to 0.4 and 0 mean larvae at days 5 and 8, respectively (Table I). By comparison the susceptible genotype only decreased 4-fold by day 3 ($t = 3.006$, $P < 0.001$) and

TABLE I. *Variation in development of larvae of Brugia malayi in susceptible (BLACK EYE) and refractory (ROCK) genotypes of Aedes aegypti tested at various time intervals.*

Strain	Time interval	Total no. larvae	Mean larvae per female ± SE
BLACK EYE	2 hr	590	29.5 ± 7.02
	1 day	460	19.3 ± 7.12
	2 days	425	17.0 ± 7.46
	3 days	164	6.8 ± 1.44
	4 days	222	6.9 ± 1.99
	5 days	208	8.0 ± 1.67
	8 days	69	3.9 ± 0.57
ROCK	2 hr	961	38.4 ± 9.20
	1 day	604	25.9 ± 9.38
	2 days	44	1.8 ± 0.41
	3 days	36	1.7 ± 0.77
	4 days	0	0.1 ± 0.11
	5 days	6	0.4 ± 0.22
	8 days	0	0 ± 0

showed a significantly greater number of larvae than the refractory genotype at days 5 and 8 (t = 4.536, $P < 0.001$ at 5 days; ROCK = 0 L_3's at 8 days).

Our results indicate that a susceptible mosquito genotype will maintain a higher infection (88%) and support a greater number of larvae (overall mean = 13.06) than a refractory genotype (44% susceptible; mean larvae = 9.76). Both genotypes initially show high infections (BLACK EYE = 100% susceptible; ROCK = 88%). However, significant differences in filarial development occur 2–3 days post-infection, with the refractory genotype demonstrating a much greater decrease in susceptibility and number of developing larvae. Eight days post-infection several larvae will approach complete development in a susceptible genotype. By contrast, most larvae are eliminated by the fifth day in a refractory genotype (Table I). Similar changes in filarial development were also obtained by Morison (1973, M.S. Thesis, Univ. Texas Schl. Publ. Hlth, Houston, Texas) and by Rodriguez et al. (unpubl. data) when specific susceptible and refractory genotypes of *Ae. aegypti* were infected with *B. pahangi*.

The authors thank Prof. George B. Craig, Jr., Director of the WHO International Reference Centre for *Aedes*, University of Notre Dame, for supplying the BLACK EYE and ROCK strains. The strain of *B. malayi* was from infected jirds obtained from Dr. John McCall, University of Georgia through the U.S.-Japan Co-operative Medical Science Program-NIAID. This study was supported in part by NIH Research Grant No. RR-08194-04.

with radiation dose but decreased in proportion to the stage of development reached.

This investigation attempted to determine the differential effects of a monofunctional alkylating agent (EMS) and a trifunctional alkylating agent (thiotepa) on 3 unique stages in the development of the PUGU strain of *Ae. aegypti*. Two principal lines of inquiry were followed. First, the differential chemosensitivity of eggs, pupae and adults were investigated. Secondly, the comparative developmental effects of the 2 (a monofunctional and a trifunctional) alkylating agents were studied.

MATERIALS AND METHODS

All test populations originated from the same colony of a strain of *Aedes aegypti* designated the PUGU strain. It is a heterozygous geographic stock and was obtained from Professor George B. Craig, Jr, Director of the WHO International Reference Centre for *Aedes,* University of Notre Dame. As reported by Rodriguez & Craig (1973), this strain was initially collected as larvae from tree holes in the Pugu Forest, 32 km W of Dar Es Salaam, Tanzania, East Africa, in December 1968.

Mosquitoes were reared according to methods outlined by Craig & VandeHey (1962) and Esparza & Rodriguez (1975) in both a reach-in incubator (Scientific Systems, Baton Rouge, La.) and a walk-in environmental chamber (Environator, Calumet Scientific, Inc., Elk Grove Village, Ill.) at a temperature of 27 ± 1°C and 85 ± 10% RH. Eggs were hatched in 475-ml (1-pint) cardboard containers with deoxygenated water—approximately 375 ml of distilled water plus 5 ml of a solution of liver powder (Nutritional Biochemical Corp., Cleveland, Ohio).

Two or 3 days after hatching, the larvae were transferred to round white enamel pans (General Housewares Corp., Terre Haute, Ind.) containing 2 liters of tap water and 5–10 ml of liver powder solution. Heavy populations of larvae were given additional water and liver medium to minimize the effects of overcrowding. The pans were covered with a clear polyethylene plastic (Glad Wrap) and secured to prevent any contamination of the cultures. Pupation generally occurred 5–7 days after hatching, and the pupae were separated according to sex. Mosquito cages, constructed from 3.8-liter (1-gal.) paper cartons, were used to rear the adults. The adults were fed on sugar cubes and water.

One hundred mosquito eggs, 100 pupae, and 100 adults were exposed to various doses of the alkylating agents ethyl methanesulfonate (EMS) and thiotepa (*N, N′, N″*-triethylene thiophosphoramide). EMS is a monofunctional alkylating agent and was obtained from Sigma Laboratories, St. Louis, Mo.; thiotepa is a trifunctional chemomutagen which was supplied by Lederle Laboratories, Pearl River, N.Y. Eggs and male and female pupae were exposed to 0.025%, 0.050%, 0.075% and 0.10% concentrations of EMS or thiotepa. These concentrations are comparable to 10, 20, 30, and 40 parts per million, respectively. The highest concentration (0.10%) of either chemical was initially prepared by dissolving 30 mg of thiotepa or 0.3 ml of EMS in 30 ml of deionized water. The remaining 3 concentrations (0.025%, 0.050%, and 0.075%) were made by dilutions from either of the 0.10% solutions using the concentrations vs volume relationship ($C_1V_1 = C_2V_2$).

Exposure of eggs or pupae was accomplished by placing 10 ml of the appropriate chemical concentration in a 475-ml (1-pint) carton with 235 ml of water and 5 ml of liver medium. The actual treatment was administered for 24 h. Subsequently, the eggs or pupae were removed by filtering through Whatman #4 filter paper and were transferred to rearing pans containing 2 liters of tap water and 5–10 ml of liver powder mixture. One group of eggs and 1 culture of pupae (controls) received no exposure to either EMS or thiotepa. Various matings, derived from those individual eggs or pupae surviving chemical exposure, were established in either 1.9-liter (1/2-gal.) or 3.8-liter (1-gal.) mosquito cages.

One hundred male and 100 female adult mosquitoes were treated orally by allowing them to feed freely for 5 days on sugar cubes containing a given concentration of either of the 2 chemomutagens. Again, 4 different concentrations were utilized, 0.025%, 0.050%, 0.075%, and 0.10%. Approximately 0.25 ml of each of the chemical solutions was applied to each of 4 sugar cubes for a total of 1 ml per experimental cage. One group of male and female mosquitoes received no treatment (controls). Each culture consisted of 25 pairs of mosquitoes. All matings were done in duplicate. To maximize egg production in all experimental groups, the females were offered a blood meal using anesthetized mice (Nembutal, Abbott Laboratories, Chicago, Ill., diluted to a 10% solution). The mosquitoes were fed twice a week and eggs were collected at 7-day intervals for a period of 3 weeks

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DEVELOPMENTAL EFFECTS OF ETHYL METHANESULFONATE AND THIOTEPA IN *AEDES AEGYPTI* (DIPTERA: CULICIDAE)[1]

By **Patricia A. Thompson and Paul H. Rodriguez**[2]

Abstract: The effects of various concentrations of a monofunctional alkylating agent, ethyl methanesulfonate (EMS), and a trifunctional alkylating agent, thiotepa, on 3 stages in the development of the PUGU strain of *Aedes aegypti* were tested. Eggs, pupae, and adult populations were treated with 0.025%, 0.050%, 0.075%, and 0.10% concentrations of the chemomutagens. Viable adults derived from the treated eggs and pupae were cross-mated, as were the treated adults. Egg production, percentage hatchability, viability, and productivity in the F_1 generation were employed to measure the developmental effects. Treatment with EMS did not significantly reduce egg production, percentage hatchability, or total productivity when administered at any of the 3 stages. However, thiotepa induced sterility at all concentrations when male and female adults were treated. Sterility was induced in eggs treated with thiotepa at concentrations of 0.050% and 0.075%; a significant decrease in productivity was shown at both 0.025% and 0.10% thiotepa. Significant reductions in total mean F_1 progeny were found at the 0.050%, 0.075%, and 0.10% concentrations of thiotepa when the pupae were treated. Overall, EMS was less effective in altering the life cycle of *Ae. aegypti* than was thiotepa.

Although several species of insects such as the pomace fly, *Drosophila melanogaster,* or the screwworm, *Cochliomyia hominivorax,* have been utilized to study the effects of chemomutagens on diploid systems (Alderson 1965, Cattanach et al. 1968, Crystal 1964, 1971, Fahmy & Fahmy 1955, 1961), relatively few chemomutagen studies have been conducted on mosquitoes. Also, few investigators have been concerned with the chemosensitivity of various stages in the development of mosquitoes.

Alkylating agents are known chemomutagens (Auerbach 1967) and have been reported to induce sterility. Such chemomutagens have also been shown to affect productivity and development in various insects (Vogel 1972, Esparza & Rodriguez 1975). Crystal (1963), for example, induced sterility in male screwworm flies using several chemosterilants, including thiotepa. Working with *Aedes aegypti,* Rai (1964) found numerous chromosomal abberations caused by apholate, a polyfunctional alkylating agent which can inhibit DNA and lactic dehydrogenase synthesis (Fishbein et al. 1970). Apholate also produced sterility in adult male and female *Ae. aegypti* in concentrations as low as 0.10% (Weidhaas et al. 1961). Bertram (1963) used thiotepa to induce sterilization in both *Anopheles gambiae* and *Ae. aegypti.*

Recently, treatment of adult males of *Ae. aegypti* with thiotepa was shown to induce sterility at concentrations of 0.025%, 0.050%, and 0.075% (Esparza & Rodriguez 1975). Other investigations (Rodriguez, unpubl. data) indicate that the productivity of F_2 offspring derived from parental females treated with 0.001% and 0.025% thiotepa is also affected significantly.

Ethyl methanesulfonate (EMS) has caused dominant lethal mutations in various organisms. Epler (1966) found significant numbers of complete lethals and mosaic lethals after injecting male flies with doses of EMS ranging from 0.01 to 0.04M. A similar study by Jenkins (1967) also revealed induced mosaic and complete "dumpy" mutants in *Drosophila*. A study by Lim & Snyder (1968) detected only a single translocation in more than 2000 EMS-treated gametes. Induced dominant lethals have also been reported in mice when males were treated orally with EMS (Cattanach et al. 1968, Soares & Crenshaw 1974).

