Berkala Ilmu Kedokteran
Jil. XXV, No. 1, Mar 1993

Antifilarial Activity of Diethylcarbamazine in *Brugia Pahangi–Aedes Togoi* Model Infection

By: Sugeng Juwono Mardihusodo

Laboratory of Parasitology, Faculty of Medicine
Gadjah Mada University, Yogyakarta

INTISARI

Sugeng Juwono Mardihusodo – Aktivitas antifilarial dietilkarbamasin pada infeksi model *Brugia pahangi–Aedes togoi.*

Sejak tahun 1947 dietilkarbamasin (DEC) diketahui khasiatnya sebagai antifilarial. Penelitian lanjutannya lebih banyak mengarah kepada khasiatnya sebagai mikrofilarisida dan makrofilarisida pada Vertebrata. Kejelasan aktivitas DEC terhadap stadia perkembangan cacing filaria dalam nyamuk vektornya sangat sedikit diketahui, yang sebenarnya juga penting untuk mengungkap makna obat itu dalam pengendalian penularan filariasis. Penelitian ini bertujuan untuk mengungkap lebih rinci tentang daya DEC sebagai filarisida pada nyamuk *Aedes togoi* yang diinfeksi dengan *Brugia pahangi.*

Penelitian dilaksanakan dengan memberikan pakan secara langsung kepada *Ae. togoi* yang dikolonisasi dengan larutan DEC dalam air sukrose 10% dengan konsentrasi 200, 100, 50 dan 0 mg%. Angka mortalitas (AM), angka infeksi (AI), angka infektif (AIn), jumlah larva per nyamuk terinfeksi (JLPN), jumlah larva infektif per nyamuk terinfeksi (JLIPN), penyebaran larva *B. pahangi* dalam tubuh nyamuk, dan ukuran (panjang badan) larvae, setelah nyamuk *Ae. togoi* dikolonisasi selama 12 hari setelah pemberian pakan darah infeks dari kucing yang terinfeksi *B. pahangi*, di tetapkan.

Pertambahan AM nyamuk *Ae. togoi* yang tanpa maupun dengan infeksi *B. pahangi* tidak bermakna, dan tidak bergantung kepada konsentrasi DEC, menunjukkan bahwa DEC tidak bersifat insektisidal. Perbedaan di antara AM nyamuk tanpa dan dengan infeksi filarial diduga karena daya merusak filaria parasit selama perkembangan dan migrasi dalam tubuh nyamuk.

AI dan AIn nyata menurun pada hari ke 12. Larva II (L2) *B. pahangi* masih ditemukan dalam tubuh *Ae. togoi* yang diberi pakan larutan DEC selama 12 hari; hal ini mengesankan bahwa DEC menghambat perkembangan sebagian larvae *B. pahangi*. Selama 12 hari persentase larvae *B. pahangi* di bagian kepala nyamuk *Ae. togoi* nyata lebih rendah daripada yang pada nyamuk pembanding; hal ini menunjukkan bahwa DEC juga menghambat migrasi sebagian larvae filaria. Disimpulkan bahwa DEC nyata berkhasiat parsial sebagai antifilarial pada infeksi model *B. pahangi - Ae. togoi.*

Key Words: diethylcarbamazine – *B. pahangi* – *Ae. togoi* – insecticidal effect – inhibitory effect

0126-1312/93/2501-0023$01.00

INTRODUCTION

Diethylcarbamazine or 1-diethylcarbamyl-4-methylpiperazine hydrochloride (DEC) was proved to be filaricidal by Hewitt *et al.* (1947) in cotton rats *Sigmodon hispidus* infected with *Litomosoides carinii*, and by Santiago-Stevenson *et al.* (1947) in human patients with *Wuchereria bancrofti*. Further studies showed that the effect of the drug on the filarial parasites in vivo apparently depends on the species and stage of the filaria and its vertebrate host (Hawking, 1978).

