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A PRELIMINARY REPORT OF THE TOXICITY AND THE ASSOCIATED BLOOD CONCENTRATIONS OF PALUDRINE* IN LABORATORY ANIMALS

BY

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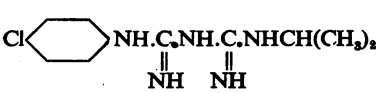
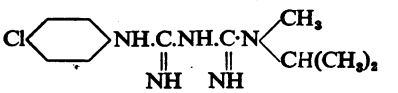
During the preparatory work before paludrine (4888) was administered to human beings the usual investigations were made of its toxicity in various laboratory animals. Early in the work we recognized that different species of animals behaved differently towards it; the rat and the mouse, for example, appeared more susceptible than the chick. We also recognized that the differences were possibly not due to a difference in susceptibility to unchanged drug, but more likely to a difference in its metabolism and the liberation of greater or less amounts of toxic by-products. Sufficient work was done to justify giving the drug to human beings, and the Liverpool workers (Adams, Maegraith, King, Townshend, Davey, and Havard, 1945) pursued their investigations on the assumption that human beings might react like the mouse and the rat, which are amongst the most susceptible of the laboratory animals. It was for this reason that, in the beginning, paludrine was given only twice daily, and doses were progressively increased by amounts not greater than 25 mg. Quite quickly it was shown that man must be classed amongst the least susceptible animals.

The laboratory results have not been published earlier because it was hoped that a fuller investigation would be made. Unfortunately, this will take longer than was planned, and because paludrine has now been sent to many laboratories in different parts of the world we are making the preliminary results available without further delay for the convenience of other workers.

The constitution of paludrine (base) is given below.



paludrine



4430

Two salts were used in the experiments, the monoacetate and the monohydrochloride. The former contains 81 per cent by weight of the base and is soluble

* Paludrine is the registered name for N_1-*p*-chlorophenyl-N_5-*iso*propylbiguanide.

to the extent of about 2 per cent in water; the latter contains 87.4 per cent by weight of the base and is about half as soluble. Solutions of either salt are stable when boiled. The figures quoted in the text, unless it is stated to the contrary, refer to the salts. The monoacetate was used in the intravenous and intraperitoneal tests, the monohydrochloride in the oral tests.

Acute toxicity

This was measured in the usual way. Solutions were administered orally by means of a catheter tube, and intravenously or intraperitoneally by rapid (3 sec.) injection. The results are given in Table I. For most species of animal three sets of figures are quoted which give, respectively, approximately the largest dose permitting all animals to live (LD0), the dose which kills approximately half the experimental animals (LD50) and approximately the smallest dose which kills all (LD100).

TABLE I

ACUTE TOXICITY OF PALUDRINE IN LABORATORY ANIMALS

Species	Route	LD0 (mg./kg.)	LD50 (mg./kg.)	LD100 (mg./kg.)
Chick (wt. 50 g.) ..	Oral	200	400–600	
	i.v.	40	60–80	100
Mouse (wt. 18–22 g.) ..	Oral	50	60–80	100
	i.v.	10	20–30	40–50
	i.p.	10	20–30	40–50
Rat (wt. 100 g.) ..	Oral	80	100–150	
	i.v.	20	40	60
	i.p.	20	40	60
Rabbit (wt. 1.5 kg.) ..	Oral		*circa* 150	
	i.v.	30	*circa* 50	

The intravenous or intraperitoneal injection of paludrine into both rats and mice is associated with delayed deaths, a point which is of much interest. It is best emphasized by comparing the results of an intravenous test using this drug with one using a closely related substance (4430), which differs only by a methyl group (see formula above). The results are given in Table II.

