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

PART I. SOME FACTORS INFLUENCING THE ELIMINATION OF 4:6 DINITRO-*o*-CRESOL FROM THE BLOOD OF THE RAT

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INTRODUCTION

KING and Harvey[1] have shown that the rat, the rabbit and man eliminate dinitro-*o*-cresol at widely differing rates. This observation suggested that further work should be carried out to investigate, first, the influence of various factors, for example, age, weight, sex and environmental temperature on the elimination rate from the blood of some commonly used laboratory animal, and secondly, the elimination of this and analogous nitro-compounds by different species of animal.

The present communication reports the results of the first of these investigations, namely, the effect of some common variables on the elimination of dinitro-*o*-cresol by the rat.

EXPERIMENTAL

The methods employed were essentially similar to those described by King and Harvey[1]. Hooded rats of the same strain were used throughout. Statistical analysis for variance, and the method employed for the determination of regression lines (*b*) were essentially as described by Emmens.[2]

RESULTS

These are given in Tables I and II and in Figure 1. Table I summarises the effects of various common factors on the slope value *b*. This shows that the decay of dinitro-*o*-cresol from the blood is exponential and that the range of 17 values is − 0·010 to − 0·022. Table II compares values of *b* following tail bleeding and cardiopuncture (after ether anæsthesia) methods of obtaining blood, and demonstrates that the former method results in values of *b* that are about 30 per cent. smaller than the latter.

Figure 1 is a diagrammatic representation of the results in terms of the extreme limits of time necessary to eliminate dinitro-*o*-cresol almost completely from the blood of the rat when the initial blood level is 50 to 60 μg./g.

DISCUSSION

The overall range of elimination rates derived from 17 experiments is − 0·010 to − 0·022, or 120 per cent. variation. This is equivalent to saying that an initial blood level of about 60 μg./g. will be eliminated almost completely from the blood in 82 to 182 hours (Fig. 1).

However, this range includes at least 3 abnormal variables, namely,

TABLE I

EFFECT OF VARIOUS FACTORS ON THE ELIMINATION OF DINITRO-*o*-CRESOL FROM THE BLOOD OF RATS

Serial number	Description of factor	Weight g.	Number of animals	Dose mg./kg.	Mode of administration	$b \pm$ SE b (a)	Per cent. difference of b between pairs. (Based on lower value)	Remarks
	(A) Sex and Size							
1	Female	150–200	4	9 × 20	Oral	− 0·010 ± 0·0004	− 7	All doses given at normal temperature, i.e., 18° to 20° C and relative humidity 50 to 60 per cent.
2	Female	”	5	1 × 30	”	− 0·011 ± 0·0006		
3	Male	”	5	1 × 20	Intraperitoneal inj.	− 0·017 ± 0·001	− 53	
4	Female	”	6	1 × 20	” ”	− 0·013 ± 0·0003	− 30	
5	Large (♂ + ♀)	350–400	4	1 × 20	” ”	− 0·017 ± 0·0008	− 12	
6	Small (♂ + ♀)	40–50	16	1 × 20	” ”	− 0·019 ± 0·0008		
	(B) Mode of Administration and Environmental conditions							
7	Female	150–200	6	2 mg./cu.m/5 hrs.	Aerosol	− 0·010 ± 0·0009	− 0	25° to 30° C and increasing relative humidity
8	Male	”	6	1 × 5	Intraperitoneal inj.	− 0·010 ± 0·0005	− 7	
9	Male	”	6	1 × 10	” ”	− 0·011 ± 0·0005	− 40	37° C and relative humidity 50 per cent.
10	Male	”	6	1 × 10	” ”	− 0·014 ± 0·0007		Pretreated at 37° C and relative humidity 50 per cent. for 7 days before administration of dinitro-*o*-cresol
11 (a)	Mixed (♂ + ♀)	”	6	1 × 15	” ”	− 0·011 ± 0·003	− 36 } 64	Given 7 days rest before oral administration
(b)		”		1 × 15	Oral	− 0·015 ± 0·005	− 15	
12 (a)	Mixed (♂ + ♀)	”	6	1 × 15	”	− 0·013 ± 0·0005		Given 7 days rest before intraperitoneal injection
(b)		”		1 × 15	Intraperitoneal inj.	− 0·018 ± 0·0007	− 38	
	(C) Metabolic Depressant—Methylthiouracil							
	Mixed (♂ + ♀)	150–200	60	1 × 15	Intraperitoneal inj.			Divided into 3 groups (serials 13, 14, 15) of 20 each.
13			20		” ”	− 0·018 ± 0·0009	22 } 6	1 × 15 mg./kg. of methyl thiouracil by intraperitoneal injection
14			20		” ”	− 0·022 ± 0·0009	30	3 × 15 (1 daily) thiouracil by intraperitoneal injection
15			20			− 0·017 ± 0·0008		Control—No methylthiouracil

