THE EFFECT OF TETRACYCLINE TREATMENT ON FILARIAL SUSCEPTIBILITY IN MEMBERS OF THE *AEDES SCUTELLARIS* COMPLEX[1]

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ABSTRACT. Larvae of *Aedes polynesiensis* and *Ae. malayensis* were treated with tetracycline for 2 generations. After treatment, females were screened for susceptibility/refractoriness to infection by subperiodic *Brugia malayi*. No change was seen in the normally refractory nature of *Ae. malayensis*. Several refractory females were found in the normally susceptible *Ae. polynesiensis*. In addition, the number of infective filarial larvae was much lower in the treated group when compared with an untreated control.

Rickettsial symbionts in the *Culex pipiens* complex are believed to be responsible for patterns of incompatibility seen in several crosses among members of the complex (Yen and Barr 1973). The presence of similar rickettsia has been reported in some members of the *Aedes scutellaris* complex (Beckett et al. 1978, Wright and Wang 1980), and patterns of incompatibility in crosses between members of the complex are similar to patterns reported in *Cx. pipiens* Linn. (Woodhill 1949, Tesfa-Yohannes and Rozeboom 1974, Macdonald 1976). Tetracycline treatment has been shown to be effective in restoring the compatibility of crosses in the *Cx. pipiens* complex (Yen and Barr 1973). Similar treatments have had mixed results in the *Ae. scutellaris* complex. Macdonald (1976) reported that attempts to use tetracycline to restore compatibility in the *Ae. scutellaris* complex were not totally successful. Trpis et al. (1981) have restored the compatibility of some crosses using antibiotic or heat treatments.

Because of the importance of some members of the *Ae. scutellaris* complex as vectors of filariasis, efforts have been made to cross closely related susceptible and refractory species in attempts to determine the genetics of susceptibility (Macdonald 1976, Trpis et al. 1981). The incompatible nature of crosses between *Ae. polynesiensis* Marks females and *Ae. malayensis* Colless males was the origin of the present study. It was hoped that treatment with tetracycline would restore the compatibility of the cross. Such attempts proved unsuccessful (Duhrkopf, unpublished data). However, regardless of the ability of the treatment to restore compatibility in the cross, no information was known on the effect of treatment with tetracycline on the normal susceptible nature of *Ae. polynesiensis* and the normal refractory nature of *Ae. malayensis* when infected by *Brugia malayi*.

MATERIALS AND METHODS

All mosquitoes used in the present study were obtained from colonies maintained in the Laboratories of Medical Entomology at The Johns Hopkins University School of Hygiene and Public Health. *Aedes malayensis* BANG strain was originally obtained in 1969 from the SEATO Laboratories in Bangkok. *Aedes polynesiensis* APIA strain was collected by Barry Engber in Apia, Western Samoa. It has been shown to be highly susceptible to infection by subperiodic *Brugia malayi* (Duhrkopf and Trpis 1980).

All mosquitoes were reared in a controlled environment of 26 ± 1°C and 80 ± 10% R.H. on a 16:8 light:dark reg-

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imen. Larvae were reared 100 per liter of water and were fed on diluted liver powder. Adults were kept in cylindrical paper containers 18 cm high × 18 cm in diameter, and were provided with cellucotton soaked with honey at the top of the cage.

Three experimental and 2 control groups were used in the experiment. Tetracycline treatment in the experimental groups consisted of adding tetracycline hydrochloride at a concentration of 0.25 mg/ml in the larval rearing medium for 24 hr during the fourth instar of the first generation and for 24 hr during the first and 24 hr during the fourth instars of the second generation. The 5 groups of mosquitoes were: 1. Untreated *Ae. malayensis*, 2. Treated *Ae. malayensis*, 3. Untreated *Ae. polynesiensis*, 4. Treated *Ae. polynesiensis*, and 5. Treated *Ae. polynesiensis* tested one generation after treatment.

Five days after emergence, the females were given a blood meal from a gerbil (*Meriones unguiculatus*) infected with subperiodic *Brugia malayi*. The rate of microfilaremia in the peripheral blood of the gerbil was 74 microfilaria per 20 μl. From each group, 50 blood fed females were removed and isolated for 10 days. At that time, the surviving females were dissected by separating the proboscis, head, thorax, and abdomen and teasing each apart in a drop of saline. The number of infective larvae in each female was then recorded. It should be noted that the second and fourth groups were infected immediately after tetracycline treatment, and the fifth group was infected after one generation of larval growth without treatment.

RESULTS

The results of the study can be seen in Table 1. Tetracycline treatment had no apparent effect on the normally refractory *Ae. malayensis* females. The treatment did have an effect on the normal susceptible nature of the *Ae. polynesiensis* females. The control group showed 100% susceptibility. The group tested immediately after treatment was 72% susceptible, and the group tested after relaxation of treatment was 96.4% susceptible. Thus, the tetracycline treatment apparently resulted in an increase in refractory females immediately after treatment which returned to essentially normal levels of susceptibility after one generation without treatment.

With respect to the numbers of infective filarial larvae recovered, the tetracycline treatment apparently had a significant effect. The analysis of variance (Table 2) shows highly significant differences in the number of infective larvae in the groups of *Ae. polynesiensis*. Mean separation procedures showed nonsignificant differences between the control and relaxed treatment groups (groups 3 and 5), but significant differences between the control and treated

Table 2. Analysis of variance for levels of infection.