Apparently, the effect of chemomutagen treatment is dependent on the stage of development at which the chemical is administered. Both somatic cell and chromosomal damage, for example, were induced with apholate when the 2nd-instar larvae of *Ae. aegypti* were treated (Rai 1964). Sharma et al. (1973) treated male pupae of *Culex pipiens fatigans* with thiotepa to induce sterility. Crystal (1964) treated adults, prepupae, and pupae of the screwworm with alkylating agents and found sterility induced when prepupae were immersed in 10% thiotepa for 30 s and when pupae were immersed in 10% thiotepa for 120 s. Sterility was induced in adults treated orally with 0.50% thiotepa.

Recently, Asman & Rai (1972) published a comprehensive study on the radio-sensitivity of all stages in the development of *Ae. aegypti.* The first 3 larval instars were most highly sensitive due to their mitotic activity. Mutational damage increased

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TABLE 3. Total productivity of adult *Aedes aegypti* (PUGU) when treated orally with EMS and thiotepa.

% CONCENTRATION	TOTAL NO. PROGENY	TOTAL MEAN PRODUCTIVITY ± SE
0	488	81.3 ± 58.6
0.025 EMS	1468	244.7 ± 77.5
0.050 EMS	1492	248.7 ± 69.6
0.075 EMS	1247	207.8 ± 74.5
0.10 EMS	1744	290.7 ± 113.1
0.025 thiotepa	0	—
0.050 thiotepa	1	0.16 ± 0.16
0.075 thiotepa	0	—
0.10 thiotepa	0	—

tration, no statistically significant differences were obtained. The control group produced a mean of 153.7 F_1 progeny. The 0.025%, 0.050%, 0.075%, and 0.10% EMS-treated populations produced 84.3, 36.2, 58.3 and 61.1 F_1 offspring, respectively.

However, a significant decrease in mean productivity was found when the pupae were treated with concentrations of 0.025%, 0.050%, 0.075%, and 0.10% thiotepa. The total egg production of the 0.075% thiotepa-treated populations was also significantly lower than that of other groups ($F = 8.30$, $P < 0.01$).

Eighty-six to 98% of the pupae treated with thiotepa emerged. The number of eggs deposited in the control group was 2027 as compared with 1789, 2195, 817 and 1837 for 0.025%, 0.050%, 0.075% and 0.10% thiotepa-treated groups, respectively. Except for the control which showed a 48.4% hatch and the group treated with 0.025% thiotepa (hatch = 40.7%), the number of pupae recovered was 20% or less of the total number of eggs produced.

Mean productivity for the control populations was 153.7, while the groups treated with 0.025%, 0.050%, 0.075% and 0.10% thiotepa had 107.8, 35.0, 24.0 and 27.2 mean F_1 progeny, respectively (TABLE 2). A monofactorial analysis of variance test showed that the total productivity of all groups was significantly different ($F = 5.48$, $P < 0.01$). A Student-Newman-Keul's multiple range test (Steel & Torrie 1960) showed significant decreases in total mean progeny between the control and all the treated groups except the 0.025% thiotepa-treated population.

Effects of adult exposure

Treatment of male and female adult mosquitoes with 4 different doses of EMS gave no significant differences in either total egg production or total mean F_1 progeny. In the control population, 34% of the 1458 eggs hatched. The groups treated with EMS had an average percentage hatch of 50.5%. The control group had a mean productivity of 81.3 while the treated populations (0.025%, 0.050%, 0.075% and 0.10% EMS) had means of 244.4, 248.7, 207.9 and 290.7, respectively. TABLE 3 contains a summary of the data with regard to total mean F_1 progeny.

Sexual sterility was induced by all doses of thiotepa when orally administered to both male and female adult mosquitoes. Of the 57 eggs produced in the group treated with 0.050% thiotepa, only 1 pupa was recovered. In the other groups (0.025%, 0.075% and 0.10% thiotepa-treated), 80, 301, 113 eggs were produced. However, none of them hatched (TABLE 3). High mortality occurred in all groups of treated adults during the 2nd and 3rd weeks.

DISCUSSION

Differential chemosensitivity

The Bergonie-Tribondeau law states that the radiosensitivity of cells is in direct proportion to their dividing activity (Kaufman 1954). In the development of *Ae. aegypti,* it would be expected that the developing embryo and other larval instars would be highly susceptible to radiation damage, while pupal and adult stages would be less sensitive. An investigation by Asman & Rai (1972) found this to be the case. The present study attempted to apply this relationship to chemosensitivity. However, no discernable trend was observed with either EMS or thiotepa treatment at the egg, pupal or adult stage.

Treatment with the monofunctional chemomutagen EMS did not significantly reduce egg production, percentage hatchability, or total productivity when administered at any of the 3 stages (FIG. 1). By contrast, thiotepa significantly reduced productivity at all 3 stages. When compared to the adults and eggs, the pupae seemed to be the most chemoresistant (FIG. 2).

Eggs hatched in this study were at least 2 weeks old, thus, fully embryonated. After a 24-h treatment with EMS, there was no reduction in the number of eggs hatched or in the number of viable emerging adults. Asman & Rai (1972) also found that older embryonated eggs were more resistant than any of the larval instars. No doubt delayed hatching reduces the sensitivity of the eggs due to the quiescent state of the cells. However, treatment with thiotepa did cause a significant reduction in viability.

TABLE 1. Total productivity of adult *Aedes aegypti* (PUGU) derived from eggs treated with EMS and thiotepa.

% CONCENTRATION	TOTAL NO. PROGENY	TOTAL MEAN PRODUCTIVITY ± SE
0	446	74.3 ± 14.3
0.025 EMS	723	120.5 ± 33.2
0.050 EMS	750	125.0 ± 28.2
0.075 EMS	295	49.1 ± 18.9
0.10 EMS	575	95.8 ± 30.7
0.025 thiotepa	87	14.5 ± 8.6
0.050 thiotepa	0	—
0.075 thiotepa	0	—
0.10 thiotepa	139	23.2 ± 12.2

TABLE 2. Total productivity of adult *Aedes aegypti* (PUGU) derived from pupae treated with EMS and thiotepa.

% CONCENTRATION	TOTAL NO. PROGENY	TOTAL MEAN PRODUCTIVITY ± SE
0	922	153.7 ± 47.6
0.025 EMS	506	84.3 ± 30.2
0.050 EMS	217	36.2 ± 9.9
0.075 EMS	350	58.3 ± 42.9
0.10 EMS	370	61.7 ± 14.8
0.025 thiotepa	647	107.8 ± 31.5
0.050 thiotepa	210	35.0 ± 25.5
0.075 thiotepa	144	24.0 ± 9.4
0.10 thiotepa	163	27.2 ± 6.5

following the 1st blood meal. Numbers of eggs laid, pupae (male and female) and viable adults (male and female) were recorded for each group.

RESULTS

Effects of egg exposure

Aedes aegypti eggs treated with 4 different doses of EMS (0.025%, 0.050%, 0.075%, and 0.10%) showed no significant reduction in either egg production or total productivity. The treated eggs hatched normally. The control group had a 50% hatch while the EMS-treated eggs showed a 49% to 58% hatch. Ninety percent of the adults were recovered from these cultures and were mated as mentioned previously. The control group produced a total of 917 eggs, whereas the populations exposed to 0.025%, 0.050%, 0.075% and 0.10% concentrations of EMS produced 1327, 1311, 672, and 1102 eggs, respectively. The percentage hatch for the control and experimental populations ranged from 49.5% to 61.1%. The percentage hatch was determined by the number of viable pupae collected over a period of 4–8 days posthatch.

Based on an analysis of total mean productivity over a period of 3 weeks, the mean number of male and female F_1 progeny showed variable results. The control group produced a mean of 74.3 F_1 progeny per population. In contrast, both the 0.025% and 0.050% EMS-treated groups showed higher means of 120.5 and 125.0. The 0.075% and 0.10% EMS-treated populations produced means of 49.1 and 95.8 F_1 progeny (TABLE 1). An analysis of variance, however, showed no significant differences between the groups ($F = 1.73$, $P > 0.05$).

Sexual sterility was induced by treatment of the eggs with 0.050% and 0.075% thiotepa. Concentrations of 0.025% and 0.10% thiotepa produced significant reductions of both egg production ($F = 5.60$, $P < 0.01$) and the total mean number of F_1 progeny ($F = 11.50$, $P < 0.01$). Eggs treated with thiotepa had a percentage hatch ranging from 25% to 42%, in contrast to the control, which had a 50% hatch. More than 90% of the pupae emerged in the control and 0.025%-treated populations. For the 0.050%, 0.075%, and 0.10% thiotepa-treated groups, less than 50% of the pupae became viable adults.

A representative group of the surviving adults was mated and those treated with 0.075% thiotepa produced no eggs over a 3-week period. The 0.050% thiotepa-treated group produced 6 eggs, none of which hatched. The 0.025% and 0.10% thiotepa-treated populations produced 329 eggs and 526 eggs, respectively. Approximately 28% of the eggs from these groups hatched, whereas the control had 49.5% hatch. Based on total productivity for a 3-week period, the populations treated with 0.025% and 0.10% thiotepa showed a significant decrease in the mean number of F_1 progeny ($F = 11.50$, $P < 0.01$). The control groups had a mean of 74.2 offspring; the 0.025% and 0.10%-treated populations had means of 14.5 and 23.2 offspring (TABLE 1).

