Brit. J. Pharmacol. (1951), **6**, 630.

DEPENDENCE OF THE TOXICITY OF ANTRYCIDE METHYLSULPHATE IN MICE ON THE VOLUME OF A DOSE INJECTED SUBCUTANEOUSLY

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(Received June 4, 1951)

When a drug is injected subcutaneously, its concentration in the blood stream depends on the rate of its absorption. If, therefore, the drug is one that causes immediate toxic effects, their severity will also depend on its rate of absorption. The trypanocidal drug "Antrycide" provides an excellent example. When the soluble methylsulphate is injected subcutaneously in mice, absorption is rapid and most mice will die within a few hours after a dose of 0.8 mg. per 20 g. body weight. When, however, the very insoluble chloride is given, absorption from subcutaneous tissues is so slow that mice will tolerate a dose 150 times as great (Curd and Davey, 1950). Using only the soluble methylsulphate, we have found that its toxicity (as determined by percentage mortality) when injected subcutaneously in mice is significantly influenced by the volume of fluid in which it is given. We believe this to be due to different rates of absorption when different volumes are injected, and therefore to afford another illustration of the dependence of the toxicity of a rapidly acting compound on its rate of absorption.

The subcutaneous dose used, per 20 g. body weight, was 0.75 mg. dissolved in 0.1, 0.2, 0.4, and 0.8 ml. distilled water. The mice were young adults of either sex, ranging in weight from 18 to 26 g. The Table and graph (Fig. 1) below show the results of two similar experiments (on different days), each involving 120 mice, obtained from a different source for each experiment. In each experiment the mice were divided into four equal groups for subcutaneous injection with the volumes of 0.1, 0.2, 0.4, and 0.8 ml. respectively. Care was taken to ensure that each group was treated under the same conditions (except for the difference in dose-volume). The mice had not been fed since the previous day, and, as far as possible, they were all injected at the same time, 10 with one dose-volume, then 10 with the next, and so on, repeating this cycle until all 30 in each group had been treated in each experiment. Signs of toxicity appeared within a few minutes of the injection and consisted of accelerated breathing and general activity and excitability, sometimes followed by a more passive condition than normal but without any obvious paralysis. About three-quarters of the ensuing deaths occurred within an hour of the injection, and were preceded by the sudden onset of violent convulsions a minute or two before the end. No death occurred more than five hours after the injection, and the next day all the survivors appeared to be perfectly normal.

TABLE I

NUMBER OF MICE KILLED, IN RELATION TO NUMBER TREATED, BY SUBCUTANEOUS INJECTION OF 0.75 MG./20 G. ANTRYCIDE METHYLSULPHATE IN VARYING VOLUMES OF AQUEOUS SOLUTION

Volume, in ml.	Exp. 1	Exp. 2	Totals
0.1	$\frac{16}{30}$ (53%)	$\frac{16}{30}$ (53%)	$\frac{32}{60}$ (53%)
0.2	$\frac{10}{30}$ (33%)	$\frac{20}{30}$ (67%)	$\frac{30}{60}$ (50%)
0.4	$\frac{9}{30}$ (30%)	$\frac{13}{30}$ (43%)	$\frac{22}{60}$ (37%)
0.8	$\frac{6}{30}$ (20%)	$\frac{12}{30}$ (40%)	$\frac{18}{60}$ (30%)

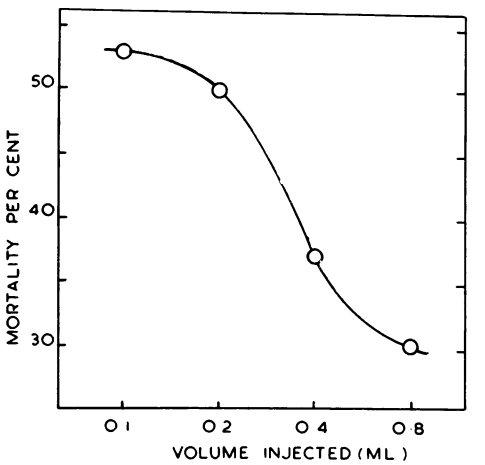

FIG. 1

It will be seen from the Table, which shows the numbers and percentages of deaths, that the mice of Exp. 2 were, on the whole, more affected than those of Exp. 1. This illustrates the well-known variability of results to be expected in toxicity tests with mice obtained from different sources of supply (and treated at different times). There was, however, in each experiment, the same trend from higher mortality in the groups injected with the smaller volumes to lower mortality in those treated with the larger volumes. When the results of the two experiments are combined, as in the last column of the Table and on the graph, the mortalities range from 53 per cent for mice treated with 0.1 ml. to 30 per cent for those treated with 0.8 ml. Applying the probit transformation to the percentages in the last column of the table, the regression coefficient (b) of probits on log volume injected is found to be -0.707, with a standard error of 0.246. The ratio of b

