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ABSORPTION AND PERSISTENCE OF ANTRYCIDE

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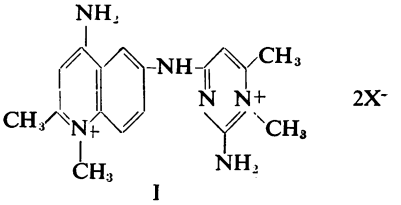
"Antrycide"* (I) is a powerful trypanocide developed in these laboratories by Curd and Davey (1949, 1950) and their associates. They found that the chloride (I, X = Cl) and the methylsulphate (I, X = CH_3SO_4) had different pharmacological properties.



I

When both salts were given subcutaneously to mice, increase in dose of the chloride beyond that involving administration of dispersed solid as well as solution did not raise the toxicity significantly, and at such doses the chloride was much less toxic subcutaneously or intramuscularly than the methylsulphate. There was no difference in toxicity after intravenous administration. When the chloride was given subcutaneously to mice in doses of 25 mg./kg. it conferred protection against subsequent infection by *T. congolense* for at least six weeks. The same dose given by slow intravenous injection conferred protection only for a few days. In the rabbit it was shown that a subcutaneous deposit of the chloride became encapsulated and could be detected for several weeks after the injection. Removal of the residue resulted in loss of prophylactic activity. Curd and Davey (1950) thought that these and analogous results could be ascribed to the very different solubilities in water of the chloride and methylsulphate, which are of the order of 0.1 and 30 per cent (w/v) respectively. They suggested that the dissolved part of the chloride was rapidly absorbed, and that the undissolved part remained behind to form a reservoir from which slow seepage into the blood stream could occur. They also suggested that the prophylactic activity of the methylsulphate, which is less than that of the chloride, might be due to its partial precipitation at the injection site by interaction with body anions such as chloride. Persistence owing to formation of a subcutaneous reservoir of sparingly soluble or slowly absorbed material was first demonstrated by Browning and co-workers (Browning, Cohen, Cooper, Ellingworth, and Gulbransen, 1933; Browning and Gulbransen, 1934), who were able to detect a highly

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coloured styrylquinoline derivative at the injection site for more than a year after it was administered, and to associate this phenomenon with prolonged prophylactic action against trypanosomal infections.

When Curd and Davey first made these suggestions the only analytical method available was relatively insensitive, detecting about 1 mg. of antrycide per litre by direct fluorimetry (Spinks, 1950). However, it was possible to demonstrate that antrycide was rapidly eliminated from the plasma after intravenous injection, and therefore that its prolonged prophylactic action was probably not conferred by its persistence in the blood stream, unless this persistence was at a very low concentration. Later a more sensitive method was developed (Spinks, 1949, 1950) which allowed the detailed examination of absorption and persistence. The results are described below.

Experimental

Analytical methods.—The indirect fluorimetric method (Spinks, 1949, 1950) was used throughout. Concentrations of antrycide are expressed in terms of the diacidic cation, because it is fully ionized at physiological *p*H and cannot be supposed to exist as a particular salt in body fluids or tissues.

Animals.—Albino mice and albino or chinchilla rabbits were used. Blood samples from calves were provided by Mr. J. S. Steward, F.R.C.V.S., to whom the author's thanks are extended.

Conditions of dosing.—All doses of the methylsulphate and low doses of the chloride were given as solutions in distilled water. Higher doses of the chloride were given as dispersions in an aqueous solution of Dispersol OG. and Cellofas WLD. Doses of x mg. chloride were compared with doses of $1.32x$ mg. methylsulphate to compensate for the difference in molecular weight.

