ON THE METABOLISM OF SOME AROMATIC NITRO-COMPOUNDS BY DIFFERENT SPECIES OF ANIMAL

PART II. THE ELIMINATION OF VARIOUS NITRO-COMPOUNDS FROM THE BLOOD OF DIFFERENT SPECIES OF ANIMAL

BY D. J. LAWFORD, E. KING AND D. G. HARVEY
From the Department for Research in Industrial Medicine, Medical Research Council, Hampstead

Received April 15, 1954

INTRODUCTION

KING and Harvey[1] have shown that the rat can eliminate dinitro-*o*-cresol from the blood at different rates and that these depend on several normal and artificial variables. The present communication records the elimination rates of some aromatic nitro-compounds from several species of animal.

EXPERIMENTAL

Four substances were chosen for experiment, partly because of their chemical relationship and partly because they present some occupational risks. The first, *p*-nitrophenol, is a moiety of the *p*-nitrophenyl diethyl thiophosphate molecule (parathion, E605). This is widely used as a pesticide. The second substance, 2: 4-dinitrophenol, was once used as an explosive in the First World War. It was also used therapeutically as a metabolic stimulant, and is very similar to the third substance 4: 6-dinitro-*o*-cresol. The latter has been studied in the rat, rabbit and man, and considerable information exists on its elimination rates by the 3 species (King and Harvey[2,3]). The fourth substance, 2: 4-dinitro-α-naphthol was once used as its sodium salt as a colouring pigment, more commonly known as Martius Yellow, but the discovery of its toxic properties prevented its further employment in this respect. All 4 compounds possess the *p*-nitrophenolic group, but more important, 3 of them possess the 2: 4-dinitrophenolic group which appears to be essential for a specific form of biological activity. The animals selected for this study were the mouse, the rat, the guinea-pig and the rabbit. All of these animals are employed in laboratories where toxicological assays are performed. Also a few small West African monkeys were used, but the observations made on these were not sufficient for detailed statistical evaluation. *p*-Nitrophenol was estimated by the method of Lawford and Harvey[4], dinitrophenol and dinitro-α-naphthol were estimated essentially by the method of Parker[5] modified by Harvey[6] for dinitro-*o*-cresol in whole blood. The wavelengths at which solutions of the sodium salts of these compounds in methyl ethyl ketone were measured were 4200 Å for dinitrophenol, 4300 Å for dinitro-*o*-cresol, 4500 Å for dinitro-α-naphthol. Solutions for administration by oral feeding or by intraperitoneal injection were made by dissolving the substances in 0·5 per cent. sodium chloride solution and 0·5 per cent. sodium bicarbonate solution to give final concentrations of the substance of 0·5 or 1·0 per cent. In the case of dinitro-α-naphthol the administration was carried out at about 37° C.

because of the relative insolubility of its sodium salt and its tendency to separate out in solutions stronger than 0·5 per cent. LD50 values were determined essentially by the method employed by Harvey[7,8], in studies on dinitro-*o*-cresol and associated compounds. As far as possible similar strains of animal were used throughout. This rule was not easy to observe in the case of rabbits. These consisted very largely of "scrub" groups, usually lop-eared or half lop-eared types obtained from various sources. Statistical analyses for variance, and the method employed for the determination of regression lines—elimination rates—(*b*) were essentially as described by Emmens[9].

Results

These are given in Tables I, II and III, and in Figure 1. Table I gives the absolute slope values (*b*) of the nitro-compounds for the mouse, rabbit, guinea-pig and rat following administration of the substances by stomach tube (oral) and by intraperitoneal injection. Table II summarises the results in the form of ratios of *b*, with rat = 1 or dinitro-*o*-cresol = 1. Table III compares LD50 values with absolute slope values and their ratios. Figure I summarises the experiments carried out on the monkeys and compares them with those of the rat.

