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The Rate of Breakdown of Methyl Methanesulphonate, Dimethyl Sulphate and *N*-Methyl-*N*-nitrosourea in the Rat

By P. F. SWANN*
Medical Research Council Toxicology Research Unit, Carshalton, Surrey

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1. The rates of decomposition of methyl methanesulphonate, dimethyl sulphate and *N*-methyl-*N*-nitrosourea in the rat were measured. 2. Dimethyl sulphate is no longer detectable in the blood of the rat 3min. after an intravenous dose (75mg./kg. body wt.). Methyl methanesulphonate is only just detectable in the blood 1½hr. after an intravenous dose (100mg./kg. body wt.). *N*-Methyl-*N*-nitrosourea is no longer detectable in the blood 15min. after an intravenous dose (100mg./kg. body wt.). 3. The exhalation of $^{14}CO_2$ after an intragastric dose of *N*[^{14}C]-methyl-*N*-nitrosourea (100mg./kg. body wt.) is appreciably slower than after an intravenous dose, from which it is estimated that the lifetime in the rat is 2–3hr.

It is possible that the carcinogenic activity of the nitrosamines may be caused by the alkylation of cellular components, which occurs in their metabolism. In the preceding paper (Swann & Magee, 1968) the amount of alkylation produced in the rat by doses of two nitrosamines and two alkylating agents was compared with the carcinogenic activity of these four compounds. Before this could be done it was necessary to know the approximate rate of decomposition of each compound in the rat. The rate of metabolism of dimethylnitrosamine by the rat was already known (Heath, 1962), and the present paper reports the lifetime of the other compounds, dimethyl sulphate, methyl methanesulphonate and *N*-methyl-*N*-nitrosourea.

MATERIALS AND METHODS

Animals and chemicals. These were similar to those used in the preceding paper (Swann & Magee, 1968). In addition, 4-*p*-nitrobenzylpyridine was a gift from the Chemical Defence Experimental Establishment (Porton, Wilts.) and [^{14}C]methanol was purchased from The Radiochemical Centre (Amersham, Bucks.).

Measurement of the rate of breakdown of dimethyl sulphate in vitro. The rate of decomposition of dimethyl sulphate was followed by measuring the release of titratable groups produced in hydrolysis. The measurement was made with a Radiometer Titrigraph TTT1 pH-stat (Radiometer, Copenhagen, Denmark). To 20ml. of water 1ml. of 0·05M-potassium phosphate buffer was added. The pH of this was adjusted to 7 with the pH-stat, which had been charged with 0·1N-NaOH. Then dimethyl sulphate solution in acetone (0·5ml.; 0·1–1·0M) was added and the rate of decomposition was followed by the addition of NaOH needed to maintain the pH.

Measurement of the rate of breakdown of dimethyl sulphate and methyl methanesulphonate in vivo. Dimethyl sulphate and methyl methanesulphonate were determined with 4-*p*-nitrobenzylpyridine (Epstein, Rosenthal, & Ess, 1955; Klatt, Griffin & Stehlin, 1960).

Before being given dimethyl sulphate, rats (170g.) were anaesthetized by intraperitoneal injection of sodium pentobarbital. Dimethyl sulphate (75mg./kg. body wt. in 0·5ml. of 0·1M-sodium citrate buffer, pH7·4) was injected into the tail vein. After increasing time-intervals, blood (1ml.) was withdrawn from the abdominal aorta and pipetted into glass tubes cooled in ice. Acetone (4ml.) was added and the mixture was centrifuged for 5min. at 0°. The supernatant (2ml.) was diluted with buffer (1ml. of 0·05M-potassium hydrogen phthalate buffer, pH4·5) and 4-*p*-nitrobenzylpyridine (0·5ml. of a 5% soln. in butan-2-one) was added. The solution was heated at 65° for 25min. After cooling, 2ml. was mixed with triethylamine (2ml.; 50% in acetone) and E_{570} was read at once. 4-*p*-Nitrobenzylpyridine is not very reliable for the quantitative determination of alkylating agents, so in these experiments three samples of blood were taken. Two were used for determination of the alkylating agent and a known quantity of dimethyl sulphate was added to the third as a positive control. In all cases this third sample gave the expected extinction.

