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SHORT COMMUNICATIONS

Metabolism of compounds related to ethyl mercaptan

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THE striking *in vivo* antitubercular action of ethyl mercaptan and compounds closely related to it has been established for some years (Davies *et al.*,[1] Brown *et al.*[2]). Recently, Davey and Hogerzeil[3] have reported the success of Etisul (diethyl dithiol*iso*phthalate), an ethyl mercaptan derivative, in the treatment of leprosy. Perhaps the most intriguing facets of these observations are (a) the activity is confined to the ethyl mercaptan series, it is not shown by homologous thiols, (b) the antitubercular activity *in vitro* is small and no greater than that of other homologous thiols (Davies *et al.*[1]). This latter fact suggests that the antitubercular action might not be due to ethyl mercaptan *per se* but to some metabolite.

The distribution, excretion and metabolism of ^{35}S-ethyl mercaptan derivatives has been investigated by Snow[4] who showed that following oral dosing of either ethyl thiolbenzoate or diethyl disulphide to guinea-pigs the radioactivity is excreted mainly in the urine and approximately 50 per cent of the administered ^{35}S appeared in the urine as sulphate. Two organic metabolites were also detected, one was ethyl methyl sulphone, the other was not identified. However, neither ethyl methyl sulphone nor a crude mixture containing both metabolites showed any antitubercular activity when tested *in vivo*. The main object of the present work was to study the fate of the carbon moiety of ethyl mercaptan.

^{14}C-Ethyl iodide was converted to the 3:4:5-trichlorobenzoic acid salt of ethyl*iso*thiourea (activity 225 μc/g. salt) from which ^{14}C-ethyl mercaptan was generated by treatment with alkali. The resulting ethyl mercaptan was either oxidized with solid I_2 in alkaline solution to give ^{14}C-diethyl disulphide or benzoylated with benzoyl chloride to give ^{14}C-ethyl thiolbenzoate. Both compounds were purified by distillation. Compounds were dissolved in arachis oil and dosed orally to guinea-pigs through a stomach tube.

For collection of CO_2 and ethyl mercaptan, animals were housed in a metabolism jar of the type described by Berlin *et al.*[5] CO_2-free air was drawn through the apparatus; respiratory ethyl mercaptan was trapped as the $HgCl_2$ complex and CO_2 as K_2CO_3. To minimize losses due to volatilization all tissues were placed in solid CO_2 immediately they were removed from the animal. A small portion of the tissue was then oxidized to CO_2 in a modified van-Slyke type apparatus (Calvin *et al.*[6]) The modification consisted of an extra limb fused on to the oxidation tube at right angles. The CO_2 was collected in N NaOH, precipitated as $BaCO_3$, filtered and counted as such. In most cases, counts were continued for sufficient time to give a reliable error of 2·3 per cent. All counts were corrected to infinite thickness. For absolute determination of ^{14}C a polymer reference source (1 μc/g) was used.

Guinea pigs were killed 48 hr after dosing with ^{14}C-diethyl disulphide and their respiratory gases, urine, faeces and various tissues analysed for ^{14}C. Table 1 shows typical results; it can be seen that approximately 85 per cent of the ^{14}C was recovered, 50 per cent of the administered ^{14}C appeared in the breath as CO_2 and from 3–7 per cent as ethyl mercaptan. The urine contained from 15–25 per cent; the remaining activity was spread throughout the tissues with the liver containing the largest amount.

The nature of the radioactivity in the urine was next studied. No loss of activity occurred when urine was passed through Deacidite FF (OH) but a slight loss was noted after passing through Zeo-Karb 225 (H) indicating the presence of some ^{14}C-cations. After deionisation, samples of urine were chromatographed on paper (ascending) using various solvent systems. The best separations achieved were obtained using n-propanol, n-butanol, water (280 ml, 470 ml, 250 ml). This established that the urine contained two metabolites with R_F 0·40 and 0·75. Snow[4] had previously shown that ethyl methyl sulphone had an R_F 0·6 in aqueous butanol/propanol. It seemed most likely that the spot with R_F 0·75 might also be due to the substance. Since 50 per cent of the activity given to the animal appeared in the breath as CO_2, incorporation of CO_2 into urea was anticipated. Comparison of the chromatograms of urine and urine treated with urease and deionized showed that the latter treatment

removed material with R_F 0·40. Estimation of the ^{14}C in the urea was made, this revealed that 1 per cent of the ^{14}C was incorporated.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY FOLLOWING ORAL DOSING OF ^{14}C-DIETHYL DISULPHIDE TO GUINEA-PIGS

	Experiment 1	*Experiment* 2
Weight of animal	360 g	480 g
Dose of diethyl disulphide	17 mg (7·83 μc)	51 mg (23·5 μc)
Recovery of ^{14}C		
(a) Respiratory CO_2	3·91 μc (50 per cent)	12·81 μc (54·5 per cent)
(b) Breath as EtSH	0·59 μc (7·5 per cent)	0·64 μc (2·7 per cent)
(c) Urine	1·24 μc (15·8 per cent)	5·92 μc (25·2 per cent)
(d) Faeces	0·19 μc (2·5 per cent)	*
(e) Liver	0·16 μc (2·1 per cent)	*
(f) Other tissues (Blood, Spleen, Heart, Lung, Kidney, Stomach, Intestine)	0·53 μc (6·7 per cent)	*
Total Recovery	6·62 μc (84·6 per cent)	19·37 μc (82·4 per cent)

* not measured.