TABLE I

Absolute rates of elimination (*b*) of the four nitro-compounds

Animal	Method of dosage	Substance mol. wt. solubility in water g./100 ml.	*p*-nitrophenol 139 1·6 (25° C.)	2:4-dinitrophenol 184 0·56 (18° C.)	4:6-dinitro-*o*-cresol 198 0·024 (19° C.) (a)	2:4-dinitro-α-naphthol 234 0·004 (18° C.)
Mouse	Oral	S (b)	30	36	44	
		T	30	36	44	(c)
		b	− 0·90 ± 0·06	− 0·098 ± 0·033	− 0·036 ± 0·004	
	Intra-peritoneal	S	24	24	28	20
		T	24	24	28	20
		b	− 1·24 ± 0·12	− 0·21 ± 0·014	− 0·04 ± 0·002	− 0·012 ± 0·006
Rabbit	Oral	S	4	6	6	4
		T	64	24	30	16
		b	− 0·43 ± 0·036	− 0·010 ± 0·02	− 0·045 ± 0·001	− 0·061 ± 0·02
	Intra-peritoneal	S	5	6	3	4
		T	45	24	15	20
		b	− 0·78 ± 0·006	− 0·22 ± 0·0009	− 0·077 ± 0·0109	− 0·087 ± 0·02
Guinea-pig	Oral	S		16	16	20
		T	(c)	16	16	20
		b		− 0·12 ± 0·017	− 0·032 ± 0·001	− 0·051 ± 0·004
	Intra-peritoneal	S		16	20	16
		T	(c)	16	20	16
		b		− 0·135 ± 0·017	− 0·021 ± 0·003	− 0·04 ± 0·004
Rat	Oral	S	4	6		4
		T	32	24	−0·01	24
		b	− 0·190 ± 0·012	− 0·062 ± 0·009		− 0·015 ± 0·0006
	Intra-peritoneal	S	5	6	(d)	4
		T	25	24	−0·02	20
		b	− 0·80 ± 0·06	− 0·122 ± 0·008		− 0·021 ± 0·001

Notes.—(a) See Harvey[7]. (b) S = number of animals; T = number of blood samples. These were always arranged in equal groups. Thus when S = 4 and T = 64, there were 16 equal sample groups. All mice and guinea-pigs gave one sample each so S = T. Groups were spaced fairly evenly over time necessary for total elimination. This was determined approximately by preliminary experiments involving a small number of animals. (c) Values too scattered to give a satisfactory regression line. (d) See King and Harvey[1].

TABLE II

COMPARISON OF RATES OF ELIMINATION (*b*)

(A) By Animal Species : Rat = 1

Substance		Mouse		Rabbbit		Guinea-pig		Rat
p-nitrophenol	Oral	4·9	>	2·3	>	(N.A.)	>	1·0
	Intra-peritoneal	1·5	>	1·0	≃	(.NA.)	≃	1·0
2:4-dinitro-phenol	Oral	1·5	<	1·6	<	2·0	>	1·0
	Intra-peritoneal	1·7	<	1·8	>	1·1	>	1·0
4:6-dinitro-*o*-cresol	Oral	3·6	<	4·5	>	3·2	>	1·0
	Intra-peritoneal	2·0	<	3·8	>	1·0	=	1·0
2:4-dinitro-α-naphthol	Oral	(N.A.)		4·0	>	3·4	>	1·0
	Intra-peritoneal	6·0	>	4·1	>	1·9	>	1·0

(B) By Compounds : dinitro-*o*-cresol = 1

Animal		*o*-nitrophenol		2:4-dinitrophenol		2:4-dinitro α-naphthol		4:6-dinitro-*o*-cresol
Mouse	Oral	25·0	>	2·7	>	(N.A.)	>	1·0
	Intra-peritoneal	31·0	>	5·2	>	3·0	>	1·0
Rabbit	Oral	9·5	>	·22	>	1·3	>	1·0
	Intra-peritoneal	10·0	>	3·0	>	1·3	>	1·0
Guinea-pig	Oral	(N.A.)		3·9	>	1·6	>	1·0
	Intra-peritoneal	(N.A.)		6·5	>	2·0	>	1·0
Rat	Oral	19	>	6·2	>	1·5	>	1·0
	Intra-	40	>	6·1	>	1·1	>	1·0

TABLE III

COMPARISON OF LD50 VALUES AND ELIMINATION SLOPE RATIOS

(A) For dinitro-*o*-cresol for the four species

	Mouse		Rabbit		Guinea-pig		Rat
Absolute LD50 values (mg./kg.)	24·0 24·2 (b) 26·0		23·5		22·5		28·5 (b)
Ratio (Rat = 1)	0·8	<	0·7	=	0·7	<	1·0
Elimination slope ratio	2·0	<	3·8	>	1·0	≃	1·0

(B) For all 4 nitro-compounds for mice and rats (all in mg./kg.)