TABLE II

COMPARISON OF THE RESULTS FOLLOWING THE RAPID INTRAVENOUS INJECTION OF 4430 AND PALUDRINE INTO MICE

Dose	Results	
	4430	Paludrine
100 mg./kg. ..	6/6 mice dead within 3 min.	12/12 mice dead within 3 min.
80 mg./kg. ..	5/6 mice dead within 3 min; survivor alive 5 days later	9/18 mice dead within 3 min.; remaining 9 died 1 to 24 hours after the injection
60 mg./kg. ..	6/6 survived 5 days	No immediate deaths; 12/12 mice died 2 to 24 hours after the injection

At the time these experiments were carried out the blood concentrations of paludrine had not been measured, and it was thought possible that the delayed deaths were caused by unusually prolonged retention of the drug in the blood, and therefore that an additive effect might be produced by further intravenous injections.

The idea was tested by giving a second injection, after various intervals, of an amount (20 mg./kg.) that, by itself, produced very few deaths. Seventy-two mice were injected in the beginning; 12 were kept as controls and the remainder were divided into five further groups of 12 which were given a second injection 1, 3, 6, 24, and 48 hours respectively, after the first injection. The results are given in Table III. (The results of a second, similar experiment are given in parentheses in the Table.)

TABLE III

MORTALITY IN MICE AFTER A SECOND INTRAVENOUS INJECTION OF 20 MG./KG. PALUDRINE FOLLOWING A FIRST INJECTION OF THE SAME AMOUNT

Figures in parentheses are the results of a second experiment

Group		Mortalities				Total dead after 5 days
		0–1 hr.	1–5 hr.	5–24 hr.	24–48 hr.	
I	Control ..	—	—	—	1/12 (1/12)	1/12 (1/12)
	2nd injection after					
II	1 hr.	—	1/12	9/12	2/12	12/12
III	3 hr.	—	—	9/12	—	9/12
IV	6 hr.	—	—	11/12 (6/12)	—	11/12 (6/12)
V	24 hr.	1/12	--	4/12 (1/12)	2/12 (2/12)	7/12 (3/12)
VI	48 hr.	—	—	—	1/12	1/12

In mice, therefore, the second injection clearly exerts an additive effect. Similarly, a second injection of paludrine into rats also produces an additive toxic effect (Table IV), and in them, too, its parenteral injection is associated with delayed deaths. On the other hand, in chicks which have received paludrine intravenously, deaths occur within about 15 min. or not at all, and in them a second injection does not produce an additive effect.

TABLE IV

MORTALITY IN RATS AFTER A SECOND INTRAVENOUS INJECTION OF 25 MG./KG. PALUDRINE FOLLOWING A FIRST INJECTION OF THE SAME AMOUNT

Group		Mortalities					Total Deaths
		0–1 hr.	1–4 hr.	4–8 hr.	8–24 hr.	24–48 hr.	
I	Control	—	—	—	—	—	0/6
	2nd injection after						
II	1 hr.	2/6	—	1/6	3/6	—	6/6
III	3 hr. (1/6 dead before 2nd injection)	—	—	2/5	2/5	—	4/5
IV	6 hr.	—	—	2/6	3/6	—	5/6
V	24 hr.	—	—	—	—	—	0/6

Although these results tended to support the suggestion that paludrine might be highly persistent in rats and mice, measurements of blood concentrations soon disproved this, and we now find it difficult to believe that delayed deaths or additive effects are due to unchanged paludrine. We are therefore searching for a metabolite in the hope that the properties of the latter may provide an explanation.

Chronic toxicity

(*a*) *In mice.*—Two types of experiment were done. In the first, mice weighing 18 to 22 g. were arranged in groups of 10 and dosed twice daily for 5 days with the test solutions. The LD50 in this experiment is about 25 mg./kg.; at 12.5 mg./kg. no animals die; at 50 mg./kg. they all die.

In the second type, young mice weighing 14 to 16 g. were dosed twice daily for 14 days. Growth appeared normal amongst those receiving 12.5 mg./kg.; deaths occurred at higher doses.

(*b*) *In rats.*—Newly weaned rats, weighing about 40 g., and selected from as few litters as possible, were arranged in groups of 10. Sexes and litter mates were distributed equally among the various groups. Food (standard cubes made to a formula of the Rowett Institute) was given to the animals immediately after the daily weighing at 10 a.m.; water was always available.