Studies on the action of DEC as an antifilarial were mostly carried out experimentally in a filarial parasite – vertebrate host model infection to reveal its activity either as a microfilaricide or a macrofilaricide (Hawking, 1978). Very few studies, however, were carried out on the detail action of DEC on mosquitoes infected with filarial parasites which might be useful to elucidate the value of the drug in controlling filariasis transmission.

The present paper reports series of experimental work using *Brugia pahangi* – *Aedes togoi* model infection to study the effect of DEC of various concentrations on:

(a) the mortality rate of *Ae. togoi* reared in the insectarium;

(b) the mortality rate of *Ae. togoi* after being infected with *B. pahangi*, and

(c) the development and migration of *B. pahangi* larvae in *Ae. togoi* after twelve days respectively reared in the insectarium under the same conditions, beginning from the blood meal. It was aimed at testing the proposed hypothesis that DEC in any way affect the filarial infection in mosquitoes.

MATERIALS AND METHODS

Materials

B. pahangi. This filarial parasite was obtained from an experimentally infected domestic cat *Felis catus*. The microfilarial density of the cat was 11.5 ± 2.8 mff per ml of peripheral blood.

Ae. Togoi. This mosquito species was used as the experimental vector of *B. pahangi*, due to its high susceptibility to infection of *Brugia* and other filarial worms (Ramachandran *et al.*, 1963). The mosquito colonization was maintained according to a method described by Gerberg (1970) under insectary conditions.

Drug. The drug tested for its antifilarial activity was diethylcarbamazine citrate. It was ground finely and dissolved at three concentrations, namely 50, 100 and 200 mg/100 ml of 10% sucrose-water solution.

Methods

The method used in the study was direct administration of the DEC solutions to *Ae. togoi* by allowing the mosquito to ingest the drug by imbibing from cotton pads soaked in the drug solutions in sucrose-water. Such procedure was much similar to that carried out

by previous workers, *e.g.* Sucharit *et al.* (1978). The experiments composed of two parts : first dealed with drug testing on non-infected *Ae. togoi* and second on *B. pahangi* - infected *Ae. togoi.*

DEC testing on non-infected Ae. togoi. This work was aimed at observing the effect of the tested compound on the mortality rate of the mosquito. The manipulation of individual batches of mosquitoes in the two series of experiments was standardized in the following procedure: During the first 4 to 7 days after eclosion, about 500 females and 250 males mosquitoes were housed in one foot-cube screened cage, with free access to a 10% sucrose-water-saturated cotton pad put in a vial. After being starved for about 8 hours, usually from 8 a.m. to 4 p.m. by taking the vial out of the cage, the females were allowed to feed upon a gerbil kept in a small wire bobbinet that was placed in the cage for about 1 to 2 hours. During this time the fully engorged females were randomized into groups of 50 mosquitoes which were kept in separate paper cup of about 250 ml with the opening screened. These mosquito-cups were subdivided into 4 subgroups, each consisting of 2 cups with 50 mosquitoes per cup, and treated as the following : the first was maintained by placing a sucrose-water-soaked cotton wool pad on the cup's screen; the second, third and fourth subgroup were provided with a cotton pad saturated with 10% sucrose-water in which the drug was dissolved at the desired concentrations within 12 days experimental periods. These were meant as the control non-infected group.

The number of mosquitoes were counted daily and the dead ones were recorded during the treatment period, and summed up at the end of each experiment (calculated as the mortality rate (%) of the mosquitoes under study).

DEC testing on B. pahangi *infected* Ae. togoi. This work was dealt with observations on the effects of the tested drug both upon the mortality of *Ae. togoi* and developmental stages of *B. pahangi.* The procedures followed were almost the same with that carried out for setting the control non-infected group. The only difference was that instead of using non-infected gerbils as the source of blood meal, a *B. pahangi*-infected cat was used to feed the mosquitoes, after previously anesthetized with pentobarbitalum natrium (vetaranol r/, Veterinaria AG Zurich) by intraperitoneal injection in a dosage of 0,1 ml per kg of body weight. Attempts were made to eliminate variables as much as possible by feeding all the mosquitoes used in any series of experiments on the same cat at the time of day, usually in late afternoon, in a darkened room. Prior to feeding microfilarial level of the cat was estimated by taking 20 ml of blood from its ear, smeared on a glass slide, air dried overnight at from temperature, and then stained with Giemsa, following a procedure recommended by WHO (1974).

