THE EFFECT OF DIETHYLCARBAMAZINE TREATMENT ON THE DEVELOPMENT OF BANCROFTIAN MICROFILARIAE IN *CULEX P. FATIGANS*

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INTRODUCTION

Diethylcarbamazine (DEC) is an effective agent against microfilariae (MF) and is widely used for filariasis control in endemic areas. However, DEC treatment does not always achieve a complete clearing of MF in all individuals (Hawking, 1962) and in many of control programmes a considerable proportion of populations continue to have MF in their blood (Hewitt *et al.*, 1950; Ciferri *et al.*, 1969; Mahoney and Kessel, 1971; Desowitz and Southgate, 1973; Fan *et al.*, 1974).

The present experiments were undertaken to determine whether residual MF in the blood of DEC-treated carriers are capable of developing into infective larvae after being ingested by vectors, and to establish whether residual MF after DEC treatment will be a possible hindrance to control programmes.

MATERIALS AND METHODS

Source of mosquitoes: *Culex p. fatigans* were obtained from the NAMRU-2 laboratory in Taipei. They were initially colonized from specimens collected on Kinmen (Quemoy) and maintained in the insectary since 1972.

Source of MF: Three bancroftian carriers from Kinmen were used in the experiment. One was a 41-year-old farmer who had received 3 courses of DEC (total dosage of 12.9 gram) and MF reappeared in the blood 115 days after the termination of the last treatment. The second carrier was a 15-year-old student who had received only one course of DEC (total dosage of 5.0 gram) and in whom MF were redetected 46 days after treatment. The third carrier was a 35-year-old housewife who had received no DEC treatment and she served as the control.

Mosquito feeding: Mosquitoes were allowed to feed on the subjects for 1 hour (2200-2300 hours) in Kinmen and the engorged mosquitoes transferred to the insectary in Taipei. The temperature of insectary was maintained at 25 ± 1°C and relative humidity at 70% to 80% throughout the course of study.

MF density: The MF density for each carrier was determined 10 minutes before and after feeding by counting 5 quantitated (20 c.mm) blood smears.

RESULTS

In preliminary experiment MF from both DEC-treated and untreated carriers were found to reach the infective stage at least 14 days after taking the blood meal. Subsequently, the infectivity of MF was determined 18 days after the mosquito feed. As shown in Tables 1 and 2, the DEC-treated MF from the individuals were found to be able to develop to the infective stage larvae in mosquitoes. The number of infective larvae per mosquito in the treated group were nearly one half that

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of the untreated group (Table 1). Although there was no significant difference ($p > 0.3$) pertaining to the MF density, there was a highly significant difference ($p < 0.005$) in the infection rates in mosquitoes. Although infective larvae were recovered from the head, thorax, abdomen, wing and leg, most were found in the head of the mosquitoes. No significant difference was observed between mosquitoes from the treated and untreated groups on the distribution of infective larvae in the mosquitoes (Table 2).

DISCUSSION

Previous workers demonstrated that MF which persisted in the blood of carriers following treatment with DEC could successfully develop to the infective stage in their intermediate host (Hawking *et al.*, 1950; Kume *et al.*, 1954; Kanda *et al.*, 1967). Our results supported these findings and showed further that the MF can even survive 3 courses of DEC and are still able to reach the infective stage in mosquitoes. The number of larvae

Table 1

Effect of diethylcarbamazine treatment on infectivity of bancroftian microfilariae in *Culex p. fatigans.*

Subject	Mean No. mf/20 c.mm	*Culex p. fatigans*						
		No. used	No. engorged	No.* dissected	Infection No.	Infection %	Infective larvae No.	Infective larvae Ave. No./mosq.
Treated	6.0 ± 3.3	1,200	226	182	99	54.4**	259	2.6
Untreated	5.8 ± 3.2	600	141	110	80	72.7	388	4.9

* Mosquitoes were dissected 18 days after blood feeding.
** Significant lower ($p < 0.005$) than value of untreated subject.

Table 2

Distribution of filarial larvae in *Culex p. fatigans* after feeding on diethylcarbamazine treated and untreated subjects.

Subject	Locality (organs)	Mature larvae No.	Mature larvae %	Immature larvae No.	Immature larvae %
Treated	Head*	164 (78)***	63.3	7**	100
	Thorax	59 (41)	22.8		
	Abdomen	35 (26)	13.5		
	Wing & leg	1 (1)	0.4		
Untreated	Head*	248 (65)	63.9	3**	100
	Thorax	110 (38)	28.4		
	Abdomen	26 (17)	6.7		
	Wing & leg	4 (3)	1.0		

* Including the probosics, palps and antennae.
** The second stage larvae.
*** Number of infected mosquitoes in parenthesis.

developing from treated host, however, is much lower than that of the untreated host. The drug affected the number of MF in the blood as well as the ability of the larvae to develop to maturity in the vector.

The fact that MF can survive and develop to the infective stage in intermediate hosts after treatment of a filarial carrier should be taken into consideration in the endemic areas when implementing control measures.

SUMMARY

Bancroftian microfilariae survived after one to three courses of diethylcarbamazine 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 carrier. The fact that surving MF can develop to infective stage in their vector indicates that such filarial carriers may be important sources for transmission of filariasis in the endemic areas after suspension of control measures.

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AN AUTOCIDAL OVITRAP FOR THE CONTROL AND POSSIBLE ERADICATION OF *AEDES AEGYPTI*†

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INTRODUCTION

Dengue haemorrhagic fever (DHF) has replaced malaria as the most important and serious mosquito-borne disease throughout tropical Asia and the Western Pacific. Hundreds to thousands of people die of the disease almost every year. After some 20 years of its history, no effective drug or vaccine has yet been developed for its treatment. Its control still depends largely on the control of the *Aedes* vectors, particularly *Ae. aegypti*, the principal vector.

Ae. aegypti can be controlled effectively by integrated measures (Chan, 1973a). In Singapore the *Aedes* premise index is reduced to 2%-5% by source reduction, health education, legislation and fogging of areas with more than 5% premise index (Chan and Ng, 1975). Further reduction does not seem possible in the absence of additional measures.

On a long term basis, the conventional method of controlling *Ae. aegypti* by using insecticides should be discouraged. This is because the use of insecticides has many disadvantages and undesirable side effects, e.g. insecticides are only palliative and temporary 'stop-gap' measures; they cannot suppress vector populations for long periods and must be used repeatedly for year-round control; their constant repetitive and unending use causes problems of environmental pollution and development of resistance in the vector; indiscriminate and careless use of insecticides leads to accidental poisoning to both the applicators and the public, and to unwarranted destruction of beneficial non-target insects and other animal life. Alternative methods that are safe, effective and economical should therefore be found. The use of ovitraps in *Ae. aegypti* control is one such method.

The ovitrap or oviposition trap was first developed in the United States for the surveillance of *Ae. aegypti* (Fay and Perry, 1965; Fay and Eliason, 1966). It was subsequently used as a surveillance device for detecting the presence of *Ae. aegypti* in low density areas in the *Aedes aegypti* eradication programme in that country (Jakob and Bevier, 1969; Thaggard and Eliason, 1969).

The American ovitrap was first modified and used in 1969 as a supplementary measure for the control of *Aedes* vectors in the Singapore Paya Lebar International Airport (Chan, 1973b). It was so effective that *Ae. aegypti* became eradicated from the airport one year after its introduction (Chan, 1973b). For six consecutive years, *Ae. aegypti* has not reappeared in the airport within the 800 metres boundary.

A new type of ovitrap specially designed and developed for the control of *Aedes* vectors in urban premises was recently experimented in two Singapore city areas (Chinatown and Rochor) with high *Aedes* density and DHF incidence. This paper presents the findings of one aspect of this study, namely the use of the attractiveness of the ovitrap for the possible eradication of *Ae. aegypti* in the Rochor experimental area.

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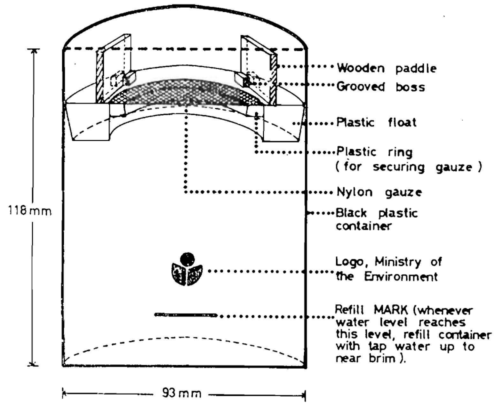

Fig. 1—Longitudinal section of autocidal ovitrap.

MATERIALS AND METHODS

The American surveillance ovitrap was modified into a control trap for *Aedes* vectors. Several models were designed and tested in the laboratory until the final model (Fig. 1) became autocidal, i.e. capable of continuously destroying the progeny of *Aedes* vectors from eggs laid, without human intervention, except for periodic refilling of the ovitrap with water when it has become dry.

The autocidal ovitrap (Fig. 1) comprises 3 principal components:

(i) a black plastic container for attracting gravid and ovipositing *Aedes* female mosquitoes, black being the colour most attractive to *Aedes (Stegomyia)* species;

(ii) a plastic ring or float with air trapped inside its sealed hollow space, and with fine nylon netting to prevent the escape of emergent adult mosquitoes should this happen; and

(iii) two pieces of hard-board oviposition paddles which serve as suitable substrates for gravid ovipositing females and which are held in a vertical position by grooved bosses on the topside of the float.

