Alkylating Esters VII. The Metabolism of Iso-Propyl Methanesulphonate and Iso-Propyl Iodide in the Rat

The structural similarity between dimethylmyleran (I) and *iso*-propyl methanesulphonate (IMS, II, which has been termed[1] 'Half-dimethylmyleran'), has led to the assumption[2] that they share the same in vivo mechanism of action, one of unimolecular aklylation, which accounts for their similar biological effects on spermatogenesis[3] and the haemopoietic system[1]. In assessing the action of alkylating agents on the haemopoietic system, Elson introduced[4] a classification based on the growth curves of rats treated with lethal doses. Compounds acting by a unimolecular mechanism cause rapid death during the single initial weight loss phase, and those acting by a bimolecular process result in delayed death during the second period of weight loss. In our studies with methanesulphonate esters, we examined the weight responses of rats to both dimethylmyleran and IMS, and the results (Figure 1) suggest that, by this criterion, the mechanisms of action of these 2 compounds might be different. Dimethylmyleran has subsequently been shown to react in vivo by a bimolecular process[5], though the in vivo mode of action of IMS remains uncertain.

A comparison betwen the metabolism of IMS and *iso*-propyl iodide (III), which is known to react by a bimolecular process[6], reveals differences in their in vivo reactivities. I.p. administration of ^{14}C-IMS (75 mg/kg) to rats produced 2 urinary metabolites, *S-iso*-propyl cysteine (V, R=H) and the corresponding mercapturic acid, *S-iso*-propyl cysteine-*N*-acetate (V, R=$COCH_3$) amounting to 30% of the administered dose. This contrasts with the metabolism of ^{14}C-*iso*-propyl iodide (88 mg/kg, i.p.), in which only trace amounts (2–3% of the dose) of the cysteine conjugate (V, R=H) were detected in the urine, and *iso*-propyl bromide, which is reported[7] to give rise to no detectable sulphur-containing meta- bolites.

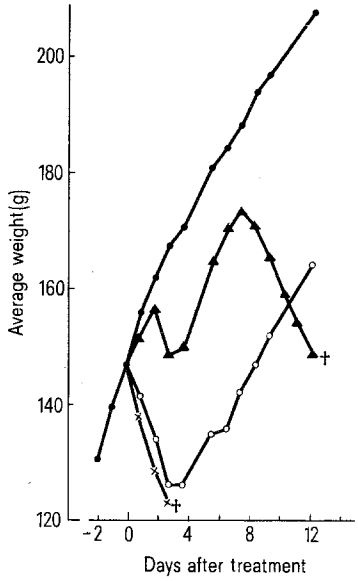
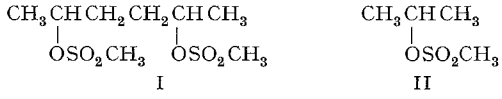


Fig. 1. Weight responses of immature rats to *iso*-propyl methanesulphonate (IMS) and dimethylmyleran. Single i.p. doses of the compounds in arachis oil were given on day 0. ▲—▲, dimethylmyleran 1×6 mg/kg; ○—○, IMS 1×200 mg/kg; X—X, IMS 1×500 mg/kg; ●—●, controls; †, death.



Excretion of radioactivity in the urine and expired gases over 24 h from rats receiving equi-molar doses of compounds

Compound	Dose (mg/kg)	Urine	Carbon dioxide
^{14}C-*iso*-propyl methanesulphonate	75	38	20
^{35}S-*iso*-propyl methanesulphonate	75	100	—
^{35}S-methanesulphonic acid	50	100	—
^{14}C-*iso*-propyl iodide	88	10	3
^{14}C-*iso*-propyl alcohol	32	10	5

Activity, expressed as percent of the administered dose, was assessed as previously described[15]. ^{14}C-*iso*-propyl iodide and ^{14}C-*iso*-propyl alcohol were obtained from the Radiochemical Centre, Amersham. ^{14}C-IMS was prepared from ^{14}C-*iso*-propyl alcohol and methanesulphonyl chloride in pyridine at −5° and ^{35}S-IMS from *iso*-propyl alcohol and silver ^{35}S-methanesulphonate (Radiochemical Centre, Amersham) by the method of EMMONS and FERRIS[16]. ^{35}S-methanesulphonic acid was obtained by base hydrolysis of ^{35}S-IMS. Compounds were administered i.p. as suspensions in arachis oil, except for *iso*-propyl alcohol and methanesulphonic acid, which were given in aqueous solution.

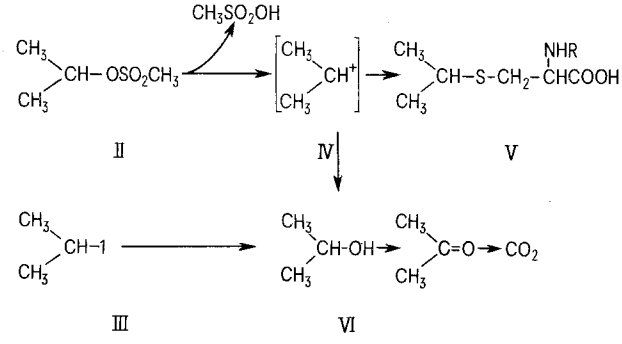


Fig. 2. Major metabolic pathways of IMS and *iso*-propyl iodide in the rat. The cysteine conjugates were isolated from urine as previously described[13] and their chromatographic mobilities compared with authentic compounds[7]. On silica gel G plates (0.1 mm) in butanol glacial acetic acid water (4:2:1), the Rf values were 0.63 for (V) R=H and 0.86 for (V) R=$COCH_3$. The metabolites were interconverted either by acetylation (acetic anhydride) or hydrolysis (5 *N* HCl at 95 °C for 1 h, or acylase at pH 7.4 and 37 °C for 3 h), as well as being oxidised to *S-iso*-propyl-cysteine-*S*-oxide (Rf 0.42). Methanesulphonic acid (Rf 0.27) was identified by gas-liquid chromatography as the methyl ester[14].