Effects of pupal exposure

Treatment of mosquito pupae with the 4 doses of EMS yielded no significant deviation from the control. Even after a 24-h treatment with EMS, more than 90% of the pupae in all populations emerged and became viable adults. Matings within each population produced the following counts of eggs: control, 2027; 0.025% EMS-treated, 1797; 0.050% EMS-treated, 851; 0.075% EMS-treated, 1402; and 0.10% EMS-treated, 1047. Forty-eight percent of the control eggs hatched, while percentage hatchability in the EMS-treated groups ranged from 26% to 40%. Although the number of F_1 progeny derived from pupae treated with EMS decreased with an increase in EMS concen-

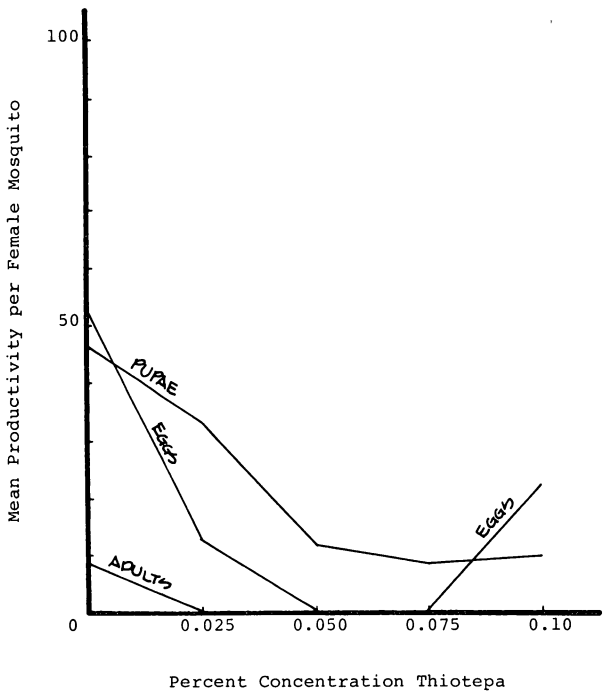

FIG. 2. Comparison of the mean productivity per ♀ mosquito of 3 developmental stages of *Aedes aegypti* treated with 4 concentrations of thiotepa.

Comparative chemomutagen effects

Evidence from cytological studies regarding the induction of dominant lethals supports the observation that an increase in the number of functional groups of a chemomutagen yields a proportionate increase in the number of gross chromosomal changes. Since the degree of lethality correlates directly with chromosomal damage, those chemomutagens producing greater numbers of gross chromosomal mutations should yield proportionately greater damage in an experimental organism. Fahmy & Fahmy (1961) showed that the presence of 2 active groups favored the induction of a higher number of complete breaks, as well as a higher frequency of small deficiency recessive lethals, when compared with the monofunctional analogue. A more comprehensive study (using mono-, di-, tri-, and tetrafunctional alkylating agents) demonstrated that the frequency of dominant lethals increased with the number of functional groups present in the mutagen (Vogel 1972). In contradistinction, monofunctional agents produced few small deletions but a high number of mosaic mutations, which were expressed in subsequent generations (Snyder & Oster 1964, Alderson 1965).

The number of transitional changes and possible depurinations of DNA caused by the concentrations of EMS used (0.025%, 0.050%, 0.075%, and 0.10%) was probably not sufficient to induce visible mutagenic abnormalities. Thiotepa, however, was apparently able to cross-link DNA strands (primarily in germinal cells), which caused substantial chromosomal breakage with subsequent decrease in fertility and fecundity.

The stage of development reached has been shown to be an important factor in determining the sensitivity of *Ae. aegypti* to mutagenic agents. The current study failed to support that conclusion when differential chemomutagen tests were performed on eggs, pupae, and adults. A significant difference was recorded, however, between the action of EMS and thiotepa. These results strongly reinforce the observation that there is a major qualitative difference between the effects of monofunctional and polyfunctional alkylating chemicals (Lim & Snyder 1968). Expanding the investigation of chemosensitivity to include the larval instars of this mosquito, as well as higher dosages of EMS, could probably yield additional positive results.

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Apparently, somatic cell damage induced by thiotepa at the egg stage caused a decrease in hatchability. Of those pupae that did develop, less than 50% emerged. When the surviving adults were mated, egg production was either significantly decreased or, as in the case of treatment with 0.075% thiotepa, totally absent. Radiation given in earlier stages also produced marked effects in the reproductive potential of this organism (Asman & Rai 1972). Eggs which did escape lethal somatic damage showed minimal productivity or sterility. One replicate of 0.10% thiotepa was responsible for all the offspring in that group and probably escaped lethal mutations due to an environmental influence. It is doubtful that this resistance was induced by the mutagen. Both somatic and germinal cell damage occurred when the embryonated egg was treated with thiotepa.

Treatment of male and female pupae with EMS had no effect on development. Matings of viable adults showed reduced egg production as well as a decrease in mean F_1 progeny, but the differences were not statistically significant. Perhaps higher doses of this chemomutagen would have induced visible effects, including chromosomal damage.

The pupal stage of several insects has been shown to be resistant to somatic damage but susceptible to lethal germinal mutations. Sterility, for example, was induced in male *Culex pipiens fatigans* by exposing male pupae to 0.6% thiotepa for 3 h, but sterility was not accompanied by reduced vigor of the adults (Sharma et al. 1973). Grover et al. (1972) reported an absence of significant damage in the testes of young adult mosquitoes, *Cx. p. fatigans,* after pupal treatment with 3580 ppm tepa. This was probably due to decreased mitotic activity during the advanced stages of spermatogenesis.

Failure of EMS to affect the production of offspring in *Ae. aegypti* was probably due to the low concentrations that were administered. EMS-induced dominant lethals were reported by several investigators in both *Drosophila* (Fahmy & Fahmy 1961, Alderson 1965, Epler 1966, Jenkins 1967) and mice (Cattanach et al. 1968, Soares & Crenshaw 1974). However, a study by Vogel (1972) showed that 2000 μm of the monofunctional A_{137} (2-ethyleneimino-5,6,7,8-tetrahydronaphthoquione-1,4) was ineffective in producing dominant lethals in mature and immature oocytes of *Drosophila melanogaster.* In addition, only a single translocation was detected in a study of over 2000 EMS-treated gametes of *Drosophila* (Lim & Snyder 1968). Perhaps future studies on EMS-treated adults should incorporate the effects of recessive lethal mutations, mosaics, and chromosomal damage in the F_2 and subsequent generations.

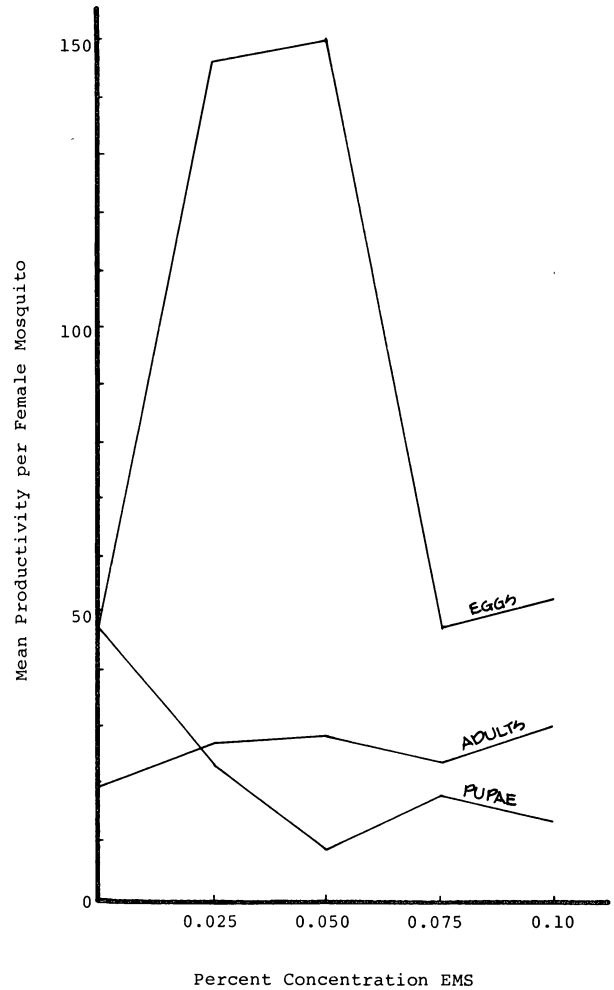

FIG. 1. Comparison of mean productivity per ♀ mosquito of 3 developmental stages of *Aedes aegypti* treated with 4 concentrations of EMS.

In the present study, thiotepa treatment of adults induced sterility. Fecundity was also reduced. These data support various studies in which sterility was induced in insect populations using different polyfunctional alkylating agents (Weidhaas et al. 1961, Bertram 1963, Rai 1964, Esparza & Rodriguez 1975). In general, it required higher doses of the chemomutagen to produce sterility in the female mosquito than in the male. Matings in which both male and female mosquitoes were treated have produced even greater reduction in fecundity and fertility (Rai 1964). This compound effect would account for the complete sterility found at even the lowest dose (0.025% thiotepa) in the present study.

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J. Med. Entomol. Vol. 14, no. 4: 493–494 24 December 1977

EFFECTS ON THE PRODUCTIVITY OF IRRADIATED MALE POPULATIONS OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE)[1]

Although several species of insects such as *Drosophila melanogaster* or *Habrobracon juglandis* have been utilized to study the effects of ionizing radiation on diploid systems (Grosch, 1962, Annu. Rev. Entomol. **7:** 89–106), relatively few radiation studies have been conducted on mosquitoes. Also, few investigators have been concerned with the genetic effects on fitness produced by ionizing radiation in mosquito populations. In 1953, Terzian (J. Immunol. **72:** 202–06) demonstrated that X rays can induce mortality in *Aedes aegypti*. Subsequently, Terzian & Stabler (1958, Biol. Bull. **115:** 536–50) showed that gamma radiation can affect fertility and fecundity in this species of mosquito. Irradiation has also been used to induce mutations (VandeHey & Craig, 1959, Bull. Entomol. Soc. Am. **5:** 113) as well as sterility in *Ae. aegypti* and other mosquitoes (McCray, Jenson & Schoof, 1961, Proc. N. J. Mosq. Ext. Assoc. 48th Annu. Meet., p. 110–15; Morlan, McCray & Kilpatrick, 1962, Mosq. News **22:** 295–300; Weidhaas, Schmidt & Seabrook, 1962, Mosq. News **22:** 283–91). Cytogenetic damage (Rai, 1963, Caryologia **16:** 595–607), radiation-induced translocations (McDonald & Rai, 1970, Science **168:** 1229–30; Rai, McDonald & Asman, 1970, Genetics **66:** 635–51; Lorimer, Hallinan & Rai, 1972, J. Hered. **63:** 159–66), and inversions (McGivern & Rai, 1972, J. Hered. **63:** 247–55) have also been reported in *Ae. aegypti*. Recently, Asman & Rai (1972, J. Med. Entomol. **9:** 468–78) showed that ionizing radiation can cause developmental changes in this mosquito.

The present paper reports the genetic effects on fitness of gamma radiation given to male adults derived from heterozygous populations. Quantitative techniques, such as those of Crenshaw (1965, Science **149:** 426–27), were employed. Productivity or the average number of progeny born to heterozygous carriers of irradiated chromosomes per unit time was used to assess the radiation effects. All test populations originated from a wild-type laboratory strain (ROCK) of *Ae. aegypti*, which was obtained from Prof. George B. Craig, Jr, Director of the W.H.O. International Reference Center for *Aedes*, University of Notre Dame. Mosquitoes were reared according to methods published by Craig & VandeHey (1962, Ann. Entomol. Soc. Am. **55:** 47–58) and Esparza & Rodriguez (1975, J. Hered. **66:** 172–74) in an environmental chamber (Scientific Systems Model 148) with a temperature of 27 ± 1°C and 85% ± 10% RH.