About 2 months after preliminary observations in the Rochor study area ovitraps were placed inside 115 houses, 3 to each house, except for two houses where only single ovitraps were used. The *Aedes* premise index, breteau index, and density of larval and adult were measured weekly throughout the study period.

RESULTS

Habitats positive for breeding: Table 1 shows the percentage of habitats that were positive for *Aedes* breeding in the 115 premises examined weekly. It will be seen that there was much higher breeding of *Ae. aegypti* than *Ae. albopictus* in domestic habitats as well as in autocidal ovitraps. Autocidal ovitraps were about 81 times more attractive than habitats to gravid ovipositing *Aedes* females, as shown by the ratio of attractiveness between the two types of habitats (Table 1).

Fig. 2 shows graphically the same comparison between the attractiveness of autocidal ovitraps and domestic habitats in terms of percentage habitats found positive for *Aedes* vector breeding. The percentage of breeding in domestic containers fluctuated at a low level, with a mean of 0.2%. The percentage of breeding in autocidal ovitraps fluctuated at a considerably higher level after an initial buildup. By April 1975, the percentage of positive autocidal ovitraps had stabilized, and fluctuated at a mean of 18.4%. Again, it is apparent that *Ae. aegypti* was much more attracted to the autocidal ovitraps than was *Ae. albopictus*. The autocidal ovitraps were also much more attractive to *Ae. aegypti* than were domestic containers.

Premises positive for breeding: Fig. 3 shows a comparison between premises that had *Aedes* breeding in domestic containers and in autocidal ovitraps.

Table 1

Percentage of habitats positive for *Aedes* breeding in the Rochor study area.

Date of survey	Domestic habitats					Autocidal ovitraps					Ratio of attract-iveness (ovitrap: natural)
	No. exam.	No. breeding		% positive		No. exam.	No. breeding		% positive		
		aeg.+ alb.	aeg.	aeg.+ alb.	aeg.		aeg.+ alb.	aeg.	aeg.+ alb.	aeg.	
1974											
Dec. 16	482	7	7	1.5	1.5	-	-	-	-	-	-
26	482	2	1	0.4	0.2	-	-	-	-	-	-
1975											
Jan. 2	482	2	1	0.4	0.2	-	-	-	-	-	-
6	480	5	4	1.0	0.8	-	-	-	-	-	-
14	480	4	4	0.8	0.8	-	-	-	-	-	-
21	477	5	5	1.1	1.1	-	-	-	-	-	-
27	467	5	5	1.1	1.1	-	-	-	-	-	-
Feb. 3	488	3	3	0.6	0.6	-	-	-	-	-	-
17	482	1	1	0.2	0.2	-	-	-	-	-	-
24	479	3	3	0.6	0.6	338	2	1	0.6	0.3	0.9:1
Mar. 3	482	0	0	0	0	341	21	18	6.2	5.3	-
10	479	1	1	0.2	0.2	337	26	23	7.7	6.8	36.8:1
17	482	1	1	0.2	0.2	341	50	47	14.6	13.8	69.6:1
24	479	1	1	0.2	0.2	338	66	56	19.5	16.6	93.0:1
31	482	3	3	0.6	0.6	338	62	60	18.3	17.8	29.6:1
Apr. 7	482	2	2	0.4	0.4	338	61	56	18.1	16.6	43.0:1
14	462	1	1	0.2	0.2	326	50	43	15.3	13.2	69.7:1
21	475	1	1	0.2	0.2	335	75	60	22.4	17.9	106.6:1
28	475	2	2	0.4	0.4	335	63	55	18.8	16.4	44.8:1
May 5	480	5	5	1.0	1.0	338	68	59	20.1	17.5	19.3:1
12	467	1	1	0.2	0.2	329	68	56	20.7	17.0	98.4:1
19	478	1	1	0.2	0.2	335	53	46	15.8	13.7	75.3:1
27	472	3	3	0.6	0.6	329	54	46	16.4	14.0	25.7:1
June 2	459	0	0	0	0	329	61	50	18.5	15.2	-
9	480	1	1	0.2	0.2	338	69	60	20.4	17.8	97.2:1
16	477	1	1	0.2	0.2	335	68	59	20.3	17.6	96.7:1
23	472	0	0	0	0	335	61	55	18.2	16.4	-
30	482	1	1	0.2	0.2	341	66	56	19.4	16.4	92.2:1
July 7	482	0	0	0	0	341	76	66	22.3	19.4	-
14	482	1	1	0.2	0.2	341	77	69	22.6	20.2	107.5:1
21	460	0	0	0	0	326	64	62	19.6	19.0	-
28	482	0	0	0	0	341	61	58	17.9	17.0	-

Table 1 (cont'd)

Date of survey		Domestic habitats No. exam.	No. breeding aeg.+alb.	No. breeding aeg.	% positive aeg.+alb.	% positive aeg.	Autocidal ovitraps No. exam.	No. breeding aeg.+alb.	No. breeding aeg.	% positive aeg.+alb.	% positive aeg.	Ratio of attract-iveness (ovitrap: natural)
Aug.	4	475	0	0	0	0	335	56	50	16.7	14.9	-
	11	472	0	0	0	0	332	73	59	22.0	17.8	-
	18	461	0	0	0	0	329	70	60	21.3	18.2	-
	25	458	0	0	0	0	323	56	46	17.3	14.2	-
Sep.	1	480	1	1	0.2	0	338	40	31	11.8	9.2	59.2:1
	8	467	1	1	0.2	0	329	55	51	16.7	15.5	83.0:1
	15	441	0	0	0	0	311	49	42	15.8	13.5	-
	22	461	0	0	0	0	323	40	39	12.4	12.1	-
	29	448	0	0	0	0	320	59	55	18.4	17.2	-
Total	(A)	15113	31	31	0.21	0.21	10665	1820	1594	17.1	15.0	81.3:1
	(B)	12712	25	25	0.20	0.20	8970	1655	1449	18.5	16.2	97.1:1

(A) = 24 Feb. 75-29 Sep. 75, (B) = 31 Mar. 75-29 Sep. 75.

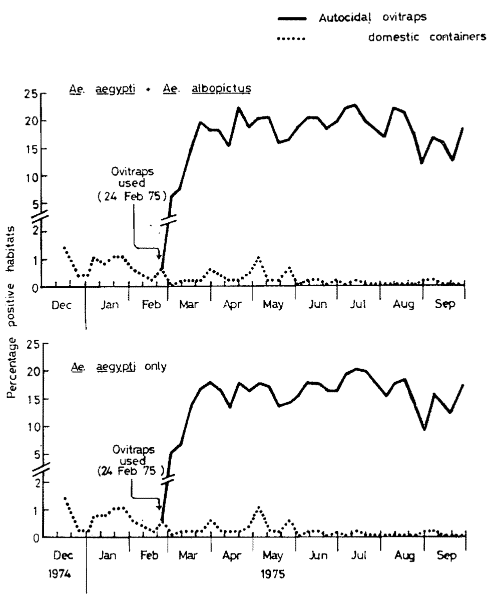

Fig. 2—Percentage of positive autocidal ovitraps and domestic containers, Rochor study area.

The usual premise index or percentage of premises positive for *Aedes* breeding,

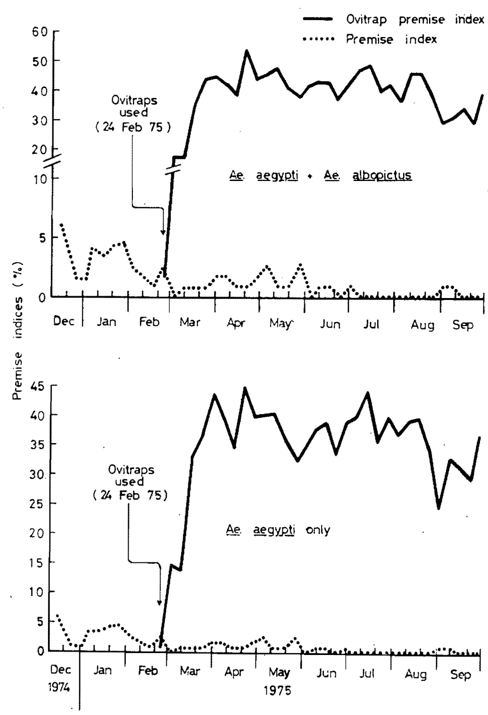

Fig. 3—Premise indices in Rochor study area.

fluctuated between 6.1 % initially in December 1974 and 0% towards July 1975, averaging at 1.3%.

The ovitrap premise index, i.e. percentage of premises with at least one autocidal ovitrap positive for *Aedes* breeding, fluctuated at 41.3% by the end of March 1975 after an initial buildup from 2% in February when it was introduced, thus showing that the use of ovitraps had increased the premise index by 31 times, i.e. from 1.3% to 41.3%. The autocidal ovitrap is thus again shown to be much more attractive than domestic habitats.

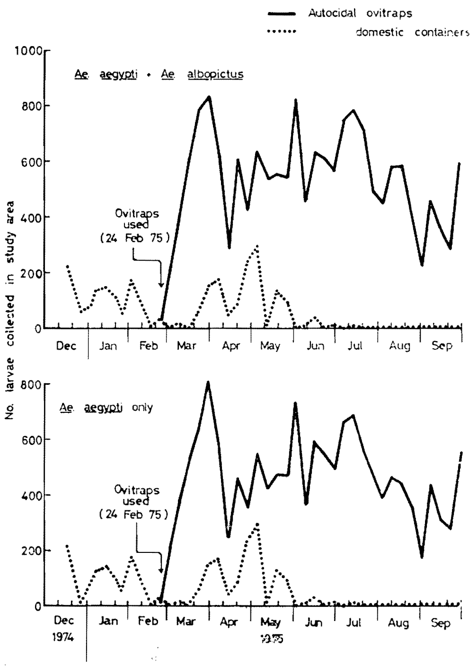

Fig. 4—No. larvae collected in autocidal ovitraps and in domestic containers, Rochor study area.

