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VARIATIONS OF MICROSOMAL OXIDASE ACTIVITY IN MALE RATS AS SHOWN BY RATES OF DIMETHYLNITROSAMINE METABOLISM

D. F. HEATH

Toxicology Research Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey

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Abstract—In one set of experiments (Series I) male rats metabolized dimethylnitrosamine rapidly, and pre-dosing with dimethylformamide increased time to death and LD_{50} value, all as with female rats (Heath[1]). In a second set (Series II) begun five months later a quarter of the males metabolized dimethylnitrosamine as fast as in Series I, but the rest metabolized it only half as fast. After lethal doses they died more slowly than in Series I, and neither time to death nor LD_{50} value was affected by dimethylformamide. Rats of the same strain but from another colony behaved metabolically as in Series II. The differences between Series I and II cannot be explained with certainty. They show that the activity of one, somewhat atypical microsomal oxidase can change rapidly in a closed rat colony with random mating, can be highly variable, and may be bimodally distributed, with obvious implications when results depend upon metabolic rates. For many experiments with dimethylnitrosamine female rats are better, as they show much less variation.

DIMETHYLNITROSAMINE, $Me_2N.NO$, is oxidized *in vivo* and *in vitro* by a microsomal oxidase (Magee and Vandekar,[2] Brouwers and Emmelot,[3] Mizrahi and Emmelot,[4] and Orrenius, Ericsson and Ernster[5]). Heath[1] showed that its rate of metabolism in rats could be determined from the rate of $^{14}CO_2$ excretion after i.v. or i.p. injection of ^{14}C-dimethylnitrosamine. In female rats the rate showed little variability. It was also found that the LD_{50} value was increased slightly and the time to death after a lethal dose was increased considerably either by giving the dose in three portions, separated by about 12 hr, or by pre-dosing with dimethylformamide, which acted as a competitive inhibitor of dimethylnitrosamine metabolism. Both treatments about tripled the period during which toxic metabolites were formed and acted.

Some analogous experiments on male rats are reported here, in which considerable variations in metabolic rates are demonstrated.

MATERIALS AND METHODS

Materials and methods were as described by Heath,[1] except that a smaller metabolism chamber was used, and only one rat was placed in it at a time.

Rats. The rats used were albino males, wt. 170–180 g, of Porton Wistar strain, which had been maintained as a closed colony with random mating for the previous 6–7 yr. Rats were taken from the colony as they reached the correct weight. In

consequence most groups contained rats from several litters. Heath[1] used females from the same colony. For one experiment nine male rats were obtained from the original colony at C.R.D.E., Porton, Wilts.

Errors. The rates of $^{14}CO_2$ excretion were estimated in terms of the injected label with a coefficient of deviation of less than 3 per cent. This includes errors in injection (intravenous), timing, and CO_2 and ^{14}C estimations. Relative rates should therefore be reliable to better than $\pm 4{\cdot}5$ per cent ($3\sqrt{2}\%$). The rates were converted to rates of metabolism by the use of the factors described by Heath[1] and fully tested on female rats. The $^{14}CO_2$ excretion curves from both sexes were so similar that no serious error can have arisen from using the same conversion factors for both, but a small systematic error may have been introduced.[1] The emphasis throughout is on relative rates, which are not affected by this possibility.

RESULTS

Two series of experiments were conducted, separated by five months.

Series I (October 1957–January 1958). In this series male rats metabolized dimethylnitrosamine at rates closely similar to female rats (Table 1). Dimethylformamide reduced the rates similarly, and had similar effects on the sequelae of toxic doses, i.e. it delayed death in all experiments and slightly but significantly ($P < 0{\cdot}01$) raised the LD_{50} value (Table 2).

TABLE 1. RATES OF METABOLISM OF DIMETHYLNITROSAMINE IN SERIES I

Rates in females are given for comparison, and the effects of pre-dosing with dimethylformamide (DMF) 0·5–1 hr before dimethylnitrosamine. Standard deviations are given.