Methyl methanesulphonate (100mg./kg. body wt.; 0·5ml. of solution in 0·9% NaCl) was injected into the tail vein of unanaesthetized rats. Before blood was taken the rats were given sodium pentobarbital by intraperitoneal injection. The method of estimation was the same as for dimethyl sulphate except that sodium acetate buffer, pH4·5, was used.

The amount of alkylating agent was found by comparison

*Present address: Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W. 1.

with standard curves constructed by adding known amounts of the alkylating agent to fresh rat blood.

Determination of N-*methyl*-N-*nitrosourea* in vivo. Female rats (each 100g.) were given 100mg. of *N*-methyl-*N*-nitrosourea/kg. body wt. After increasing time-intervals, the rats were anaesthetized and blood was taken. Ice-cold 5% (w/v) sulphosalicylic acid (9ml.) was added to each sample of blood (1ml.) and the mixture was centrifuged at 0°. The amount of *N*-methyl-*N*-nitrosourea in the supernatant was determined by polarography (PO4 Polarograph; Radiometer). Polarographic estimation of nitroso compounds directly in the acid supernatant from blood is not generally possible and preliminary distillation is necessary (Heath & Jarvis, 1955). However, the half-wave potential (−0·75v) of *N*-methyl-*N*-nitrosourea under these conditions is lower than that of dimethylnitrosamine (−0·92v) and polarography on the acid supernatant is possible.

Rate of exhalation of $^{14}CO_2$ *after a dose of* N[^{14}C]-*methyl*-N-*nitrosourea*, [^{14}C]*methanol and* [^{14}C]*methyl methanesulphonate*. The $^{14}CO_2$ was collected in NaOH in a continuous collector based on a design by Dr Sprott (Unilever Ltd., Colworth House, Sharnbrook, Bedford). Samples (2ml.) of the NaOH were diluted with 0·2M-Na_2CO_3 (1ml.) and water (4ml.). The carbonate was precipitated with 0·4M-$BaCl_2$ (2ml.) by the method of Heath & Dutton (1958). The precipitate was centrifuged, washed with water and suspended in scintillator (Bruno & Christian, 1961) containing 4% Cab-O-Sil, and the radioactivity was determined. Urine was collected from the animals while they were in the CO_2 collector and the radioactivity of this was measured. At the end of the collection period the animals were killed and frozen whole in liquid N_2. The frozen animal was pulverized and then pulped in water (2l.) in a Waring Blendor. The pulp was freeze-dried to give approx. 50g. of powder. Two samples (2·5g.) were dissolved in 50ml. of Hyamine 10-X (1M in methanol) and the radioactivity in the solution was determined.

RESULTS

Dimethyl sulphate. There was a very rapid fall in the concentration of this alkylating agent in the blood of the rat, and it was no longer detectable 5min. after the injection (Fig. 1). The half-life in 2·5mM-phosphate buffer, pH7, as measured by the release of titratable groups, was about 4½hr., but the release of titratable groups from a solution containing 0·25m-mole of dimethyl sulphate was increased from 1·2μequiv./min. in phosphate buffer at pH7 to 3·3μequiv./min. in 5mM-cysteine and to 4·9μequiv./min. in 5mM-GSH.

N-*Methyl*-N-*nitrosourea*. Polarographic measurement showed that an intravenous dose of the compound had broken down in about 15min. (Fig. 2). The exhalation of $^{14}CO_2$ after an intravenous dose of *N*-methyl-*N*-nitrosourea (0·9m-mole, 90mg./kg. body wt.) continued for a similar time to that after [^{14}C]methanol (0·85m-mole, 25mg./kg. body wt.), and in both cases most of the $^{14}CO_2$ was exhaled within 4hr. of the dose; but after an intragastric dose the exhalation was much slower (Fig. 3).