The infected mosquitoes which were also subdivided into batches of 50 per cup, were reared in the insectarium with 10% sucrose only (serving as control infected goup), and with DEC of desired concentrations in 10% sucrose-water.

The number of the mosquitoes were counted daily and the dead ones were recorded and summed up at the end of each experiment. After four, eight and twelve days of rearing in the insectarium all the mosquitoes were dissected with the following procedure: The survivors were anesthetized with chloroform, the legs and the wings were removed, and then placed on a glass slide. The head, thorax and abdomen were separated, each part was placed in a few drops of normal saline solution and the tissues were gently teased apart under a dissecting microscope. Actively moving larvae encountered were immobilized by the addition of a few drops of Bles' fluid on the slide to facilitate the establishment of larval stages and measurements. The stages were identified on the bases

of their morphological figures as indicated by Schacher (1962). All stages of the filarial larvae together with the number found in the body parts of the dissected mosquitoes were recorded and expressed as the average number of larvae per infected mosquito (NLPM) and number of infective larvae per infected mosquito (NILPM). The body length and width of all the larvae detected were measured under a compound microscope with a calibrated ocular micrometer. The width of larva was measured at fixed point: for the first stage at the nerve ring, while for the second and third at the esophagointestinal junction. The tests were carried out on two replicates.

Data analysis and interpretation. Appropriate statistical tests were used to analyse the data obtained, such as: (1)χ^2-test, for mortality rate, infection rate and infective with equal variance, *i.e.* number of larvae per infected mosquito, number of infective larvae per infected mosquito, and size (body length) of larvae (Steel & Torrie, 1976). The value of either χ^2 or t of the observed data are considered significant when they exceed the critical value at the significant level (α) of 0.05.

RESULTS

Rearing of the non-infected mosquitoes during 12 days with the three doses DEC apparently produced no effect on mortalities of the mosquitoes. The mortality rates of mosquitoes ingesting the drug of 50, 100 and 200 mg% were 17%, 18% and 21% respectively, whereas that of the controls (0 mg%) was 15% (TABLE 1).

TABLE 1. – The effect of various concentrations of diethylcarbamazine on the mortality rates (MR) of non-infected *B. pahangi* infected *Ae. togoi* reared in the insectarium for 12 days after the cat's blood meal. Observed in two replicates (R_1 and R_2) of 100 mosquitoes.

Ae. togoi mosquito		Drug Dosage (mg%)			
		0	50	100	200
Non-infected					
No. mosquito dead	R_1	12	16	19	19
	R_2	18	18	17	23
	$\bar{X}$	15.0	17.0	18.0	21.0
	MR[1] (%)	15.0	17.0	18.0	21.0
B. pahangi infected					
No. mosquito dead	R_1	30	31	39	40
	R_2	26	37	39	44
	$\bar{X}$	28.0	34.0	39.0	42.0
	MR[2] (%)	28.0	34.0	39.0	42.0
	χ^2	5.007	7.600	10.821	10.219
	p	<0.05	<0.05	<0.05	<0.05

MR[1] $\chi^2 = 0.872$; df = 3; $p > 0.05$
MR[2] $\chi^2 = 4.909$; df = 3; $p > 0.05$

A phenomenon similar to that observed on 4- and 8-day insect tests was also found in this experiment in which *B. pahangi* infection caused significantly higher mortalities of the mosquitoes, *i.e.* 28% compared with 15% in case of non-infected one ($p<0.025$). The mortality rates of infected mosquitoes under the three doses of drug were 34%, 39% and 42% respectively (TABLE 1). they are not significantly different from the control.