Summary of experiments.—Doses of 0.5–10 mg./kg. of the chloride, and the molecular equivalent of the methylsulphate, were administered subcutaneously to rabbits, and plasma concentrations of antrycide were estimated at intervals using blood drawn from a marginal ear-vein. The desired dose for 1 kg. was contained in 1 ml. After doses of 10 mg./kg., concentrations were estimated in some organs; animals were killed by air embolism at selected intervals. Doses of 5 and 10 mg./kg. were administered subcutaneously to groups of 6 mice, and plasma from the pooled heart blood of each group was analysed. The dose for 20 g. was contained in 0.2 ml. Individual calves (200–400 kg.) received 5 mg./kg. subcutaneously; the dose for 200 kg. was contained in 10 ml. Excretion in urine was examined in rabbits that received 10 mg./kg. subcutaneously.

Results and Discussion

1. *Absorption of antrycide*

Typical plasma concentration–time curves obtained after the subcutaneous administration of equivalent molecular doses of the chloride (10 mg./kg.) and methylsulphate are shown in Fig. 1. The highest concentrations given by the two salts were in the ratio of 1/20. Elimination was apparently rapid; in both experiments the concentration fell to a value too small to measure (0–10 μg./litre) within 24 hours. These results showed that the methylsulphate was much more rapidly or completely absorbed than the chloride. They were confirmed and extended by constructing the corresponding curves for a range of doses of the two salts. In Fig. 2 the highest concentrations from these curves are plotted against dose. The

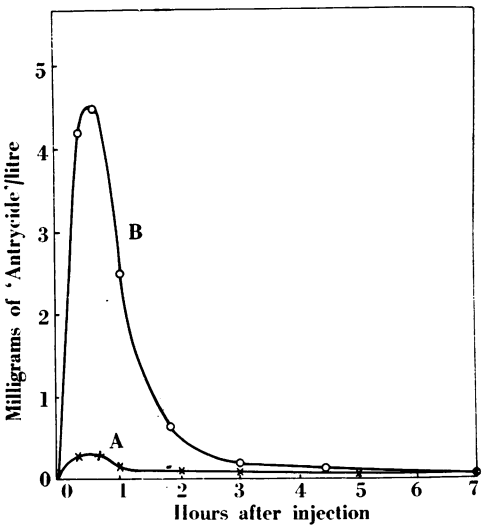

FIG. 1.—Concentrations of antrycide in rabbit plasma after subcutaneous administration of 10 mg. chloride (A) and 13.2 mg. methylsulphate (B) per kg.

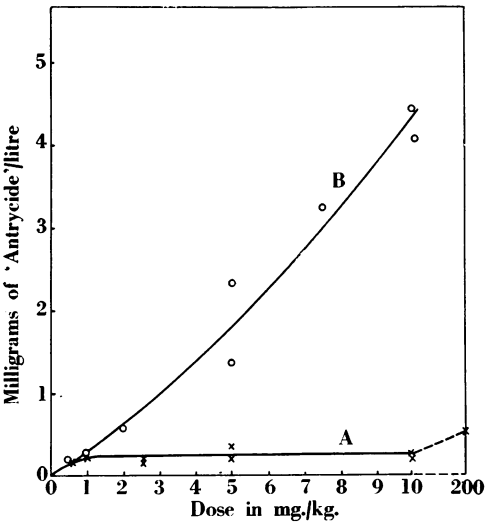

FIG. 2.—Maximum plasma concentrations of antrycide in rabbits after subcutaneous administration of various doses of the chloride (A) and equivalent doses of the methylsulphate (B) per kg.

main features of the results were as follows. First, when the dose of methylsulphate was increased, the maximal plasma concentration increased in a regular manner. The plot of log. concentration (mg./l.) against log. dose (mg./kg.) was a straight line which fitted the equation $4.92\ C = (D)^{1\cdot16}$; that is, in the range of doses studied, doubling the dose rather more than doubled the concentration. This effect might be due either to the metabolism of a decreasing proportion of the drug as the dose was increased, or to the precipitation of a decreasing proportion at the injection site, the amount of anion available for such precipitation being presumably fairly constant. Second, at doses above 1 mg./kg. the concentration attained after chloride administration was approximately constant and independent of the dose. This constancy was preserved over the range from 1 to 10 mg./kg., and a dose of 200 mg./kg. gave an only slightly higher concentration. Third, at doses equal to or less than 1 mg./kg. methylsulphate and chloride gave approximately the same concentration.