The growth of rats for the first few weeks after weaning is linear and, with careful matching, all the groups in experiments such as the ones being described can be made to follow the same straight line. Seven days were allowed for the line to become established, and then treatment with paludrine was commenced. It was given orally, once daily.

It was found that a dose of 50 mg./kg. caused an immediate alteration in the slope of the growth curve, although not sufficient to reduce it to zero. Scattered deaths also occurred with this treatment. With a dose of 40 mg./kg. a slight deviation of the curve was caused sometimes immediately, sometimes later. With a dose of 30 mg./kg. growth was normal over the whole period of treatment (two months in some experiments) and there were no deaths.

Rats which died, and the survivors of all groups, were subjected to a pathological examination, but nothing of significance was found.* It is noteworthy that in none of these toxicity tests has any symptom been produced in any of the mammalian species that would lead one to suppose that the drug had affected the central nervous system. Chemical estimations confirm that the amount of drug which can be recovered from the brain of rats and rabbits is insignificant (see below and Spinks, 1947).

Blood concentrations†

The rat growth test just described is probably one of the most sensitive toxicity tests available in the laboratory and it was regarded as important to determine the concentrations of paludrine in the blood associated with the doses,

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*We are indebted to Dr. J. R. M. Innes for this information. The organs examined were brain, kidney, liver, pancreas, spleen, lung, intestine, and thyroid.

† All concentrations, whether in plasma, whole blood or tissue, are given as mg./l. or mg./kg. of the free base.

50 mg./kg. and 30 mg./kg. per day respectively, which delimited the toxic region. They were measured by the method of Spinks and Tottey (1946). Measurements on whole blood were made, in different experiments, after the first dose and after the seventh dose. At least three rats were used in the determination of each point. A curve for the concentrations reached on the seventh day is given in Fig. 1.

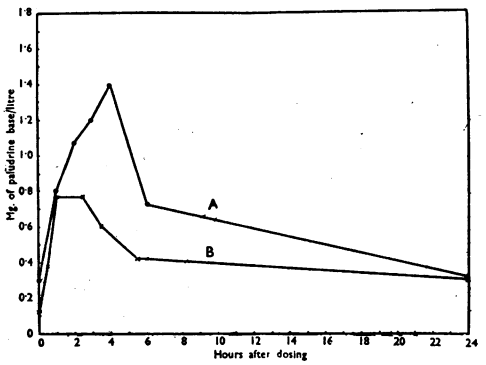

FIG. 1.—Concentrations of paludrine in the whole blood of rats after the seventh dose of 50 mg./kg. (A) and 30 mg./kg. (B) once daily.

The most interesting feature of the results is the fact that the blood concentration associated with a dose of 50 mg./kg. once daily, which produces deaths in some rats, is comparatively low and has a peak of only about 1.4 mg./l. That such a concentration should be toxic in rats is of interest, because we believe it to be readily tolerated by human beings; 700 mg. of paludrine have been administered twice daily in man with only mild toxic effects (Adams *et al.*, 1945), while plasma concentrations of about 0.5 mg./l. have been recorded twelve hours after doses of 500 mg. twice daily (Maegraith *et al.*, 1946). The maximal concentration in the plasma following the latter dose was found to be about 0.7 mg./l. in one subject, who, however, showed minimal concentrations rather lower than normal (Maegraith *et al.*, private communication). Since the whole blood concentration in man is between 2 and 3 times the plasma concentration (Maegraith *et al.*, 1946), it is reasonable to assume that blood concentrations between 1 mg. and 1.5 mg./l. are attained following the administration of 500 mg. twice daily. The comparison can also be made on the basis of plasma concentrations. The maximal plasma concentration given in rats by the (toxic) dose of 50 mg./kg. daily is 0.236 mg./l. (Table V). Concentrations much higher than this have been frequently observed in man. A further point of difference between man and rat is the lower persistence of paludrine in the latter, illustrated by the low minimal concentrations, and by the fact that the concentrations determined after only one dose are very similar to those determined after 7 doses.

It now became important to determine the blood concentrations associated with chronic toxic effects in other species. We chose to examine the mouse and the chick because the mouse behaved like the rat in the matter of delayed deaths after parenteral injection, whereas the chick did not. Curves for whole blood concentrations in these two species are given in Figs. 2 and 3.