From the observations on the development of *B. pahangi* in *Ae. togoi* it was found that in the control mosquitoes all larvae had developed to infected stages, the infective rates being 65.8%. This rate tends to decrease due to the increasing dose of the treatment. In the treated groups, the infective rates were 58.3%, 57.4% and 47.4% respectively, which are not significanttly different from that of the controls (65.8%). Likewise the drug of 50, 100 and 200 mg% did not affect the infection rates of the mosquitoes, namely: 50.6%, 62.3% and 53.4% respectively, while that of the control was 65.8%. Nevertheless some larvae were still in the second stage in all the treated ones (TABLE 2).

TABLE 2. – The effect of various concentrations of diethylcarbamazine on the development of *B. pahangi* larvae in *Ae. togoi* reared for 12 days after the cat's blood meal. Observed in two replicates (R_1 and R_2).

Ae. togoi mosquito		Drug Dosage (mg%)			
		0	50	100	200
No. mosquitoes dissected	R_1	70	69	61	60
	R_2	74	63	61	56
	$\bar{X}$	72	66	61	58
No. (%) of mosquioes with filarial larvae					
Stage I (L_1)	R_1	-	-	-	-
	R_2	-	-	-	-
	$\bar{X}$				
	%				
Stage II (L_2)	R_1	0	0	1	2
	R_2	0	2	3	1
	$\bar{X}$	0	1	2	1.5
	%		1.5	3.3	2.6
Stage III (L_3)	R_1	45	36	37	26
	R_2	49	39	29	26
	$\bar{X}$	47	38	33	26
	IR (%)	65.8	57.6	54.1	44.8
All stages	R_1	45	36	38	28
	R_2	49	41	32	27
	$\bar{X}$	47.0	38.5	35.0	27.5
	InR (%)	65.8	58.3	57.4	47.4

IR (Infective Rate): $\chi^2 = 2.049$; df = 3; $p > 0.05$
InR (Infection Rate): $\chi^2 = 2.019$; df = 3; $p > 0.05$

During the period of incubation the infective larvae in large proportion had migrated out of the thoracic muscles achieving the abdomen and the head areas of the two groups of mosquitoes. The percentage of larvae found in the head area of the control mosquitoes was 61.1%, while those in mosquitoes treated with 50, 100 and 200 mg% were 55.6%, 49.4% and 47.2% respectilvely; the latter two are significantly different from the control ($p<0.05$). The three concentrations of the drug seemed not to exert marked effect on the number of larvae per infected mosquito of the treated groups; the number of larvae per infected mosquito of the control was 11.5 while that of the groups dosed with 50, 100 and 200 mg% DEC were 9.8, 9.0 and 7.9 respectively (TABLE 3).

In case of the infective larvae, no significant effect of the drug on the number of infective larvae per infected mosquito and the migration rate to the head of the mosquitoes. Out of the respective total third stage larvae, the percentage found in the head of control mosquitoes was 61.1%, while that in mosquitoes reared with 50, 100 and 200 mg% DEC were 55.6%, 51.7% and 48.8% respectively, the latter two are significantly different from the control ($p<0.05$). The number of infective larvae per infected mosquito of control groups was 11.5, whereas that of mosquitoes treated with 50, 100 and 200 mg% DEC were 9.8, 8.7 and 7.7 respectively (TABLE 4).

Result of measurements of length and width of *B. pahangi* larvae found in both untreated and treated *Ae. togoi* were presented in TABLE 5. Without treatment the size of third stage larva was $1646.9 \pm 166.2 \times 23.8 \pm 2.9$ µm, while those with the drug of 50. 100 and 200 mg% were $1600.6 \pm 130.3 \times 23.6 \pm 2.1$ µm, $1500.6 \pm 130.6 \times 23.6 \pm 2.1$ µm and $1544.4 \pm 135.9 \times 23.2 \pm 2.3$ µm respectively. They are not significantly different from that in the controls (TABLE 5).

