EFFECT OF DIETHYLCARBAMAZINE ON *SETARIA CERVI IN VITRO*

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Summary: Diethylcarbamazine (DEC) produced an initial stimulation followed by depression of the movements of the intact worm and nerve-muscle preparation of *Setaria cervi*. The effective concentration of DEC was reduced to one hundredth in the nerve-muscle preparation as compared to the whole worm, suggesting that the cuticular barrier is highly effective in preventing the penetration of the drugs. The depressant effect of DEC was concentration dependent and was not reversed even after repeated changes of the bath fluid. The worms consumed 7.7 *mg*$\pm$0.2 glucose/g wet weight/hr. The consumption of glucose was directly proportional to its motor activity; it increased during the stimulant phase with low doses of DEC and decreased during the depressant phase.

Key words: diethylcarbamazine *Setaria cervi* glucose consumption

Diethylcarbamazine (DEC) requires a concentration of 750 $\mu g/ml$ to reduce the survival time of 100% of a group of adult *Setaria cervi* from 75 to 24 hr in a continuous perfusion experiment (13). Other species of filarial worm, too, are scarcely affected by the drug *in vitro*. Thus DEC reduced the survival of *Litomosoides carinii* from 17 days to 3, 5 and 14 days at the respective concentrations of 500, 200 and 100 *mg/ml* of the medium (4). Although the *in vivo* lethal concentrations of DEC are immensely high to be of any therapeutic value, it would still be interesting to investigate the nature of this lethal action of the drug on the adult *Setaria cervi*.

MATERIALS AND METHODS

Adult *Setaria cervi* (Nematoda-Filarioidea) were picked up from the peritoneal cavity of the freshly slaughtered cattle and brought to the laboratory in a vacuum flask containing "modified Ringer" (13) solution at 37°C.

The effects of DEC on the spontaneous movements of the intact worm and on the nerve-muscle preparation were studied by the methods described elsewhere (11, 12).

Live adult *S. cervi* were placed on filter paper for one min in order to wipe off the excess of Ringer fluid. A group of 10 worms was then transferred onto another piece of tissue paper and weighed. Each group was placed in 100 *ml* Ringer solution at 37°C. From each beaker, 0.05 *ml* samples were drawn with the help of a pipette at hrly intervals up to 3 hr. The concentration of glucose was estimated in all the samples by the colorimetric method (6).

To study the effect of DEC on glucose consumption, worms were placed in Ringer solution containing different concentrations of the drug (5, 10, 25, 50 and 100 $\mu g/ml$). Samples from each beaker were taken at hrly intervals and concentrations of glucose estimated. Each dose of the

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drug was tested on five groups of 10 worms each for three consecutive hr. Mean glucose consumption was calculated on the basis of their body weight and expressed as *mg* glucose/g wet weight of worms/hr.

RESULTS

Effect of DEC on the spontaneous movements:

Fig. 1 shows the effect of DEC on the movements of the intact worm (upper and middle panel) and on the nerve muscle preparation (lower panel).

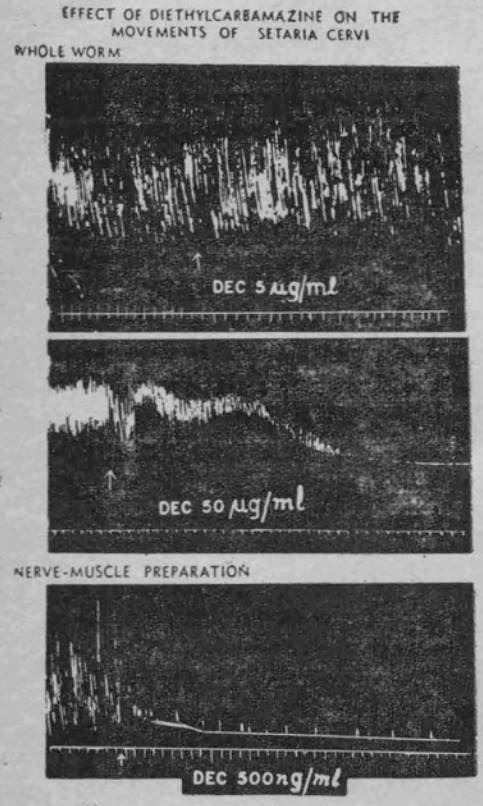

Fig. 1: Effect of DEC on the spontaneous movements of the whole worm (top and middle panel) and the nerve-muscle preparation (bottom panel) of *S. cervi*. Arrows indicate the time of addition of DEC to the bath. A concentration of 5 $\mu g/ml$ increased the amplitude of contractions of the whole worm (top panel) while a higher concentration of 50 $\mu g/ml$ produced a biphasic response consisting of an initial stimulation characterized by increase in tone and subsequent irreversible paralysis (middle panel). On the nerve-muscle preparation, DEC (500 *ng/ml*) produced an initial short lasting (about 1 min) stimulation characterized by increase in tone followed by irreversible paralysis. Time mark, 1 min.