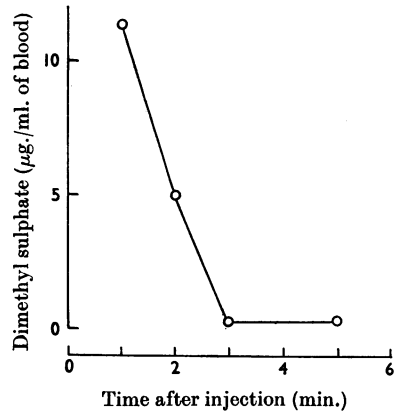

Fig. 1. Rate of disappearance of dimethyl sulphate from the blood of the rat after intravenous injection. Male rats (each 175g.) were each given 75mg./kg. body wt. in 0·5ml. of 0·1M-citrate buffer, pH7·5, by injection into the tail vein. The amount of dimethyl sulphate in the blood was estimated with 4-*p*-nitrobenzylpyridine by the technique described in the Materials and Methods section. The result for 1min. is the mean of measurements on six rats, that for 2min. on two rats, that for 3min. on two rats and that for 5min. on five rats.

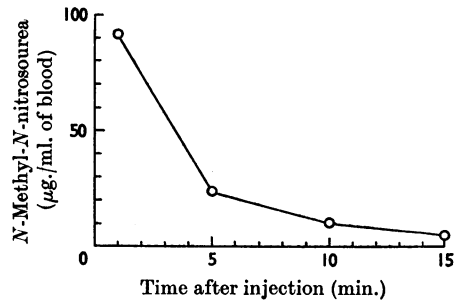

Fig. 2. Rate of disappearance of *N*-methyl-*N*-nitrosourea from the blood of rats after an intravenous injection. Female rats (each 100g.) were each given 100mg./kg. body wt. The blood proteins were precipitated with sulphosalicyclic acid and the amount of *N*-methyl-*N*-nitrosourea in the supernatant was determined by polarography. Each point is the mean of the results from three rats.

Methyl methanesulphonate. The reaction with 4-*p*-nitrobenzylpyridine showed that an intravenous dose of methyl methanesulphonate was broken down in the rat in about 1½hr. (Fig. 4). The exhalation of $^{14}CO_2$ from a dose of [^{14}C]methyl methanesulphonate was rapid for about 16hr. and, though the rate then decreased, it was still appreciable at 30hr. (Fig. 5). About one-fifth of the dose of radioactivity was excreted in the urine. This was relatively much greater than the amount from

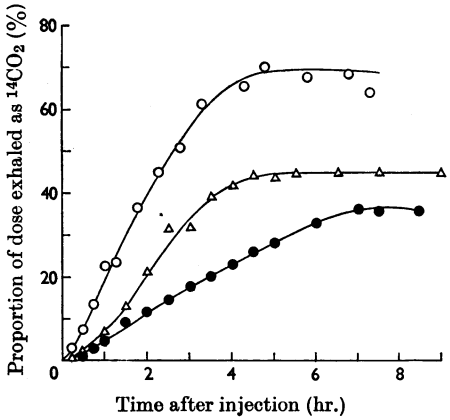

Fig. 3. Exhalation of $^{14}CO_2$ after doses of [^{14}C]methanol and *N*[^{14}C]-methyl-*N*-nitrosourea. The methods of trapping the $^{14}CO_2$ and measuring the radioactivity are given in the Materials and Methods section. ○, Methanol (25mg./kg. body wt.; intravenous); ●, *N*-methyl-*N*-nitrosourea (90mg./kg. body wt., intragastric); △, *N*-methyl-*N*-nitrosourea (90mg./kg. body wt., intravenous).