In conclusion, DEC did not increase the mortality rates of *Ae. togoi* both non-infected and infected with *B. pahangi*, but adversely affected the moltings of *B. pahangi* larvae and inhibited the migration of infective larvae to the head of the mosquito.

DISCUSSION

The results of these experiments indicated that although DEC of 50 to 200 mg% was continously administered for 12 days to *Ae. togoi* non-infected or infected with *B. pahangi* the drug did not show any insecticidal action on the mosquitoes, as no significant difference was found between the mortality rates of the treated groups and that of untreated one. The effects were not dose dependent. The mortality rates of mosquitoes ingesting the cat's blood containing *B. pahangi* microfilariae of 11.5 ± 2.8 per ml were significantly higher from those of the non-infected ones. It may be due to injurious effect of the filarial parasite during the migration and development. The notable increase in the mosquito mortalities was during the first 48 hours after the infective meal corresponding with migration of microfilariae from the gut to the thoracic muscles (Ramachandran, 1966), and when the larvae reached maturity due to overcrowding effects on the mature larvae (Wharton, 1957). Ramachandran (1966) found that in *Ae. aegypti* the adverse effects became apparent when the mosquitoes were fed on cats with *B. malayi* microfilarial densities above 5.1 per ml in peripheral blood.

TABLE 3. – The distribution of *B. pahangi* filarial larvae of stage I, stage II and stage III in the body of *Ae. togoi* treated with various concentrations of diethylcarbamazine for 12 days after the cat's blood meal. Observed in two replicates (R_1 and R_2).

Ae. togoi mosquito				Drug Dosage (mg%)			
				0	50	100	200
No. (%) positif for larvae			R_1	45	36	38	28
			R_2	49	41	32	37
			$\bar{X}$	47	38.5	35	27.5
No. (%) of *B. pahangi* larvae in the body of *Ae. togoi*							
Head	Stage III	(L_3)	R_1	341	204	159	106
			R_2	319	214	149	100
			$\bar{X}$	330	209	154	103
			%	61.1	55.6	49.4	47.2
Thorax	Stage I	(L_1)	R_1	-	-	-	-
			R_2	-	-	-	-
			$\bar{X}$				
			%				
	Stage II	(L_2)	R_1	-	-	5	8
			R_2	-	3	17	6
			$\bar{X}$		1.5	11	7
			%		0.4	3.5	3.2
	Stage III	(L_3)	R_1	161	132	120	78
			R_2	157	145	135	103
			$\bar{X}$	159	138.5	127.5	90.5
			%	29.4	36.8	40.9	41.5
Abdomen	Stage III	(L_3)	R_1	57	31	22	19
			R_2	45	26	17	16
			$\bar{X}$	51	28.5	29.5	17.5
			%	9.5	7.5	6.4	8.0
All sites			R_1	559	367	306	211
			R_2	521	385	318	225
			$\bar{X}$	540	376	312	218
			%	100	100	100	100
No. of larvae per infected mosquito (NLPM)			R_1	12.4	10.2	8.1	7.5
			R_2	10.6	9.4	9.9	8.3
			$\bar{X}$	11.5	9.8	9.0	7.9
			t	-	1.726	1.964	3.655
			p		>0.05	>0.05	>0.05

χ^2 of larvae in the head = 17.5; df = 3; $p < 0.05$

From the observations on *B. pahangi* infected *Ae. togoi* dosed with 50 to 200 mg% DEC in 10% sucrose water for 12 days it was found that there was no significant effect of the drug on the infection and infective rates, the number of larvae and the number of in-

TABLE 4. – The distribution of *B. pahangi* infective larvae in the body of *Ae. togoi* treated with various concentrations of diethylcarbamazine for 12 days after the cat's blood meal. Observed in two replicates (R_1 and R_2).