Larvae in breeding habitats: Fig. 4 shows the number of larvae collected weekly from domestic habitats and from autocidal ovitraps. The weekly total number of larvae collected from domestic habitats in the study area of 115 houses fluctuated between zero and 300 per collection with an average of 48. The weekly total number of larvae collected from autocidal ovitraps, on the other, fluctuated at a much higher level. From 20 at the time of introduction, it increased sharply to 834 about one month later and thereafter fluctuated between 834 and 286, with a mean of 550.

Thus, the superior attractiveness of the autocidal ovitrap is again demonstrated. That it is particularly attractive to *Ae. aegypti* is also abundantly clear.

DISCUSSION

In the control of DHF, the control of the principal vector, *Ae. aegypti*, is most important. Any device or method employed for its control should be directed principally at this vector.

Ae. aegypti is a highly anthropophilic mosquito. In Singapore, it is fully domesticated and dependent on man for its survival. All its activities such as breeding, feeding, resting and mating, are carried out in the domestic environment. Man is the chief, in most cases, the only, source of its blood meal and the provider of its breeding habitats. Its control thus depends largely on man changing his habits of storing water in and around the house. For this, health education and legislation are important means for preventing and controlling its breeding. However, it is Singapore's experience that these and other integrated measures, including fogging of areas with more than 5% premise index, cannot suppress the premise index below 2%. Further suppression must involve the use of additional supplementary measures, such as the ovitrap.

The use of the ovitrap in the surveillance of *Aedes* vectors is recommended by the World Health Organization (WHO, 1972). It is a sensitive and accurate device for detecting the presence of *Ae. aegypti* in port and airport areas. It is also recommended for the detection of this species in certain urban situations such as schools and hospitals and is recommended for use in low density areas (WHO, 1975). However, it has not been recommended for use in the control of *Aedes* vectors, whether under low density areas or in special situations such as ports, airports, schools and hospitals.

The first documented use of the ovitrap as a control device for *Aedes* vectors was that reported by Chan (1973b) in the Singapore Paya Lebar International Airport. This was also the first documented account of the eradication of *Aedes aegypti* from an airport by this device.

The present study using the improved ovitrap or autocidal ovitrap is also the first documented account of using this device for the control and possible eradication of *Ae. aegypti* in an urban environment. The present study has shown that the autocidal ovitrap, designed by the authors, is superior to all other domestic containers including ant traps and jars, the most preferred breeding habitats of *Ae. aegypti* and *Ae. albopictus* in nature (Chan *et al.*, 1971). Its superior attractiveness is due to the combination of two characteristics preferred above all others, by *Aedes (Stegomyia)* species, namely, the black colour of the container and the special properties of the hard-board paddles as oviposition substrates.

The present study shows that through gradual trapping and killing of the progeny of *Ae. aegypti*, and through gradual reduction of the mosquito population, eradication of this mosquito is possible, as was demonstrated at the Paya Lebar International Airport (Chan, 1973b). Eradication would theoretically be most rapid when domestic habitats are first reduced to a minimum by intensified integrated control. Total eradication of the species may not, however, be simple. There will always be the problem of constant re-introduction of the species through air, sea and land travel.

It is unlikely that the autocidal ovitrap could be used for the eradication of *Ae. albopictus* as this species is little affected. Being less domesticated than *aegypti*, it still utilizes many natural habitats such as tree holes, fallen leaves, leaf axils, etc, in forested areas. The control and eradication of *Ae. albopictus* is thus more difficult.

The use of autocidal ovitraps may be compared to the use of genetic measures. Like genetic methods, it works best when the natural mosquito population, in this case the number of domestic habitats is first reduced to a minimum. The number of natural domestic habitats must be significantly less than the number of autocidal ovitraps used. The ratio between these two types of habitats at which eradication is possible has not yet been determined.

The use of autocidal ovitraps in an integrated control programme has many advantages. First, unlike insecticides and biological agents, such a mechanical device does no apparent harm to the environment. Second, it is specific, being effective against only container breeders like the *Aedes* vectors, particularly *Ae. aegypti*. Third, it creates no environmental pollution or insecticide resistance problems. Fourth, it can be easily implemented in the home. All that is required is the periodic replacement of water that is evaporated. However, the removal of any of its parts must first be made fool-proof before it could be implemented on a country-wide basis. Otherwise it would itself become a major breeding habitat. Fifth, it is economical, each trap costing only about S$ 2 and, except for the paddles which would rot with time, would last almost indefinitely if the plastic parts are properly constructed. Sixth, it can be placed anywhere in the home to achieve maximum control, e.g. in the kitchen and bathroom and near water sources where the adult mosquitoes rest. Seventh, it saves manpower required in the routine inspection of premises by public health officers.

SUMMARY

A revolutionary, autocidal ovitrap has been developed as a promising weapon for the control and possible eradication of *Ae. aegypti*, the principal vector of dengue haemorrhagic fever (DHF), in urban high DHF endemic areas. A study carried out with this trap in a highly endemic area for DHF in Singapore city, namely the Rochor area, has shown this trap to be superior to all other domestic habitats in attractiveness to ovipositing *Ae. aegypti* females.

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EVALUATION OF DURSBAN[R] AND DOWCO 214 AS MOSQUITO LARVICIDES IN RICE-FIELDS

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INTRODUCTION

Among the mosquito-borne diseases, malaria and filariasis have been the most important diseases of mankind for centuries. This is true in many parts of the tropics and subtropics (Kessel, 1966; Johnson, 1973). In Malaysia, malaria is the most common mosquito-borne disease, especially so in the rural areas. Several species of *Anopheles* including *Anopheless campestris* are the main vectors of malaria in West Malaysia (Cheong, 1972). Filariasis caused by *Brugia malayi* is endemic in Malaysia (Ramachandran, 1972), the main vectors being the *Mansonia* species and several species of *Anopheles* particularly *Anopheles campestris* (Reid *et al.*, 1962; Ramachandran and Dondero, 1973). In Malaysia, antibodies to Japanese B encephalitis, another mosquito-borne disease which is epidemic in Japan, Korea and Taiwan, is widespread. A few cases involving children are seen throughout the year although the number of deaths are not known. *Culex tritaeniorhynchus* and *Culex gelidus* are the main vectors of Japanese B encephalitis (W.H.O., 1969; Reisen *et al.*, 1976).

Rice fields, especially those in Asia, are common breeding grounds for vector mosquitoes (Bram, 1967; Reid, 1968; Reisen *et al.*, 1976). On Penang Island, *Anopheles campestris*, *Culex tritaeniorhynchus* and several other species of *Anopheles* and *Culex* are commonly found in rice-fields. The control of these mosquitoes have so far largely depended upon the use of adulticides such as DDT (Cheong, 1972). No serious attempt was made to control the mosquitoes at the immature stages simply because of the dirth of information on larvicide field trials.

More recently, a few organophosphorous compounds have been found to be selective and effective as mosquito control agents (Schoof, 1966; W.H.O., 1972). Among the newer insecticides, Dursban[R] and Dowco 214 have been found to be effective mosquito larvicides and adulticides (Ludwig and McNeill, 1966; Kitagaki *et al.*, 1973).

In earlier laboratory tests, Dursban[R] (0, 0 diethyl 0-3, 5, 6-trichloro-2-pyridyl phosphorothioate) has been found to be a broad-spectrum insecticide against many agricultural and stored-product insect pests (Kenaga *et al.*, 1965). Its effectiveness as a larvicide against mosquitoes has been confirmed in laboratory tests (Ludwig and McNeill, 1966; Schoof, 1966; Yap *et al.*, 1968; Kitagaki *et al.*, 1973; Yap and Sulaiman, 1976) as well as in field trials (aerial and ULV applications) in many subtropical regions (Ludwig *et al.*, 1968; Mulla *et al.*, 1970; Washino *et al.*, 1972; Cooney and Pickard, 1974). Dursban has been found to be particular effective in controlling mosquito immatures in polluted water (Schaefer and Dupras, 1970b; Lowe and Parakarn, 1971). Various polymer control-release formulations of chlorpyrifos (Dursban) have been tested as mosquito larvicides (Miller *et al.*, 1973; Nelson *et al.*, 1973; Roberts *et al.*, 1973; Evans *et al.*, 1975; Nelson *et al.*, 1976). Adulticides effects of Dursban have also been investigated (Ludwig *et al.*, 1968; Husted *et al.*, 1975). At the dosages used for mosquito control, the biological effects of Dursban appear to be minimum (Ludwig *et al.*, 1968; Hurlbert *et al.*, 1970).

The insecticidal effects of Dowco 214 (0,0 dimethyl 0-3, 5, 6-trichloro-2-pyridyl phosphorothioate), a dimethyl analog of Dursban currently known commercially as Reldan[R], are less studied. Laboratory tests indicate that Dowco 214 is promising as larvicides and adulticides against *Aedes*, *Anopheles* and *Culex* mosquitoes (Kitagaki *et al.*, 1973) and as larvicide against *Mansonia* mosquitoes (Yap and Sulaiman, 1976).

The present investigation concerns the field evaluation of Dursban and Dowco 214 as mosquito larvicides in some rice-fields of Penang. The physico-chemical conditions of the study sites were determined.