	p-nitro-phenol		2:4-dinitro-phenol		4:6-dinitro-*o*-cresol		2:4-dinitro-α-naphthol
MICE:							
Absolute LD50 values (mg./kg.)	107·6		26·0		24·0 24·2 26·0 (b)		55
Ratio (dinitro-*o*-cresol = 1)	4·3	>	1·0	=	1·0	<	2·2
Elimination slope ratio (dinitro-*o*-cresol = 1)	31·0	>	5·2	>	1·0	<	3·0
RATS:							
Absolute values	97·0		32·7		28·5		47·5
Ratio (dinitro-*o*-cresol = 1)	3·4	>	1·3	>	1·0	<	1·7
Elimination slope ratio (dinitro-*o*-cresol = 1)	40·0	>	6·1	>	1·0	<	1·1

NOTES.—(a) All substances administered by intraperitoneal injection for slope ratios and for determination of LD50 values.

(b) See Harvey.[7]

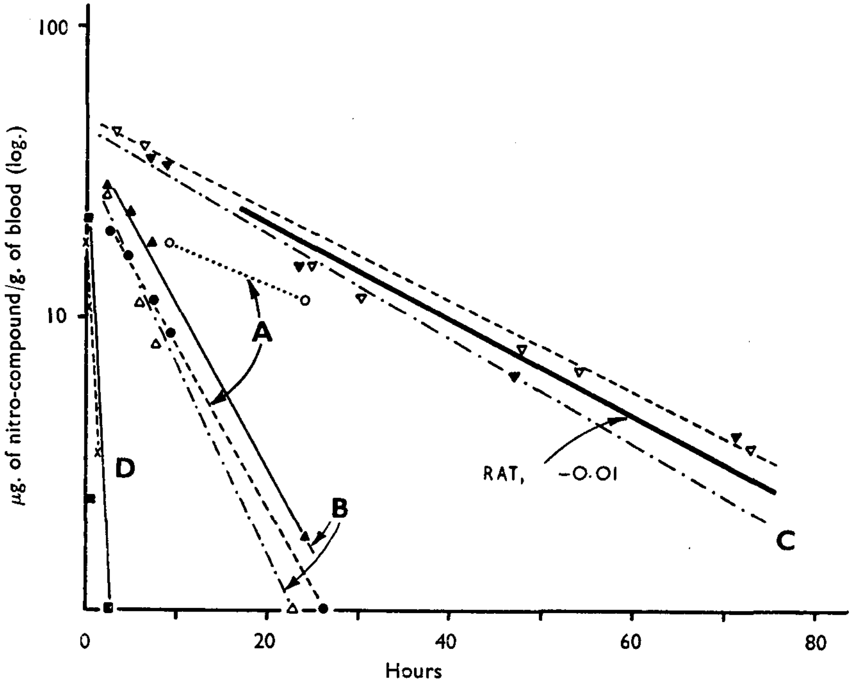

FIG. 1. Elimination of nitro compounds by the monkey.

A. Dinitro-α-naphthol
B. Dinitrophenol
C. Dinitro-*o*-cresol
D. *p*-Nitrophenol

× - - - ×	20 mg./kg. *p*-nitrophenol oral	one monkey
▪——▪	20 mg./kg. *p*-nitrophenol intraperitoneal	
△-·-·-△	15 mg./kg. 2:4-dinitrophenol oral	one monkey
▲——▲	15 mg./kg. 2:4-dinitrophenol intraperitoneal	
○····○	15 mg./kg. dinitro-α-naphthol oral	one monkey
●- - - -●	15 mg./kg. dinitro-α-naphthol intraperitoneal	
▽ - - - ▽	20 mg./kg. dinitro-*o*-cresol oral	one monkey
▼-·-·-▼	20 mg./kg. dinitro-*o*-cresol intraperitoneal	one monkey