In preparation for irradiation, pupae were isolated and sexed, and the males which emerged were aged for 2 days. These were randomly alloted to 5 groups of 75 each, and then placed in plastic vials (100 mm × 30 mm) lined with a small piece of wet paper toweling. Three of the groups were irradiated, but with different unfractionated doses of 1000, 3000, and 5000 rads at 54.73 rads per min.; the other 2 groups were kept as untreated controls. The radiation source was a Picker reactor containing cobalt-60, located at the Bexar County Hospital in San Antonio, Texas. After irradiation, 5 groups of 20-pair matings (including 2 controls) were established in 1-gal. (3.8-liter) mosquito cages. All the treated and untreated males were mated to untreated females. Subsequently, the F_1 male progeny of the 2nd untreated control group were backcrossed to the F_1 female progeny of all the other groups (1 untreated, 3 treated). Again, each culture consisted of 20 pairs of animals and all matings were replicated once. To ensure maximum egg production, female mosquitoes were given blood meals using anesthetized mice (Nembutol, Abbott, diluted to 10%) at 3-day intervals. The number of live F_2 male and female adults produced during a 3-week test period was recorded.

TABLE 1. Total productivity over a 3-week test period of F_2 *Aedes aegypti* (ROCK) derived from ♀♀ heterozygous for irradiated chromosomes.

Radiation dose (rads)	Sex	Total no. progeny	Total mean productivity ± SE
0	♂♂	4059	338.25 ± 50.60
	♀♀	3912	326.00 ± 42.90
1000	♂♂	3320	276.67 ± 41.28
	♀♀	3201	266.75 ± 29.62
3000	♂♂	3558	296.50 ± 43.02
	♀♀	3140	261.67 ± 37.32
5000	♂♂	2725	227.08 ± 31.13
	♀♀	2551	212.58 ± 24.65

Based on total productivity for a 3-week test period, the mean number of F_2 progeny showed a general decrease with increased radiation exposure (TABLE 1). A factorial analysis of variance test indicated that the effect in productivity due to treatments or radiation dose was statistically significant (df 3,88; $F=2.868$; $P<0.05$). However, the observed differences between male and female groups were not significant statistically (df 1,88; $F=0.434$; $P>0.05$). A Student-Newman-Keuls multiple range test (Sokal & Rohlf, 1969, *Biometry*, W. H. Freeman & Co., San Francisco, 776 p.) showed that only populations in which parental males were exposed to 5000 rads gave a significant decrease in productivity when compared to the control population. At this level of gamma radiation,

TABLE 2. Mean productivity during a 3-week period of F_2 *Aedes aegypti* (ROCK) derived from ♀♀ heterozygous for irradiated chromosomes, ♂♂ and ♀♀ of F_2 progeny inclusive.

Treatment	Mean weekly productivity ± SE		
	Week 1	Week 2	Week 3
Control	446.38 ± 66.11	299.25 ± 43.00	250.75 ± 34.24
1000 rads	378.75 ± 39.07	269.75 ± 23.29	166.63 ± 28.19
3000 rads	289.25 ± 38.08	354.00 ± 44.54	194.00 ± 49.67
5000 rads	244.88 ± 37.28	258.63 ± 17.72	156.00 ± 33.71

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only a mean of 219.83 F_2 males and females per culture was produced. By comparison, the control groups produced a total mean of 332.13 F_2 progeny per culture. Populations in which parental male mosquitoes were exposed to 1000 rads and 3000 rads produced a total mean of 271.71 and 279.08 male and female F_2 offspring, respectively.

Mortality was high for the F_1 females having an irradiation background of 5000 rads. Of the 40 females used in the backcross populations, only 24, or 60%, survived after 3 weeks. The control populations, however, showed a viability rate 13% higher than that of the 5000-rad group. Seventy-three percent of the mosquitoes in the 1000-rad and 3000-rad groups also survived the testing period.

TABLE 2 summarizes the mean weekly productivity of the F_2 progeny. Although an increase in productivity was observed for the 3000-rad and 5000-rad populations from week 1 to week 2, a higher mean number of F_2 offspring were recorded for the control and other experimental groups during the 1st-week period. Analysis of the data, however, indicates that significant differences between the 5000-rad experimental and control populations occurred during this same 1-week period. The control culture gave a mean of 446.38 offspring as compared to a mean of 244.88 for the 5000-rad population ($t=2.655$, $P<0.05$). Other 2-sample t-tests (Sokal & Rohlf, 1969, loc. cit.) demonstrated that the observed differences in productivity among the 1000-rad and 3000-rad groups for each week were not significant statistically when compared to the control population ($P>0.05$ in all cases; $t=0.881$ and 2.060, $t=0.603$ and 0.884, $t=1.897$ and 0.941, respectively, for weeks 1, 2 and 3).

Productivity of F_2 offspring derived from parental males exposed to 5000 rads of gamma radiation showed a significant decrease in comparison with the controls. Exposure of parental males to 1000 and 3000 rads reduced productivity but not significantly so when compared statistically with the control population. These observations indicate that polygenic mutations at doses of 5000 rads reduced fitness or reproductive potential in *Ae. aegypti*. Alternatively, reproductive potential could have been reduced by an increased number of chromosomal aberrations induced at this level of gamma radiation. Lorimer et al. (1972, loc. cit.) have reported the isolation and identification of 2 reciprocal translocation homozygotes by exposing young *Ae. aegypti* males to doses of 1800 and 3500 rads of gamma irradiation. The fact that the 5000-rad F_1 population displayed a higher mortality rate than did the others could also be correlated with a reduction in F_2 productivity. Also, high doses of radiation stress could affect the longevity of *Ae. aegypti* males. Under normal circumstances the ROCK strain females have been shown to have a mean lifetime of 21.91 days; the mean expected lifetime for males of the same strain was 6.77 days (Crovello & Hacker, 1972, Evolution **26:** 185–96).

I am grateful to Prof. George B. Craig, Jr for supplying the ROCK strain of *Ae. aegypti*. I also wish to thank Dr Robert G. Waggner for his assistance during the radiation processes.—**Paul H. Rodriguez,** Division of Allied Health and Life Sciences, The University of Texas at San Antonio, San Antonio, Texas 78285, U.S.A.

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DOMINANT LETHAL EFFECTS OF THIOTEPA IN MALE *AEDES AEGYPTI* (DIPTERA: CULICIDAE)[1]

Abstract. The chemosterilizing effects of low concentrations of thiotepa on *Aedes aegypti* were tested. Three-day-old males were treated with 0.001, 0.0025, and 0.005% thiotepa by allowing them to feed freely for 5 days on sugar cubes containing the chemical mutagen. Egg production, percent hatchability, and productivity (average number of adult progeny surviving over 1 week) were used to assess mutagenic effects. Although egg production was higher in thiotepa-treated groups, the effects were not statistically significant when compared to untreated populations. By contrast, hatchability and the mean number of F_1 progeny were reduced at concentrations of 0.001 and 0.0025%. Male sterility was induced with 0.005% thiotepa.

Thiotepa (*N,N′,N″*-triethylenethiophosphoramide, Lederle Laboratories) has been used to study chemosterilant effects in several insects including the house fly, *Musca domestica* L. (La Brecque, Adcock & Smith, 1960, J. Econ. Entomol. **53:** 802–05; Painter & Kilgore, 1964, J. Econ. Entomol. **57:** 154–57), the screwworm, *Cochliomyia hominivorax* (Coq.) (Crystal, 1963, J. Econ. Entomol. **56:** 468–73), and the mosquitoes *Anopheles gambiae* Giles and *Aedes aegypti* (L.) (Bertram, 1963, Trans. R. Soc. Trop. Med. Hyg. **57:** 322–35). Esparza & Rodriguez (1975, J. Hered. **66:** 172–74) reported the effects of thiotepa on productivity in male *Ae. aegypti* of a heterozygous laboratory strain (ROCK). Oral treatments of 0.025, 0.050, and 0.075% thiotepa induced sterility in males. Moreover, the number of F_2 progeny derived from parental males treated with 0.001% of the chemical mutagen was significantly reduced. Thompson & Rodriguez (1979, J. Med. Entomol. **15:** 115–21) described the effects of thiotepa on eggs, pupae, and adults of *Ae. aegypti.* Thiotepa induced sterility in adults and infertility in eggs treated with 0.050 and 0.075%. In addition, significant reductions in total mean F_1 progeny were demonstrated when, using a pupal dip method (Rodriguez & De La Peña, 1979, J. Med. Entomol. **16:** 465–69), pupae were treated with 0.050, 0.075 and 0.10% of the chemical mutagen.

The present paper reports the detection of dominant lethals when adult male *Aedes aegypti* (ROCK) were treated orally with low levels of thiotepa, an alkylating agent. Quantitative estimates often used for genetic fitness effects, and similar to those of Crenshaw (1965, Science **149:** 426–27), Esparza & Rodriguez (1975, loc. cit.), and Thompson & Rodriguez (1979, loc. cit.), were employed. Egg production, percent hatchability, and the average number of male and female F_1 adults surviving in a population over 1 week (productivity) were used to assess the mutagenic effects of thiotepa. All test populations originated from the same colony of a wild-type laboratory strain (ROCK) of *Ae. aegypti,* which was obtained from Prof. George B. Craig, Jr, Director of the WHO International Reference Centre for *Aedes,* University of Notre Dame. Mosquitoes were reared according to methods described by Craig & VandeHey (1962, Ann. Entomol. Soc. Am. **55:** 47–48) and Esparza & Rodriguez (1975, loc. cit.) in a walk-in environmental chamber (Environator, Calumet Scientific, Inc., Elk Grove Village, IL) at a temperature of 27 ± 1 °C and 85 ± 10% RH.

