SERUM LEVELS OF A FILARICIDE, DIETHYLCARBAMAZINE CITRATE, IN CATS FOLLOWING DIFFERENT ROUTES OF ADMINISTRATION

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INTRODUCTION

Diethylcarbamazine citrate (DEC) is the current drug of choice in the treatment of lymphatic filariasis, a helminthic infection affecting millions of people in the tropics. Although the drug has been used for many years in extensive control programs, there is no general agreement on the optimal regimen for mass treatment nor for possible prophylactic measures. In an effort to gain a more detailed basis for predicting the effectiveness of DEC when given at different intervals and via different routes of administration, we quantitated serum levels in domestic cats after varying treatment regimens.

MATERIALS AND METHODS

Diethylcarbamazine citrate (DEC) used in the preparation of aqueous and mineral oil solutions was a gift from the Union Carbide Corporation, Houston, Texas, U.S.A. All DEC concentrations refer to the citrate salt. A physiological saline solution containing 100 mg DEC/ml was used for intraperitoneal injection. Two preparations of DEC were used for topical (transepidermal) application. A 5% preparation of DEC in Nivea cream was obtained from Dr. Maurice Langham, Johns Hopkins University. When we discovered that a substance in the cream interfered with quantitation of the DEC, we prepared a 5% solution of DEC in mineral oil. This was accomplished by dissolving DEC in as small an amount of ethyl acetate as possible. Mineral oil was then added and mixed until homogeneous and the ethyl acetate evaporated by bubbling dry N_2 through the mixture. After one hour no more ethyl acetate could be detected by odor, and the preparation was considered to be ready for use.

DEC used for oral administration was a dry formulation "Filaribits" received from Norden Laboratories, Inc., Lincoln, Nebraska, U.S.A. The tablets were ground into a fine powder with a mortar and pestle and the appropriate amount, by weight, was added to commercial fish flavored cat food. If not all the food was eaten within 10 min. an estimate was made of the food remaining.

Adult male and female domestic cats were obtained from the University Animal Care Center and were under the care of qualified veterinarians. Cats receiving topical applications of DEC for the first time were tranquilized with ketamine hyrochloride and the hair on the left hind leg and foot were removed with animal clippers. From 0.5 to 1.0 ml Nivea cream or mineral oil containing DEC was rubbed on the leg and foot.

At various times after DEC administration, cats were tranquilized by intramuscular injection of 20 mg/kg ketamine and bled from the jugular vein. Although cats partially recovered from the effects of the ketamine between bleedings, they were not observed to lick any of the DEC preparations from their legs. Blood was collected in serum separation

tubes, allowed to stand at room temperature for several hours to facilitate clotting and placed in the refrigerator overnight. Sera were generally harvested the following morning and stored at -70°C until analyzed for DEC content.

DEC levels in serum were determined by the gas chromatographic method of Allen *et al.*, (1979). A Varian model 1700 gas chromatograph equipped with a thermionic specific detector and a 2% Carbowax 20M, 5% KOH, on Chromosorb GAW DMCS (100-120 mesh) column was used. Phenmetrazine was added to the samples as an internal standard. The eluted peak areas were measured using a Neumonics graphic digitizer. The ratio of the area of the DEC peak to the phenmetramzine peak was used to determine the DEC content of the sample. Prior to adopting the assay method that we used, the colorimetric method of Ramachandran (1973) was tried; this method proved to be insufficiently sensitive for these studies. We also tried several variations on the gas chromatographic method which would have been more convenient or less expensive, but no other detector or column packing was found which produced acceptable results.

RESULTS

In preliminary studies we showed that storage of serum samples for one week in the refrigerator at 4°C resulted in no significant loss of DEC concentration. However, storage at room temperature resulted in a 50% loss of DEC in one week. We also determined that storage in a -70°C freezer preserved the original DEC levels (Fig. 1). Thus, in this study, serum samples were either refrigerated and analyzed within a few days after they were obtained, or they were kept frozen until DEC determinations were made.

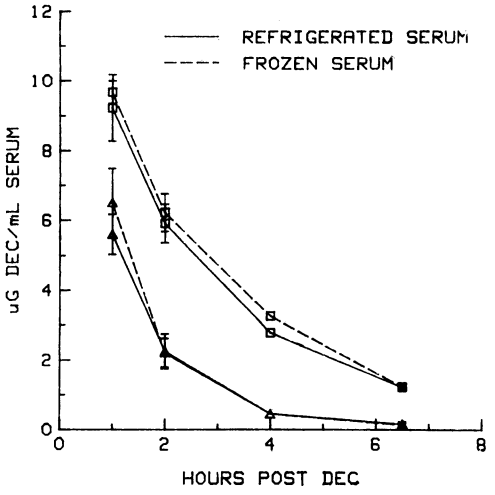

Fig. 1—Comparison of DEC concentrations in refrigerated and frozen serum at various times after a single intraperitoneal injection in cats.