These findings can be correlated quantitatively with the solubilities of the two salts. The solubility of the chloride is about 1 mg./ml. and that of the methylsulphate about 300 mg./ml. Therefore, the methylsulphate was completely dissolved at all doses, whereas the chloride was so only at doses below 1 mg./kg. (volume of injected fluid 1 ml./kg.), the higher doses being administered in the form of a saturated solution (ca. 1 mg./ml.) containing dispersed solid. The obvious conclusions are that the dissolved part of the chloride is equally as rapidly absorbed as the methylsulphate, and that the undissolved part is left unabsorbed at the injection site (cf. Curd and Davey, 1950).

A consequence of these conclusions is that the quantitative difference between the two salts must vary according to the size of the animal to which they are given,

if a conveniently small volume of fluid is injected. This has been confirmed experimentally (Table I, Figs. 3 and 4). The virtual identity of the curves obtained after subcutaneous administration of the two salts to mice shows clearly that dissolved chloride is absorbed equally as rapidly as the methylsulphate. The very poor absorption of the chloride in the calf shows that the quantitative difference between the two salts is dependent on the proportion of chloride undissolved. This effect is a very important one because antrycide is administered therapeutically in large

TABLE I

DEPENDENCE OF DIFFERENCE BETWEEN CHLORIDE AND METHYLSULPHATE ON SIZE OF ANIMAL (CHLORIDE 5 MG./KG., METHYLSULPHATE 6.6 MG./KG., BOTH SUBCUTANEOUSLY)

Animal	Volume of injected fluid ml.	Concn. of soln. or dispersion g./100 ml.	Percentage of salt in solution		Ratio of max. concns. CH_3SO_4/Cl
			Cl	CH_3SO_4	
Mouse (20 g.)	0.2	0.05	100	100	0.7
Rabbit (2 kg.)	2	0.5	24	100	11
Calf (200 kg.)	10	10	1.2	100	65

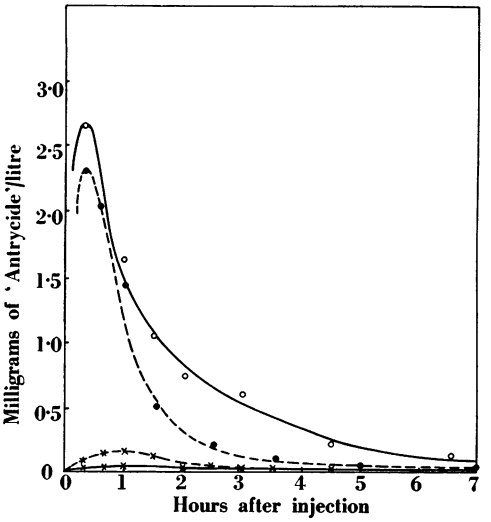

FIG. 3.—Plasma concentrations of antrycide in the calf (continuous lines) and rabbit (broken lines) after subcutaneous administrations of 5 mg. of chloride (×) and 6.6 mg. of methylsulphate (O) per kilogram.

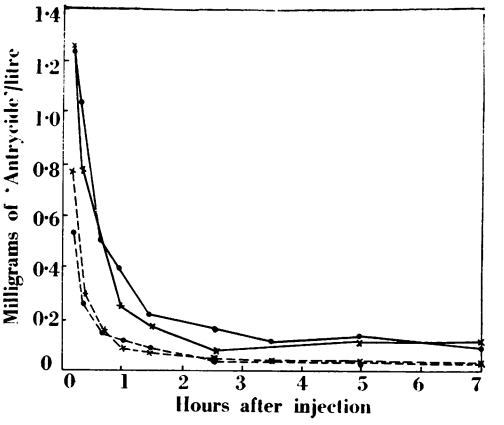

FIG. 4.—Plasma concentrations of antrycide in mice after subcutaneous administration of 10 (×——×) and 5 (×- - -×) mg. of chloride and 13.2 (●——●) and 6.6 (●- - -●) mg. of methylsulphate per kilogram.

animals, particularly the adult cow or ox and the camel. Clearly, the chloride would be expected to have little curative activity in these species, if curative action is exerted by a high peak concentration rather than a low, sustained one (see below).