Ae. togoi mosquito		Drug Dosage (mg%)			
		0	50	100	200
No. (%) positif for larvae	R_1	45	36	38	28
	R_2	49	41	32	27
	$\bar{X}$	47	38.5	35	27.5
No. (%) of *B. pahangi* larvae in the body of *Ae. togoi*					
Head	R_1	341	204	159	106
	R_2	319	214	149	100
	$\bar{X}$	330	209	154	103
	%	61.1	55.6	51.2	48.8
Thorax	R_1	161	132	120	78
	R_2	157	145	135	103
	$\bar{X}$	159	138	127.5	90.5
	%	29.4	36.7	42.4	42.5
Abdomen	R_1	57	31	22	19
	R_2	45	26	17	16
	$\bar{X}$	51	29	20	18
	%	9.5	8.1	7.3	9.7
All sites	R_1	559	367	301	203
	R_2	521	385	301	219
	$\bar{X}$	540	376	301	211
	%	100	100	100	100
No. of infective larvae per infected mosquito (NILPM)	R_1	12.4	10.2	7.9	7.3
	R_2	10.6	9.4	9.4	8.1
	$\bar{X}$	11.5	9.8	8.7	7.7
	t	-	1.726	2.433	3.858
	p		>0.05	>0.05	>0.05

L_3 in the head : $\chi^2 = 15.529$; df = 3; $p < 0.05$

fective larvae per infected mosquito, the filarial larval growth and migration in the mosquitoes. In the case of infective rate, the results confirm with those obtained by Denham *et al.* (1978), reporting that feeding DEC at levels up to 10^4 mg/liter (1.000 mg%) in 10% sucrose water did not decrease the infective rate of *Ae. aegypti* infected with *B. pahangi* after rearing for 10 days. They recorded that the proportion of treated mosquitoes harboring infective larvae (expressed as the percentage of the control group) was 74% at 0.1 mg%, 103 mg% at 10 mg%, 108% at 100 mg%, and 79% at 1.000 mg%; none of those differences were significant from the control mosquitoes. In the present study, the corresponding proportion was about 81% at 50 mg%, 70% at 100 mg%, and 55% at 200 mg%.

TABLE 5. - The size (um) of *B. pahangi* larvae in *Ae. togoi* reared with various concentrations of diethylcarbamazine for 12 days after the cat's blood meal. Observed in two replicates (R_1 and R_2)

Drug Dosage (mg%)		0	50	100	200
		No. Lenght (x) / Width (x)	No. Lenght (x) / Width (x)	No. Lenght (x) / Width (x)	No. Lenght (x) / Width (x)
Stage I	(L_1)				
	R_1	-			
	R_2	-			
	$\bar{X}$ lenght / width				
Stage II	(L_2)				
	R_1	-	20 1080.6± 102.4 / 28.0±2.3	30 1010.2±101.9 / 28.6±2.2	25 980.2±91.9 / 28.8±2.0
	R_2	-	10 1092.0±98.3 / 28.0±2.1	30 990.1±104.4 / 28.1±1.8	30 950.4±98.6 / 26.6±1.9
	$\bar{X}$ length / width		1086.3±1000.4 / 28.3±2.2	1000.6±95.3 / 28.9±2.1	965.3±95.3 / 27.7±1.9
Stage III	(L_3)				
	R_1	50 1673.7±189.9 / 23.9±2.6	50 1610.5±129.3 / 23.7±3.5	50 1580.7±121.9 / 23.8±2.2	50 1590.3±130.2 / 23.2±2.4
	R_2	50 1620.0±192.4 / 23.6±3.1	50 1590.8±131.4 / 23.5±2.6	50 1540.4±139.3 / 23.4±2.1	50 1498.4±141.6 / 23.1±2.3
	$\bar{X}$ length / width	1646.9±166.2 / 23.8±2.9	1600.6±130.3 / 26.6±2.5	1500.6±130.6 / 23.6±2.1	1544.4±135.9 / 23.1±2.3
	t		1.621	2.577	1.926
	p		>0.05	>0.05	>0.05

The number of larvae as well as the numbers of infective larvae per infected mosquito were not significantly decreased due to the drug administration. Similar results were noted by Denham *et al.* (1978), in the comparable levels of treatment; the average number of infective larvae per infected mosquito (expressed as the percentage of that of the control group) was 69% at 10 mg% and 63% at 100 mg%, while in the present study it was 85% at 50 mg%, 76 at 100 mg% and 67% at 200 mg%.