MATERIALS AND METHODS

Field study was carried out in the rice-fields adjacent to Jalan Kampung Masjid in the township of Telok Kumbar, situated on the southern coastal alluvial plain of Penang Island, Malaysia. Two rice-field sites were chosen for study from May to July, 1975. In the first site, fifteen plots totalling 0.284 hectare were used. The plot sizes range between 0.008 and 0.049 hectares. The second site lies adjacent to the first one, and consists of six plots totalling 0.162 hectare (ranging from 0.028 to 0.045 hectares). Air and water temperature and daily rainfall were recorded at these two sites for the entire study period.

Samples of mosquito larvae and adults were collected from the various plots prior to the larvicidal treatment and subsequently identified (Bram, 1967; Reid, 1968).

The chemistry of the semi-lotic water from all the plots within these two rice-field sites was studied. Water analysis was carried out on filtered samples taccording to standard methods (A.P.H.A., 1965; Golterman and Clymo, 1969). pH was measured at 25° ± 1°C with a pH-meter reading to ± 0.02 pH units. Alkalinity was determined using B.D.H. 4.5 as indicator. Total and calcium hardness were determined by means of sodium versenate (EDTA) technique. Specific conductance was measured with a conductivity meter. Orthophosphate was determined colorimetrically using stannous chloride-molybdate method.

The insecticides tested were in the forms of emulsifiable concentrates of Dursban (75 E.C., 7.5% chlorpyrifos, a.i. w/v) and Dowco 214 (22% chlorpyrifos-methyl, a.i. w/v). Dursban was tested at dosages of 14, 28 and 56 gm a.i. per surface hectare (i.e. 0.0125, 0.0250, 0.0500 lb a.i. per surface acre), while Dowco 214 was tested at 56 gm a.i. per hectare only. Two plots were used for each insecticide concentration in the first trial but in the second trial, one plot was used. The rest of the plots were used as control (untreated). The plots were chosen randomly.

Insecticides were diluted with tap-water and sprayed using a 2-gallon Hudson knapsack sprayer equipped with lance and nozzle (TEE JET, HSS 8002E). Two to four liters of insecticide - water mixture were sprayed per plot depending on the size of the plot. A dipper constructed of a light-colour enamelled plate (diameter 15.5 cm) with a 30-cm long handle was used to sample the mosquito larval population along the levees of the plots. Samples were taken immediately before and after 1, 2, 3, 7, 15, 30, 45 and 60 days after insecticide applications. Both the field trials were initiated immediately after the planting of rice on 2 May and 28 May 1975 respectively.

RESULTS

Physico-chemical conditions: The mean air temperature for Penang Island is 27.2°C (range: 23.3 - 31.1°C). At the study sites, the rice plots are totally exposed to direct insolation and as such the mean surface water temperature is high with mean values ranging between 29.1°C in the morning and 32.3°C in the afternoon. The lowest and highest water temperature recorded during this study are 25.5°C and 42.0°C respectively. Water depth varies between 7 and 15 cm.

The average annual rainfall for the last seventy five years is about 330 cm (130 in.). The daily rainfall record for the test sites over the study period (May-July 1975) is shown in Table 1. The total monthly rainfall for May, June and July are 31.2, 24.6 and 25.1 cm respectively. Spraying of insecticides was carried out on the relatively dry first and last week of May.

Some aspects of the chemical composition of the rice-field water are shown in Table 2. As a whole, the water is characteristically soft, weakly acidic and weakly buffered as indicated by the low bicarbonate alkalinity values. The ionic content is moderately high with mean specific conductance of 45.4 and 33.2 μmho cm^{-1} for Sites I and II respectively. Orthophosphate concentration varies between 0.015 and 0.034 ppm with means of 0.027 and 0.022 ppm for Sites I and II respectively.

Mosquito species: From the samples of mosquitoes collected from the rice-fields, at least three species of *Anopheles*, namely *An. campestris*, *An. jamesii* and *An. sinensis*, were encountered. *An. campestris*, the dominant species, is also a vector of malaria and filariasis in Penang. *Culex tritaeniorhynchus* is the most common *Culex* mosquito found here, the other being *Cu. bitaeniorhynchus*. *Cu. tritaeniorhynchus* is associated with the transmission of Japanese B encephalitis while *Cu. bitaeniorhynchus* is known to be naturally infected with *Brugia malayi* (Carter, 1948).

Larvicidal effects of Dursban and Dowco 214: The results of the field evaluation of the larvicidal effects of Dursban and Dowco 214 on

Table 1

Rainfall over the study area in Telok Kumbar, Penang.*

Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
May	O	O	S	O	O	O	O	S	S	M	S	S	S	M	H	M	M	O	O	O	H	M	H	M	H	S	O	M	O	O	O
June	M	O	O	S	O	O	O	O	O	M	O	H	O	O	H	M	O	O	O	O	S	O	O	O	O	O	O	O	O	H	-
July	O	O	O	O	O	S	S	O	M	O	O	O	O	O	O	O	O	O	H	O	O	O	O	O	O	H	H	H	O	O	O

* O = No rain; S = Slight rain (less than 1.27 cm); M = Moderate rain (1.27-2.54 cm); H = Heavy rain (more than 2.54 cm).

Table 2

Chemical content of the rice-field water in experimental areas.
All values are mean ± S.E. from seven determinations of water samples collected from various plots throughout the experimental period.

Site No.	pH	Specific Conductance (μmho. cm^{-1})	Alkalinity (ppm HCO_3)	Total Hardness (ppm $CaCO_3$)	Calcium Hardness (ppm $CaCO_3$)	Orthophosphate (ppm)
I	6.8 ± 0.9	45.4 ± 6.8	13.9 ± 2.0	5.4 ± 0.8	3.7 ± 0.5	0.027 ± 0.004
II	6.9 ± 1.0	33.2 ± 4.9	11.4 ± 1.8	4.7 ± 0.7	3.6 ± 0.5	0.022 ± 0.003

larvae of *Anopheles* and *Culex* are shown in Table 3. The *Anopheles* larvae appear to be more sensitive to Dursban and Dowco 214 than the *Culex* larvae. At the concentrations used, both achieved 100% control of the *Anopheles* larvae for at least two days. The dosage-mortality relationship of Dursban to *Anopheles* is well defined in Site I. Dursban, at concentrations of 14, 28 and 56 gm hectare^{-1}, kept the paddy plots free from *Anopheles* for at least two, three and seven days respectively (Table 3). Mosquito control by Dursban at Site II appears to be less effective even at a concentration of 56 gm hectare^{-1}. With respect to *Culex*, the results are more erratic (Table 3). The few *Culex* larvae that re-appeared in the first three days following larvicide application are of the fourth instar stage, which have apparently survived the insecticide treatment. From the seventh post-treatment day onwards, mosquito larvae were found in the treated plots but at densities generally lower than those of the untreated plots. The gradual reduction in larval densities in both the treated and untreated plots towards the end of the study period is attributable to the gradual drying up of the rice plots. Dowco 214, applied at a dosage of 56 gm hectare^{-1}, appear to be less effective against *Anopheles* larvae than Dursban at the same dosage.

DISCUSSION

As stated earlier, the mosquito larviciding and adulticiding effects of Dursban have been investigated using aerial and ultra low volume (U.L.V.) applications as well as various other types of polymer formulations involving large-scale study sites. The present study on

Table 3

Larvicidal effects of Dursban and Dowco 214 against *Anopheles* and *Culex* mosquitoes in rice-fields at Site I and Site II.

Number of days after treatment			0*	1	2	3	7	15	30	45	60
Treatment received (gm/hectare)	Plots	Mosquitoes	Mosquito larvae per twenty five dips per plot								
SITE I.											
Control (untreated)	7	*Anopheles*	23.7	11.6	13.1	18.9	10.9	12.3	14.1	4.4	2.7
Dursban (14)	2	,,	24	0	0	0.5	19	9	2.5	5	0
Dursban (28)	2	,,	18	0	0	0	0.5	7.5	6.5	0.5	0
Dursban (56)	2	,,	11.5	0	0	0	0	5	2.5	4	4
Dowco-214 (56)	2	,,	13	0	0	0	6	6	6.5	19	10.5
Control (untreated)	7	*Culex*	17.4	32	46.7	15.7	8.4	15	55.4	2.1	1.2
Dursban (14)	2	,,	21.5	4	0.5	0.5	5	7.5	4.5	3.5	0
Dursban (28)	2	,,	15	1	0	0	6.5	2	12	14.5	1
Dursban (56)	2	,,	18.5	0.5	0	0	5.5	7.5	13	5	1.5
Dowco-214 (56)	2	,,	7.0	0	0	0.5	0.5	7	9	20.5	31.5
SITE II.											
Control (untreated)	2	*Anopheles*	13.8	20.5	29	28.5	24.5	6.5	5	7.5	D**
Dursban (14)	1	,,	17	0	0	2	0	3	0	3	D
Dursban (28)	1	,,	16	0	0	0	9	22	3	12	D
Dursban (56)	1	,,	46.5	0	4	12	68	13	8	11	D
Dowco-214 (56)	1	,,	2.5	0	0	7	20	5	3	4	D
Control (untreated)	2	*Culex*	9.3	22	38	40.5	20	9.5	4.5	8.5	D
Dursban (14)	1	,,	9	0	0	0	0	0	4	0	D
Dursban (28)	1	,,	21.5	0	0	0	29	1	3	2	D
Dursban (56)	1	,,	20	0	5	0	6	0	0	0	D
Dowco-214 (56)	1	,,	27.5	0	0	3	33	0	0	0	D

* Mosquito larval count before spraying. ** Paddy field dried.

the larviciding effects of Dursban and Dowco 214 was carried out on small plots using emulsifiable concentrates and Hudson knapsack sprayer. The dosages (i.e. 14, 28 and 56 gm hectare^{-1}) tested in this study are below the normal application rates (112 gm hectare^{-1}) commonly used for organophosphorous larvicides (Schaefer and Dupras, 1970a). The results indicate that both Dursban and Dowco 214 have good potentials as mosquito larvicides in tropical rice-fields.