5 $\mu g/ml$ of DEC caused a short-lasting (about 15 min) increase in the amplitude as well as the tone (upper panel). The frequency of movements was not visibly affected. 50 $\mu g/ml$ of DEC caused an initial stimulation of the spontaneous movements characterised by an increase in tone only (middle panel). The amplitude and the frequency of movements were also reduced. About 15 min later the tone started decreasing till it reached well below the original level. Simultaneously there was reduction in the amplitude as well and the movements ceased completely about 25 min after the addition of the drug. Movements could not be restored even after repeated washings.

With 500 *ng/ml* DEC the initial stimulation produced was of short duration (about 1 min) and was characterised by increase in tone only. This was followed by decrease in amplitude, frequency and the tone of spontaneous movements. After about 10 min the movements became infrequent and ceased completely in another 15 min. Repeated changes of the bathing fluid were ineffective in restoring the movements (lower panel). Thus 500 *ng* of DEC produced an effect in the nerve muscle preparations which was greater than that produced by 50 μg of the drug in the intact worm.

Effect of DEC on glucose consumption of adult S. cervi:

Table I shows the average glucose consumption of adult *S. cervi* and its alteration by addition of DEC in varying concentrations. The worms consumed 7.7 *mg* glucose/g wet body weight/hr in the Ringer solution.

Table I: Effect of diethylcarbamazine on the glucose consumption of adult *Setaria cervi in vitro*.

Drug	*Glucose consumption mg/g/hr (mean±S.E.)*	*P value*	*Percentage change from the control value*
Control*	7.70±0.2		—
Diethylcarbamazine**			
5	8.47±0.31	<.02	+10
10	7.12±0.18	<.001	— 7
25	5.80±0.43	<.001	—25
50	0.80±0.12	<.001	—90
100	—		—

* Figure represents the mean of 30 observations carried out with 10 groups of 10 worms each for 3 consecutive hr.
** Figures represent the mean of 15 observations carried out with 5 groups of 10 worms each for 3 consecutive hr.

5 μg/*ml* DEC increased the glucose consumption of the worms by 10 per cent. Further increase in the concentration decreased the consumption of glucose which was dose-dependent. Thus, decrease in glucose consumption was 7%, 25%, 90% and 100% at concentrations of 10, 25, 50 and 100 μg/*ml* of DEC, respectively. At the concentration of 100 μg/*ml* the worms were paralysed and no detectable amount of glucose was consumed by them.

DISCUSSION

The stimulation of the spontaneous movements with a low concentration (5 μg/*ml*) of DEC in the intact adult worm (top record in Fig. 1) was much less in duration as well as in intensity in the nerve-muscle preparation, although the sensitivity of the nerve-muscle preparation was 100 times more than the whole worm. This stimulant effect, therefore, appears to be a non-specific irritant effect exerted on the subcuticula. The increased physical activity of the worm is reflected in the increase in glucose consumption at this concentration of DEC (Table I).

The other effect consisting of an initial transitory stimulation followed by depression (middle

and lower panels of Fig. 1) was similar in both the intact as well as the nerve-muscle preparation. Since this effect occurred inspite of the removal of the cuticula and subcuticula, it appeared to be exerted upon the nerve-muscle complex and may therefore be termed as "specific" effect. However, the concentration required to produce this effect in the intact worm was 100 times higher than in the nerve-muscle preparation suggesting that the cuticular barrier is highly effective in preventing the penetration of the drug. The existence of cuticular barrier has been shown for other drugs as well (12).

The depressant effect of DEC on the worm was concentration dependent (Fig. 1) and as the spontaneous activity decreased, a parallel reduction occurred in the consumption of glucose (Table I). The normal glucose consumption of 7.7 *mg/g* body weight of *S. cervi* is lower than that found in *L. carinii* by Bueding (3) which was 21 *mg/g*.

Great variations in the rate of glucose consumption are found in different species of nematodes. The factors playing a decisive role in limiting the glucose utilization include the size, metabolic pattern and the habitat of the parasite. Thus, besides being smaller in size *L. carinii* differs from *S. cervi* in habitat as well. The former resides in the pleural cavity of cotton rat under a rich supply of oxygen and the latter in the peritoneal cavity where the oxygen availability is less. The differences also exist in the metabolic pattern of the two parasites especially regarding the enzymes of phosphoenol pyruvate succinate pathway (1).

The paralysis caused by 50 $\mu g/ml$ DEC was complete and irreversible indicating death of the worm (Fig. 1). This is a high concentration and is not likely to be achieved inside the body with usual doses. The finding lends support to an earlier observation (10) of recovering live worms in the peritoneal cavity of rats following oral administration of 100 *mg/kg* DEC for 6 days. It is also in agreement with similar earlier reports that DEC is devoid of lethal effect on the adult worm *in vivo* in *L. carinii* (4, 5) in rat and *O. volvulus* and *Loa loa* (2) in man. Further, the finding explains the recurrence of microfilaremia in patients cured of symptoms by the drug. The elimination or suppression of microfilariae from the peripheral blood following DEC administration in experimental (9, 10) and clinical infestation is therefore suggested to be due either to an action on the circulating microfilariae or on the reproductive potential, rather than due to any lethal effect on the worm. Such a suggestion has also been put forward by many workers (4,7,8,14).

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