FATE OF METHYL METHACRYLATE IN RATS

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Summary.—Up to 88% of a single dose of methyl[14C]methacrylate in rats is expired as 14CO2 in 10 days (65% in 2 h), irrespective of the route of administration and of the specific labelling of the propylene residue of the molecule. The implications of this observation, and of the excretion of small amounts of [14C]methylmalonate, [14C]-succinate and probably of [14C]β-hydroxyisobutyrate and 2-formylpropionate, and of the formation of [14C] normal, physiological metabolites that may be accounted for by anabolism both from 14CO2 and from [14C]acetate emergent from the citric acid cycle, are that the metabolic pathway concerned involves intermediary metabolism and relates to mitochondrial function. Present findings are discussed in relation to the imputations of a report of carcinogenic risk.

Methyl methacrylate and its homologous esters seem to be quite innocuous substances. Thus, Deichmann (1941) and Spealman et al. (1945) found that, in small laboratory animals, these substances were less acutely toxic than ethyl acetate with an s.c. LD50 of 5000 mg/kg in rats, and that the most characteristic effects, caused by prolonged exposure to methyl methacrylate vapour, were degenerative changes of the liver. Chronic long-term toxicity testing of methyl methacrylate in dogs and rats by oral route (Borzelleca et al., 1964) failed to reveal an increased mortality amongst the animals, or any histological changes. The dogs were given gelatin capsules containing methyl methacrylate, in solution in corn oil, for a period of 2 years, in amounts equivalent to 10, 100 and 1000–1500 parts/106 in the diet, and the rats received concentrations of 6–7, 60–70 and 2000 parts/106 in their drinking water.

However, the present, widespread usage of methyl methacrylate polymer for acrylic sheet and moulding material, and a report (Singh, Lawrence and Autian, 1972) that its i.p. administration to female rats on Days 5, 10 and 15 of gestation caused haemangiomas in some of the pups, make a better understanding of the biological fate of this monomer in mammals desirable.

MATERIALS AND METHODS

Chemicals.—Methacrylic acid and succinic acid reference compounds were obtained from Koch-light Laboratories Limited, and were of Analar grade.

All reagents and solvents were of Analar grade or of the next highest quality available.

Methylmalonic acid, m.p. 135°C, was prepared by alkylation of diethyl cyanohydrin, and with 98% chemical and radiochemical purity exceeding 98%, was synthesized from [1,3-14C]acetone via the cyanohydrin, and methyl[2-14C]-propylene-2-carboxylate with sp. act. 0.398 mCi/mmol, and with a chemical and radiochemical purity exceeding 98%, was synthesized similarly from [2-14C]acetone, by our colleague Mr D. Greenslade of Imperial Chemical Industries Limited, Petrochemicals Division, Billingham, Cleveland. On account of the risk of polymerization, both radioactive chemicals were supplied in spectro-
scopig-grade ethanol solution, stabilized with < 50 parts/10⁶ of hydroquinone. These solutions were stored at −20°C.

Experiments in animals.—Adult male rats (about 2 months old, 200 g body wt) were used (Alderly Park strain (Wistar-derived), specific-pathogen-free), and kept on a standard pellet diet.

(a) For the purpose of establishing the excretion-retention pattern of ¹⁴C over a 10-day period, 3 rats were each administered intragastrically a 5-7 mg/kg body weight dose containing 4 µCi of methyl [1,3-¹⁴C]propylene-2-carboxylate in corn-oil solution. The animals were kept singly in glass metabolism cages (Jencons of Helens Hempstead, Herts.). Unrestricted food and water were supplied, and the urine and faeces were collected separately in the dark, and frozen at −20°C. The exhaled air was drawn through a gas train, comprising successively two Dreschel bottles, each of which contained 75 ml of ethanol at −70°C to remove unchanged methyl[¹⁴C]-methacrylate, and two CO₂ absorber Nilox columns (Jencons of Hemel Hempstead) each containing 500 ml of 2N NaOH. Products of excretion were collected for 10 days.