Direct sunlight and temperature fluctuation may enhance the hydrolysis of organophosphorous insecticides such as Dursban and Dowco 214. It is probable that these two factors are operational in this study and thus affect the results to some extent. Since rainfall was negligible during the first week following each treatment (Table 1), overflowing or washing away of insecticides did not occur. Under the existing chemical conditions of the rice-field water, the breakdown rates of Dursban and Dowco 214 should be moderate.

Dursban at the dosages used for mosquito control has been reported to be safe against non-target freshwater organisms. When applied at rates below 0.1 lb acre^{-1} (112 gm hectare^{-1}) it causes less than 10% mortality to the caged mosquito fish *Gambusia affinis* and does not affect fish reproduction (Hurlbert *et al.*, 1970). However, application of Dursban at the same dosage in combination with the herbicide propanil in rice fields has been shown to trigger photo-toxic response in the rice plants (Craven and Steelman, 1968). The persistence of Dursban in pond waters has been shown to be relatively short, but its residues in the mud layer have been shown to increase gradually after application (Hurlbert *et al.*, 1970). The longer persistence of Dursban in polluted water is attributable to the fact that Dursban is absorbed onto the organic matter and thus is protected from hydrolysis (Schaefer and Dupras, 1970b).

Like most organophosphorous insecticides, Dursban and Dowco 214 are blood cholinesterase inhibitors. Repeated exposure to these compounds, even at low dosages, may cause cholinesterase depression. The oral toxicity values (LD_{50}s) of chlorpyrifos (Dursban) and chlorpyrifos-methyl (Dowco 214) to white rats are 97-276 and 941-2,140 mg kg^{-1} respectively. However, the dermal toxicity values for both are 2,000 mg kg^{-1}. This relatively low dermal toxicity makes both the safer organophosphorous compounds to be used for mosquito control (W.H.O., 1972; Kenaga and End, 1974).

With the tremendous increase in the prices of petroleum products of which the anti-malaria oil is no exception, alternatives are being sought to replace the often-used anti-malaria oil. Abate appears to be a good alternative with its good larviciding property and also from the point of view of safety (Cyanamid, 1969; Yap, 1976). From this present study, Dursban and Dowco 214 appear to be promising as mosquito larvicides. They could be used as substitutes for the anti-mosquito oil particularly so in polluted waters.

SUMMARY

Emulsifiable concentrates of Dursban^{R} (chlorpyrifos) and Dowco 214 (chlorpyrifos-methyl) were tested as mosquito larvicides using Hudson knapsack sprayers on small plots of rice-fields on Penang Island. The mosquitoes found in these rice-fields are predominantly *Anopheles campestris* and *Culex tritaeniorhynchus*. At dosages of 14, 28 and 56 gm hectare^{-1}, Dursban is effective in maintaining the rice-fields free of *Anopheles* larvae for at least 2, 3 and 7 days respectively. Dowco 214 at 56 gm hectare^{-1} is able to keep the fields free of all mosquito larvae for at least 2 days.

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DDT RESIDUES IN ADIPOSE TISSUE OF PEOPLE IN RANGOON AREA

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INTRODUCTION

In Burma DDT is the most widely used organochlorine insecticide all over the country in connection with public health programs to control vector insects of human diseases. The Malaria Eradication programme has introduced DDT in Burma since 1954; DDT is also commonly used by the people for domestic control of insect pests.

Thus, it is obvious that considerable amount of DDT residues may have accumulated in our environment as well as in our bodies over these many years. Yet, no residue investigation of this persistent pesticide has been made. Accordingly, the aim of this work is to determine the levels of DDT type residues in the body fat of Burmese people. However, due to limited facilities it was not feasible to conduct a nation-wide survey; thus, a preliminary investigation was carried out on fat samples of post mortem subjects that lived in the Rangoon area.

MATERIALS AND METHODS

Adipose tissue samples, a total of forty-three, were randomly collected from the Rangoon General Hospital, the Children's Hospital and the Insein General Hospital, all situated within the Greater Rangoon area. Samples were subcutaneous fat from abdominal area of subjects who died of natural or accidental death; none of the persons had known occupational exposure to organochlorine insecticides.

Wherever possible samples were analyzed immediately; when this was not permissible, they were stored in plastic bags in deep freeze. Extraction of lipid was carried out according to de Faubert *et al.*, (1964). Adipose tissue (10 gm) was mixed with acid-washed sand and anhydrous sodium sulphate and ground in a mortar to obtain a dry granular mass. The latter was extracted four times with a total of 100 ml of petrol. Petrol extract was evaporated to a small volume and partitioned with dimethylformamide (DMF) to effect the transfer of organochlorine residues into DMF, saturated with petrol. The DMF was then shaken with 2% aqueous sodium sulphate solution leaving petrol extract of organochlorine residues associated with a small amount of lipid.

Crude organochlorine residues were purified on a Florisil column as described by Mill *et al.*, (1972). The column was successively eluted with 6% and 15% diethyl ether in petrol; the eluates, fraction-1 and fraction-2 were evaporated. Residues were analyzed by TLC as described by Fishbein (1974), using ethanolic solution of 0.5% silver nitrate as chromogenic reagent with subsequent UV irridiation. Spots were visually assessed against standards containing known quantities of authentic compounds run on the same plate along with fraction-1 and fraction-2. Detection limit in routine analyses was 0.1 ppm for DDT, 0.2 ppm for DDE and 0.2 ppm for endrin when present.

RESULTS AND DISCUSSION

DDT and DDE residues were found in all 43 samples analyzed (Table 1).

Table 1

Concentration of organochlorine insecticides in adipose tissue of people in Rangoon area (ppm).

Compound	Age group / No. of cases	1-4 / 7	5-24 / 9	25-44 / 15	45+ / 12
DDT	Range	0.20 – 1.00	0.10 – 0.79	0.1 – 0.99	0.1 – 1.11
	Mean ± SD	0.69 ± 0.12	0.29 ± 0.07	0.34 ± 0.10	0.46 ± 0.14
DDE	Range	0.20 – 5.00	0.20 – 4.80	0.2 – 5.9	0.2 – 5.5
	Mean ± SD	2.30 ± 3.29	1.69 ± 3.62	1.49 ± 2.77	2.21 ± 3.54
Total DDT	Range	0.42 – 6.06	0.32 – 5.76	0.32 – 7.36	0.32 – 7.05
	Mean ± SD	3.26 ± 4.88	2.17 ± 5.66	1.99 ± 4.24	2.92 ± 5.85
Per cent DDE		70.6	77.9	74.9	75.7

All throughout this work no attempt was made to separate DDT and DDE into their ortho- and para- isomers since a satisfactory separation was difficult to achieve in routine analyses by TLC. Again, DDD which is normally present at vanishingly small levels in adipose tissue was not detected. Endrin was detected in two cases: at 2.2 and 2.0 ppm, respectively; detection limit for this compound was 0.2 ppm. This may be due to limited use of endrin as an insecticide, especially with edible crops, in Burma.

Levels of DDT type residues in fat samples were found to be 0.10 to 1.10 ppm for DDT and 0.20 to 5.90 ppm for DDE, respectively. These ranges are of the order of magnitude found in a non-agricultural country such as U.K. (Abbott *et al.*, 1972) where organochlorine compounds are not extensively used for the control of agricultural pests. Although Burma, unlike U.K. is an agricultural country, pesticide use is not extensive in Rangoon area. This is reflected in the low levels of DDT, DDE and endrin residues in the body fat of the people living in that area compared with those found in the populations in neighbouring agricultural countries such as Thailand (Wassermann *et al.*, 1972) and India (Ramachandran *et al.*, 1973).

Mean values together with standard deviations for DDT, DDE and total DDT for males and females, respectively, is shown in Table 2.

To study whether there is any significant difference between sexes in the content of DDT type residues " t " test was carried out. It revealed that no significant difference was present ($P > 0.80$). No significant difference between males and females has also been observed in India by Ramachandran *et al.*, (1973); on the other hand, Wassermann *et al.*, (1972) found a significant difference between sexes in the population of Thailand.

The distribution of DDT, DDE and total DDT residues in different age groups was also studied. For this purpose four age groups were divided as shown in Table 2. No attempt was made to divide males and females separately, since in doing so sufficient number of subjects would not be available in some age groups. It was found that there was no significant difference of DDT, DDE or total DDT content among the four age groups. Level of DDE in total DDT for all age groups was around 70%; again, there was no significant difference of DDE level in total DDT load between males and females. In conclusion it may be stated that levels of DDT and endrin residues in the body fat of people living in Rangoon area are low when compared with those found in the populations living in

Table 2

Concentration of organochlorine insecticides in adipose tissue of males and females in Rangoon area (ppm). Number of cases in parenthesis.