NOTE.—Serials 6, 13, 14 and 15 bled by cardiopuncture—the remainder by tail.

high environmental temperatures and relative humidities and the effect of a metabolic depressant. Comparison of some smaller sub-groups of values indicates that the differences are less. For example, the values of b for series 1, 2, 11b and 12a following oral administration are − 0·01, − 0·011, − 0·015, − 0·013 (mean − 0·0124 ± 0·001), and for serials 3, 4, 5, 11a and 12b following intraperitoneal injection are − 0·017, − 0·013, − 0·017, − 0·011 and − 0·018 (mean − 0·015 ± 0·003).

TABLE II

EFFECT OF VARIATION OF MODE OF OBTAINING BLOOD ON THE ELIMINATION OF DINITRO-*o*-CRESOL BY THE RAT

32 hooded male rats; 83 to 117 g., mean 102 g. ± 1·9, given a single dose of 15 mg./kg. by intraperitoneal injection. They were then divided into 2 groups; (A) of 6 for tail bleeding, and the remaining 28 (B) for cardiopuncture under ether anæthesia. These animals were killed after the blood samples were obtained.

GROUP A—TAIL BLEEDING

Individual Analysis

Rat No.	$b \pm$ SE b	
1	− 0·0143 ± 0·00063	
2	− 0·0179 ± 0·00049	
3	− 0·0167 ± 0·00098	Mean slope b =
4	− 0·0145 ± 0·00191	− 0·0158 ± 0·00064
5	− 0·0171 ± 0·00147	
6	− 0·0145 ± 0·00131	

Block Analysis

$\bar{x} = 37 \quad \bar{y} = 0{\cdot}92895$

	S. squares	D.F.	Mean square	F.
Between Times	3·92648	3	1·30883	262
Linear Regression	3·91544	1	3·91544	783
Departures	0·01104	2	0·00552	1
Within Times	0·10085	20	0·005	

From this $b \pm$ SE b = − 0·0158 ± 0·00064

GROUP B—CARDIOPUNCTURE

Block Analysis

$\bar{x} = 37, \quad \bar{y} = 0{\cdot}08920958$

	S. squares	D.F.	Mean square	F.
Between Times	7·14692	3	2·3823	45
Linear Regression	7·00081	1	7·00081	134
Departures	0·14611	2	0·07306	1·4
Within Times	1·04110	20	0·05206	

From this $b \pm$ SE = − 0·021 ± 0·0018

From this observation it can be calculated that:

b_B is 31 per cent. greater (faster) than b_A

$\sqrt{\text{SE}\, b_A + \text{SE}\, b_B} = 0{\cdot}0018$, or

the difference between b_A and b_B is significant.

Two general conclusions can be made from these observations. First, that variations such as sex, magnitude and frequency of the dose of dinitro-*o*-cresol do not have any very marked effects on its elimination rate. Secondly, that any of the values of b in the smaller ranges or even in the complete range will permit at least a semi-quantitative comparison with values obtained under similar conditions for other species of animal, e.g., the rabbit (King and Harvey[1]).

The methods employed in administering dinitro-*o*-cresol and in collecting the blood gave different results. Up to the present no theory can be advanced to explain why b values calculated from concentrations in blood samples obtained by cardiopuncture following intraperitoneal injection are significantly greater (elimination rate more rapid) than those obtained by oral administration or by inhalation.