DISCUSSION

Three main conclusions can be made from the results. First, that the 4 substances are eliminated in the following order of descending speed: *p*-nitrophenol, dinitrophenol, dinitro-α-naphthol, and dinitro-*o*-cresol. Secondly, that the animals eliminate these compounds from the blood in the following descending order of efficiency: mouse, rabbit, guinea-pig, rat and monkey. Thirdly, that the ratios of the LD50 values are smaller and more uniform in rats and in mice for all 4 compounds than are the corresponding ratios of their elimination rates. An attempt was made to correlate eliminating power with species and with the substances studied. thus at first it was thought that there might be a correlation between the animals' basal metabolism and their ability to detoxicate the substances administered. Brodie, Proctor and Ashworth[10], in an extensive study of the basal metabolic rates of mature animals of different species, have shown that the basal metabolism tends to vary with the 0·73 power of the body weight, also that there is a linear relation between the logarithm of the body weight and the logarithm of the basal metabolism of the animal. The value increases with increasing size. Thus, the order of the animals studied would be mouse, rat, guinea-pig and rabbit. Nevertheless, the present study indicates that no such relationship exists. Quite clearly, an explanation of the quantitative differences observed will have to be investigated in a further study of the specific excretion and detoxication mechanisms of the 4 species. Although the elimination rates appear to increase with diminishing molecular weight and with increasing solubility, there is a reversal in the case of dinitro-*o*-cresol and dinitro-α-naphthol. On the whole the former compound is more slowly excreted than the latter.

Of the four substances studied dinitro-*o*-cresol is undoubtedly the most important from the occupational-hazard aspect. *p*-Nitrophenol can be placed next to dinitro-*o*-cresol in importance because of its derivation from parathion and because of the wide use of this pesticide. The ready release of this last substance by the hydrolysis of parathion *in vivo* suggested that its estimation in the blood of workers at risk to parathion or its analogues might be a useful monitoring device.

However, the results of the work reported in the present communication indicate that the removal of *p*-nitrophenol from the blood of animals is very rapid. In fact most doses are eliminated completely from the blood within 2 hours of administration. No information is available on the clearance of *p*-nitrophenol from the blood of man, but it seems probable that unless estimations are made immediately after exposure the results will not be very valuable. A more practical approach appears to be in the estimation of urinary *p*-nitrophenol. This has been applied by Lieben, Waldman and Krause[11] in environmental studies on tobacco pickers. The results of these workers, although interesting, do not contain sufficient information from which to derive excretion rates. Clearly, further information on this aspect is highly desirable.

The evidence presented in this and the previous communication (King and Harvey[1]) emphasises that it is essential to include elimination experiments of the type described in any survey of a toxic substance. This is particularly important if one of the ultimate objects of the assay is to determine whether the substances will accumulate in man as the result of small but repeated exposures (Harvey[12]).

Summary

1. Four aromatic nitro-compounds have been studied, and it has been found that they are eliminated from 4 species of laboratory animals in the following descending order of speed: *p*-nitrophenol > 2:4:dinitrophenol > 2:4-dinitro-α-naphthol > 4:6-dinitro-*o*-cresol.

2. In the 4 species of animals employed the eliminating efficiency in descending order of speed is mouse > rabbit > guinea-pig > rat.

3. A few experiments on West African monkeys reveals that the elimination rates of the 4 substances are of the same order as for the rat.

4. A comparison of the ratios of the LD50 values and of the elimination rates indicates that the LD50 values are much closer numerically than are the elimination rates. The latter exhibit some divergence.

Our thanks are due to Miss Jean Peal for her constant and reliable assistance and to Miss Audrey Mackrill and Miss June Welsher for help with the animals, also to Mr. H. Garling of the Human Nutrition Research Unit, Medical Research Council, for assistance in the preparation of the two diagrams in this and the previous paper.

References

1. King and Harvey, *J. Pharm. Pharmacol.*, 1954, **6**, 529.
2. King and Harvey, *Biochem. J.*, 1953, **53**, 185.
3. King and Harvey, *Biochem. J.*, 1953, **53**, 196.
4. Lawford and Harvey, *Analyst*, 1953, **77**, 63.
5. Parker, *Analyst*, 1949, **74**, 646.
6. Harvey, *Lancet*, 1952, **262**, 796.
7. Harvey, *J. Pharm. Pharmacol.*, 1952, **4**, 1062.
8. Harvey, *ibid.*, 1953, **8**, 197.
9. Emmens, C. W., *Principles of Biological Assay*, 1st. Ed., Chapman and Hall, London.
10. Brodie, Proctor and Ashworth, *Missouri Agricultural Experiment Station, Res. Bull.*, No. 220, 1934.
11. Lieben, Waldman and Krause, *Arch. Industr. Hyg. & Occup. Med.*, 1953, **7**, 93.
12. Harvey, *Brit. J. industr. Med.*, 1953, *II*, 58.