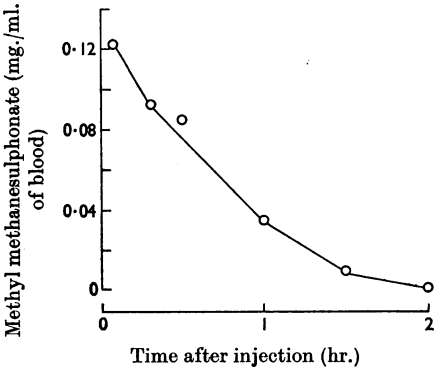

Fig. 4. Rate of disappearance of methyl methanesulphonate from the blood of the rat after intravenous injection. Male rats (each 200g.) were each given methyl methanesulphonate (100mg./kg. body wt. in 1ml. of 0·9% NaCl), and the amount of methyl methanesulphonate remaining in the blood after each interval was measured with 4-*p*-nitrobenzylpyridine. The results for 5min. and 20min. are each the mean of measurements on three rats, those for 30 and 90min. each on two rats and those for 60min. and 120min. each on four rats.

N-methyl-*N*-nitrosourea or methanol (Table 1) or from dimethylnitrosamine (Heath, 1962).

DISCUSSION

Within 1min. after intravenous injection the concentration of dimethyl sulphate in the blood of the rat is less that one-sixth of the amount that would have been expected had the compound been evenly distributed in the body water (Fig. 1), and the methylation of the nucleic acids in the lungs and brain, both of which receive a relatively large proportion of the cardiac output, is much higher than in the liver or kidney (Swann & Magee, 1968). Therefore it seems likely that the compound does not equilibrate throughout the body but breaks down in the first organs that it penetrates.

The breakdown of dimethyl sulphate in the rat (Fig. 1) is faster than might have been expected from its half-life (4½hr.) in water at pH 7, but it is a very reactive compound and its decomposition is accelerated by the addition of a reactive group such as the thiol group; no doubt *in vivo* there is rapid reaction with these and other reactive groups.

N-Methyl-*N*-nitrosourea is more stable than dimethyl sulphate even when given as an intravenous dose. Polarographic estimation showed that the concentration in blood falls to about 5% of the initial concentration within 15min. of the injection (Fig. 2). It seems likely that *N*-methyl-*N*-nitrosourea given into the stomach is not immediately broken down but mainly absorbed into the bloodstream unchanged, for there is considerable methylation of the nucleic acids not only of the gastrointestinal tract but also of other organs such as the liver and kidney (Swann & Magee, 1968). The compound would be expected to be more stable in the acid environment of the stomach than at neutral pH, since its half-life at pH 6 is 24hr.,

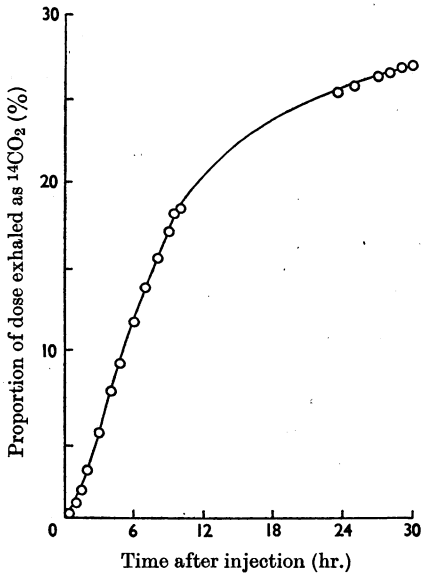

Fig. 5. Exhalation of $^{14}CO_2$ after an intravenous injection of [^{14}C]methyl methanesulphonate (2·5μc; 120mg./kg. body wt.).