In preparation for chemical exposure, pupae were isolated and sexed, and males that emerged were aged for 3 days. These were then randomly allotted to 4 groups of 50 each and placed in 3.8-litre (1-gal) paper-carton mosquito cages. Three of the groups were exposed to concentrations of 0.001, 0.0025, or 0.005% thiotepa by allowing them to feed freely for 5 days on sugar cubes containing a total of 1 ml of the chemical solution per experimental cage. One group, with sugar cubes containing 1 ml of deionized water as solvent, was kept as untreated controls. After treatment with thiotepa, 1 untreated and 3 treated groups of 10 mosquitoes of each sex were established in reusable 1.9-litre (½-gal) mosquito cages. All treated and untreated males were exposed to untreated virgin females. Also, all matings were repeated. To ensure maximum egg production, female mosquitoes were given 2 blood meals before mating and at 3-day intervals after mating using anesthetized mice (Nembutal, Abbott Laboratories, Chicago, IL, diluted to a 10% solution). The number of eggs produced, percent egg hatch, and the number of F_1 male and female adults surviving for 1 week were recorded.

Untreated females mated with treated males exhibited greater egg production. However, 2-sample *t*-tests (Sokal & Rohlf, 1969, *Biometry,* W.H. Freeman & Co., San Francisco, 776 p.) indicate that these differences are not significant. The control group laid 1012 eggs, while the 0.001 and 0.0025% groups produced 1562 and 1512 eggs, respectively ($t = 1.159$, $P > 0.05$; $t = 2.106$, $P > 0.05$). Although also not significant ($t = 2.562$, $P > 0.05$), the 0.005% group produced the highest number of eggs (1642). By contrast, percent hatchability was significantly reduced in all experimental populations. In the control population, 815 (81%) of the eggs hatched. The 0.001% group gave rise to 446 larvae, or 29% egg hatch; at 0.0025% only 99 larvae, or 7% hatch, were obtained. Sterility was induced at 0.005%, since no larvae (0% hatch) were produced. Based on total number of adults surviving (total productivity) for 1 week, the mean number of F_1 progeny decreased with increased concentration of thiotepa (Table 1). Mean productivity for the control population was 199.5, while the groups treated with 0.001, 0.0025, and 0.005% thiotepa had 103.0, 22.5, and 0 mean F_1 progeny, respectively. Two-sample *t*-tests (Sokal & Rohlf, 1969, loc. cit.; Ryan, Joiner &

[1] This study was supported in part by grant number RR-08194-04 from the General Research Support Branch, Division of Research Resources, National Institutes of Health.

TABLE 1. Total number of F_1 progeny produced over a 7-day test period after adult ♂ *Aedes aegypti* were fed thiotepa and mated to untreated virgin ♀♀.

% CONCENTRATION	SEX OF PROGENY	TOTAL NO. PROGENY	TOTAL MEAN PRODUCTIVITY* ± SE
0	♂	366	183 ± 71.01
	♀	432	216 ± 51.00
0.001	♂	226	113 ± 33.01
	♀	186	93 ± 19.00
0.0025	♂	54	27 ± 1.00
	♀	36	18 ± 0.00
0.005	♂	0	0
	♀	0	0

* Average number of F_1 adults surviving over 1 week.

Ryan, 1976, *Minitab student handbook,* Duxbury Press, North Scituate, MA, 341 p.) demonstrated that the observed differences were not significant at 0.001% ($t = 2.383$, $P = 0.055$) and were statistically significant at 0.0025% ($t = 4.779$, $P < 0.01$). Figure 1 summarizes the results and compares the effects of thiotepa as measured by the mean number of eggs, larvae, and adults (male and females) produced in the F_1 generation.

These observations indicate that dominant lethal mutations induced by low levels of thiotepa reduced reproductive potential in *Ae. aegypti.* Simultaneously, administration of thiotepa might have induced other lethal physiological or morphological abnormalities during spermatogenesis. In addition, lethals are manifest during larval and adult development (Fig. 1). Earlier studies (Esparza & Rodriguez, 1975, loc. cit.; Thompson & Rodriguez, 1979, loc. cit.) reported sterility and reduction of reproductive potential in *Ae. aegypti* ingesting 0.025, 0.050, and 0.075% thiotepa. Recently, Rodriguez & Torres (unpubl. data) showed that direct pupal exposure to 0.0025, 0.005, and 0.025% thiotepa induced sterility in males of this mosquito. Also, direct pupal exposure to 0.01% thiotepa still produced a significant decrease in reproductive potential in the 2nd generation. A 1-wk test period was used in experiments described herein because parental males and females were about 15 days old by the end of the study period. Moreover, a previous study on the effects of tretamine in *Ae. aegypti* adults (Rodriguez & McCreless, 1985, J. Med. Entomol. **22:** 38–42) gave poor survival of males by the end of a 14-day test period.

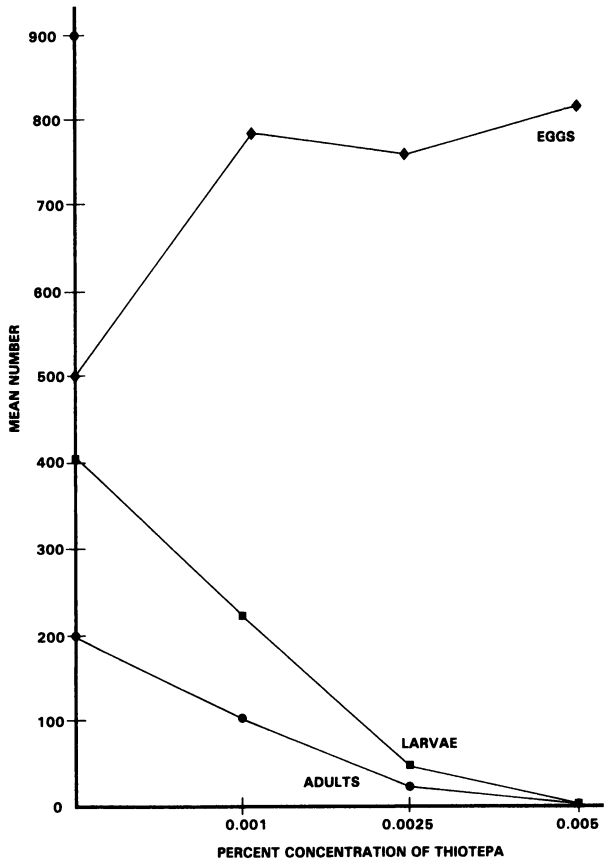

FIG. 1. Relationship between percent concentration of thiotepa and mean numbers of eggs, larvae, and adults (♂ and ♀) of F_1 *Aedes aegypti* (ROCK).

We are grateful to Prof. George B. Craig, Jr, for supplying the ROCK strain of *Ae. aegypti.* We also thank Mrs Karen P. Hoskins for typing the manuscript. The junior author submitted part of this work as a Science Fair Project at Pat Neff Middle School, San Antonio, Texas.—**Paul H. Rodriguez** and **Karl A. Rodriguez,** Division of Life Sciences, The University of Texas at San Antonio, San Antonio, Texas 78285, USA.

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DEVELOPMENTAL EFFECTS OF *BRUGIA PAHANGI* (NEMATODA: FILARIOIDEA) TO HIGH TEMPERATURE IN SUSCEPTIBLE GENOTYPES OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE)[1]

By **Paul H. Rodriguez**[2]

Abstract: The sensitivity of developing *Brugia pahangi* larvae to high temperature stress was tested in 3 known susceptible genotypes of *Aedes aegypti*. Three-day-old female mosquitoes were fed on infected jirds (*Meriones unguiculatus*), then subjected to 27° (control), 32°, and 37°C. Mosquitoes were dissected 8 to 10 days after infection and the number of active, fully developed 3rd-stage larvae per female mosquito was analyzed. All 3 strains demonstrated an average decrease of 17% in susceptibility when maintained at 37°C. Both the PUGU and SOFI strains were decreased by 34% and 11%, respectively. The BWAMBA strain gave a slight decrease of 7%. However, the mean number of infective larvae (L_3) per female supported at 37°C was reduced significantly. BWAMBA and PUGU displayed a 2-fold decrease in L_3; SOFI gave a 1-fold decrease, which was also significant ($t = 2.945$, $P<0.01$). Although both the BWAMBA and SOFI strains showed slight increases in susceptibility and mean L_3 at 32°C, these differences were not statistically significant when compared to the controls at 27°C.

Variation in the susceptibility of vectors of filariasis may be partially caused by environmental factors (Macdonald 1967, 1971). In 1967, Beam demonstrated that temperature influenced the development of *Dirofilaria immitis* (Leidy) in *Aedes sollicitans* (Walker). Brunhes (1969) showed that temperature affects the susceptibility to as well as the developmental rate of *Wuchereria bancrofti* (Cobbold) in both *Anopheles gambiae* Giles and *Culex pipiens fatigans* Wiedemann. The optimum temperature for the development of this filarid was 30°C. At 20°C, larvae did not become infective until the 27th day. El-Dine & Habib (1969), working with *C. pipiens*, also demonstrated that the susceptibility to and the developmental rate of *W. bancrofti* were dependent on temperature. At 18° to 22°C, microfilariae did not develop beyond the sausage stage throughout a 4-week period.

Rodriguez & Thompson (1974) have recently reported that high temperatures can affect the development of *Brugia pahangi* (Buckley & Edeson) in a highly susceptible laboratory strain (BLACK EYE) of *Aedes aegypti* (L.), originally derived from selection experiments (Macdonald 1962). Female mosquitoes maintained 8 to 10 days postinfection at 30° and 35°C showed a 20% decrease in susceptibility when compared to others placed in normal temperatures of 27°C. Likewise, the mean number of infective larvae per female supported at

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the higher temperatures was reduced significantly. Infected BLACK EYE mosquitoes placed in temperatures of 37°C gave similar but more significant results.

This paper presents additional information on the quantitative effects of high temperature stress on the development of *B. pahangi* in known susceptible genotypes of *A. aegypti*. Three geographic strains, previously shown to have the f^m factor for susceptibility to *B. pahangi*, were used, and the females were subjected to different temperatures following simultaneous infections.

MATERIALS AND METHODS

Clawed jirds, *Meriones unguiculatus*, infected with *Brugia pahangi* were obtained from the University of Georgia through the U.S.-Japan Cooperative Medical Science Program—NIAID. The strains of *Aedes aegypti* used were obtained from Professor George B. Craig, Jr., Director of the W.H.O. International Reference Centre for *Aedes*, University of Notre Dame. Their history and place of origin have been reported elsewhere (Rodriguez & Craig 1973).

Mosquitoes were reared according to methods outlined by Rodriguez & Craig (1973) and Craig & VandeHey (1962). Briefly, conditioned eggs were hatched using deoxygenated water. Larvae were cultured in round, white enamel pans (General Housewares Corp., Terre Haute, Ind.) containing 200 ml of water and fed on a solution of liver powder (Nutritional Biochemical Co.). Special attention was given to ensure that cultures were not crowded. Pupation generally occurred on days 5 and 6 after hatching. Adults, placed in gallon (3.8-liter) paper cages, were fed on sugar cubes and water.