The nature of the spot with R_F 0·75 was next investigated. Urine was collected from guinea pigs dosed orally with ^{14}C-ethyl thiolbenzoate. In all, 243 mg of material (=145 μc ^{14}C) was administered and 470 ml urine collected. Paper chromatography of this urine again revealed two spots with R_F 0·40 and 0·75. Urea and electrolytes were removed from the urine which was then concentrated by distillation at low temperature (<30°) and under reduced pressure. The resulting urine concentrate was extracted with $CHCl_3$ and this extract concentrated to 40 ml. This solution was distributed between $CHCl_3$–H_2O in a 50-tube Craig machine. The contents of each tube were concentrated under reduced pressure and low temperature (<30°) and made up to 2·5 ml for counting. Using an analytical procedure developed by Levi[7] it was shown that the distribution of radioactivity could be represented by the summation of theoretical curves for three substances having K ($CHCl_3$–H_2O) 0·69 (1 part), 2·6 (0·65 part) and 4·9 (0·1 part) (Fig. 1). The difference between the height of the experimental curve in tubes 20 and 21 and the theoretical curve is not fully understood but could be due to the volatility of ethyl methyl sulphone; greater percentage loss occurring in the tubes containing the smaller amounts of this substance. The distribution of ethyl methyl sulphone between $CHCl_3$ and H_2O had previously been shown by Snow[4] to be 0·69. K ($CHCl_3$–H_2O) for ethyl methyl sulphoxide was measured and gave a value of 4·9. Clearly these compounds account for two of the metabolites found in urine. The partition coefficients ($CHCl_3$–H_2O) of β-hydroxyethyl methyl sulphone and β-hydroxy ethyl methyl sulphide were measured: these were found to be 14 (approx.) and 0·6 respectively, thus ruling out the possibility of either of these substances being the third urinary metabolite. The nature of this metabolite is still unknown. Snow[4] found a substance with K($CHCl_3$–H_2O) 2·7 in guinea-pig urine after the animals had been dosed with either ^{35}S ethyl thiolbenzoate or diethyl disulphide. The partition coefficient found in the present work (K = 2·6) is in good agreement with Snow's figure. Moreover, the fact that both substances are neutral, extracted by the same procedure and only appear in the urine after dosing either ethyl thiolbenzoate or diethyl disulphide leaves little doubt that these substances are identical.

These results are in close agreement with those of Snow.[4] There is complete absence of any evidence in the present work suggesting a direct metabolite from the carbon moiety differing from those in which the C–S link is intact. Removal of hydrogen sulphide by desulphydrase action leaving a two-carbon residue seems a possible metabolic path for ethyl mercaptan. Subsequent oxidation of the hydrogen sulphide and metabolism of the two-carbon unit would account for the equivalent production of CO_2 and SO_4^{2-}. The desulphydrases appear to be of general occurrence among higher animals (Fromageot[8]) and the oxidation of hydrogen sulphide to sulphate has been demonstrated to occur

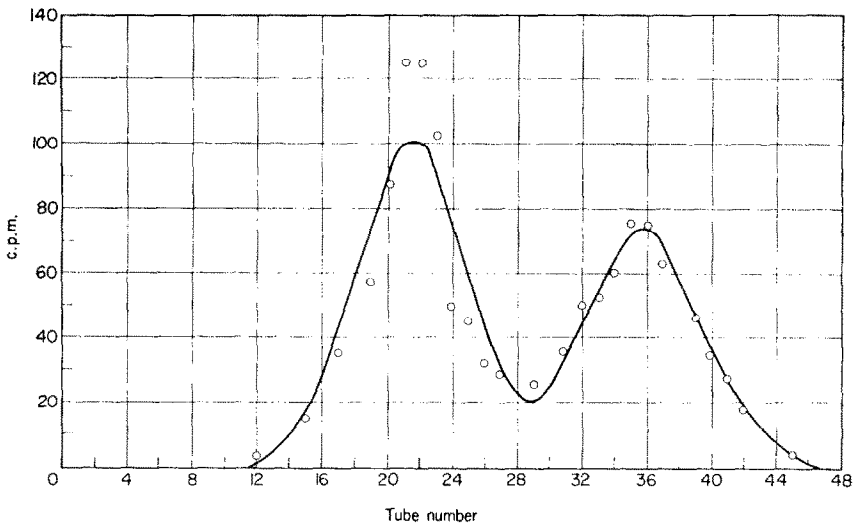

FIG. 1. Counter-current separation of ^{14}C-material. 49 Transfers between $CHCl_3$-H_2O. Points represent determined radioactivity in individual tubes. Curve is theoretical for mixture of three solutes. $K = 0{\cdot}69$ (1 part); $K = 2{\cdot}6$ (0·65 parts); $K = 4{\cdot}9$ (0·1 parts).

in the dog (Dennis and Reed[9]) and rat (Dziewiatkowski[10]). This proposed metabolic route agrees with the findings of Canellakis and Tarver[11] who studied the metabolism of methyl mercaptan in rats. They showed that the sulphur appeared as urinary sulphate while the carbon atom was incorporated into the methyl groups of choline and methionine and into the β-carbon of serine. Failure to find evidence of acidic urinary metabolites suggests that direct oxidation to sulphate via ethane sulphinic and sulphonic acids is not a likely possibility. Snow[4] has previously suggested that the sulphone may arise by methylation of ethyl mercaptan to ethyl methyl sulphide and subsequent oxidation to the sulphone. The finding of small amounts of urinary ethyl methyl sulphoxide agrees with this scheme.

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