(b) Another 3 rats were each injected in a femoral vein with a 5-7 mg/kg body weight dose containing 4 µCi of methyl[1,3-¹⁴C]-propylene-2-carboxylate in ethanol solution (50 µl), and the excretion-retention pattern of ¹⁴C was investigated over a similar period.

(c) Three animals were each injected in a femoral vein with a 6.8 mg/kg body weight dose containing 4 µCi of methyl[2-¹⁴C]-propylene-2-carboxylate in ethanol solution (50 µl), and the excretion-retention pattern of ¹⁴C was studied over a 10-day period.

(d) Two rats were each given a single intragastric dose of methyl [2-¹⁴C]propylene-2-carboxylate (120 mg/kg; 84 µCi) as a corn-oil solution, and the excretion-retention pattern of ¹⁴C was again studied over a 10-day period. More than 90% of the urinary ¹⁴C was excreted during 48 h, and this urine was used for [¹⁴C]metabolite identification.

Measurement of radioactivity.—An automated and computerized Intertechnique Model SL30 Liquid Scintillation Spectrometer was used for measurement of ¹⁴C, making use of standard channels-ratio quench-correction curves. Liquid samples were admixed with standard scintillator and radio-assayed direct, and samples of faeces were burnt in an Intertechnique "Oxymat", solid-sample oxidizer.

Characterization of unchanged methyl methacrylate in the expired air.—Ethanol from the cold traps was slowly evaporated under N₂ < 15°C, and samples of the concentrate were examined with a Pye model 104 gas chromatograph that was equipped with flame-ionization detection and coupled to an E.S.I. Nuclear 504 Radiogas detector. The column effluent was split in the ratio of 9 : 1 between the Radiogas detector and the flame-ionization detector. This gas chromatograph was fitted with glass columns (1.5 m long × 4 mm internal diameter), which were packed with 10% (w/w) of squalene on Chromosorb P (80–100 mesh size) and which were run at 75°C. All the columns were operated at a 30 ml/min flow-rate of a (95 : 5, v/v) Air–CO₂ mixture. Under these operating conditions, methyl methacrylate has a retention time of 5-8 min.

Systematic separation of the urinary metabolites into fractions of chemically similar substances.—A 50-ml sample of the combined urine from the 2 rats [(d) above] was evaporated to dryness under reduced pressure, at < 35°C. A solution of the residue in 5 ml of 0.1N KOH was percolated (20 drops/min) through a column (bed volume, 100 ml) of Amberlite IRA410 anion-exchange resin (14–52 mesh size, 1-40 mg-equiv/ml) in the CH₂CO₂⁻ cycle, and the column was then washed (60 ml/h) with 250 ml of de-ionized water. The total eluate, which contained 54% of the urinary ¹⁴C, was retained. 500 µl of 3N acetic acid, when percolated through the column, stripped the remaining (46%) urinary ¹⁴C from the anion-exchange resin.

Thin-layer chromatography.—A methanol solution of the evaporate from the bulked aqueous washings was spotted on SiO₂-gel GF thin-layer plates, which were run with butan-1-ol : acetic acid : water (4 : 1 : 1, v/v). A major [¹⁴C] spot with an R₂ value of 0.27 was identified by chromatography and co-chromatography with reference material, as [¹⁴C] urea and gave a characteristic yellow colour with Ehrlich's reagent. (Identification was confirmed by reverse isotope-dilution analysis of [¹⁴C]urea nitrate in an aliquot portion of the methanol solution of the evaporate from the bulked aqueous washings). A minor [¹⁴C] spot with an R₂ value of 0.14 on the developed plate was unidentified, but the presence of H¹⁴CO₃⁻ was established in further aliquot portions of the acidified urine in Conway-diffusion units.

Radioactivity in the 3N acetic acid fraction
was not retained by percolation through a column (bed-volume, 50 ml) of Dowex 50W-X8 cation-exchange resin in the H⁺ cycle. Accordingly, a methanol solution of the evaporate from the bulked effluent was spotted on SiO₂-gel GF thin-layer plates, which were run with the same solvent system. Chromatographic characteristics of the resulting [¹⁴C] acidic metabolites of methyl [¹⁴C]-methacrylate were exemplified in Table II. Radioactivity corresponding to each metabolite was measured in the eluate from the separate spots.