Compound		Male (25)	Female (18)
DDT	Range	0.10 – 0.99	0.10 – 1.11
	Mean ± SD	0.38 ± 0.31	0.47 ± 0.40
DDE	Range	0.20 – 5.90	0.20 – 5.50
	Mean ± SD	2.02 ± 1.75	1.66 ± 1.83
Total	Range	0.32 – 7.36	0.32 – 7.05
	Mean ± SD	2.62 ± 2.18	2.31 ± 2.3
Per cent DDE		77.1	71.4

Calcutta (Ramachandran *et al.*, 1973) and Bangkok (Wassermann *et al.*, 1972).

SUMMARY

Necropsy fat samples from subjects living in Rangoon area have been analyzed by TLC for their DDT, DDE and endrin contents. DDT and DDE were found in all cases; endrin, in excess of 0.2 ppm, was detected in two cases only.

There was no significant difference in DDT, DDE or total DDT content in various age groups or between males and females. Levels of these residues were found to be low when compared with those observed in the populations living in Calcutta and Bangkok. This may be due to limited use of DDT and endrin for the control of agricultural pests in Burma.

ACKNOWLEDGEMENTS

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STUDIES ON THE LIFE HISTORY OF *MEGASELIA SCALARIS* (LOEW) IN THAILAND

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INTRODUCTION

Two species of *Megaselia* cause myiasis in man and animals, *M. scalaris* and *M. rufipes*, but there are only a few cases and reports of human myiasis caused by this fly in the world. Patton (1921, 1922) recorded some cases of intestinal, ophthalmic, and wound myiasis in man and animals in which this fly was involved, either alone or in association with *M. rufipes* and *Chrysomyia bezziana*. In Thailand, Priyanond *et al.*, (1973) reported the first case of acute urethral obstruction in a female patient caused by the larvae of this species. The purpose of this study is to describe the life history and to discuss the possible importance of this fly to public health.

MATERIALS AND METHODS

The work was divided into morphological, life-cycle, and experimental studies. The species were identified and reared in the laboratory. Recognition characters at each morphological stage were studied. For studying the life cycle, the adults were reared in vials. Blood agar, made from deer blood and used as a rearing medium, was cut into small pieces and placed at the bottom of the vials. Nine pairs of male and female flies were transferred from a big jar, used for rearing colonies, to the vials, one pair to a vial, and the vials were then corked tightly. Each vial was checked daily until the flies completed their life cycle.

Experimental studies : Three infection experiments were done. In the first, white rats were orally exposed to the larvae. In the second, the female genital organs of the animals were exposed to larvae, and in the third, white rats were injected intraperitoneally. Three white rats and newly hatched larvae were used for each test. The infected animal faeces were collected and checked every day for 7 days in the first test. In the second and third tests, infected white rats were sacrificed 7 days after infection and checked for types of myiasis.

RESULTS

Morphological study : In general appearance, the adult was a small, hump-backed fly, about 2-3 mm in length. The male was usually a little smaller than the female and the body of both sexes was yellowish-brown. The head was of the phorid type with a short proboscis. The thorax was humped and the wings were well-developed, with a forked vein R_S on each wing. The legs were yellowish-brown with hairy tibia. The abdomen was banded with brown colour. The egg was small, canoe shaped, creamy white in colour. The mature larva was creamy white, about 4-5 mm in length. The head had one pair of toothed mandibles in place of the usual hooks. Each body segment was provided with short fleshy processes, not hairy, on the dorsal and lateral surfaces. The pupa was more robust than the larva. The anterior spiracles were extended into rodlike processes. The general appearance of the adults, larvae, and pupae are shown in Figs. 1, 2 and 3 respectively.

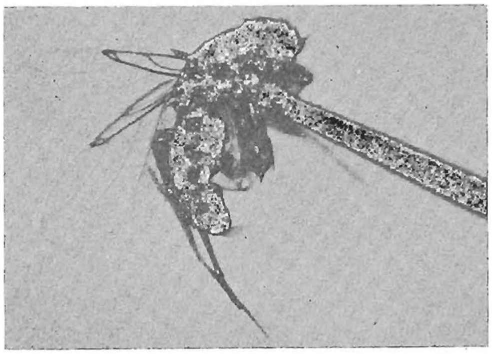

Fig. 1—Adult of *Megaselia scalaris* x 25.

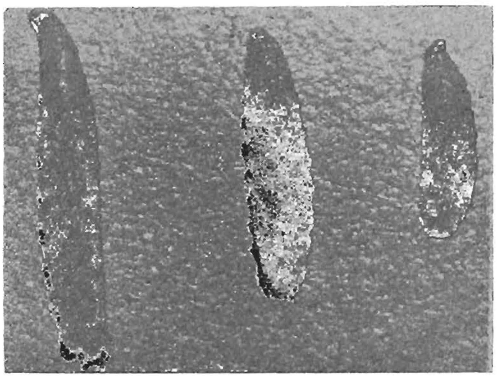

Fig. 2—Larvae of *Megaselia scalaris* x 25.

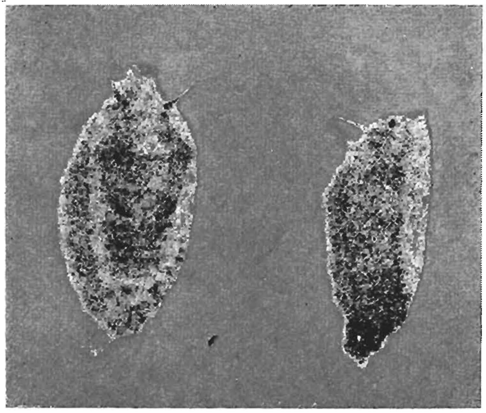

Fig. 3—Pupa of *Megaselia scalaris* x 25.

Life cycle : The following life cycle was based on room temperature set at 27 ± 1°C and 81 ± 3% relative humidity. Mating occurred within one day after the adults had emerged. Two days later each female deposited the eggs singly on the media, usually in the afternoon. Two to three days after the first oviposition, each female deposited eggs once more before she died. The number of eggs laid each time varied from 9 to 26. The life span of male and female flies was 2 and 7 days respectively. The eggs hatched within

Table 1

The life cycle of *M. scalaris*.

No. each pair of adults	No. eggs deposited		% hatch	Larvae (day)	Pupae (day)	Adults (day)		Life history (day)	
	1st time	2nd time				Male	Female	Male	Female
1	13	13	85.7	5-6	6-7	3	6	15-17	18-20
2	26	14	95.0	5-7	6-7	2	4	14-17	16-19
3	15	17	85.0	5-6	6-8	3	5	15-18	17-20
4	25	13	85.0	5-1	6-8	3	5	15-18	17-20
5	9	12	90.1	5-6	6-7	3	5	15-17	18-19
6	22	18	80.0	5-9	6-7	3	7	15-20	19-24
7	19	14	93.9	5-7	6-7	2	5	14-17	17-20
8	11	16	81.7	5-6	6-9	3	6	15-19	18-22
9	23	10	77.8	5-6	6-7	3	4	15-17	16-18

one day. The larvae passed 3 instars in 5 to 9 days and then pupated. The pupal stage required 7 to 9 days (see Table 1).

In experimental studies no artificial myiasis occurred in the infected white rats at all. This might be because the experimental animals were very small or unsuitable conditions were involved.

DISCUSSION

A total of 58 countries in every region of the world have been cited in the geographical distribution of this species (James, 1947). In Thailand, the flies are commonly found everywhere, usually on human and animal excrement, decaying and stale meat, milk, etc. (Sucharit *et al.*, 1976). This species can cause many types of myiasis, especially urinary myiasis. It is believed that in the majority of cases, infestation is probably a result of natural exposure, such as might come from the use of unsanitary outdoor toilets or from sleeping during the daytime with the body exposed. Or the female fly may be attracted by discharges, especially those of an albuminous nature. The ultimate stopping place of the larvae is the site that attracted the parent flies, namely the urethra or bladder (Chevrel, 1909). This species can complete its life cycle in a short time, therefore the population can increase rapidly at times. Parasitization of man by this species is accidental but it is nevertheles one of the medically important species to man since infestation may result in severe injury to the patient.

SUMMARY

The morphological, life-cycle, and experimental studies of *Megaselia scalaris* were reported. This fly is commonly found both in urban and rural areas in Thailand. It is easily identified and the humped thorax was the most distinct characteristic of the adult. The egg to the adult stage and the life span required 15 to 20 days for the male and 16 to 22 days for the female. Experimental attempts to induce myiasis infection in laboratory animals were unsuccessful.

ACKNOWLEDGEMENTS

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AN ENTOMOLOGICAL SURVEY ON THE MOSQUITOES OF WUVULU ISLAND, PAPUA-NEW GUINEA

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INTRODUCTION

An epidemiological survey on arboviruses was carried out on Wuvulu Island, in August 1975 and 1976.

Wuvulu (or Matty) Island is located 142 miles north of Wewak, in the Sepik district of New Guinea, 260 miles west of Manus Island (Bismarck archipelago) and more than 600 miles south of Caroline Island. Thus, it is a very remote, coralline and small size island (3,700 acres).

The climate is of oceanic type : the average temperature is between 70° and 95°F; the trade winds, whose force is always moderate, blow north-westward from June to September and south-eastward from November to February ; the annual rainfall is between 80 and 120 inches quite evenly distributed throughout the year. The vegetation is a very dense primary forest, rich in epiphytes, cleared in some places for coconut palm plantation.

The islanders (about 500), of Micronesian origin and culture, are distributed in two villages, Auna (sunrise) and Onne (sunset), settled on the west coast where they are two miles apart. Traditionally, they live from their fishing and grow taros, coconuts and sweet potatoes. Work on the plantation is done by a small group of Melanesians from the West Sepik district, living apart from both villages so they have but few contacts with the native population (Gaxotte, 1975).