Three-day-old female mosquitoes were starved for 24 hr and infected using jirds having a mean microfilarial density of 157 per 20 mm^3 of blood. The same 2 jirds were used in each of 3 separate feeding experiments. In each experiment, 350 to 375 female mosquitoes were exposed to infection. In the course of 2 replicates, 100 fully engorged females were selected, then placed in 1 of 3 incubators at 27° (controls), 32°, and 37°C, respectively, with relative humidities as high as 80% ± 10%. To minimize desiccation, 2 oviposition cups, having cotton balls and lined with paper toweling, were placed in each of the 6 cages used per strain or experiment.

Mosquitoes were dissected 8 to 10 days postinfection in 2 to 3 drops of *Aedes* physiological saline (Hayes 1953) under a stereoscopic microscope. The number of active, fully developed 3rd-stage larvae per female mosquito was recorded.

RESULTS

TABLE 1 summarizes results from 3 separate experiments wherein susceptible genotypes were infected, then subjected to different temperatures. Likewise, 3 different strains, BWAMBA, PUGU, and SOFI, were tested. A previous study (Rodriguez & Craig 1973) established that the f^m factor for susceptibility to *B. pahangi* had frequencies as high as 0.73, 0.61, and 0.57, respectively, for the 3 strains.

Mosquito mortality for all 3 strains was high at 37°C. Of the 325 females subjected to this temperature postinfection, only 228 or 70% survived. Some 84% survived at both 27° and 32°C. Of the 3 strains, BWAMBA showed a mortality rate about 13% higher than the other 2 strains at both 27° and 32°C. At 37°C, BWAMBA gave a higher mortality of 21%.

Females maintained at 37°C demonstrated an overall decrease of 17% in susceptibility (27°C = 43%; 32°C = 44%; and 37°C = 27%). However, both the PUGU and SOFI strains gave more marked decreases at 37°C. Susceptibility for PUGU was decreased by 34%; SOFI showed an 11% decrease. Both BWAMBA and SOFI displayed slight increased susceptibility rates at 32°C.

TABLE 2 shows variation in the development of infected, viable, 3rd-stage larvae when susceptible genotypes were maintained at different temperatures. The mean number of infective larvae per female supported at the higher temperatures was markedly reduced in all 3 strains. For both BWAMBA and PUGU, the mean number of infective larvae was twice as great at 27°C than at 37°C. Two-sample *t*-tests (Sokal & Rohlf 1969) indicate that these differences are significant ($t = 2.708$, $P<0.01$; $t = 4.947$, $P<0.001$, respectively, for

TABLE 1. Effects of temperature on the development of *Brugia pahangi* in susceptible genotypes of *Aedes aegypti* fed on jirds with an average 157 mf/20 mm^3 of blood.

Strain	Temperature (0°C)	♀♀ tested: No.	♀♀ tested: % mortality	♀♀ tested: % showing L^3
BWAMBA	27	95	22	41
	32	89	22	48
	37	71	43	38
PUGU	27	90	10	49
	32	96	4	44
	37	75	25	15
SOFI	27	94	6	38
	32	85	15	41
	37	82	18	27

TABLE 2. Variation in the development of infective larvae of *Brugia pahangi* in susceptible genotypes of *Aedes aegypti* subjected to different temperatures.

Strain	Temperature (0°C)	No. infective larvae per ♀	
		Range	Mean ± S.E.
BWAMBA	27	1–19	3.43 ± 0.591
	32	1–24	3.66 0.591
	37	1–11	1.61 0.320
PUGU	27	1–15	2.21 0.376
	32	1–20	2.18 0.372
	37	1–4	0.29 0.096
SOFI	27	1–19	1.65 0.322
	32	1–19	1.80 0.380
	37	1–8	0.60 0.153

BWAMBA & PUGU). At 37°C, the decrease for SOFI was 1-fold and also significant ($t = 2.945$, $P<0.01$). Although slight increases in the mean L_3 were obtained at 32°C for the BWAMBA and SOFI strains, these differences are not statistically significant ($P>0.05$ in both cases; $t = 0.275$, $t = 0.301$, respectively, for BWAMBA and SOFI).

DISCUSSION

Although various studies have been conducted on the influence of environmental temperature upon vertebrates and invertebrates (Prosser 1973), relatively few experiments have been reported on the effect of high temperature stress on the susceptibility and development of a parasite in a vector host. Most important among the latter are studies performed on cestodes. Voge & Turner (1956) reported that high temperature affects the development of cysticercoids of *Hymenolepis diminuta* in *Tribolium confusum*. Temperatures below 15°C and above 37°C proved to be unsuitable. Voge & Heyneman (1958) and Voge (1959) described structural abnormalities in these larvae when grown in confused flour beetles at supraoptimal temperatures of 38.5° and 40°C. Comparable temperature effects were demonstrated for the larval development of *H. nana* (Voge & Heyneman 1958) and *H. microstoma* (Voge 1963) in *Tribolium confusum*.

The results of the present work show that high temperature stress affects the development of *Brugia pahangi* in geographic strains of *Aedes aegypti* of known susceptible genotypes. These data support those of earlier publications which showed sensitivity of developing cysticercoids and microfilariae in vector hosts subjected to high temperature. Likewise, the effects of such an environmental stress (37°C at 8 to 10 days postinfection) are quantitatively similar to those reported by Rodriguez & Thompson (1974) for a laboratory-synthesized, susceptible strain. In both studies, percentage susceptibility and the number of 3rd-stage larvae supported per female were significantly reduced at 37°C.

Exposure to 32°C gave favorable results in the 3 strains tested. Susceptibility rates and the number of infective larvae per female were similar to those obtained for infected mosquito females maintained at 27°C (TABLE 1, 2). Although not statistically significant, the BWAMBA and SOFI strains showed slight increases in susceptibility and mean L_3. Based on these studies and those with the BLACK EYE strain (Rodriguez & Thompson 1974), it appears that favorable development of *B. pahangi* is accomplished at 27° to 32°C. Temperatures of 35°C and above may be unsuitable.

Variability in the development of *B. pahangi* in the 3 strains at 27° and 32°C could be attributed to differences in gene frequency for the f^m factor. These data support those of an earlier publication (Rodriguez & Craig 1973) which showed genetic variation in susceptibility of *A. aegypti* to *B. pahangi*.

The fact that the BWAMBA strain displayed a much higher mortality rate than the other strains could possibly be attributed in part to a change in microfilarial density. The same 2 jirds, originally with microfilarial densities of about 105 per 20 mm^3 of blood, were used throughout the study to keep environmental factors constant. Mean microfilarial densities for the PUGU and SOFI experiments were 110.5 and 97.0, respectively. For the BWAMBA experiment, however, a density of 257 mf per 20 mm^3 of blood was recorded.

The higher mortalities obtained at 37°C for all 3 strains were probably due to the lowered humidities produced at this temperature. Working with various strains of *Aedes aegypti*, Machado-Allison & Craig (1972) have shown that African strains of feral origin (subspecies *A. a. formosus* Walker) display low resistance to desiccation. Both BWAMBA and PUGU gave higher mortalities when exposed to decreased relative humidities. The SOFI strain, also of feral origin, was not tested for resistance to desiccation. Humidities were kept as high as 80 ± 10% and the oviposition cups were constantly refilled with water. The latter, however, lost moisture more rapidly at 37°C.

High temperature stress might have induced lethal physiological or morphological abnormalities in developing *B. pahangi* larvae. Various studies (Prosser 1973) have reported that high temperature is limiting or lethal by protein and lipid denaturation and by the acceleration of some reactions relative to others. As mentioned above, exposure to high temperature has been shown to induce structural

abnormalities in developing cestode larvae (Voge & Heyneman 1958, Voge 1959). Currently, other studies are being pursued to substantiate this hypothesis. Likewise, detailed experiments will be conducted on the effects of temperature on infectivity and development of *B. pahangi* in the vertebrate host.

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DIFFERENTIAL DEVELOPMENT OF *BRUGIA PAHANGI* IN LABORATORY STRAINS OF *AEDES AEGYPTI*[1]

By **Paul H. Rodríguez**[2]

Abstract: Six inbred lines and 7 marker strains of *Aedes aegypti* were tested for susceptibility to *Brugia pahangi*. Five-day-old female mosquitoes were fed on infected jirds, *Meriones unguiculatus;* 9 to 12 days after infection, mosquitoes were dissected. Mosquitoes were considered susceptible if they produced viable 3rd stage larvae. As a control, each strain was tested along with REFM, a synthetic laboratory stock where 43% of the females tested were susceptible. Nine of the 13 laboratory strains of *A. aegypti* were completely refractory. Among the refractory strains were 4 of the inbred lines and 5 of the marker stocks. A line inbred for 86 generations had a very low susceptibility rate (2%). However, a line which originated from Uganda showed 23% and 31% after 7 and 8 generations of inbreeding. Of the marker strains, 1 (PLS) was 7% susceptible; another (BLACK EYE) gave 95%. The former was derived from a line previously selected for refractoriness to *Plasmodium gallinaceum*, whereas the latter originated from a stock selected for filarial susceptibility.

Although Faust (1949) listed *Aedes aegypti* (L.) as a natural vector for *Wuchereria bancrofti* (Cobbold), this mosquito is generally not considered an important intermediate host for human filariasis. However, it has been infected in the laboratory with Bancroftian microfilariae (Henrard et al. 1946), and is frequently used as an experimental vector for various filariae (Kartman 1953, Schacher 1962, Nelson et al. 1962, Ash & Schacher 1971).

Research is currently being conducted in our laboratory on the genetic basis of filarial susceptibility in *A. aegypti*. *Brugia pahangi* (Buckley & Edeson) is being used as the filarioid model. As demonstrated by Ash & Riley (1970), the jird *Meriones unguiculatus* has proved to be an efficient vertebrate host for this nematode.

The present study was undertaken to establish more information on the development of filariae in different laboratory strains of *A. aegypti*. Several inbred stocks and genetic marker strains were tested for infections to *B. pahangi*.

MATERIALS AND METHODS

Jirds, *Meriones unguiculatus*, infected with *B. pahangi* were first obtained from Dr Paul E. Thompson (School of Veterinary Medicine, University of Georgia), through the U.S.—Japan Cooperative Medical Sciences Program. Thereafter, uninfected jirds, from 6 weeks to 3 months old and maintained in our laboratory, were infected with infective filarial larvae by techniques similar to those reported by Ash & Riley (1970).