Gas chromatography.—Gas chromatography of the methyl esters of the foregoing [¹⁴C] acidic metabolites was undertaken on the previously described gas chromatograph that was equipped with flame-ionization detection and coupled to an E.S.I. Nuclear 504 Radio-gas detector. In this case, the gas chromatograph was fitted with glass columns (2 m long × 4 mm internal diameter), which were packed with 6% (w/w) of OV-101 on Supelcoport (80–100 mesh size) and which were run at 100°C. All the columns were operated at a 30 ml/min flow rate of a (95 : 5, v/v) Air-CO₂ mixture. Retention times of the principal methyl esters are shown in Table II.

Analysis of triglyceride fat and cholesterol esters.—A sample of lipid was saponified with alcoholic KOH (1 ml of 33% aq. KOH + 9 ml of ethanol/g of lipid), under N₂ in the dark in a sealed vessel, on a water-bath at 55°C for 30 min, when the cooled solution was extracted with ether to remove cholesterol and glycerol. The soaps were converted into free fatty acids by acidification, and the acid solution was re-extracted with n-hexane. The two solvent extracts were evaporated under reduced pressure, and the residues were dissolved in small volumes of n-hexane. Extracts were spotted separately on SiO₂-gel GF thin-layer plates, which were run either with n-hexane : diethyl ether : glacial acetic acid (90 : 20 : 1, v/v) or with chloroform : acetone : 5% NH₃ (10 : 80 : 10, v/v).

RESULTS AND DISCUSSION

A high proportion of a dose of methyl methacrylate is fully oxidized in rats, and in an early investigation (Deichmann, 1941) no urinary metabolites were found. Thus, 84–88% of a single dose (5.7 mg kg) of methyl[1,3-¹⁴C]propylene-2-carboxylate was expired as ¹⁴CO₂ in 10 days, whether by parenteral or enteral administration (Table I), and up to 65% as ¹⁴CO₂ in 2 h. About half the remainder of the dose was excreted in the urine and the rest was retained by the body tissues at 240 h. Pulmonary excretion of unchanged methyl methacrylate accounted for less than 1.0% of the dose. The same pattern of excretion was observed when animals were dosed another labelled form of methyl methacrylate, viz. methyl[2-¹⁴C]propylene-2-carboxylate (Table I). Within the limitations of the experiment, the rate of the initial phase of pulmonary excretion of ¹⁴CO₂ after an i.v. injection of methyl[1,3-

| Form of ¹⁴C label | Route of administration | Dose (mg/kg) | Urine | Faeces | ¹⁴CO₂ | Unchanged methyl[¹⁴C] methacrylate | Carcass plus skin | Total |
|-------------------|------------------------|-------------|-------|--------|--------|----------------------------------|------------------|------|
| Methyl[1,3-¹⁴C]-propylene-2-carboxylate | By stomach tube | 5.7 | 4.7 | 2.7 | 88.0 | 0.1 | 4.1 | 99.6 |
| | i.v. | 5.7 | 6.6 | 1.7 | 84.0 | 0.7 | 6.6 | 99.6 |
| Methyl[2-¹⁴C]-propylene-2-carboxylate | i.v. | 6.8 | 7.2 | 1.8 | 84.1 | 1.0 | 6.6 | 100.7 |
| | By stomach tube | 120.0 | 6.0 | 3.0 | 76.4 | 1.4 | — | — |

* 10 days after administration.

† The data displayed are representative of that obtained in several animals.

‡ Unmeasured.
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100
75
50
25
0

Time (h)

Fig. 1.—Cumulative plots for the initial phase of pulmonary excretion of \(^{14}\)CO\(_2\) in representative rats that had been dosed different radioactively labelled forms of methyl methacrylate. Each animal was given a single injection of either—methyl[1,3-\(^{14}\)C]propylene-2-carboxylate (5.7 mg/kg) ○ or methyl[2-\(^{14}\)C]propylene-2-carboxylate (6.8 mg/kg) □ in a femoral vein at zero time (for details, see Methods section).

