ALTERATION IN *AEDES TOGOI* SUSCEPTIBILITY TO *BRUGIA PAHANGI MICROFILARIAE INDUCED BY AEDES ALBOPICTUS* THORACIC HOMOGENATE

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INTRODUCTION

The finding of aborted or undeveloped microfilariae of *Wuchereria bancrofti* and *Brugia spp* in the thoraces of certain species of mosquitoes have led many investigators to study the refractory mechanism(s). In 1976, MacDonald demonstrated that the refractoriness of the filarial infection in mosquitoes was controlled by sex-linked dominant traits. To understand the mechanism(s) which the genetic constituents affect the development of the filarial larvae, many factors that might influence the filarial susceptibility in the mosquitoes have been studied and some of those factors have shown their contributions. Experiments along this line involved the introduction of refractory factors obtained from the refractory strains into the susceptible species and *vice versa*. Different mosquito-parasite systems have been investigated (Owen, 1979). Here in this paper, we report the inhibition of the development of *Brugia pahangi* microfilariae in the normally susceptible *Aedes togoi* mosquitoes which had been injected with thoracic homogenates of a highly refractory *Aedes albopictus*.

MATERIALS AND METHODS

Mosquito strains and filarial parasites : *Aedes togoi*, Taiwan strain, were from the colony maintained in an insectorium at the Department of Medical Entomology, Faculty of Tropical Medicine, Bangkok, Thailand since 1965. A colony of *Aedes albopictus* was established from a wild caught mosquitoes at Kanchanaburi Province, Thailand. The *Brugia pahangi* worms originally isolated from infected cats in Malaysia have been maintained at the Faculty of Tropical Medicine since 1973.

Preparation of mosquito thoracic homogenates : Crude thoracic homogenates (CTH) were prepared from either 6–8 day old *Aedes togoi* or *Aedes albopictus* by grinding 50 thoraces of the mosquitoes in 2 ml of ice-cold steriled normal saline solution (NSS). The preparation was then centrifuged at 1,500 rpm at 4 ° C for 10 minutes. The supernatants were collected and were used as the CTH for passive transfer experiments (see below). The CTH was always prepared just before use. Protein content of the supernatant was determined by Folin-Ciocalteu method (Kabat and Mayer, 1961) and were standardized to the same concentration for every CTH preparation. Some preparations of the CTH were further subjected to Sephadex G–200 column chromatography which the 0.05 M Tris–HCl pH 8.6 buffer was used throughout the fractionation procedure. Optical densities of the 50 drop fractions of the Sephadex column were determined at 280 nm against the Tris–HCl buffer. Pool of each protein profile was dialysed thoroughly against distilled water (DW) overnight at 4 ° C then lyophilized. The profiles were rehydrated to the standard protein concentration and were also used in the passive transfer experiments.

Passive transfer experiments : Female mosquitoes of 4–5 days old were starved for 12–18 hours then they were anaesthetized with ether and placed in lateral position on a glass slide under a dissecting microscope. Heterologous or homologous homogenate or NSS was introduced into the mesothorax of the mosquito via the post-spiracular area using a tiny-calibrated pasteur pipette connected to a small rubber bulb (Nelson, 1962; Townson, 1975; Sucharit and Choochote, 1982). Each mosquito received a volume of 0.3 μl of an appropriate solution.

Infection of mosquitoes with Brugia pahangi microfilariae : After injection with either thoracic homogenate or NSS, and immediately after the treated-mosquitoes recovered from the anaesthesia, they were fed on the blood of *Brugia pahangi* infected cat for 12 days as the followings: the infected cat of known microfilarial density (previously determined by multiple samplings of blood from ear vein) was anesthetized with Nembutal (0.4 mg/kg body weight) by intraperitoneal injection. Hair on the lateral side of the cat was shaved and the animal was placed laterally (with the shaved side downward) on three cages of the starved-treated mosquitoes i.e. the cages contained mosquitoes injected with heterologous thoracic homogenate, homologous thoracic homogenate and NSS control, respectively. The mosquito feeding period was three hours each day. Normal, untreated *Aedes togoi* and *Aedes albopictus* were also fed with blood of the same infected cat in the similar manner. Representatives of the infected mosquitoes of all treatments were dissected on days 2, 4, 8 and 12 after the last feeding. The numbers of dead and alive microfilariae and their developmental stages were recorded.

RESULTS

Our preliminary experiments indicated that *Ae. togoi* were highly susceptible to infection with *Brugia pahangi* as a total of 135 larvae were recovered from 40 mosquitoes dissected at days 2, 4, 8 and 12 after the last feeding on blood of the infected cat. Among them, only 6 of the larvae were dead and the living ones were at three different developmental stages i.e. stages I, II and III, respectively. On contrary, all of the 56 filarial larvae recovered from 36 infected *Ae. albopictus* were dead and did not show any sign of

development. The average numbers of the larvae recovered and the percentage of living larvae found in the normal untreated mosquitoes of both species are shown in Table 1.

