

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

C.C. CHEN and P.C. FAN

Department of Medical Biomorphics, National Defense Medical Center; Department of Parasitology, National Yang-Ming Medical College, and Department of Medical Research, Veterans General Hospital, Taipei, Taiwan.

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 re-

ceived 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 $25 \pm 1^\circ\text{C}$ 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

This study was supported in part by U.S. Army Research and Development Group (Far East) (Grant No. DA-RDRF-S92-544-75-G222) and by National Science Council, Republic of China (Grant No. NSC-63B-0412-06-29).

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	<i>Culex p. fatigans</i>						
		No. used	No. engorged	No.* dissected	Infection		Infective larvae	
					No.	%	No.	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		Immature larvae	
		No.	%	No.	%
Treated	Head*	164 (78)***	63.3		
	Thorax	59 (41)	22.8	7**	100
	Abdomen	35 (26)	13.5		
	Wing & leg	1 (1)	0.4		
Untreated	Head*	248 (65)	63.9		
	Thorax	110 (38)	28.4	3**	100
	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 surviving 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.

ACKNOWLEDGEMENTS

The authors are indebted to Drs. John H. Cross and O.K. Khaw of U.S. Naval Medical Research Unit-2 and Dr. S.W. Huang of National Defense Medical Center, Taipei, for their suggestions and comments; to Mr. Y.P. Hsu for his technical help during this study.

REFERENCES

- CIFERRI, F.E., SILIGA, N., LONG, G. and KESSEL, J.F., (1969). A filariasis control program in American Samoa. *Amer. J. Trop. Med. Hyg.*, 18 : 369.
- DESOWITZ, R.S. and SOUTHGATE, B.A., (1973). Studies on filariasis in the Pacific. 2. The persistence of microfilaremia in diethylcarbamazine-treated population of Fiji and Western Samoa: diagnostic application of the membrane-filtration technique. *Southeast Asian J. Trop. Med. Pub. Hlth.*, 4 : 179.
- FAN, P.C., WANG, Y.C., LIU, J.C. and HSU, JAMES, (1974). Filariasis on Kinmen (Quemoy) Islands, Republic of China. 1. Parasitological investigation. *Southeast Asian J. Trop. Med. Pub. Hlth.*, 5 : 211.
- HAWKING, F., SEWELL, P. and THURSTON, J.P., (1950). The mode of action of hetrazan on filarial worm. *Brit. J. Pharmacol.*, 5 : 217.
- HAWKING, F., (1962). A review of progress in the chemotherapy and control of filariasis since 1955. *Bull. WHO*, 27 : 555.
- HEWITT, R.I., WHITE, E., HEWITT, D.B., HARDY, S.M., WALLACE, W.S. and ANDUZE, R., (1950). The first year's results of a mass treatment program with hetrazan for control of bancroftian filariasis on St. Croix, American Islands. *Amer. J. Trop. Med.*, 30 : 443.
- KANDA, T., TASAKA, S. and SASA, M., (1967). The effects of diethylcarbamazine treatment on the viability of microfilariae ingested by intermediate hosts. *Jap. J. Exp. Med.*, 37 : 149.
- KUME, S., OISHI, I. and NAKAZAWA, K., (1954). Development of the microfilariae who had tolerated the microfilaricidal or filaricidal chemicals, in the body of mosquito. *Jap. J. Sanit. Zool.*, 4 : 54.
- MAHONEY, L.E. and KESSEL J.F., (1971). Treatment failure in filariasis mass treatment programmes. *Bull. WHO*, 45 : 35.