Source	D.F.	M.S.
Treatments	2	732.6215**
Error	102	75.9813

** $P < 0.01$.

Table 1. Results of treatment with tetracycline.

Group*	Species	Treatment	Number infected	Number dissected	Percent susceptible	No. L_3 $\overline{X}$	No. L_3 (S.E.)
1	*Ae. malayensis*	Untreated	50	44	0	—	—
2	*Ae. malayensis*	Treated	50	43	0	—	—
3	*Ae. polynesiensis*	Untreated	50	38	100.0	14.16	(1.38)
4	*Ae. polynesiensis*	Treated	50	39	72.0	5.95	(1.14)
5	*Ae. polynesiensis*	Treated/Relaxed	50	28	96.4	12.61	(1.99)

* See text for treatment data.

group and between the treated and relaxed treatment groups. So, tetracycline treatment not only decreased the number of susceptible females but also decreased the numbers of infective larvae found in the remaining susceptible females.

DISCUSSION

The present study demonstrates that the normally susceptible nature of *Ae. polynesiensis* females is affected by treatment with tetracycline. However, that normally susceptible nature returned in one generation after relaxation of treatment. Two possibilities might account for this.

The first is that the treatment may directly affect development of the filarial larva. It seems likely that residual tetracycline in the tissues of the mosquitoes might affect the development of the parasite. The data support this idea in that the group tested immediately after treatment shows the greatest decrement in amount of susceptibility and in the levels of infection supported. The relaxation in treatment would then result in a clearing of the residual tetracycline and a return to the previous amounts of susceptibility and levels of infection.

The second possibility is that the treatment has an indirect effect. It is possible that the rickettsia provide some necessary component for the development of the filarial parasite. Reductions in the numbers of rickettsia through tetracycline treatment would then result in reductions in the amount of susceptibility and levels of infection. Direct evidence for such a reduction is not available. However, Wright and Wang (1980) report that tetracycline treatments failed to clear the rickettsial infection from the ovaries of *Ae. polynesiensis* Tafahi strain (= *Ae. kesseli* Huang and Hitchcock). (No data were given which might indicate a reduction in levels of rickettsial infection.) In addition, Wright and Wang (1980) differentiate between cured and partially cured strains, implying a reduction rather than elimination of the rickettsia. Relaxation of treatment for one generation would then allow a normal recurrence of the rickettsia and a return to previous amounts of susceptibility and levels of infection.

Some additional support for the second possibility might come from our recent determination that the susceptibility to infection in the *Ae. scutellaris* complex is inherited in a non-Mendelian manner (Trpis et al. 1981). Since rickettsia are transmitted transovarially, the inheritance of a rickettsial symbiont would follow the same type of non-Mendelian pattern of inheritance and the present data.

It has been difficult to perform a definitive test of this hypothesis. One such test would be to isolate a rickettsia-free population from a proven susceptible strain of *Ae. polynesiensis.* If that population was then refractory, the hypothesis would be directly supported. However, attempts to isolate a population have been unsuccessful. Further support could come from comparisons of rickettsial and filarial infection levels. Such data have also proven difficult to obtain. Light microscopy methods have been ineffective (Beckett et al. 1978) except for a recent report on a modification of Giemsa staining (Wright and Wang 1980). Electron microscopy methods have been ineffective because of the amount of tissue involved.

The hypothesis that the rickettsia are involved in the process of filarial infection is consistent with both the present findings and the non-Mendelian pattern of inheritance. However, until a rickettsia-free population can be isolated and tested, or until a better method of assaying levels of rickettsial infection can be developed, the hypothesis is only indirectly supported.

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TOLERANCE OF *AEDES AEGYPTI* LARVAE TO SYNTHETIC SEWAGE

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ABSTRACT. Tolerance of *Aedes aegypti* larvae to synthetic sewage was investigated in 8 strains of the mosquito. First instar larvae, 4 hr after hatching, were raised in a range of concentrations of sewage, mortality being scored on day 4. There was significant heterogeneity between strains. Tolerance was particularly low in 2 longstanding laboratory strains suggesting laboratory selection for clean water breeders. Tolerance also seemed partly related to the geographical origins of strains, being consistently high in 3 from Africa.

The sex ratio in survivors of exposed individuals did not differ significantly. The tests are discussed in relation to reported cases of breeding by *Ae. aegypti* in soakage pits, drains and similar sites.

INTRODUCTION

Aedes aegypti (Linnaeus) is generally considered to be a clean water breeder (Christophers 1960). However, some observers have found *Ae. aegypti* in dirty water, larvae having been found by Boyce (1910) in drains, by MacFie and Ingram (1916) thriving in liquids rich in animal and vegetable debris, by Reed, Carroll and Agramonte (1901, quoted by Horsfall 1955), in pools and drop buckets of privies, and by Chinery (1969) in drains, latrines and septic tanks. Recently, Curtis (1980) recovered *Ae. aegypti* adults from a soakage pit in Dar es Salaam, Tanzania.

This paper is a report of preliminary experiments to compare strains of *Ae. aegypti* from various parts of the world for tolerance to synthetic sewage (Stoveland et al. 1979) in the laboratory.