Certain practical aspects of these experiments have a bearing on the design of toxicological assays. Obviously there is considerable value in determining some index of the elimination rate of a toxic material, and it is essential to design experiments that will embody as many natural conditions of environment and of exposure as possible. Clearly it is better to administer a substance *via* the alimentary canal or through the lungs and to obtain blood by tail bleeding than to adopt the less natural

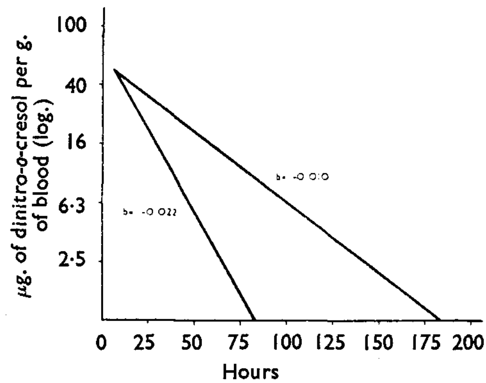

FIG. 1. Elimination of dinitro-*o*-cresol by the rat, range values of *b*.

procedures already referred to. Of necessity cardiopuncture may have to be employed, for example, if growing rats or small animals (for example, mice) are used, but such a procedure will involve a greater waste of animal life. By use of a few (for example, 6) animals for some preliminary experiments, considerable information can be obtained on elimination rates from the blood. There is no doubt that the results obtained will give some guide in assessing any possible accumulation of the substance in the animal following repeated exposures.

Several facts emerge from this study which may have some practical applications in maintaining safe conditions for spray operators and others handling dinitro-*o*-cresol. First, it is clear that a sudden increase in the environmental temperature and in the relative humidity is unlikely to cause any marked changes in the elimination rate. In fact the actual rates of elimination of 2 groups of rats given 5 and 10 mg./kg. dinitro-*o*-cresol are identical (— 0·01). This value is numerically the lowest of the range, and therefore represents the slowest rate of elimination. Secondly, acclimatisation to these two environmental factors results in a somewhat faster elimination rate and one that is more comparable with that obtained under normal temperatures and humidities. The exacerbating effect of high environmental temperatures on the action of dinitro-*o*-cresol is well known (Bidstrup and Payne[4]; Parker, Barnes and Denz[4];

King and Harvey[5]), but it has been demonstrated (King and Harvey[5]) that heat does not alter significantly the highest blood concentration in intoxicated animals.

These observations add further emphasis for the need for maintaining the strictest safety measures among men handling dinitro-*o*-cresol, especially in hot weather. Not only does heat cause an increase in toxicity but it fails to assist its elimination from the blood. Thirdly, methylthiouracil has been suggested as a therapeutic agent in reducing high metabolic rates caused by excessive doses of dinitro-*o*-cresol (Siedek and Hoffman Credner[6]). The present studies indicate that the elimination rate from man is unlikely to be altered greatly by the employment of methylthiouracil. In other words it is at all times essential to encourage the natural elimination from man by removing him from exposure once early toxic symptoms appear (Bidstrup, Bonnell and Harvey[7]), and by carrying out the recognised treatment, including bed-rest in cool conditions, on persons who are seriously poisoned (Pollard and Filbee[8]).

Summary

1. Elimination rates of dinitro-*o*-cresol from the blood of hooded rats have been determined under normal and abnormal (high environmental temperature, metabolic depressant) conditions. The effects of varying the methods of administration and of obtaining the blood have also been studied. Seventeen values of b ranged from $-$ 0·010 to $-$ 0·022.

2. Elimination rates are faster when dinitro-*o*-cresol is administered by intraperitoneal injection than by mouth or by inhalation of an ærosol, and when blood is obtained by cardiopuncture rather than by tail bleeding.

3. Sudden application of high environmental temperatures to animals poisoned by dinitro-*o*-cresol caused a slight slowing of the elimination rate. Acclimatisation for 7 days under the same conditions resulted in a faster elimination rate.

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