Concentrations given by the same dose of methylsulphate in calf and rabbit were similar, those in the mouse were much lower. This may explain its lower toxicity in the mouse. The LD50 for the mouse was given by Curd and Davey (1950)

as 25 mg./kg. subcutaneously, and the author found that about four out of ten rabbits given 13.2 mg./kg. died. The concentrations after this dose shown in Figs. 1 and 2 are therefore selected, and may be lower than the true mean values. Indeed, post-mortem blood of the more susceptible animals contained higher concentrations (see section 4). However, this could have been due to failure of the eliminating mechanisms before death, while absorption was continuing.

2. *Distribution of antrycide*

Data on the distribution of antrycide have to be considered in relation to deficiencies in the method of analysis. One of the main difficulties met during the development of the method (Spinks, 1950) was the coprecipitation of antrycide with proteins. Plasma could be precipitated with trichloroacetic acid at a dilution of 1/10, and the recovery of antrycide was constant at about 77 per cent of theory down to amounts of about 0.02 μg. Blood gave very poor results at this dilution but could be precipitated at 1/30 dilution to give about 65 per cent recovery of antrycide. Recoveries of antrycide from liver, lung, spleen, kidney, heart, intestinal wall, muscle, pancreas, brain, and fat were also examined, and it was found that all gave recoveries of about 70 per cent when large amounts of antrycide were extracted by trichloroacetic acid at an overall dilution of 1/60. The smallest amount of antrycide that could be accurately measured varied with the tissue but was usually about 0.2 to 1.0 μg. Large proportions of smaller amounts were lost on the precipitate. Since not more than 0.2 g. of tissue could be used (because of the necessity of precipitating at 1/60 dilution) this limitation means that tissue analyses can only be accepted with

TABLE II

CONCENTRATION OF ANTRYCIDE IN TISSUES OF RABBITS (AFTER THE SUBCUTANEOUS INJECTION OF 10 MG. CHLORIDE OR 13.2 MG. METHYLSULPHATE/KG.)

Time	Concentration of antrycide (mg./kg.) after s.c. injection of								
	Chloride				Methylsulphate				
	Plasma	Spleen	Liver	Kidney	Plasma	Blood	Spleen	Liver	Kidney
20 min. ..					4.98	5.86	2.0	16	80*
40 „ ..					3.16	2.67	1.4	21	35
1 hr. ..	0.07	tr.	3.7	9.2*	1.43	0.95	tr.	38	88
1½ „ ..					0.53	0.50	tr.	38	78*
2½ „ ..					0.44	0.31	tr.	35	57
3½ „ ..	0.03	tr.	tr.	11*	0.08	0.06	tr.	38	76
5 „ ..					0.03	tr.	tr.	45	78
7 „ ..	tr.	tr.	3.9	21*	tr.	tr.	tr.	42	—
1 day ..			13	35*			tr.	51	94
2 days ..			13	23*				54	19
7 „ ..			28	3.1*				3	11
2 weeks ..			10	2.3*				3.0	tr.
4 „ ..			33	tr.				tr.	tr.
8 „ ..			5.5	tr.					
12 „ ..			tr.	tr.					

Concentrations in heart, lung, muscle, perirenal fat, brain, pancreas, and duodenal wall were too low for accurate measurement (<2–5 mg./kg.). tr. = traces (see text).

* Results in individual rabbits. The others are means of results in two rabbits.

confidence when the concentration measured is above 1–5 mg./kg. Accordingly, results given in this section of the paper are recorded as " traces " when they fall below the limit applicable to the particular tissue.