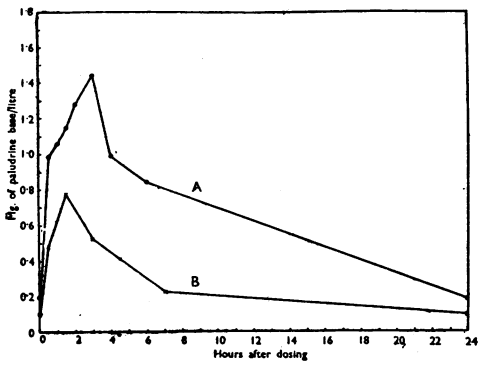

FIG. 2.—Concentrations of paludrine in the whole blood of mice after the third dose of 30 mg./kg. once daily (A) and the fifth dose of 12.5 mg./kg. twice daily (B).

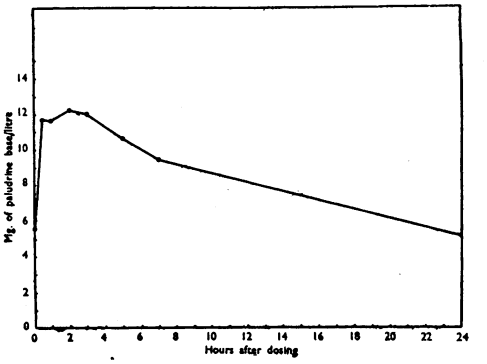

FIG. 3.—Concentrations of paludrine in the whole blood of chicks after the fourth dose of 60 mg./kg. twice daily.

The curves for mice were determined on one group receiving 12.5 mg./kg. twice daily, a treatment which is apparently harmless, and on another receiving 30 mg./kg. once daily, a treatment which produces scattered deaths. In the first group measurements were made after the fifth dose, in the second after the third dose. It will be seen that there is a good parallelism between the concentrations toxic for mice and those toxic for rats, and that the general form of the curves is similar, the build-up being negligible. It will also be apparent that the same dose given to mice and rats on a weight basis will produce higher concentrations in the mice.

Curves for chicks were obtained from animals receiving 60 mg./kg. twice daily. Scattered deaths occur with this regime although about 30 to 50 per cent of the animals will survive indefinitely treatment given for 5 days. Measurements were made after the fourth dose. It will be seen that the peak concentrations associated with a potential lethal effect in chicks are about 10 times as high as concentrations associated with lethal effects in rats and mice. Also the build-up is considerable, a residue of about 5 mg./l. being left after the third and fourth doses. The contrast between chicks on the one hand, and rats and mice on the other, is also well shown by the results of other experiments in which only one dose of 50 mg./kg. was given to chicks. Peak concentrations of paludrine in the blood rose to 3–4 mg./l. and after 24 hours concentrations of 1.5 mg./l. were recorded.

DISCUSSION

Clearly a fundamental difference must exist between the metabolism of paludrine in rats and mice and its metabolism in chicks (and probably man). Certainly, the grosser aspects of distribution which can be measured chemically do not account for the differences in results. In all species examined so far the concentration of the drug in the plasma is about a third to a fifth that in whole blood, and the ratios between tissue and plasma concentrations (which vary from 10 to 100/1 depending on the tissue) are similar. We sought to emphasize the distinction between the chick and the rat, and the fact that the tissue distribution of the drug does not reveal any explanation of it, by comparing the concentrations found in the tissues of rats and chicks following the seventh dose of

50 mg./kg. once daily. This treatment is lethal for some rats, but tolerated by chicks. The blood and tissues of 3 rats or 6 chicks were used at each time interval. The results are shown in Tables V and VI.