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Apart from detoxification by the alkylation of thiol groups, some degree of hydrolysis must occur, since neither IMS nor *iso*-propyl iodide are excreted unchanged, and the excretion of methanesulphonic acid from ^{35}S-IMS parallels that of ^{35}S-methanesulphonic acid itself (Table). The hydrolysis product, *iso*-propyl alcohol (VI), and its metabolite[8] acetone, were not detected from these compounds but as both *iso*-propyl alcohol and acetone are partially oxidised in vivo[9], it is probable that the expired ^{14}C-carbon dioxide (Table) represents this hydrolytic pathway.

As the excretion patterns of *iso*-propyl iodide and *iso*-propyl alcohol are almost identical, hydrolysis of the former probably represents the major detoxification route, alkylation reactions such as conjugation with cysteine (glutathione) representing only a minor pathway. The different pattern of excretion of radioactivity from IMS indicates that although in vivo hydrolysis is rapid (half-life at 37 °C is 13 min at pH 7)[1], the alkylation reaction is a major pathway (Figure 2). This can be interpreted as reaction of the compounds by 2 different mechanisms; bimolecular for *iso*-propyl iodide (and presumably the bromide) and unimolecular for IMS. The production of the highly reactive dimethylcarbonium ion (IV) from IMS by a unimolecular reaction is consistent with a rapid degree of alkylation both in the detoxification route and in the reaction of IMS with DNA in vitro[10].

Whereas the different biological actions of alkylating agents have been attributed to their mechanisms of alkylation[11], present and recent[12] studies indicate that, at least for methanesulphonate esters, this may not be true. IMS and dimethylmyleran react by different mechanisms yet possess similar biological activities suggesting that in some instances, the mechanism of alkylation may not be an important factor in their mode of action.

Zusammenfassung. Nachweis, dass die im Rattenharn auftretenden Metaboliten Isopropyljodid und Isopropylmethansulfonat für einen unterschiedlichen Wirkungsmechanismus der Substanzen in vivo sprechen. Aus Isopropylmethansulfonat entsteht durch einen monomolekularen Prozess das äusserst reactive Dimethylcarboniumion, während Isopropyljodid aufgrund einer bimolekularen Reaktion haupsächlich durch Hydrolyse entgiftet wird.

A. R. Jones and K. Edwards

Pharmacology Department, The University, Manchester M13 9PT., and Paterson Laboratories, Christie Hospital, Manchester M20 9BX. (England),
3 November 1972.

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Acceleration of Red Cell Glycolysis by Citrate due to Intracellular pH Enhancement

Since the introduction of sodium citrate for blood preservation[1], it has been used as an important ingredient for blood preservation media: acid dextrose citrate (ACD) and citrate phosphate dextrose (CPD) solutions. Citrate has been added to preservation media as an anticoagulant and the effect of the anion on red cells has not been thoroughly studied. Although citrate anion is known to be practically impermeable to red cell membrane[2], its high concentration is expected to exert some influence on red cell metabolism. Recently we have found that the intracellular pH (pH_i) of red blood cells stored in ACD medium is higher than the extracellular pH (pH_e) of the suspension[3]. This finding urged us to study the glycolysis of red cells in the presence of citrate.

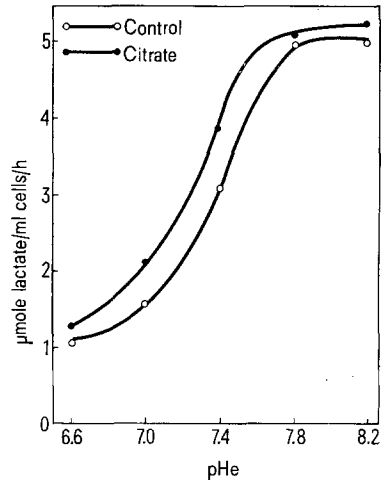

Fig. 1. The pH-curves of lactate formation in red cells in presence and absence of 33 m*M* citrate.

Methods. One-day-old ACD blood was obtained from a local blood bank and red cells were washed thoroughly with isotonic saline. The cells were suspended in a solution (120 m*M* NaCl, 5 m*M* KCl, 1 m*M* $MgCl_2$, 1 m*M* inorganic phosphate and 10 m*M* glucose) and incubated at 37°C. During incubation, the pH was kept constant by a pH-stat with the addition of 0.2 *M* NaOH. After 2 h preincubation, sodium citrate solution was added to a final concentration of 33 m*M* and incubated for further 2 h. Samples were taken out at intervals for analyses.

Results and discussion. More than 20% increase of the lactate formation was observed when citrate was added to the cell suspension at pH 7.4. The increase by the citrate addition depended on the pH of the suspension as shown in Figure 1. Shift of the pH curve was observed by the addition of citrate, which suggests the increase of the intracellular pH. The possibility of pH increase inside the cells was further supported by the changes of the glycolytic intermediates. Hexose monophosphates decreased

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