The results of tissue analyses are shown in Table II. Concentrations in seven out of ten of the tissues other than blood examined were too low for accurate measurement. In so far as discrimination among the results with these tissues is justifiable it is probable that concentrations in brain, fat, duodenal wall, and muscle were particularly low (up to 1 mg./kg.) and those in heart, lung, and pancreas possibly slightly higher (up to 3 mg./kg.). There was certainly a marked difference between all these tissues and spleen on the one hand, and liver and kidney on the other. Antrycide was selectively localized in liver and kidney and could readily be detected in them for several weeks after administration. Concentrations in kidney reached very high levels, as much as 120 mg./kg. in one or two samples.

The selective localization of antrycide in liver and kidney is an interesting phenomenon in several respects. It might have a bearing on the elimination of antrycide, although the superficial conclusion that the latter is mobilized for excretion and metabolism in these two tissues is unjustifiable, first because many drugs known to be eliminated by them are not similarly localized, second because it is doubtful whether the organism can be said actively to mobilize an unfamiliar substance for a particular purpose. However, localization might well facilitate the elimination of antrycide, which is excreted in part in the urine, and is probably degraded to a considerable extent.

Localization will confer a certain degree of persistence on antrycide, irrespective of other reasons for persistence that are discussed later. It can be shown that if a drug *A* is uniformly distributed and a drug *B* distributed so that liver and kidney concentrations are a thousand times those in the rest of the body, then the plasma concentration of *A* will be about fifty times that of *B*, when the total amounts of each present are the same. If *A* and *B* are eliminated only by way of the plasma and at the same rate (i.e. if the volumes of plasma cleared of drug in unit time are the same), then *A* should be eliminated from the body fifty times as rapidly as *B*. This is a large difference but probably not sufficient to explain the prolonged prophylaxis conferred by antrycide. Moreover, the localization of *B* in two tissues known to be particularly concerned with excretion and metabolism might well reduce this assumed difference. The subsidiary importance of localization is particularly indicated by the fact that it must clearly be independent of the nature of the salt administered, since antrycide chloride and methylsulphate are fully dissociated at *p*H 7 (cf. Table II). The duration of prophylaxis, if it depended mainly on localization in tissue, should therefore also be independent of the nature of the salt administered. However, it is in fact markedly dependent thereon.

Localization of antrycide in liver and kidney allows quantitative comparison of the two salts as regards persistence of the base in the body. Examination of plasma concentrations did not allow this. The results in Table II show that antrycide can be measured in liver for about eight weeks after chloride and for about two weeks after methylsulphate administration. Since the difficulty of measuring it subsequently may be due to the poor recovery of very small amounts the actual retention in the body might have been more prolonged than these figures indicate. However, they agree quite well with the known duration of prophylaxis. Davey

found that break-throughs in rabbits occurred 8–12 weeks after administering 5 mg. of the chloride per kg. (Davey, private communication). It is therefore likely that the substance measured as antrycide is the active one (see below).

Localization of antrycide in tissue was expected to occur since it is a property common to a large variety of basic compounds (e.g. mepacrine, proguanil, chloroquine, benadryl, etc.). However, its highly selective nature is noteworthy: nothing exactly comparable seems to have been demonstrated for any other drug, although many compounds, e.g. mepacrine (Oldham and Kelsey, 1945), reach much higher concentrations in liver than in other tissues.

3. *Excretion of antrycide*

One rabbit of a pair received 10 mg. chloride per kg. subcutaneously, the other 13.2 mg. methylsulphate. Urine was collected daily and analysed. The experiment was repeated three times and the averages of the four sets of data are shown in Fig. 5. Much larger amounts of antrycide appeared in the urine immediately after