Dose mg/kg	No. of rats	DMF mg/kg	Rates of metabolism, mg/kg/hr males	females[1]
53·5	4	0	5·3 ± 0·6	5·4 ± 0·6
14·2	7	0	4·9 ± 0·4	4·0 ± 0·7
14·2	3	570	0·45 ± 0·06	0·33
14·2	1	59	1·85	1·98

TABLE 2. EFFECTS OF DIMETHYLFORMAMIDE ON THE TOXICITY OF DIMETHYLNITROSAMINE

Groups of 8 rats were given dimethylnitrosamine i.v. in graded doses. Four in each group had received 700 mg dimethylformamide i.p. in water 0·5–1 hr earlier. The order of death is recorded, 'A' denoting a rat receiving only dimethylnitrosamine, 'B' a rat pretreated with dimethylformamide. The probabilityof this order on the hypothesis of null effect was calculated for each case as described by Heath and Irwin.[6] LD_{50} values were calculated as described by Weil.[7]

Dose mg/kg	Order of death	P	Dose mg/kg	Order of death	P
50	AAABBABB	0·04	45·5	AAABBB	0·13
40	AAABB	0·10	35·0	AAAABBB	0·02
32	AA	0·16	26·9	AA	0·16
25·6	A	—			

LD_{50} values and fiducial ranges: Dimethylnitrosamine only, 31·8 (28·1–36·0) mg/kg. With dimethylformamide, 41·1 (35·2–48·1) mg/kg.

Series II (*June* 1958–*January* 1959). In this series most male rats metabolized dimethylnitrosamine more slowly and at much more variable rates. There were no obvious differences except time of year between the conditions of the rat colony in the two series, so any attempt to induce more consistency was necessarily a shot in the dark. The attempts made are summarized in Table 3. All groups showed great

TABLE 3. RANGES OF METABOLIC RATES FOUND IN MALE RATS OF SERIES II SUBJECTED TO CHANGES IN ENVIRONMENT OR DIET

All rats received 14·2 mg dimethylnitrosamine/kg.

Treatment	No. of rats	Range of rate, mg/kg/hr
Controls	19	1·4–5·0
Controls in winter	8	1·6–3·8
Reared from weaning with 8 hr light/day	6	2·3–5·2
Rats from mother colony, Porton	9	1·9–7·1
Fasted 24 hr	5	2·3–5·3
Fed from weaning on: Dixon's M.R.C.41B diet (normal)	5	1·9–4·5
Rank's M.R.C. 41B diet	5	1·9–4·5
Dixon's M.R.C. 86 diet	6	0·8–3·2
Fed diet containing 250 mg thiouracil/kg for 3–4 weeks	9	0·8–3·5
25 μg thyroxine subcutaneously 2–3 times/wk for 2–3 weeks	4	2·0–3·5

variability, with no statistically significant differences. The bulked results are shown in Fig. 1. The hodograph is almost exactly that calculated if the rats came from two populations, 60 metabolizing dimethylnitrosamine at 2·5 ± 0·6 (±S.D.) mg/kg/hr, and 19 metabolizing dimethylnitrosamine at 4·5 ± 0·7 mg/kg/hr. The differences never exceed one rat in any column of the hodograph, and are therefore too small to show.

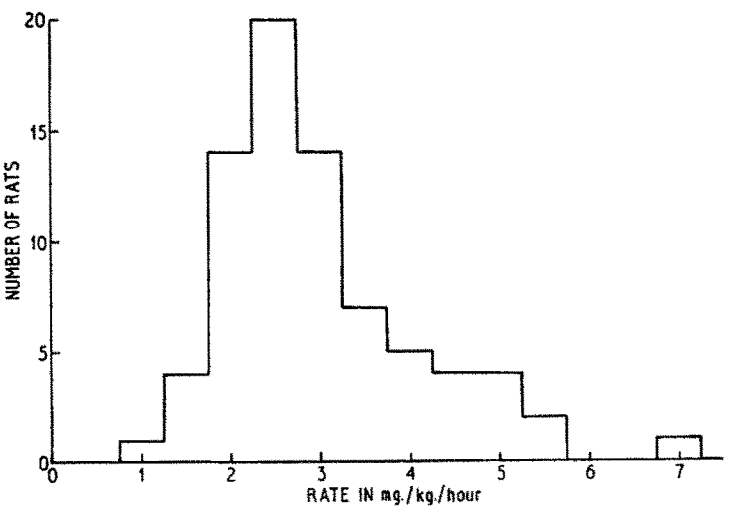

FIG. 1. Rates of metabolism of dimethylnitrosamine in rats of Series II.