The strains of *A. aegypti* used are listed in TABLE 1. These were obtained from the reference collection maintained by the W.H.O. International Reference Centre for *Aedes*, University of Notre Dame. Mosquitoes were reared according to the methods outlined by Rodríguez & Craig (1973) and Craig & VendeHey (1962).

Five-day-old female mosquitoes were starved for 24 hr and fed at random on infected jirds having microfilarial densities of 24 to 261 per 20 mm³ of blood. The jirds were anesthetized with 0.4 cc to 0.6 cc of 20% nebutal (Nembutal Sodium, Abbott Laboratories). Subsequent to infection, the female mosquitoes were supplied with apple and mated to a comparable number of males within 24 hr. The jirds' abdomens were shaved closely with animal clippers before each feeding. At the time of feedings, 20-mm³ blood smears were made by collecting blood from the jird's tail and stained with dilute Giemsa.

In each experiment, 150 female mosquitoes were exposed to infection in the course of 2 replicates with 75 each. From these, 100 engorged females were selected and held 9 to 12 days for dissection. The number of mosquitoes remaining alive after this period were dissected in 2–3 drops of *Aedes* physiological saline (Hayes 1953) with the aid of a stereoscopic microscope. The number of infective 3rd stage larvae per female mosquito was recorded. Mosquitoes were classified as susceptible if one or more active, fully developed 3rd stage larvae were present.

The various laboratory strains of mosquitoes were tested in 6 separate feeding experiments. The REFM strain (TABLE 1) was used as a control in each experiment. In most cases, 2 strains of mosquitoes were fed simultaneously on the same jird; 2 mosquito cages (gallon paper cartons) were placed in proximity with the jird overlapping each cage. The period of exposure was 45 min. and

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TABLE 1. Laboratory strains of *Aedes aegypti* used in *Brugia pahangi* susceptibility surveys.

	Strain	Place of origin	History
I.	*Inbred Strains**		
	BLPCO F_{38}	Laboratory synthesis	Originated from mutants black palp (*blp*) and compressed antenna (*co*), both isolated from a Ghana strain, GSAL. Inbred for 38 generations.
	BW F_7	Bwamba Co., Uganda	Originated from the BWAMBA strain. Inbred for 7 generations.
	BW F_8	Bwamba Co., Uganda	Originated from the BWAMBA strain. Inbred for 8 generations.
	GKEP F_{18}	Kpedome, Ghana	Originated from the GKEP strain. Inbred for 18 generations.
	NESC F_{86}	Ness Ziona, Israel	Originated from a laboratory colony; received from M. Bat-Miriam, Institute for Biological Research. Inbred for 86 generations, since 1960.
	RJB F_7	Laboratory synthesis	Originated from the ROCK strain, laboratory-reared at Rockefeller University over 25 yrs. Inbred for 7 generations.
	VN F_{20}	Saigon, Vietnam	Originated from the VIET-NAM strain. Inbred for 20 generations.
II.	*Marker Strains*		
	REFM	Laboratory synthesis	Received from W. W. Macdonald, Liverpool School of Tropical Medicine, England in 1965. Homozygous for red eye (*re*) & selected for filarial susceptibility (f^m).
	BLACK EYE	Laboratory synthesis	Received from R. Gwadz, School of Public Health, Harvard University in July 1971. Previously from A. R. Barr, UCLA. Originated from Liverpool School of Tropical Medicine; selected for filarial susceptibility (f^m) by W. W. Macdonald.
	BZ/WH	Laboratory synthesis	Contains bronze (*bz*) and white eye (*wh*) on linkage group 1.
	SMA WH/BZ	Laboratory synthesis	Contains bronze (*bz*), white eye (*wh*) and small antenna on linkage group 1.
	WHITE EYE	Laboratory synthesis	Contains white eye (*wh*) on linkage group 1.
	MYS	Laboratory synthesis	Contains Silver mesonotum (*Si*) and spot abdomen (*s*) on linkage group 2.
	PLS	Ilobi, Nigeria	Originated from the ILOBI strain. Selected by W. L. Kilama in 1970 for refractoriness to *Plasmodium gallinaceum*. Located on linkage group 2.
	MIN BLT	Laboratory synthesis	Contains mutants miniature (*min*) and black tarsi (blt) on linkage group 3.

*Lines inbred by single-pair brother-sister matings.

approximately half way through, the front end of the jird was reversed.

RESULTS

TABLE 2 shows results from 6 separate infection experiments. Inbred stocks were tested in Experiments 1, 2, and 3. In Experiments 4, 5, and 6 marker strains were analyzed. Of the inbred stocks, BWAMBA gave 23% positive in a line inbred for 7 generations and 31% after 8 generations of inbreeding. The NESC F_{86} line was only 2% susceptible. Of the marker strains, BLACK EYE was 95% susceptible and PLS was 7%. All the other marker stocks were 0%.

The control strain, REFM, was also tested in each of the 6 experiments extending over a period of approximately 7 months. The percent infections were 26, 61, 35, 50, 56, and 30. In all, 401 mosquitoes were dissected and the mean percent infection for the 6 experiments was 43%.

Mosquito mortality was quite variable. In the control REFM, a range of 20 to 72% mortality was obtained with an average of 33% for the 6 experiments. In 2 experiments with the inbred BWAMBA, there was an average of 27% susceptibility and 21% mortality. However, the BLACK EYE strain had a susceptibility of 95% and 5% mortality. The refractory marker strains ranged from 19 to 43% mortality; the refractory inbred lines showed mortality rates of 27 to 55%.

TABLE 3 summarizes variation in those laboratory strains of *A. aegypti* that did support the development of infective viable 3rd stage larvae. Although the REFM, BLACK EYE, and inbred BWAMBA

TABLE 2. Experiments demonstrating susceptibility of various inbred and marker strains of *Aedes aegypti* when fed on jirds infected with *Brugia pahangi*.

Experiment no.	Jird: Mean no. mf/20 mm³		Female mosquitoes: Strain	No. dissected	% with 3rd stage
1	ND-11	45	REFM	73	26
			BW F_7	64	23
	ND-17	125	GKEP F_{18}	67	0
			NESC F_{86}	66	2
2	ND-22	89	REFM	28	61
			BW F_8	94	31
3	ND-22	82	REFM	80	35
			BLPCO F_{38}	65	0
			RJB F_7	45	0
			VN F_{20}	73	0
4	ND-11	119	REFM	70	50
			BLACK EYE	95	95
5	ND-22	54	REFM	73	56
			BZ/WH	79	0
	ND-26A	24	SMA WH/BZ	62	0
			MIN BLT	66	0
	ND-26B	261	WHITE EYE	81	0
			MYS	57	0
6	ND-29	183	REFM	77	30
			PLS	74	7

strains showed a similar range in the number of infective larvae per female, the mean that each supported is indeed different. The BLACK EYE had a mean number of infective larvae 3 times as great as the REFM control. A non-factorial analysis of variance (ANOVA) test (Steel & Torrie 1960) indicates that this difference is highly significant ($F=46.35$; $P<0.005$). The inbred BWAMBA lines also displayed a higher mean number of infective larvae than did REFM; nevertheless, these differences proved to be statistically non-significant ($F = 0.87$; $P>0.05$). Thus, the BLACK EYE strain seems to be a much better laboratory model than REFM for the development of *B. pahangi* filariae in this mosquito. Theoretically this should hold true for human filariae as well (Macdonald & Ramachandran 1965). Were it not for the high mortality rates, the inbred BWAMBA lines could also serve as efficient laboratory models.

DISCUSSION

Macdonald (1962a) selected for an *Aedes aegypti* strain (Liverpool) susceptible to infection with semi-periodic *Brugia malayi* (Brug). In 1 generation, the infection rose from 17 to 94%. Maintaining this strain through 15 generations, he obtained a mean susceptibility rate of 85%. Genetic experiments indicated that the mode of inheritance for filarial susceptibility (f^m) was monofactorial, recessive, and sex-linked (Macdonald 1962b). Subsequently, Macdonald & Ramachandran (1965) demonstrated that the f^m factor controlled the development of both forms of *Wuchereria bancrofti*, *Brugia malayi*, and *B. pahangi*.

Analyses of the various inbred stocks and marker strains of *A. aegypti* in the present study revealed that the majority are refractory to *B. pahangi* infections (TABLE 2). Of the 6 inbred lines tested, only 1 demonstrated susceptibility (BW $F_7 = 23\%$; BW $F_8 = 31\%$). The marker strain BLACK EYE was 95% susceptible, while the PLS only showed 7%.

Both the REFM and BLACK EYE stocks now maintained in our laboratory are substrains of Macdonald's highly susceptible line. Presumably, both are homozygous for filarial susceptibility or f^m, the difference being that REFM has a red eye (*re*) marker but BLACK EYE does not (TABLE 1). Theoretically our substrains should have been similar in susceptibility to those reported by Macdonald.

The results with our BLACK EYE strain were similar to those of Macdonald. R. Gwadz (unpubl. data) and J. McCall (pers. commun.) have also reported comparable results with this strain. However, REFM gave different susceptibility rates. A range of 26 to 61% with a mean rate of 43% was obtained for all 6 experiments. These data support an earlier publication (Rodríguez & Craig 1973) in which REFM gave an overall average of 48% in 11 experiments. Susceptibility in the latter ranged from 23 to 63%. Undoubtedly the better of the 2 laboratory models for the development of microfilariae in *A. aegypti* is the BLACK EYE strain.

Aedes aegypti mosquitoes susceptible to *B. pahangi* have been shown to have a higher mortality than refractory mosquitoes (Townson 1971). Perhaps red eye in REFM acts as an additional selective pressure against this strain thereby lowering its fitness and susceptibility below that of BLACK EYE.

TABLE 3. Variation in capacity to develop infective larvae in those laboratory strains of *Aedes aegypti* showing susceptibility to *Brugia pahangi*.

Strain	Females tested: No.	% showing infective larvae	No. infective larvae per female: Range	Mean ± S. E.
BLACK EYE	95	95	1–27	8.85 ± 0.645
REFM*	70	50	1–27	2.66 0.634
BW F_7	64	23	1–28	3.59 0.916
BW F_8	94	31	1–26	3.90 0.713
PLS	74	7	1–40	0.85 0.564
NESC F_{86}	66	2	1	0.02 0.015

*Data based on Experiment 4, TABLE 2.