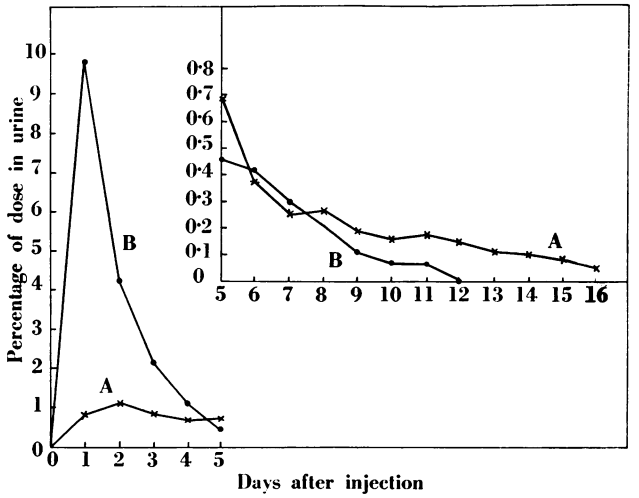

FIG. 5.—Excretion of antrycide in rabbit urine after subcutaneous administration of 10 mg. of chloride (A) and 13.2 mg. of methylsulphate (B) per kilogram.

methylsulphate than after chloride administration. The excretion of antrycide was detectable for about fourteen days after methylsulphate administration. It may have continued longer at a level too low for measurement. The excretion of antrycide after chloride administration soon exceeded that after methylsulphate administration, and was detectable for longer periods. About 6 and 19 per cent of the doses were detected in the urine after chloride and methylsulphate administration respectively.

These findings tally with those of previous sections. They lead to the conclusion that slow absorption of chloride continues for long periods after administration, the source of this chloride being presumably the solid residue left behind at the site of injection. This is confirmed by Curd and Davey's finding (1950) that the presence of the residue is essential for the maintenance of prophylaxis: its removal results in the development of susceptibility to subsequent infection. The excretion of antrycide after methylsulphate administration, and indeed its retention in tissue,

continue for a longer period than would be expected from the form of the plasma-concentration time curve. As already suggested the methylsulphate might be partly precipitated at the injection site, or possibly the localization of antrycide in the liver and kidney is sufficient to confer the degree of persistence observed. Results of intravenous administration suggest that the latter effect plays only a small part. Plasma concentrations 2 min., 7 min., 15 min., 30 min., 1 hr., 2 hr., 3 hr., 5 hr., and 7 hr. after intravenous administration to a rabbit of 2.64 mg. methylsulphate per kg. were 4,180, 2,560, 1,020, 359, 126, 73, 40, 11, and 4 μg./litre respectively. The last two concentrations were too low for accurate measurement. Excretion in the urine could be detected for about 4 days, but only 1.5–4 per cent of the dose was traced in the urine in three experiments, a much smaller amount than was traced after subcutaneous administration of 13.2 mg./kg. The last finding suggests, first, that absorption after subcutaneous administration may be fairly complete, second, that the rabbit may metabolize a larger proportion of a smaller dose, a possibility that was suggested earlier during the discussion of the dose/plasma-concentration curve (Fig. 2).

4. *Therapeutic and toxic concentrations of antrycide*

Little evidence on this topic is available. However, some speculations are interesting. It seems probable from Curd and Davey's results (1950) that a distinction must be made between curative and suppressive concentrations. It is impossible even to guess at curative concentrations, although curative doses are known for some strains of parasite and some species of host. This is because it is impossible to decide whether a peak concentration maintained only for a few minutes or a lower concentration maintained for longer periods is more likely to cure (cf. Marshall, 1949). The methylsulphate is a better curative drug than the chloride, suggesting that a high peak concentration may be needed. Nevertheless cures can be obtained with chloride alone in doses which would be expected to give much lower peak concentrations than do minimal curative doses of the methylsulphate. For example, 100 per cent eradication of the southern Sudan strain of *T. congolense* was observed in cattle with minimal doses of 4 mg. chloride or 1 mg. methylsulphate per kg. (Davey, private communication). The respective peak concentrations would probably have been of the order of 30 and 300 μg./litre (cf. Fig. 3).

The suppressive concentration is certainly below 10 μg./litre, probably below 1 μg./litre. This is assumed from the following facts: first, the concentration of antrycide falls below the former figure within 24 hours after dosing; second, it probably continues to fall, since the amount excreted in the urine falls regularly each day for several weeks; third, a single dose of 5 mg. chloride per kg. in cattle confers protection for several months (Curd and Davey, 1950). At present there seems to be little hope of measuring such minute concentrations (Spinks, 1950).