\(^{14}\)C]propylene-2-carboxylate did not differ significantly from that after i.v. injection of methyl[2-\(^{14}\)C]propylene-2-carboxylate (Fig. 1) and half the dose is expired as \(^{14}\)CO\(_2\) in 90 min. It is reasonable to suppose that on the basis of this experimental evidence, all three propylene C atoms of methacrylate were being metabolized \textit{in vivo} by the same sequence of biotransformations. Moreover, the pattern of excretion is not altered by a much larger intragastric dose (120 mg/kg) of methyl[2-\(^{14}\)C]methacrylate (Table I) but in this case the compound was metabolized more slowly, and pulmonary excretion of unchanged methyl methacrylate accounted for 1.4\% of the dose, compared with 0.1\% for the smaller dose (5.7 mg/kg).

Hence there is a strong supposition that the biological fate of methyl[\(^{14}\)C]methacrylate in mammals implicates the pathways and cycles of intermediary metabolism. The scheme that is suggested for methacrylate degradation in Fig. 2 represents the preferred pathway, in which the branched-chain methylmalonyl CoA, which is known to be formed in valine catabolism, is converted by methylmalonyl mutase into succinyl CoA. Hence, by that means all 4 C atoms belonging to methacrylate would enter the citric acid cycle simultaneously and be oxidized into CO\(_2\), but some anabolism of acetate into normal, physiological metabolites might be expected.

The fact that \textit{in vivo} the ready esterification of methacrylate by CoA directs the entry of oxygen to the carbon atom, which is in \(\beta\)-position to the carboxyl function, makes less likely the possibility of \(\alpha\)-hydroxylation, which applies to the case of acrylonitrile, and of \(\alpha,\beta\)-dihydroxylation, which has been proposed (Pantůček, 1969) for methacrylate degradation. Whilst an \(\alpha,\beta\)-dihydroxy compound is formed in the biosynthesis of valine from pyruvate, the next reaction step in this case is dehydration, and not the \(\beta\)-deformylation suggested in Pantůček’s (1969) scheme for methacrylate. In fact, Pantůček’s (1969) arsenite-inhibited \textit{in vitro} preparation may be somewhat unrealistic, and addition of small amounts of fumarate and malate to that preparation does not stimulate the citric acid cycle in
the Krebs sense. Had the scheme leading to pyruvate production applied to $^{14}$C-methacrylate degradation in rats, then small amounts of $^{14}$C-lactic acid might have been expected to have been excreted in the urine, but this was not the case.

The rest of the evidence is consistent with the scheme (Fig. 2) for the degradation of methacrylate in vivo. Thus, after administration of methyl[2-$^{14}$C]propylene-2-carboxylate, that fraction of the urine which comprises the $^{14}$C acidic metabolites (Table II) was found to contain, in addition to $^{14}$C methacrylic acid (0.8% of the dose), 1.4% of $^{14}$C methylmalonic acid ($^{14}$C isosuccinic acid), which arises by hydrolysis of methylmalonyl CoA (Fig. 2), 0.2% of $^{14}$C succinic acid from the hydrolysis of succinyl CoA (Fig. 2), and two other minor constituents, which occupy the same part of the chromatogram (Table II) as $\beta$-hydroxyisobutyric acid and formyl-2-propionic acid (methylmalonic semialdehyde) (Fig. 2). The $^{14}$CO$_2$ would appear to be the source of substantial amounts (> 2·0% of the dose) of $^{14}$Curea in the neutral fraction of the urine, as well as of a small quantity of H$^{14}$CO$_3$-. At 10 days, $^{14}$C is retained in the body only by the adipose and liver tissues, and in those biological situations $^{14}$C is associated both with the corresponding hydrolysis products and with the unsaponifiables. This evidence suggests that, in such an experi-

![Diagram of the degradation of methacrylate in mammals.](image)