The work of Langham *et al.*, (1978) and Langham, (1980) showed that DEC dissolved in Nivea lotion is effective against the microfilariae of *Onchocerca volvulus* in man. Our own experiments (Ewert *et al.*, 1983) show the effectiveness of this preparation against the developing stages of *Brugia malayi* in cats. However, in the present experiments we found that Nivea cream and several other

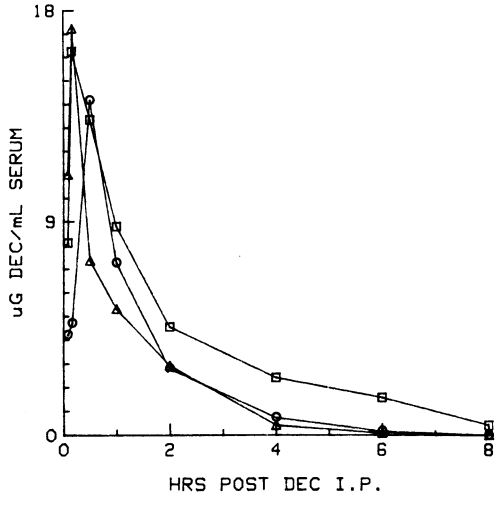

Fig. 2—Concentration of DEC in serum of three cats following a single intraperitoneal injection.

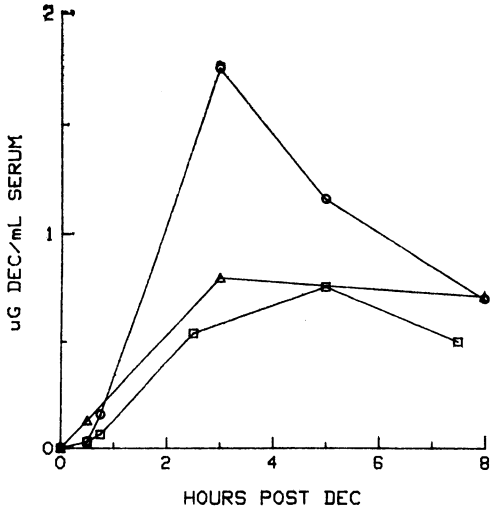

Fig. 3—Concentrations of DEC in serum after a single oral administration to cats.

commercial hand cream preparations contained a substance that co-chromatographs with DEC in our gas chromatographic system. Further experiments showed that ordinary mineral oil did not contain interfering substances. Consequently, mineral oilsolutions were used in experiments requiring determination of serum levels of DEC.

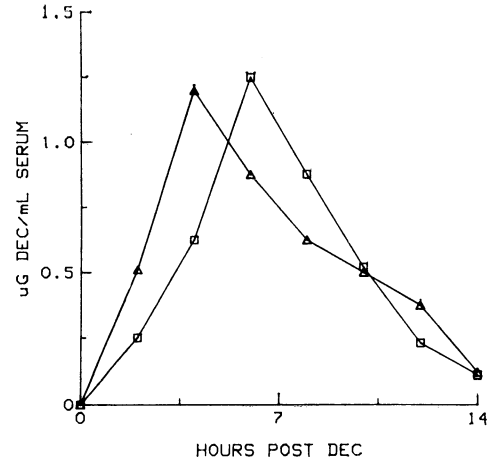

Fig. 4—Concentration of DEC in serum after a single oral administration to cats.

The serum levels of DEC were determined at various times after a single drug administration by three different routes. Fig. 2 shows a representative study in which 3 cats were given 20 mg DEC/kg body weight by intraperitoneal injection. Serum samples were taken at 5, 10 and 30 min and at 1, 2, 4, 6 and 8 hrs after DEC administration. Figs. 3 and 4 show 2 trials in which serum samples were taken at intervals for 8 and 14 hours after cats were given food containing DEC at a level equivalent to 15 to 20 mg/kg body weight. Fig. 5 shows the persistence of DEC in serum for the first 12 hours following application of 10 to 15 mg/DEC/kg in mineral oil to the lower leg and foot.

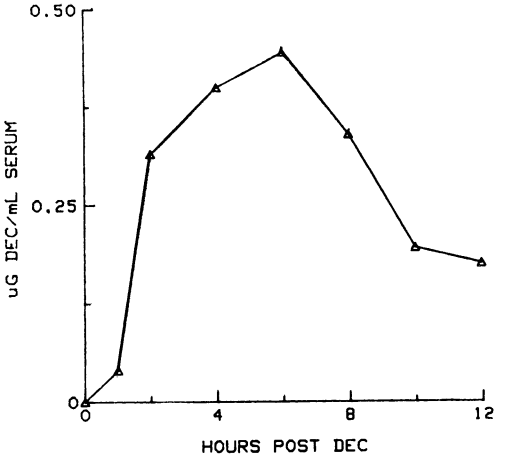

Fig. 5—Concentration of DEC in serum after a single transepidermal application to cats.