Most of these rats metabolized dimethylnitrosamine so slowly that the time to death after a lethal dose was expected to be much greater than in Series I or female rats; and also it was expected that dimethylformamide, whose inhibitory effects only last about a day[1] would have relatively little effect on the time to death or LD_{50} value. All three expectations were realized. Thirty rats were given 30 mg dimethylnitrosamine/kg after pretreatment with 1000 mg dimethylformamide/kg, and twenty-nine were given 30 mg dimethylnitrosamine/kg only. In both groups fifteen rats died, i.e. the LD_{50} values were very similar, and in both deaths were spread over the period 2–8 days after injection, whereas in Series I no rat given only dimethylnitrosamine died after the 4th day. Dimethylformamide did not affect the order of death significantly.

DISCUSSION

In Series I male rats behaved like female rats.[1] They metabolized dimethylnitrosamine rapidly and with low variability (Table 1), and in them dimethylformamide raised the time to death and LD_{50} value of dimethylnitrosamine (Table 2 and Results). In Series II, begun 5 months later, rats metabolized dimethylnitrosamine at a much more variable rate (Fig. 1). These rates fit within statistical error a log-normal distribution, geom. mean: 2·8; standard deviation range: 2·0–4·0 mg/kg/hr. There is, however, no *a priori* reason for expecting a log-normal distribution in this instance,[8] and the rates found are more closely in agreement with a bimodal normal distribution, according to which about a quarter metabolized dimethylnitrosamine as fast as in Series I (4·5 $\pm$ 0·7 mg/kg/hr), while the remainder metabolized it at half the rate (2·5 $\pm$ 0·6 mg/kg/hr). For further discussion it is mostly of no importance whether the distribution was bimodal or log-normal. Whichever was correct it was to be expected that dimethylformamide would have little effect on the toxic sequelae of dimethylnitrosamine poisoning (see Results), and this was found to be the case.

The differences between the Series cannot be explained by empirical errors in Series II. The same apparatus and methods were used subsequently by my assistant and me to estimate rates of metabolism in female rats preliminary to those experiments finally published,[1] and gave consistent results; each of us often duplicated the other's work; and the basic procedure is so simple that any major error becomes obvious in the course of an experiment. And, of course, the effects of dimethylformamide on the toxic sequelae were confirmatory.

The differences cannot be explained by chance. Consider only the estimations of metabolic rates. All fifteen rats in Series I metabolized dimethylnitrosamine quickly, and nineteen in Series II, i.e. there were thirty-four 'fast' rats. In the second series sixty decomposed dimethylnitrosamine slowly. Thus overall there were sixty 'slow' and thirty-four 'fast' rats. The chance of drawing at random from such a population the first fifteen all from the 'fast' group is about $(34/94)$,[15] i.e. about 1 in 10^6.

The differences were not seasonal. Experiments in Series II continued into the winter, like those of Series I (Table 3). Rates in female rats also did not vary with season over a 2-year period.[1]

The microsomal oxidase which metabolizes dimethylnitrosamine is atypical in that it is not greatly inhibited by monoamine oxidase inhibitors such as SKF525A (2-dimethylaminoethyl 2,2-diphenylbutyrate).[1] It also differs from the enzymes responsible for the oxidation of most of three other nitrosamines.[1] What follows

may therefore apply only to this particular oxidase. It is likely that its activity is usually very variable and perhaps distributed bimodally in male rats of the Porton–Wistar strain, as the male rats from the mother colony showed the same characteristics. Probably, therefore, there was some factor operating in Series I which raised the oxidase activities in the rats to a constant high level. In the light of more recent work I suspect contamination of the foodstuff with DDT, which has a marked stimulating effect on microsomal oxidase activity.[9, 10, 11, 12]

Whatever the explanation of the results, this study indicates three things.

(1) Microsomal oxidase activities in a rat population may change rapidly for obscure reasons, and unexpected variations in controls may be significant.

(2) The oxidase activities may be bimodally distributed, or, at least, highly variable. Large numbers of controls are required to investigate these possibilities; and if either is found certain types of experiments will be difficult to interpret. For example in experiments such as those described partial inhibition by dimethylformamide in a 'fast' rat would give results indistinguishable from no inhibition in a 'slow' rat and in the experiment with dimethylformamide the number of 'fast' rats in each group was too small (about 7 in 30) for it to make a distinctive contribution.

(3) In these experiments the overall variability of males was so much greater than of females that for studies in which the rate of metabolism of dimethylnitrosamine is important females are very much to be preferred. In subsequent studies females behaved reproducibly for over 2 years.[1]

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