MATERIALS AND METHODS

The culicids were collected at larval and adult stages. Thus, all detected potential breeding sites have been prospected; some of them were natural (swamp in the center of the island, tree holes, coconuts, shells), others were artificial (drums for water stocking). All adult mosquitoes were caught while biting man, generally near the villages or around the expedition campus.

All the collected mosquitoes were brought back to Paris to be identified.

RESULTS

Six culicid species are present in this collection, but, one of them, the identification is doubtful. So the list of Wuvulu mosquitoes is as follows :

Aedes (Stegomyia) hebrideus Edwards
Aedes (Finlaya) notoscriptus (Skuse)
Aedes (Verrallina) lineatus (Taylor)
? *Aedes (Lorrainea) dasyorrhus* King & Hoogstraal
Culex pipiens fatigans Wiedemann
Armigeres breinli (Taylor)

Aedes (S.) hebrideus : Numerous larvae and pupae collected in tree holes, coconuts, shells; adults biting man, in the late afternoon. This species, which belongs to the *scutellaris* group of *Stegomyia*, develops very often in domestic breeding sites and is usually very aggressive for man. It is known from New Hebrides where it is very abundant (Rodhain and Fauran, 1975), Solomon Islands, Santa Cruz and Torres Islands but Belkin (1965) have yet recorded it in Wuvulu in 1956.

The part played by *Ae. hebrideus* in the transmission of the dengue virus, suspected by Daggy (1944), seems likely according to the observations made in Espiritu Santo Island (New Hebrides), where, *Ae. aegypti* being absent, successive outbreaks are easily spread.

Aedes (F.) notoscriptus : Larvae and pupae in tree holes and in metallic drum in Onne and Auna. The larval ecology of this *Aedes* can be compared to the bionomics of the earlier mosquito. On the other hand, the adults are not anthropophilic, and this fact is confirmed by their absence in our collections. This species is known from a large area in the South Pacific (Australia, New Zealand, New Guinea, New Britain, New Ireland, Moluccas, Neo-Caledonian archipelago) and reported in the collection studied by Belkin (1965). It probably has no medical significance.

Aedes (V.) lineatus: Females collected on man in the whole island at the end of afternoon, in rather large numbers. This *Aedes*, of which the larvae usually breed in pools with abundant vegetation or in flooded forests (Huang, 1968) seems to be a man-biting mosquito in all its distribution area, with the largest of those of *Verrallina*: Solomon Islands, New Britain, New Ireland, Bismarck archipelago, New Guinea, Admiralty Islands, Ceram, Amboine, Australia and New Hebrides where Rageau and Vervent (1958), then Rodhain and Fauran (1975) drew attention to its abundance in forest and its aggressivity for man.

Aedes (L.) dasyorrhus : Only one female in rather bad condition seems to belong to this species, which is known in New Guinea and Solomon Islands.

Culex pipiens fatigans : Many larvae collected in drums in Auna village and one female. This species was yet known from Wuvulu (Belkin, 1965).

Armigeres breinli : Larvae collected in shells ; adult females caught on man in the whole island. Shells are classical breeding sites for the larvae of this mosquito. This species is known to bite man during daytime in the bush (Belkin, 1962). Its distribution covers New Guinea, Bismarck archipelago, Santa Cruz and Solomon Islands. It has no medical significance.

DISCUSSION

When, in 1956, David D. Bonnot made a very brief survey on Wuvulu Island, four species have been collected (Belkin, 1965) viz: *Aedes notoscriptus*, *Ae. hebrideus*, *Culex squamosus* and *C. pipiens fatigans*.

These two *Aedes* species have so been found again in 1975, with *Ae. lineatus* probably introduced since this date, and perhaps *Ae. dasyorrhus*. This survey affirms the absence of *Ae. aegypti* actually. As far as *Culex* is concerned, only *C. pipiens fatigans* is still present in the island. However, this species is certainly the first to be exposed to the anticulicid measures which are carried out from time to time. *A. breinli* lastly, probably introduced, seems to have taken root in Wuvulu Island.

No *Anopheles* species have ever been observed in the island where malaria is present. However, *Anopheles* larvae are probably present in the swampy central area and they could have escaped from the surveys.

Concerning the medical importance of these mosquitoes, only *Ae. hebrideus* could be an efficient dengue vector. The serological survey made in the islanders (Micronesian islanders and New-Guinean Melanesian imported workers) shows that dengue outbreaks which spread over South Pacific did not reach Wuvulu Island (Gaxotte *et al.*, 1975). The virus introduction anyway is still always possible, like it happened in New Britain or in Sepik district (New Guinea) in 1971-1972. Except for *C. p. fatigans*, no recorded culicid could be an efficient vector of bancroftian filariasis, the presence of which was shown by Backhouse and Heydon (1950).

SUMMARY

An entomological survey in Wuvulu Island, Papua New Guinea, in August 1975 and 1976 shows the presence of six mosquito species : *Aedes (S.) hebrideus*, *Ae. (F.) notoscriptus*, *Ae. (V.) lineatus*, *? Ae. (L.) dasyorrhus*, *Culex pipiens fatigans* and *Armigeres breinli*. The medical significance of these mosquitoes is discussed, with special reference to the problem of dengue virus transmission.

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HISTAMINE CONTENT IN 24-HOUR URINE IN PATIENTS WITH DENGUE HAEMORRHAGIC FEVER

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INTRODUCTION

Dengue haemorrhagic fever (DHF) is still a serious pediatric problem in Thailand and in other Southeast Asian countries (Udomsakdi, 1973). The present concepts indicate that immunologic mechanism plays a major role in pathogenesis of the disease (Russell, 1971). The immune complex reaction has been postulated for the mechanism of dengue shock syndrome (DSS) (Halstead, 1970, Russell, 1971) by the evidence of the rapid disappearance of dengue virus from the blood and tissue of the DHF patients (Nisalak *et al.*, 1970; Bhamarapravati and Boonyapakvanik, 1966); dengue antibody responses (Ig G) in most of severe cases being compatible with secondary infection(Halstead *et al.*, 1970) and marked reduction of serum complement during the shock phase of illness indicating complement utilization (Russell *et al.*, 1969; Suvatte *et al.*, 1973).

Precise knowledge of mechanism of shock, especially the role of mediators, provides not only better understanding of the pathogenesis of DSS but also provides a more proper treatment of the patients. The present work was conducted to study the role of histamine in DHF and to determine whether this substance is released during the course of the disease.

MATERIALS AND METHODS

The subjects include 12 normal children as a control group, there were 7 males and 5 females. Age ranged from 4-12 years. Patients with DHF who were diagnosed clinically and confirmed by serology include 12 cases. Five were males and 7 were females, and age range from 5-11 years. The severity of DHF were classified as previously described (Phitaksphraiwan *et al.*, 1961). In this study, there were 2 cases of grade I, 4 cases of grade II, 5 cases of grade III and 1 case of grade IV. Serum C_3 were measured in all patients on admission.

Twenty four hours urine of normal subjects and DHF patients were collected in the bottles with 2N.HCl and refrigerated until analysed. The amount of free and total histamine in 24 hours urine were extracted by ion-exchange-Amberlite I.R.C. 50 column chromatography (Bergström and Hansson, 1951), and then measured by four-point bio-assay method (Perry, 1970).

RESULTS

Serological study of DHF patients revealed 3 cases compatible with primary dengue infections and 9 cases compatible with secondary dengue infections.

Serum C_3 of each patient in this study are plotted, compared to the mean levels of C_3 in normal children and DHF in each grade of severity as reported by Suvatte (1973) and shown in Fig. 1.

The values of free and total histamine in normal subjects and patients (expressed as μg of histamine/kg body weight/24 hour urine) are shown in Table 1. The mean values of free and total histamine in normal subjects and patients with student 't' test between both groups are summarized in Table 2.

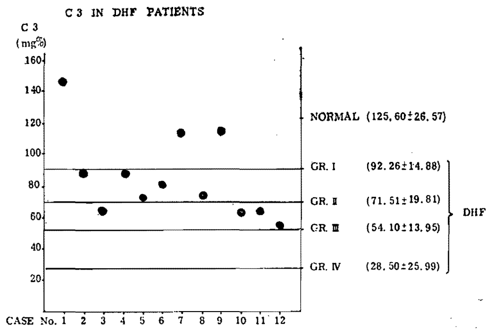

Fig. 1—Serum C_3 in each patient.

Fig. 2 shows the correlation between urinary histamine and severity of the disease. It was found that the mean values of urinary histamine definitely increased with severity of the disease.

DISCUSSION

Previous studies have demonstrated that during the shock phase of DHF, the haematocrit rises sharply (Tuchinda, 1973) and the plasma volume is markedly reduced (Suwanik *et al.*, 1967). Moreover, there are considerable amount of fluid in various serous spaces in

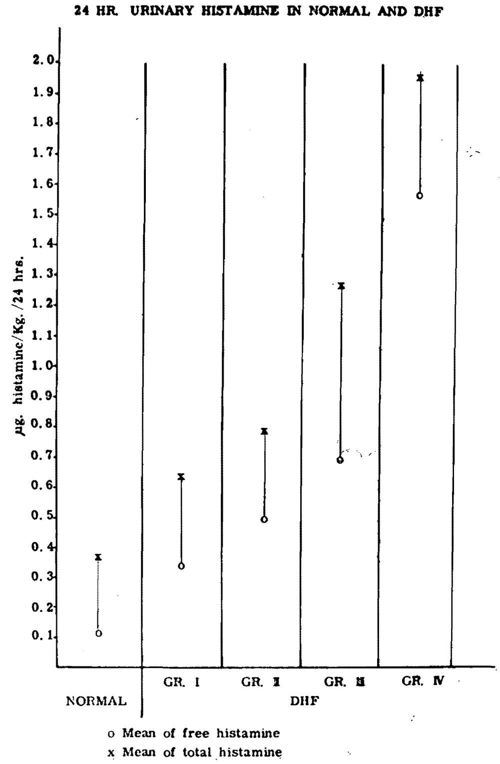

Fig. 2—The mean values of free and total histamine in normal subjects and DHF in each grade of severity.