Table 1 shows the mean serum levels of DEC in 9 cats during a ten day course of daily drug administration. A serum sample was collected each day 5 hours after drug administration. No progressive increase or decrease in the serum drug level occurred.

Table 1

DEC serum levels (ng/ml) following I.P. administration of 20 mg DEC/kg in cats.

Cat No.	Days after start of daily administration 1	2	3	4	5	8	10
81-1	125	172	172	55	141	100	70
81-2	105	71	39	80	70	120	100
855	191	207	199	135	260	355	260
859	562	242	368	310	580	670	610
879	300	ND	246	160	240	285	270
880	81	78	91	93	110	89	109
882	108	75	81	88	155	91	80
895	No	110	100	35	89	138	129
883	No	203	240	300	500	275	205

ND = not determined.

DISCUSSION

Previous work in our laboratories (Ewert and Emerson, 1975; Ewert and Emerson, 1979; Ewert, *et al.*, 1983 and unpublished data) has provided considerable information on the effectiveness of DEC against the different stages of *B. malayi* in cats. Third stage larvae are more susceptible than fourth stage larvae and adult worms. Effective drug levels can readily be obtained by intraperitoneal, oral or transepidermal routes in cats. However a number of questions still remain regarding what drug levels are needed and how long they must be sustained in order for DEC to be effective.

As was expected, intraperitoneal injection resulted in a rapidly-attained maximum serum drug level (Figs. 1 and 2). Oral administration at approximately the same dosage level (Fig. 3 and 4) resulted in a lower peak occurring from 3 to 7 hour after ingestion of the medicated food. And, while there was a fairly rapid decline in serum level in several cats, at 8 hours the level was still appreciable and even at 14 hours DEC could still be detected in the serum. It should be noted that in these experiments, DEC was mixed with a commercial cat food that contained a considerable amount of fat. It is not known to what extent, if any, this influenced drug uptake.

At a dose of 10 to 15 mg DEC/kg, an amount slightly lower than that used in intraperitoneal and oral treatment, topical application resulted in a serum level pattern similar to but lower than that seen following oral administration (Fig. 5). On the basis of these experiments the transepidermal route of application provided the lowest peak level of DEC in the serum of any of the 3 routes of administration. However, concurrent studies showed that in some instances even a single topical application of DEC was effective in killing third stage *B. malayi* larvae in cats (Ewert *et al.*, 1983).

Since DEC lotion was applied to only the left hind leg and foot but worms were also killed on the right side, we can assume that the drug was adsorbed and systemically distributed rather than concentrated in the afferent lymphatics of the left leg.

Earlier work (Ewert *et al.*, 1983) showed that weekly doses of DEC seemed to be as effective as daily doses in killing adult and microfilariae of *B. malayi* when the same total drug was given by the intraperitoneal route. Since DEC is known to be excreted rapidly (Hawking, 1962; Hawking, 1981) this observation raised the question of whether repeated administration induced a change in drug uptake or metabolism. To answer this question we gave 9 cats daily intraperitoneal injection of DEC for 10 days. At 5 hours after DEC administration we made repeated DEC serum determinations. Table 1 indicates that no uniform pattern of either increase or decrease in serum levels could be seen with repeated doses of DEC. This experiment also demonstrated that even though cats were given the same amount of DEC per body

weight by the intraperitoneal route, so that none could be vomited or lost, the serum drug levels varied considerably from animal to animal (Table 1). Since we obtained good repeatability of the serum drug levels for each cat when we analyzed duplicate samples, we assume that the differences seen represent variation among individual animals, and not lack of reproducibility in our detection system.

Our pharmacokinetic results do not differ greatly from those of other workers. Although Lubran (1950) concluded that DEC accumulates in humans during five days of administration, his data are based on a small number of human subjects and show considerable variability, as one might expect. While some of our data strongly resemble his, we do not feel that it is possible to conclude that a substantial degree of accumulation occurs. Our data on the elimination of a single DEC dose agree with, and extend, the human studies of Allen *et al.* (1979) and Lubran (1950). In future studies we anticipate incorporating determinations of DEC metabolites, since it is known (Ramachandran and Sharma, 1974) that most of a DEC dose is metabolized before it is excreted.

SUMMARY

We have reported the blood levels of diethycarbamazine (DEC) and the persistence of the drug in the circulation for several routes and protocols of DEC administration in cats. This information will be helpful in studies using the *Brugia*-cat model for studies of experimental chemotherapy.

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