Brit. J. Pharmacol. (1958), 13, 318.

ACTION OF DIETHYLCARBAMAZINE *IN VITRO* ON INFECTIVE LARVAE OF *WUCHERERIA BANCROFTI*

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(RECEIVED MAY 15, 1958)

Infective larvae of *Wuchereria bancrofti* were obtained from laboratory infected *Culex fatigans* and maintained in serum containing diethylcarbamazine. There was no difference in the survival time of these larvae and larvae maintained in control serum without diethylcarbamazine. In one of the three experiments reported the survival time of the larvae was much longer than in the other two experiments. It is suggested that this may have been due to the presence of filarial antibodies which killed the larvae in two of the sera used. In the serum which did not contain antibodies, the larvae survived very much longer.

Little work appears to have been done on the effect of diethylcarbamazine on infective larvae of *W. bancrofti*. Hawking, Sewell and Thurston (1950) found evidence of a lethal effect *in vivo* against infective larvae of *Litomosoides carinii* when the drug was given to cotton rats before and during the period of exposure to infected mites (*Liponyssus bacoti*). When given after infection, it appeared that developing males may be affected.

McGregor and Gillies (1956) suggested that the follow-up results obtained after diethylcarbamazine therapy in Gambia indicated that the drug had little effect on the ovaries of developing *W. bancrofti*.

In view of the paucity of information regarding the effect of diethylcarbamazine on infective larvae, a series of experiments to assess the effect of this drug on infective larvae of *W. bancrofti* was carried out. If the drug did have an effect on this stage of the life-cycle of the worm it might be of use as a causal prophylactic.

MATERIALS AND METHODS

Preliminary experiments indicated that infective larvae, extracted from *Culex fatigans* dissected in human sera, could be kept alive in capillary tubing for several days. Menon and Ramamurti (1941) had kept larvae alive in whole blood, changed daily, for 8 days, but considered that bacterial infection was responsible for the worms dying.

Laboratory bred *Culex fatigans* were fed on a volunteer showing microfilariae of *W. bancrofti* in his night blood. The mosquitoes which fed were kept at approximately 28° and at a relative humidity of between 80% and 100%. When infective larvae had developed the mosquitoes were dissected and the larvae picked up in capillary tubing.

The use of sterile materials—slides, capillary tubing, and dissecting needles—and the addition of penicillin to the sera in which the mosquitoes were dissected, reduced the risk of bacterial infection and enabled some larvae to remain alive for as long as 16 days. The mosquitoes themselves, however, could not be sterilized.

Lubran (1950) showed that the blood concentration of diethylcarbamazine reached a maximum approximately 4 hr. after an oral dose. A dose of 10 mg./kg. body weight produced maximum blood values of 4 to 8 μg./ml. Two hr. after 15 mg./kg., a blood value of 8.7 μg./ml. was obtained in one case. In order to obtain maximum blood concentrations in the sera to be used against the infective larvae, 500 mg. of diethylcarbamazine citrate, repeated in 3 hr., was given to 3 volunteers, one African and two Europeans. One European complained of lassitude, nausea, and vertigo after the first dose. The total dose of drug/kg. of body weight in the three experiments was 10.6 mg., 16.6 mg., and 16.6 mg. respectively. Prior to the first dose, blood was taken from each person and the sera from this specimen was used as a control. Cystalline sodium benzylpenicillin was added in a dose of approximately 400 units/ml. of serum in an endeavour to limit bacterial growth.

The mosquitoes were immobilized by stunning and dissected in the sera, control and drug containing sera being used alternately. Any infective larvae seen were taken up into capillary tubing. If there were many larvae in the mosquito, more than one sometimes inadvertently entered a tube. Care was taken to avoid any infective larvae which appeared to be damaged in any way even though their movements might suggest that the damage was unimportant.

The column of serum containing the infective larva was shaken to the middle of the tube. The ends were sealed with plasticine, which also served for the purpose of fixing the tube to a numbered slide. Under the microscope, the infective larva could be seen in the tube and its movements noted. The tubes were kept at room temperature (22° to 24°).

The activity of the larva was noted two or three times in 24 hr. When it failed to move on three consecutive occasions, it was considered to be dead. It was frequently found that after being apparently dead at one and sometimes two observations, the larva would be obviously alive when observed the next time.