The filarial growth was not apparently affected by the drug action. However the migratory movement of infective larvae to the head of mosquitoes treated with 100 and 200 mg% DEC seemed to be inhibited on day 12, and a pronounced effect of the drug on the developing filarial larvae was also noted. The drug appears to have a property of inhibiting the molting of *B. pahangi* larvae of stage I to stage II. During 12 days all larvae

had developed to infective stage in the control, while in the treated groups some second stages were still found indicating that there was inhibition of larvae stage II to develop to stage III. These findings indicated that DEC partially inhibit stage differentiation, and probaly the effects were dose related.

The results of this study might clarify those recorded by Denham *et al.* (1978) that at 1.000 mg% DEC reduced the average number of infective larvae per infected mosquito to 30% of the control group. From their experiments with *L. carinii* in cotton rats, *Dirofilaria immitis* in dogs and *W. bancrofti* in humans exposed to DEC while persisting in host's blood, Kanda *el al.* (1967) showed that a part of all three parasites ingested by the intermediate hosts could develop to mature larvae. However, some reduction in number and rate of development of mature larvae as well as considerable retardation of larval growth in the intermediate hosts could be demostrated with the human filaria at relatively small doses of the drug. Chen and Fan (1977) reported that Bancroftian filariae survived after one to three courses of the drug treatment in carriers, and the larvae able to reach the infective stage in *Culex p. fatigans*; the infection rate and the development of infective larvae per infected mosquito from DEC- treated carriers was much lower than from the untreated one.

The exact mechanism of the drug action on the mosquito stages of *B. pahangi*, as found at the present study, is still obscure. No related information could be obtained from previous works on the drug gathered by Hawking (1978). Kanda *et al.* (1967) considered that such a phenomenon was probably due to a toxic effect of the drug against the filarial larvae. In these experiments the drug was administered directly to the mosquitoes. It was very likely that the drug also rapidly absorbed from the gut, and distributed rapidly in all tissues inclusively in the thoracic muscles of the mosquito where the filarial larvae developed, similar to that found in mice by Sakuma *et al.* (cited by Hawking, 1978). Continuous exposure of the mosquitoes to the drug for least 4 days caused such a drug accumulation in the thoracic muscles and other tissues, that at certain level (easily achieved by high dose of the drug) it might affect the internal environment of the developing larvae, leading to the molting or development. The interference of the drug with the internal environment favorable for the filarial larval development in the thoracic muscles of mosquitoes might be due to the ability of DEC to derange the muscular activity of the host and or the parasite, as that observed on the microfilariae of many filarial worms, and *Ascaris* (Hawking, 1978).

Used as a prophylactic drug DEC was found to be effective in preventing the development of the infective larvae of *B. pahangi* and other filarial nematodes, such as *B. malayi, Loa loa, D. immitis* and *L. carinii* but not in the case of *Onchocerca volvulus* (Hawking, 1978).

SUMMARY AND CONCLUSION

DEC was screened for its filaricidal activity using *B. pahangi–Ae. togoi* model infection under laboratory conditions.Mosquitoes were allowed to ingest the drug by imbibing freely from cotton pads soaked in solutions of drugs of various concentrations in 10% sucrose-water. The mortality rates, the infection and infective rates of the mosquitoes, the number of larvae and infective larvae per infected mosquito together with the distribution of larvae and infective larvae in the body of mosquito, and the size of the filarial larvae were observed during 12 days beginning from the infective blood meal.