Table 1. *Distribution of radioactivity from* [^{14}C]*methanol,* [^{14}C]*methyl methanesulphonate and* N[^{14}C]-*methyl*-N-*nitrosourea*

Faeces were not collected. The carcasses were freeze-dried before estimation of the radioactivity. Recoveries are given as percentages of the dose given. i.v., Intravenous.

Substrate given ...	Methanol	Methyl methanesulphonate	*N*-Methyl-*N*-nitrosourea	
Dose (mg./kg. body wt.) and route ...	25 i.v.	120 i.v.	90 oral	90 i.v.
Period of CO_2 collection (hr.) ...	8	30	9	9
^{14}C exhaled as CO_2 (%) ...	65·7	28·6	36	45·5
^{14}C in carcass (%) ...	21·8	37	28·2	25·2
^{14}C excreted in urine (%) ...	2·6	19·6	3·4	6·7

that at pH 7 is 1·2 hr. and that at pH 8 is 0·1 hr. (Druckrey *et al.* 1967). It seems probable that most of the radioactivity from a dose of *N*[^{14}C]-methyl-*N*-nitrosourea would become methanol as the compound decomposed. The exhalation of $^{14}CO_2$ after an intravenous dose of *N*-methyl-*N*-nitrosourea is completed in a similar time as that from an equimolar dose of [^{14}C]methanol, but a smaller proportion of the dose is recovered as $^{14}CO_2$. As expected, $^{14}CO_2$ is exhaled more slowly from an intragastric dose (Fig. 3) and from this it is estimated that absorption takes 2–3 hr. Regardless of whether the dose of *N*[^{14}C]-methyl-*N*-nitrosourea was given by intragastric or by intravenous injection, only 70% of the radioactivity given was recovered. The reason for this poor recovery is not known.

There is an apparent inconsistency between the fall in the concentration of methyl methanesulphonate in the blood to zero in approx. 1½ hr. (Fig. 4) and the exhalation of $^{14}CO_2$ from [^{14}C]methyl methanesulphonate, which continues to be rapid for 16 hr. and then becomes slower, but is still appreciable 30 hr. after the dose (Fig. 5). Pillinger, Fox & Craig (1965) found that after an intraperitoneal dose the amount of radioactive metabolites in the bile rose to a maximum 1½ hr. after the dose and fell to 25% of the maximum within the next 8 hr. The amount of radioactive metabolites in the urine rose to a maximum at 6½ hr., and remained at not less than 75% of the maximum until 16 hr.; after that time it decreased rapidly but was still appreciable at 24 hr. Investigation of the metabolites in the bile and urine led to the conclusion that they were derived from *S*-methylglutathione, formed by a reaction with the GSH of the liver (Pillinger *et al.* 1965). The urine from the experiments reported in the present paper was examined by Barnsley (1968) and, though his findings differ in some respects from those of Pillinger *et al.* (1965), the overall conclusion is the same.

The correspondence between the time taken for the concentration of methyl methanesulphonate in the blood to fall to zero and the time at which Pillinger *et al.* (1965) found the concentration of metabolites in the bile to be at a maximum (both 1½ hr.) and between persistence for 16 hr. of the rapid exhalation of $^{14}CO_2$ and a high concentration of radioactivity in the urine (Pillinger *et al.* 1965) suggest that the methyl methanesulphonate is metabolized in 1½ hr., leaving a number of metabolites that are either excreted or further metabolized, and that the $^{14}CO_2$ exhalation represents the result of this secondary rather than the primary metabolism. These results (Table 1 and Fig. 5) differ in one respect from those of Pillinger *et al.* (1965), who detected only 5% of the total radioactive dose as $^{14}CO_2$. In the present experiments over 28% of the dose was recovered as $^{14}CO_2$. It is known that *S*-methylcysteine produced by the breakdown of *S*-methylglutathione can be metabolized to carbon dioxide (Horner & Kutchinskas, 1959), and methanol, which is probably also a major metabolite of methyl methanesulphonate, would be largely exhaled as carbon dioxide.

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