Table 1

A 24-hour urinary histamine in normal subjects and DHF patients (µg/kg/24 hrs).

Subject	Normal		DHF	
	Free	Total	Free	Total
1	0.0410	0.2460	0.0276	-
2	0.3030	0.6460	0.2444	0.4693
3	0.1010	0.3830	0.4070	1.039
4	0.0660	0.4190	2.1699	-
5	0.0790	0.4740	0.6302	0.7557
6	0.4983	0.6704	0.5200	1.2574
7	0.0740	0.1380	0.4667	0.8078
8	0.0840	0.5820	1.5357	1.9173
9	0.1881	0.6681	0.1930	0.4953
10	0.0271	0.0350	0.2857	0.4018
11	0.0031	0.0839	0.1907	0.4808
12	0.0118	0.1159	1.4624	1.6424

Table 2

Urinary histamine in normal and DHF.

Histamine (μg/kg/24 hrs)	Normal (Mean ± S.D.)	DHF (Mean ± S.D.)	P
Free	0.1230 ± 0.1442	0.6777 ± 0.6714	<0.02
Total	0.3714 ± 0.2418	0.9266 ± 0.5300	<0.01

fatal cases (Bhamarapravati *et al.*, 1967). These findings are strongly suggestive of leakage of intravascular fluid to extravascular spaces in DSS. Mediators released during the shock phase may play a role for increased vascular permeability. Previous study of plasma kinin system failed to provide the evidence of its significant role in the immunopathogenesis of DHF (Edelman *et al.*, 1974).

The results of this study clearly demonstrated that urinary histamine content is much more in patients with DHF than in the normal subjects. Furthermore, more histamine in urine were found in more severe cases. The more urinary histamine should reflect more histamine released in the blood in DHF than in normal subjects. The reasons of increased histamine in DHF is probably due to two mechanisms : firstly during complement activation in DHF, one of the products of C_{3a} or C_{5a} by either classical or alternate pathway or both namely "anaphylatoxin" is released. This substance has three distinct actions : contraction of smooth muscle, increased vascular permeability, and release of histamine from the mast cells, and secondly the increased histamine may be due to the release reaction of the damaged platelets, as the platelet kinetic study by Mitrakul and associates (1974) indicated increased platelet destruction in DHF.

SUMMARY

Twenty-four hour urinary histamine in 12 patients with DHF compared to 12 normal subjects in the comparable age and sex were studied. The results revealed significantly increased urinary excretion in patients with DHF than in normal subjects in both free and total forms. This finding suggests that histamine may be one, if not all, of the mediators released during the course of the disease, especially in the severe cases. Histamine may play an important role for the leakage of intravascular fluid to the various serous spaces resulting in hypovolemia and shock.

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RECAPITULATIONS ON CHANGES IN DENGUE VIRUS PROPERTIES AND THE AETIOLOGY OF HAEMORRHAGIC FEVER

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A number of significant observations were made during studies on dengue virus conducted at the Faculty of Science, Mahidol University in Bangkok, Thailand in 1973. This concerns the behavior of prototype DEN-2 New Guinea C strain in *Aedes albopictus* cell culture. Altogether the observations substantiate the hypothesis that host-induced modification of virus surface antigens occurs based on biological, immunological and electrophoretic analyses of RHA fractions and/or the products of persistently infected mosquito cells, heretofore, referred to as mosquito-adapted or modified virus. (Salazar and Thomson, 1973; Salazar, 1975).

Evidently viral maturation involves a process by which the nucleocapsid core interacts with some membranous element of the cell, thereby acquiring as it is a protein coat or envelope. Host-induced modification, therefore, appears to be the result of incorporation of host-cell membrane constituents in the viral coat.

The initial observation of host-induced modification of DEN-2 is credited to Sinarachatanant and Olson (1973) who postulated that the presence of cellular elements on the virion's surface structure accounts for its altered biological and immunological properties. These authors first demonstrated that modified virus manifest diminished lethality for suckling mice and altered plaquing characteristics. Loss of virulence *in vitro* and *in vivo* in mammalian indicator systems and diminution in immunological reactivity were likewise reported by Salazar and Thomson in the same year. Furthermore, the latter authors observed changes in electrophoretic behavior of variously derived RHA. These are summarized in Table 1.

Table 1

Changes in dengue virus properties in persistently infected mosquito cells.

	Pro-to type	Mo-dified virus
Biological		
1. Virulence to suckling mice (LD_{50}) and mammalian cells (CPE)	+	–
2. Plague variant	Large	Small
3. Virus interference	–	+
Immunological		
1. CF	+	–
2. HA:pH optimum	6.4	6.0
3. PRNT : NT_{50} titer (monkey antisera)	1:640	1:350
Electrophoretic profile:		
PAGE analysis (RHA)		
1. V_3	M.W.53,000-59,000	↓
2. V_1	M.W. 7,700- 8,700	↑
3. V_4	?	↑
4. Relative proportions of V_1 to V_2, & of V_4 to V_3	$V_1 < V_2$ $V_4 < V_3$	$V_1 > V_2$ $V_4 > V_3$

Earlier in these studies small and large plaques variants of DEN-2 were recovered from mosquitoes and only the large plaque variant from mammalian sources. In retrospect, this suggests that:

(i) there is a selection for small plaque and large plaque variants in insect and mammalian cells, respectively;

(ii) the large plaque variant in mosquitoes might represent newly acquired and potentially virulent particles from vertebrate hosts;

(iii) the small plaque variant represents host-modified virions in persistently infected mosquito cells. This variant produces progressively diminishing and ill-defined plaques that eventually disappear in carrier culture or latent virus infection. The apparently nonlethal (or avirulent) products of carrier culture induce virus interference and retain the capacity to react with specific antibody; and

(iv) latent virus, therefore, is not readily demonstrated by conventional methods of isolation i.e. virulence in mice and/or plaque formation. Their presence and identity, however, can be established by combined challenge virus resistance/neutralization tests.

The implication therefrom is that host-induced modification or the acquisition of host cellular antigens might accompany the maturation of endogenous virus in the endothelial and/or lymphoid tissues of man and subsequently elicit the production of antibody directed against these components, possibly along with the development of a viral carrier state. If so, this could predispose either to an auto-immune process i.e. an abrogation of self-tolerance, or in repeated infections with heterologous virus, the formation of antigen-antibody complexes which activate complement and cause the release of vasoactive substances (WHO, 1973).

There are at least three possible avenues which directly or indirectly predispose to the eventual collapse of the vascular system. These are:

(i) the attack by antibody on host cellular elements in the endothelial tissues;

(ii) the activation of complement by circulating antigen-antibody complexes and the release of vasoactive amines by mast cells, basophils, or platelets;

(iii) the elaboration of soluble factor(s) possibly kinins? by sensitized or infected lymphocytes/leukocytes which, in turn, stimulate platelets to release vasoactive amines. Kinins *per se* can cause lowered blood pressure and increased vascular permeability. Furthermore, antigen-antibody complexes have been shown to activate the plasma kinin system.

The hypothesis is illustrated in Fig. 1.

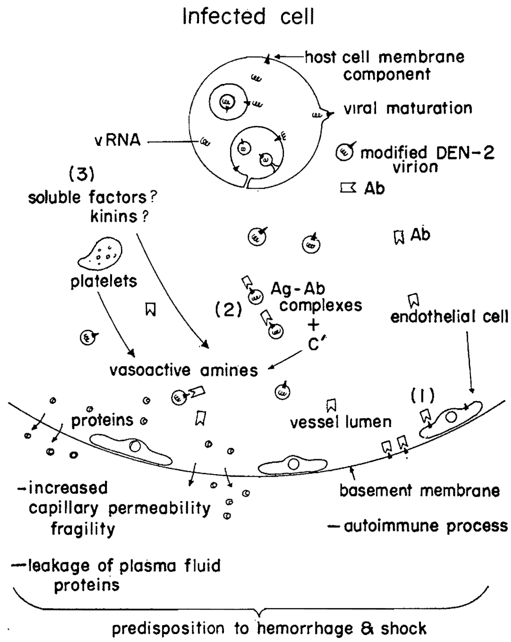

Fig. 1 Host-pathogen interactions in dengue shock syndrome.

Thus, both humoral and cellular factors in the immune response are involved in virus infection and its sequelae. By and large, these mechanisms could serve to explain the tissue damage seen in dengue shock syndrome.

The mystery of dengue haemorrhagic fever is far from being solved. (Tan *et al.*, 1968; Hammon, 1973). Instead it continues to take its toll on human life and resources.

The significance of this present knowledge in understanding the development and/or transmission of dengue virus in nature can not be overemphasized. Once more, the intriguingly diverse behavior of virus in both arthropodan and vertebrate hosts recapitulates on the phenomenon of selective adaptation wherein virus and vector might be the protagonists and man, an innocent if hapless bystander.

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