There was no significantly increased mortality rates of both non-infected and infected mosquitoes due to the drug treatment in dosages of 50 to 200 mg%, indicating that the drug had no insecticidal effect. The differences in the mortality rates of the infected and non-infected mosquitoes were due to the injurious effect of developing and migrating parasites.

There were considerably lower in the infection and infective rates as well as in the average worm load on day 12. It may be because of loss of infective larvae during feeding or due to the action the drug. The drug showed partially inhibitory effects on the development or molting of the filarial larvae, during the first and second moltings, and on the migration of the infective larvae to the head of mosquitoes. Those phenomena may be due to the drug action on the neuromuscular system of the filarial worm.

DEC definitely had a partial action on the development of *B. pahangi* in *Ae. togoi*.

REFERENCES

Chen, C. C., & Fan, P. C. 1977 The effect of diethylcarbamazine treatment on the development of Bancroftian microfilariae in *Culex p. fatigans*. *SEA J. Trop. Med. Publ. Hlth* 8(*1*): 53-5.

Denham, D. A., Suswillo, R. R., Rogers, R., & McGreevy, P. B. 1978 Studies with *Brugia pahangi*, 17. The anthelmintic effect of diethylcarbamazine. *J. Parasit.* 64(*3*):463-8.

Gerberg, E. J. 1970 Manual for mosquito rearing and experimental techniques. *AMCA Bull. 5*, AMCA Inc., Fresno, Calif.

Hawking, F. 1978 Diethylcarbamazine. A review of the literature with special reference to the pharmacodynamics, toxicity and use in the therapy of onchocerciasis and filarial infections. *WHO/Oncho/78*, 142, Geneva.

Hewitt, R. F., Kushner, S., Steward, H. W., White, E., Wallace, W. S., & Subrrow, Y. 1947 Experimental chemotherapy of filariasis. III. Effect of 1-diethylcarbamyl-4-methyl-piperazine hydrochloride against naturally acquired filarial infections in cotton rats and dogs. *J. Lab. Clin. Med.* 32 (*6*):134-9.

Kanda, T., Tanaka, S., & Sasa, M. 1967 The effect of diethylcarbamazine treatment on viability of microfilariae ingested by intermediate hosts. *Japan. J. Exp. Med.* 37(2):149-55.

Ramachandran, C. P. 1966 Biological aspect in the transmission of *Brugia Malayi* in the laboratory. *J. Med. Ent.* 3(*3*):239-43.

_____, Wharton, R. H., & Dunn, F. L. 1963 *Aedes (Finlaya) togoi* Theobald, a useful laboratory factor in studies of filariasis. *Ann. Trop. Med. Parasit.* 57(2):443-5.

Santiago-Stevenson, D., Oliver-Gonzales, J., & Hewitt, R. I. 1947 Treatment of filariasis bancrofti with 1-diethylcarbamyl-4-methylpiperazine hydrochloride ("hetrazan"). *J. Am. Med. Assn.* 135(*3*):708-715.

Steel R. G. D., & Torrie, J. H. 1976 *Introduction to Statistics*. McGraw-Hill Kogashuka Ltd., Tokyo.

Schacher, J. F. 1962 Morphology of the microfilariae of *Brugia pahangi* and of the larval stages in the mosquito. *J. Parasit.* 48(*3*):679-84.

Sucharit, S., Virabonchai, S., Panavut, N., & Harinasuta, C. 1978 Studies on the effects of tetracycline on *Brugia pahangi* infection in *Aedes togoi*. *SEA J. Trop. Med. Publ. Hlth.* 9(1):55-59.

Wharton, R. H. 1957 Studies on filariasis in Malaya: Observations on the development of *Wuchereria malayi* in *Mansonia (Mansonoides) longipalpis*. *Ann. Trop. Med. Parasit.* 51(*1*): 78-86.

World Health Organisation 1974 Expert Committee on Filariasis. Third report. *WHO Tech. Rep. Ser.* 542, Geneva.