to its standard error is 2.87, and b is therefore significantly different from zero (probability less than 0.01). There is therefore no doubt that our figures show a true relationship between the volume in which the dose was injected and the toxic effect produced.

One possible explanation of the relationship between toxicity and volume injected is that 0.8 ml. is a relatively large amount to introduce into a mouse, whose total blood volume may possibly not exceed 2 or 3 ml. The concentration in the blood that would result from the injection of a compound in such a large volume might therefore be significantly less than would result from an injection of the same dose in a smaller volume. To test this we have treated two further groups, each of 30 mice, from the same source of supply and at the same time, in the following manner. One group received 0.8 ml. saline subcutaneously, immediately followed by another subcutaneous injection, in a different part of the back, of the standard dose of antrycide methylsulphate (0.75 mg./20 g.) in 0.1 ml. water. The other, control, group received only the standard dose in 0.1 ml. water. The percentage mortalities were 57 per cent in the first group and 50 per cent in the controls, and we may therefore exclude the possibility that the lower mortality associated with the larger volumes in the main experiments is to be explained merely by the introduction of excess fluid. It is necessary for this excess to be actually a part of the solvent in which the dose of drug is administered. That delayed absorption probably accounted for the lower mortalities under these circumstances is borne out by the fact that when the compound was injected in 0.8 ml. a swelling was still easily visible at the site of injection about four hours later, while there was no obvious swelling at any time after an injection in 0.1 ml. Apart from mechanical factors, the steeper concentration-gradient between the injected mass and the surrounding tissue-fluids when the compound is given in smaller volumes no doubt increases the rate of absorption, and vital centres are accordingly subjected to the impact of higher concentrations of the drug. Another factor which might help to delay absorption when the larger volumes are used has been suggested to us by Dr. H. R. Ing. This is that the longer period during which the methylsulphate remains under the skin, when it is given in larger volumes, provides a greater opportunity for the interaction with chloride ions of the body fluids to which reference has been made by Curd and Davey (1950). There may consequently be a greater tendency to the formation and local deposition of the sparingly soluble and slowly absorbed chloride salt of antrycide.

Conclusions

Two lessons are to be drawn from these observations, one of general and the other of specific applicability. The outcome of general significance is that when quoting the subcutaneous LD50 of a substance whose lethal action is exercised quickly, it is evidently especially important to state the volume in which the dose was administered. The outcome of specific interest relates to the use of antrycide methylsulphate in veterinary practice. The customary route of administration in cattle is by subcutaneous injection, and when deaths occur they usually do so within 12 hours of the injection. In the light of the present work it might be advisable, when giving a big dose of this salt of antrycide, to inject it in as large a volume as convenient. Admittedly the peak of the resultant curve relating blood concentration to time will tend to be lower than if the dose were given in a smaller

volume, and one cannot yet be sure that this will not slightly reduce the therapeutic effect. It is significant, though, that after subcutaneous injection in the mouse, rabbit, and calf the peak has in fact been shown to be very sharp and to occur within an hour of the injection (Spinks, 1950). Undoubtedly the animal's survival depends on the height of this sharp peak. Trypanosomes, however, are destroyed by exposure to considerably lower blood concentrations than those that kill the host. For example, Spinks quotes that equal efficacy has been reported in the treatment of infected herds in the Sudan by two preparations of antrycide which would give peak plasma concentrations of 30 and 300 μg./litre respectively. It is therefore likely that when a big dose of antrycide methylsulphate is injected the practice of giving it in the largest convenient volume will increase the safety factor without significantly impairing therapeutic efficacy.

We are obliged to Dr. D. J. Finney, Lecturer in the Design and Analysis of Scientific Experiment in the University of Oxford, for advice on the statistical treatment of our results. The work was supported by a grant from the Medical Research Council to E. M. L.

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