Attempts were, then, made to induce development of the microfilariae in the refractory *Aedes albopictus* by passive transferration of *Aedes togoi* CTH to them 2 days prior to infection with the parasites. However, no significant difference on the recovery of the larvae was found in untreated and *Aedes togoi* CTH treated-*Aedes albopictus*. In contrast, the susceptible *Aedes togoi* which received the *Aedes albopictus* CTH, showed significant inhibition of the microfilarial development. The percentage of dead larvae without any sign of further development increased from 2.17% in normal, untreated *Aedes togoi* to 41.57% in *Aedes albopictus* CTH treated-mosquitoes (Table 2).

Trauma caused by intrathoracic injection did not result in any alteration of suscepti-

Table 1

Brugia pahangi larvae recovered from untreated mosquitoes.

Mosquitoes	Average no. of larvae recovered from each mosquito at				percentage of dead larvae found
	day 2	day 4	day 8	day 12	
Ae. togoi	3.1	2.04	4.8	3.0	4.44
Ae. albopictus	2.62	1.62	2.0	1.25	100

Average microfilarial density in the blood of the infected cat was 10.15 per cu.mm. of blood; no. of mosquitoes dissected per day were 10 and 8 for *Ae. togoi* and *Ae. albopictus*, respectively.

Table 2

Numbers of *Brugia pahangi* larvae recovered from untreated and heterologous CTH treated-*Aedes togoi*.

Mosquitoes	Average no. of larvae recovered from each mosquito at				percentage of dead larvae found
	day 2	day 4	day 8	day 12	
Untreated *Ae. togoi*	3.1	2.0	5.4	3.0	2.17
Ae. togoi treated with *Ae. albopictus* CTH	1.0	1.0	0.5	1.83	41.57

Average density of microfilariae in cat's blood was 10.7 per cu.mm. of blood; no. of mosquitoes dissected per day were 10 for normal, untreated *Ae. togoi* and 12 for the CTH treated ones.

bility to the microfilariae in the mosquitoes. Deliberate experiments which NSS or homologous CTH were introduced into the mesothoraces of the *Ae. togoi* revealed that there was no significant difference in the total numbers or percentages of dead and living larvae recovered from these treated mosquitoes as compared with the data from their normal, untreated counterparts.

Fractionation of the CTH of both species of the mosquitoes through Sephadex G-200 column equilibrated and eluted with 0.05 M Tris-HCl buffer pH 8.6 revealed three similar protein profiles namely P1, P2 and P3, respectively. Experiments on passive transfer of the three Sephadex profiles of *Aedes albopictus* into the susceptible *Aedes togoi* showed that P1 exerted stronger inhibitory effect to the microfilarial development than the P2 and P3. All of the larvae recovered were dead and remained in the first stage of development.

DISCUSSION

It has been known that refractoriness of certain species of mosquitoes to filarial parasites occurred in foregut, midgut or in thoracic muscles (Denham and McGreevy, 1977). In the refractory *Aedes albopictus*, large numbers of the microfilariae exsheathed after gaining entry into the mosquitoes and subsequently migrated to the thoracic muscles without any further development (Ewert, 1965a, 1965b; Oda and Wada, 1980). These evidences indicated that some factor(s) in the thoracic muscles of *Aedes albopictus* conferred the refractoriness. In 1976, MacDonald demonstrated that the refractoriness was an inherited characteristic which was controlled by sex-linked dominant genes. However, the mechanism of refractoriness is still unknown.

The fate of microfilariae in the susceptible *Aedes togoi* was also studied. Although this mosquito species lacks the cibarial armatures (Denham and McGreevy, 1977; Choochote, 1981), the other foregut structures could kill 2-22% of the ingested microfilariae (McGreevy *et al.*, 1978). However, large proportion of the filarial larvae exsheathed, migrated and developed into third stage larvae (Oda and Wada, 1980). The infection rates of *Brugia pahangi* in the susceptible *Aedes togoi* have been studied and were found to be 56-69% (Chaithong, 1976; Choochote, 1981). In our study which used the same mosquito-parasite system, the infection rate also fell into the same range.

Owen (1979) obtained a limited degree of inhibition of *Brugia pahangi* microfilarial development in the normally susceptible *Aedes tabu* by rearing the female mosquitoes on a sugar solution containing thoracic homogenate of the refractory *Aedes malayensis* mosquitoes. Only small proportion of the microfilariae were retarded in the treated mosquitoes under Owen's experimental conditions. Introduction of heterologous CTH from the refractory mosquitoes directly into the thoraces of the susceptible ones as reported in this paper conferred higher degree of inhibition to the filarial larvae. The inhibitory effect might be due to the direct toxicity of the homogenate to the larvae or the indirect activity on the parasite ribosomes which eventually resulted in a reduction in mitochondria as has been suggested by Sua-Pheng and Ben-Chuan (1973) and Sucharit *et al.* (1978).

ABSTRACT

Refractoriness to *Brugia pahangi* microfilarial infection could be induced in the normally susceptible *Aedes togoi* mosquitoes by intrathoracic injection with crude thoracic

homogenate of the refractory *Aedes albopictus* mosquitoes. The crude thoracic homogenate contained three Sephadex G-200 protein profiles of which the first profile showed strongest inhibition to the parasite development.

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