**Fig. 2.** Scheme for the degradation of methacrylate in mammals.

| Acids | R$_F$ values on SiO$_2$-gel | GC-retention times of methyl esters (min) | Relative proportions of $^{14}$C metabolites (% of dose) |
|-------|-----------------------------|------------------------------------------|-----------------------------------------------------|
|       | plates developed with butan-1-ol:acetic acid:water | Column coated with OV-101 and run at 100°C |                                                      |
|       | (4 : 1 : 1, v/v) |                                         |                                                      |
| Methacrylic acid | *0·10 | 2·0 | 0·8 |
| Unidentified acid | 0·21 | | 0·4 |
| $\beta$-Hydroxyisobutyric acid | 0·39 | 5·1 | 1·4 |
| Methylmalonic acid (isosuccinic acid) | 0·43 | | |
| Methylmalonic semialdehyde | 0·50 | 7·4 | 0·1 |
| Succinic acid | 0·53 | | 0·2 |

* Characteristically shaped, diffuse spot.
ment, some of the $^{14}$Cacetate generated has been elaborated into triglyceride fat and cholesterol esters, and in fact materials consistent with $^{14}$C free fatty acids have been detected in alkaline hydrolysates of depot fat.

Thus, the rapid oxidation of $^{14}$C-methacrylate into $^{14}$CO$_2$, which has been encountered in the present investigation, seems to be consistent with the suggested scheme (Fig. 2). That the generation of $^{14}$CO$_2$ was incomplete is due to body compartmentalization, whereby a small amount of pulmonary excretion of the unchanged ester occurred, four $^{14}$Cmetabolic intermediates were excreted in the urine, and normal, physiological metabolites were anabolized both from $^{14}$CO$_2$ and from $^{14}$Cacetate emerging from the citric acid cycle. On the assumption that the metabolic route (Fig. 2) does in fact apply in the way which has been described, it follows that exogenous methacrylate from the large amounts of administered methyl methacrylate is metabolized by the body in exactly the same way as the small amounts of endogenous methacrylate that are formed in the course of valine catabolism. This pathway is concerned with intermediary metabolism and relates to mitochondrial function.

Hence present evidence implies, but does not prove, that it is rather unlikely that methyl methacrylate metabolism would yield damaging reactive metabolites, since an acceptable pathway has been proposed which is independent of the microsomal and cytoplasmic enzymes that are usually concerned with foreign compound metabolism. The report of Singh et al. (1972) is accordingly difficult to reconcile and, in practice, i.p. injection would never be selected as the route for the testing of embryonal–foetal toxicity and teratogenicity per se (Hathway, 1975), since a compound under examination might be absorbed from the peritoneum into the foetuses direct, and not via the placenta.

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REFERENCES

BOUZELLECA, J. F., LARSON, P. S., HENNINGAR, G. R., CRAWFORD, E. M. & SMITH, E. B. (1964) Studies on the Chronic Toxicity of Monomeric Ethyl Acrylate and Methyl Methacrylate. Toxicol. appl. Pharmacol., 6, 29.

DREICHMANN, W. (1941) Toxicity of Methyl, Ethyl and n-Butyl Methacrylate. J. ind. Hyg. Toxicol., 23, 343.

HATHWAY, D. E. (1975) Embryonal and Neonatal Pharmacology. In Foreign Compound Metabolism in Mammals. Ed. D. E. Hathway. London: Chemical Society, 3, 658.

KUPIECKI, F. P. & COON, M. J. (1960) Methyl-malonie Semialdehyde. Biochem. Prep., 7, 69.

PANTČEK, M. (1969) On the Metabolic Pathway of Methyl Methacrylate. FEBS Lett., 2, 206.

SINGH, A. R., LAWRENCE, W. H. & AUTIAN, J. (1972) Embryonic–foetal Toxicity and Teratogenic Effects of a Group of Methacrylate Esters in Rats. J. Dent. Res., 51, 1632.

SPEALMAN, C. R., MAIN, R. J., HAAG, H. B. & LARSON, P. S. (1945) Monomeric Methyl Methacrylate Studies on Toxicity. Ind. Med., 14, 292.