TABLE V

DISTRIBUTION OF PALUDRINE IN RATS FOLLOWING THE SEVENTH ORAL DOSE OF 50 MG./KG. ONCE DAILY

Time	mg. base/l. or kg. in						
	Blood	Plasma	Lung	Spleen	Kidney	Liver	Brain
Before ..	0.403	0.0693	1.61	0.414	0.577	0.748	0
1 hour ..	0.802	0.0804	4.34	2.62	3.03	20.6	0
2 hours ..	1.07	0.154	11.2	4.69	9.14	32.4	trace
4 „ ..	1.40	0.236	17.9	12.3	9.80	30.1	0
6 „ ..	0.723	0.144	6.41	1.95	2.10	11.9	trace
24 „ ..	0.320	0.0671	1.82	0.711	0.947	1.07	(0.123)

TABLE VI

DISTRIBUTION OF PALUDRINE IN CHICKS FOLLOWING THE SEVENTH ORAL DOSE OF 50 MG./KG. ONCE DAILY

Time	mg. base/l. or kg. in						
	Blood	Plasma	Lung	Spleen	Kidney	Liver	Brain
Before ..	3.25	1.02	72.4	25.9	92.6	45.3	11.5
1 hour .	3.78	1.31	62.9	26.6	89.7	62.8	10.5
2 hours .	8.15	2.84	121	73.8	214	136	19.4
4 „ ..	7.59	2.58	117	57.6	246	104	17.5
6 „ ..	5.91	2.14	103	40.9	177	95.1	18.7
24 „ ..	2.95	0.809	75.6	12.4	53.9	31.6	15.1

Although the treatment is tolerated by chicks, but fatal for some rats, the drug concentrations are uniformly higher in the chicks. It would seem, too, that paludrine reaches the brain more readily in the chick than in the rat, a point which is of interest because, so far as we are aware, such a species difference has not been demonstrated for any other drug. However, this difference would hardly seem to have any bearing on the high blood concentrations in the chick or the delayed deaths in mice and rats. Surveying the results of all the experiments, we have come to the conclusion that the simplest explanation of them is to postulate that paludrine, in mice and rats, is metabolized to a substance persistent in the body and more toxic than the drug itself; in chicks, and probably in man, the metabolism is either different qualitatively or, if it is similar, the degree of degradation to the toxic substance is much less. The relevant facts can be summarized as follows:

1. Equivalent doses of paludrine give higher concentrations, and the drug is more persistent, in chicks than in mice and rats. On the other hand, it is more

toxic for mice and rats than for chicks. On the evidence so far available, it is probable that man behaves like the chick rather than like the rat or mouse.

2. Although paludrine appears to be removed so readily from the blood of mice and rats, delayed deaths may occur in both species and, after parenteral administration, an additive toxic effect can be produced by a second injection given even 24 hours after the first.

3. The tissue distribution of the drug in its grosser aspects does not account for the differences in susceptibility between chicks and rats. Concentrations are uniformly higher in the chicks.

4. The distribution of 4430 (an N_5-methyl derivative of paludrine) in the body is similar to that of paludrine (Spinks, 1946, 1947), but delayed deaths are not associated with its injection into animals.

5. Recoveries of paludrine from the faeces and urine of rats (and rabbits) are low, usually less than 30 per cent of the dose (Spinks, 1947), which contrasts markedly with what obtains in man, where they are much higher, often up to 60 per cent (Maegraith *et al.*, 1946).

SUMMARY

1. Measurements of the toxicity of paludrine for mice, rats, rabbits, and chicks are given.

2. Delayed deaths follow the intravenous injection of paludrine into mice and rats, and it is noteworthy that an additive toxic effect can be obtained by a second intravenous injection given many hours after the first. Delayed deaths do not follow the intravenous injection of the drug into chicks, and in these animals an additive toxic effect is not produced by a second injection.

3. Measurements of the concentration of paludrine in the blood of mice, rats, and chicks under various treatments showed that chicks tolerate much higher concentrations of the drug in the body than do mice and rats.

4. Gross measurements of the drug in the organs of chicks and rats on a similar treatment (50 mg./kg. once daily) did not reveal differences sufficient to account for the difference in tolerance. Because of this, and in the light of other evidence which is presented, it is suggested that the metabolism of paludrine in chicks is different from what it is in rats and mice. The facts would be explained if paludrine, in mice and rats, were degraded in large measure to a substance more toxic than the drug itself.

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