In spite of care being taken to avoid damaged larvae, some may have been injured and died in a few hours. It was impossible to say whether these early deaths were due to injury, drug, or natural causes. In an endeavour to exclude those which were injured, larvae apparently dead when first examined, and which failed to move at the next two examinations, were considered to have been damaged in some way. These have been excluded from the results. Others, living only 2 hr., have accordingly not been excluded, but the possibility of their deaths being due to damage cannot be ruled out. The time at which the larva was taken into the tube and the time at which the larva was last seen alive were used for calculating the length of life of each larva to the nearest hour. The mean length of life of the larvae in the different sera was then calculated.

Results

The results are given in Table I, from which it will be observed that there was a considerable range in the length of life of infective larvae in all sera.

In no experiment was there any significant effect of the drug on the mean life of the larvae. In Expt. 1, in which the concentration of diethylcarbamazine was lower than in the other experiments (see above), there was an apparent shortening of the life of the larvae, while in Expt. 2 the drug appeared to increase the length of life of the larvae.

It appears from these results that diethylcarbamazine has no lethal effect *in vitro* on infective larvae. If a higher dose of drug was given there might be some effect, but the doses given were near the upper limit of tolerance and it would be impracticable to give a higher dose regularly for prophylactic purposes.

These *in vitro* results cannot be taken to indicate that the drug would be of no use prophylactically. In very few cases has this drug shown any *in vitro* effect on the different stage of filarial worms (Hawking *et al.*, 1950), and it may well be that *in vivo* the drug acts indirectly by rendering the larvae more susceptible to phagocytosis.

It will be seen from Table I that the mean length of life of the infective larvae in the different experiments varied considerably.

Since it has been established that the drug had no effect on the larvae, it is possible to combine the results for the control sera and the sera containing drug in each experiment (Table I).

It is seen that the mean length of life of infective larvae in Expt. 3 was nearly double that of the larvae in Expts. 1 and 2, and this difference is statistically significant.

Since the same technique was used in the three experiments it is interesting to speculate on the possible reason for this result. There seem to be two possible explanations. First, the serum for Expt. 1 was obtained from a European with many years' exposure to filarial infection. This person was known to have a positive filarial complement fixation test. The serum used in Expt. 2 was from an African who, although showing no clinical signs of filariasis, had lived all his life in an endemic area. It is therefore very probable that the sera used in the first two experiments contained antibodies.

Serum used in Expt. 3 was from a European who had only been in East Africa for a short time and who had not been exposed to filarial infection. There would, therefore, be no antibodies in the sera, and the infective larvae might thus be expected to live longer in this serum than in the sera of the other two experiments.

The second reason for the longer life of larvae in Expt. 3 concerns the age of the larvae. In

TABLE I

THE MEAN LENGTH OF LIFE OF INFECTIVE LARVAE IN CONTROL SERA, SERA CONTAINING DRUG, AND THE TWO SERA COMBINED

The mean length of life is measured to the nearest hour.

Expt.	Control				Sera with Drug				Control and Sera with Drug Combined			
	No. of Larvae	Mean Length of Life (hr.)	Range	S.E.	No. of Larvae	Mean Length of Life (hr.)	Range	S.E.	No. of Larvae	Mean Length of Life (hr.)	Range	S.E.
1	79	34	2–155	±3	94	27	2– 81	±4	173	31	2–155	±2
2	51	23	8–69	±2	63	37	8–106	±3	114	31	8–106	±2
3	68	57	4–320	±8	60	56	5–238	±6	128	57	4–320	±5

this experiment they were removed from the mosquitoes 18 days after the infected blood meal; in Expts. 1 and 2, this time interval was 14 and 16 days respectively. If the age of the larvae was responsible one would expect a graduated increase of the survival time in the three experiments.

Further work is required to determine the influence of antibodies in this type of study, but some support for the antibody explanation given above comes from an earlier experiment in which the survival times of infective larvae maintained in sera from a newly-arrived European and from an African with elephantiasis were compared. The survival time of the larvae in the European serum was significantly greater than that of the larvae in the serum of the African.

When further work on these lines has been done, it may be possible to devise a new serological test for filariasis which might be more specific than those at present in use.

Grateful acknowledgments are made to the Administrator, E.A. High Commission, for permission to publish this work, and to Mr. Francisco Baloe and Mr. Albinus Mahunde for help in dissecting many hundreds of mosquitoes.

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