The suppressive concentration (in the plasma, if not in the tissues) is so low that it is tempting to assume that antrycide is localized also in trypanosomes. No direct evidence is available, except that trypanosomes suspended in antrycide solutions and examined in the fluorescent microscope were observed to fluoresce strongly. However, the strengths of the solutions had to be so high (because antrycide fluoresces weakly) that no definite conclusion can be drawn.

The toxic concentration of antrycide has been measured only in the rabbit. An animal receiving 13.2 mg. of methylsulphate per kg. subcutaneously usually shows symptoms of collapse, shallow respiration, and ataxia until about fifty minutes after dosing, when the concentration has fallen below about 3 mg./litre. Recovery occurs between 3 and 2 mg./litre, and no toxic symptoms have been observed at concentrations below 2 mg./litre. Plasma of 3 rabbits that were killed by subcutaneous injection of 13.2 mg. of methylsulphate per kg. contained 5.15, 7.20, and 6.48 mg. of antrycide per litre.

5. *Degradation of antrycide*

No study of the degradation of antrycide has so far been made. The amount excreted in the urine is small and most of the drug must be degraded *in vivo*. The possibility that a metabolite may be active must therefore be considered. The correlation of duration of prophylaxis with the time during which antrycide can be detected in the tissues, particularly the correlation between the activities of the two salts and their measured absorption and retention, suggests that the substance measured by the analytical method is the active material. However, it is not known whether the method distinguishes antrycide from all its possible metabolites. It presumably distinguishes antrycide from some of the excreted metabolites, since the proportion of the dose detected in the urine is so small, and it has a considerable degree of specificity as judged by its failure to detect many other bases, including some closely related to antrycide (Spinks, 1950). For example, analogues containing either or both NH_2 groups replaced by OH are not detected, although both the mono-quaternary analogues are. These compounds were examined because they are obviously potential metabolites. Unfortunately knowledge of degradative processes is at present so limited that predictions of this sort mean little. All the compounds mentioned are inactive or weakly active when administered subcutaneously. The material left at the injection site eight to ten weeks after the administration of antrycide chloride has been shown by Ainley (private communication) to be mainly unchanged chloride, mixed with unidentified colloidal material. Metabolism of antrycide before absorption appears therefore to be excluded.

SUMMARY

1. Antrycide methylsulphate is rapidly and probably almost completely absorbed. The maximal plasma concentration increases regularly with the dose.

2. Antrycide chloride is rapidly absorbed when given in solution, but any part of it that is undissolved is left behind as a drug reservoir. An increase in dose above that involving the administration of solid as well as solution does not raise the maximal concentration in the plasma to any significant extent.

3. The quantitative difference between the two salts depends on the concentration of the dosing solutions, and hence on the size of animal dosed, if conveniently small volumes are injected. There is no difference in mice at low doses, and a very large difference in calves.

4. Antrycide leaves the plasma rapidly during the first few hours after dosing. A concentration of 4,000 μg./litre falls below 10 μg./litre within 24 hours.

5. Antrycide attains high concentrations in liver and kidney. Concentrations in eight other tissues examined were found to be low. Antrycide can be detected in liver and kidney for several weeks after administration.

6. Antrycide is excreted in the urine in detectable amounts for several weeks after administration. Antrycide can be detected in urine, liver, and kidney for a longer period after chloride than after methylsulphate administration.

7. The localization of antrycide in liver and kidney is probably only a minor cause of its persistence. The major cause is probably long-continued, slow absorption from a subcutaneous reservoir of sparingly soluble chloride.

8. The substance measured by the analytical method (and referred to as antrycide above) is probably the active compound, but proof that a metabolite is not included in the measurement has not yet been obtained.

The author is greatly indebted to Dr. D. G. Davey for much helpful discussion and to Mrs. E. Powell for skilled technical assistance.

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