Recent life-table studies, in which various *A. aegypti* strains were compared, indicate that a strain with red eye is out-performed by "wild-type" or black-eyed strains (Crovello & Hacker 1972). In the present experiments, REFM displayed a higher mortality than did BLACK EYE (TABLE 2, Experiment 4).

The inbred BWAMBA line (BW F_7 and BW F_8) was derived by single-pair, brother-sister matings from a stock (BWAMBA) that proved to be 53% susceptible to *B. pahangi* (Rodríguez & Craig 1973). Presumably, this inbred line was reasonably isogenic and homozygous for the f^m locus and should have had a higher susceptibility than 31%. Macdonald (1963) has reported that the penetrance of the f^m factor is not complete and that its expressivity is variable. Obviously, this is a complex problem and was not dealt with here. However, a filarial-susceptible, isogenic line could prove useful for more penetrance studies as well as certain other genetic experiments.

The PLS strain was selected for refractoriness to *Plasmodium gallinaceum* (see TABLE 1). Genetic crosses (Kilama & Craig 1969) established that the refractory condition is controlled by a simple autosomal recessive factor (*pls*) located on linkage group 2 between the markers Silver mesonotum (*Si*) and dieldrin-resistance (*D1*). There is now also evidence that the PLS strain is relatively susceptible to *Brugia pahangi* infections. Even though PLS did display a low filarial susceptibility rate (7%), a strong selection program could produce a better laboratory model with 2 physiological markers, a sex-linked susceptible to *B. pahangi* and an autosomal refractory to *P. gallinaceum*.

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Effects of thiotepa on the productivity of male *Aedes aegypti*

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THE mutagenic effects of various alkylating agents in insects have been reported by several workers. However, few investigators have been concerned with the genetic fitness effects of induced mutations produced by such chemicals.

Administration of sublethal doses of nitrogen mustards[2], nitrogen mustard derivatives, and esters of methane sulfonic acid[8,9] have produced complete sterilization of male *Drosophila*. Oral treatment with apholate, tepa, and aphamide has been shown to sterilize male and female house flies[10]. Crystal[5,6] and Crystal and LaChance[7] have demonstrated the utility of various alkylating agents in the chemosterilization of the screwworm fly, *Cochliomia hominivorax* (Coquerel).

Bertram[3] has described the chemosterilization of *Aedes aegypti* and *Anopheles gambiae* with thiotepa. Treatment of second-stage larvae of *A. aegypti* with apholate has been shown to induce both somatic and chromosomal damage[12]. Likewise, apholate treatment of the larvae of this mosquito has been reported to produce developmental abnormalities in a variety of adult tissues[13] and pathological changes in the male accessory glands[11].

The present study concerns the genetic fitness effects of thiotepa (N, N′, N″-Triethylenethiophosphoramide, Lederle Laboratories) on *Aedes aegypti* when adult males were treated orally with the alkylating agent. Productivity or the average number of adult progeny surviving in a population per unit time was employed to estimate genetic fitness.

Materials and Methods

All test populations originated from the same ROCK strain colony of *Aedes aegypti* (L.). The strain itself is a wild-type laboratory stock and was obtained from Professor George B. Craig, Jr., Director of the WHO

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International Reference Centre for *Aedes*, University of Notre Dame. Mosquitoes were reared according to the methods outlined by Craig and Vende Hey[4] in an insectary at a temperature of 27 ± 1°C and 85 ± 10 percent relative humidity. Briefly, conditioned eggs were hatched using deoxygenated water. Larvae were placed in round white enamel pans (General Housewares Corp., Terre Haute, Ind.) containing 200 ml of water and fed on a solution of liver powder (Nutritional Biochemical Co.). Special attention was given to ensure that cultures were not crowded. Pupation generally occurred on days 5 and 6 after hatching. Adults were placed in cages constructed from gallon paper cartons and were fed on sugar cubes and water.

Two-day-old male mosquitoes were treated orally with thiotepa by allowing them to feed freely for five days on sugar cubes containing a given concentration of the chemomutagen. Approximately 0.25 cc of the chemical solution was applied to each of two sugar cubes for a total volume of 0.50 cc per each experimental cage. One group of 20 male mosquitoes was exposed to 0.001 percent thiotepa; three other identical groups were treated with concentrations of 0.025 percent, 0.050 percent and 0.075 percent, respectively. Two groups of 20 male mosquitoes (controls) received no exposure to thiotepa.

Post chemical treatment, six groups of matings (including two controls) were set up to produce the following types of control and experimental crosses: untreated females × untreated males, untreated females × treated males, and untreated females × untreated males. Subsequently, the F_1 progeny from the second untreated control group were backcrossed to the F_1 offspring derived from the parental matings of the first control and experimental groups (Table I).

Crosses were established in one gallon mosquito cages. Each culture consisted of 20 females and 20 males; a total of two replicates were established for each of the six groups. To ensurre maximum egg production, female mosquitoes were given blood meals using anesthetized mice (10 percent Nembutal solution, Abbott) at 3-day intervals. F_2 egg collections were made at 4-day intervals after the first blood meal, and a new oviposition cup was changed at the same time. The number of live F_2 male and female adults produced during a 3-week period was recorded and analyzed statistically.

Table 1. Experimental design to test the genetic fitness effects of thiotepa on populations of *Aedes aegypti* (ROCK) when male parental adults were treated orally

Groups	Percent concentration	Crosses*
	Parental	
1	0	20♀♀(u) × 20♂♂(u)
2	0.001	20♀♀(u) × 20♂♂(t)
3	0.025	20♀♀(u) × 20♂♂(t)
4	0.050	20♀♀(u) × 20♂♂(t)
5	0.075	20♀♀(u) × 20♂♂(t)
6	0	20♀♀(u) × 20♂♂(u)
	Backcrosses	
1		20 F_1♀♀(u × u) × 20♂♂(6)u
2		20 F_1♀♀(u × t) × 20♂♂(6)u

* t = thiotepa-treated; u = untreated

Results and Discussion

Doses of 0.025 percent, 0.050 percent and 0.075 percent thiotepa induced sexual sterility in the male mosquitoes. Few of the F_1 eggs that were produced from matings with untreated females hatched. Control and 0.001 percent thiotepa-treated cultures produced 150 to 200 F_1 progeny. However, both the 0.025 percent and 0.075 percent groups gave 10 or 5 percent F_1 larvae. Of these, only 2 per each group survived to adulthood. Female mosquitoes mated with males treated with 0.050 percent of the chemomutagen produced eggs that gave 7.5 percent hatch. Four of the larvae became adults. Accordingly, only the 0.001 percent experimental and the control group were tested for productivity in the F_2 generation.

Based on total productivity for a 3-week test period, populations showed a significant decrease in a mean number of F_2 progeny when the original male parents were exposed to 0.001 percent thiotepa (Table II). At this concentration only a mean of 198.04 F_2 progeny per culture was produced. By comparison the control group produced a total mean of 315.33 F_2 progeny per culture ($t = 2.641$, $P < 0.05$).

Table III summarizes the mean weekly productivity of the F_2 progeny. Analysis of the data indicate that the

Table II. Total productivity of F_2 progeny derived from adult males of *Aedes aegypti* (ROCK) males treated orally with thiotepa. Higher concentrations (0.025%, 0.050%, and 0.075%) induced sterility

Percent concentration	Sex	Total no. progeny	Total mean productivity ± SE
0	♂♂	3,804	317.00 ± 36.36
	♀♀	3,764	313.67 ± 59.20
0.001	♂♂	2,368	197.23 ± 41.14
	♀♀	2,385	198.75 ± 28.65

Table III. Mean weekly productivity of F_2 *Aedes aegypti* (ROCK) derived from thiotepa-treated male adults during a 3-week period, males and females inclusive

Mean productivity ± SE		
Week 1	Week 2	Week 3
	Control	
403.75 ± 71.17	264.25 ± 61.90	278.00 ± 27.48
	0.001% Thiotepa	
256.13 ± 73.24	234.00 ± 18.25	104.00 ± 21.45

differences between the 0.001 percent experimental and control populations occurred during the third-week period. A higher mean number of F_2 progeny were recorded for both the control and experimental groups during the first-week test period. The control culture gave a mean of 403.75 offspring as compared to a mean of 256.13 for the 0.001 percent population. During the second week, the 0.001 percent experimental group showed a slightly lower mean number (234.00) of male and female F_2 progeny than did the control group (264.25). Two-sample *t*-tests[14] demonstrated that the observed differences in productivity among groups for each week were only statistically significant during the third week ($t = 1.381$, $P > 0.05$; $t = 0.469$, $P > 0.05$; $t = 4.990, P < 0.001$, respectively, for weeks 1, 2, and 3).

Exposure to 0.001 percent thiotepa reduces fitness in F_2 progeny of treated parental male *Aedes aegypti* mosquitoes. However, higher concentrations (0.025 percent to 0.075 percent) of the chemomutagen induce sexual sterility. The latter trend supports the results obtained by Crystal[5,6] with the screwworm fly, *Cochliomyia hominivorax*. In one study[5], thiotepa was shown to be effective as a sterilant of screwworm flies by either topical application or by feeding. Specifically, oral treatments of thiotepa[6] demonstrated antifertility effects, as measured by fecundity and hatchability, at concentrations as low as 0.05 percent. Males were completely sterilized with 0.1 percent thiotepa when treated for 5 or 7 days with honey as a food carrier. Working with *Aedes aegypti*, Bertram[3] has shown that topical exposure of males to thiotepa (20 mg per square foot) for 3 hours provides only 3.2 percent hatchability when mated to untreated females.

Productivity of F_2 offspring derived from parental males treated with 0.001 percent thiotepa showed a significant decrease in comparison with the controls. These observations indicate that polygenic mutations induced at this concentration of the chemomutagen reduces reproductive potential in *Aedes aegypti*. When the experimental and control weekly means were compared, the important differences in productivity occurred during the third week. Apparently, thiotepa, as reported for other alkylating agents[1], also has a delayed mutagenic effect when administered to the parental generation male.

Currently, more detailed studies are being conducted with other mosquito strains to test these hypotheses. The differential genetic fitness effects on females and of various chemomutagens will also be examined.

Summary

The effects of various concentrations of thiotepa, a chemomutagen, on the genetic fitness of *Aedes aegypti* males was studied. Oral treatments of 0.025 percent, 0.050 percent and 0.075 percent thiotepa induced sexual sterility in the male mosquitoes. Analyses of total F_2 productivity indicate that the mean number of progeny was significantly decreased with exposure to 0.001 percent of the chemomutagen. At 0.001 percent thiotepa only a mean of 198.04 F_2 progeny per culture were produced; by comparison the control group gave 315.33.

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