## The Comparative Metabolism of Ethylene Dimethanesulphonate and Ethylene Dibromide[1]

Ethylene dimethanesulphonate (EDS, I R = $OSO_2CH_3$) possesses anti-spermatogenic activity[2] and inhibits the growth of implanted tumours in rodents[3]. Preliminary studies with $^{35}$S-EDS indicated that metabolism occurred, at a rate appearing to be species dependant, giving methane sulphonic acid as the only radioactive urinary metabolite.

Chromatography of rat urine from oral administration of 1,2-$^{14}$C-EDS (100 mg/kg) separated 2 radioactive metabolites which were identified by reference to authentic samples as N-acetyl-S-($\beta$-hydroxyethyl)-cysteine ($\beta$-hydroxyethyl-mercapturic acid, V) and N-acetyl-S-($\beta$-hydroxyethyl)-cysteine-S-oxide (VI). Detoxification of EDS by mercapturic acid conjugation is analogous to that of ethylene dibromide (EDB, I R = Br) which NACHTOMI et al. have shown[4] to be metabolized by the rat to S-($\beta$-hydroxyethyl)-cysteine (III), its N-acetate (V) and an unidentified metabolite. As the metabolic pathway of EDS and EDB appeared similar, we have re-investigated the metabolism of 1,2-$^{14}$C-EDB and confirmed that both compounds are detoxified by similar mechanisms.

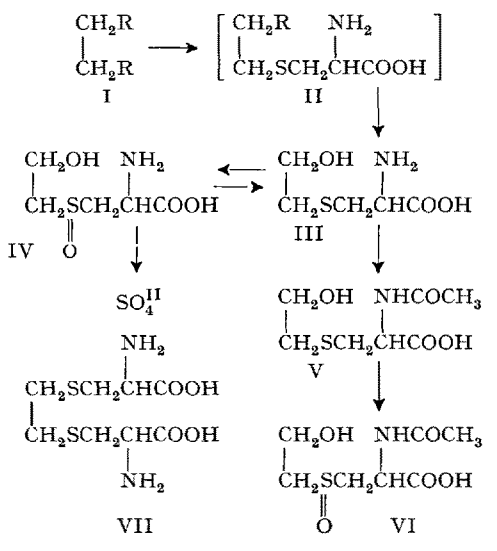
Ethylene dibromide is not excreted by the rat following a sub-lethal oral dose of 100 mg/kg whereas a similar oral dose of EDS gave 45% unchanged compound in the urine over 24 h. This difference in metabolic rate probably explains the observed metabolic patterns for each compound. Due to the in vivo stability of EDS (half-life of 12 days in both plasma and physiological saline at 37 °), slow production of III enables its conversion to V and VI. Rapid production of III by EDB results in excretion of III with an alternative pathway operating via S-($\beta$-hydroxyethyl)-cysteine-S-oxide (IV) to produce inorganic sulphate. Both pathways from III (assumed to proceed via II) are operative from EDB and as well as isolating IV as a further metabolite, we have identified VI as NACHTOMI et al.'s unidentified metabolite[4]. Compounds III, IV and V have been synthesized from $^{35}$S-cysteine and the sequence shown in the diagram confirmed by identification of their respective metabolites[5]. Biological oxidation of III to IV is of interest in that apart from being a reversible step, it appears to occur only when III is produced in large amounts indicating that the acetylation stage may be rate limiting.

WILLIAMS et al. report[8] ethylene glycol to be excreted unchanged by the rat after administration of low doses. However isotope dilution and formation of its inactive *bis*-phenylurethane derivative showed that it was not a urinary metabolite of either EDS or EDB and that hydrolysis of either compound does not occur in vivo to any appreciable extent. The isolation of radioactive carbon dioxide in 5 and 20% of administered doses of EDS and EDB respectively, suggested a more drastic degree of metabolism of both compounds. We have confirmed that 1,2-$^{14}$C-ethylene glycol (50 mg/kg) was not degraded to carbon dioxide[8] but the amounts produced by both compounds were in agreement with it being formed from their metabolites III and IV. This has been shown to be the case as III and IV, labelled with $^{14}$C in the $\beta$-hydroxyethyl moiety, gave carbon dioxide in yields of 25 and 16%. This is analogous to the fate of the methyl group of S-methyl-cysteine which HORNER and KUCHINSKAS[9] have shown to be extensively oxidized to carbon dioxide.

Although S,S'-ethylene-*bis*-cysteine (VII) is formed quantitatively by in vitro reaction of EDS and EDB with cysteine, neither it nor its *bis*-N-acetate are their urinary metabolites. This may be explained by the rapid rate of

| Compound | Synthetic Rf | Metabolite Rf |
|---|---|---|
| Ethylene dimethanesulphonate (I) | 0.85 | – |
| S-($\beta$-hydroxyethyl)-cysteine (III) | 0.34 | 0.34 |
| S-($\beta$-hydroxyethyl)-cysteine-S-oxide (IV) | 0.17 | 0.18 |
| N-acetyl-S-($\beta$-hydroxyethyl)-cysteine (V) | 0.72 | 0.71 |
| N-acetyl-S-($\beta$-hydroxyethyl)-cysteine-S-oxide (VI) | 0.46 | 0.45 |
| S,S'-ethylene-*bis*-cysteine (VII) | 0.08 | – |
| S,S'-ethylene-*bis*-(N-acetyl-cysteine) | 0.78 | – |
| Ethylene glycol | 0.64 | – |
| Sulphate | 0.00 | 0.00 |

Rf values for synthetic and isolated urinary metabolites on Whatman No. 1 paper (ascending) in the solvent system *n*-butanol-acetic acid-water (4:2:1). Detection was by ninhydrin spray, iodine vapour and autoradiography. (Metabolites were also interconverted and identified by peroxide oxidation, thioglycollate reduction[11], acid hydrolysis and treatment with hog-kidney acylase[12].

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[2] H. JACKSON, *Antifertility Compounds in the Male and Female* (Thomas, Springfield, Illinois 1966), p. 66.

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[5] Although all metabolites isolated are cysteine conjugates, it is assumed that they are formed by initial conjugation of EDS and EDB with glutathione as has been proposed in the metabolism of related sulphonic esters[6] and alkyl halides[7].

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hydrolysis[10] of the proposed intermediate II. An analogous intermediate (II, R = Cl) may also be formed in the detoxification of ethylene dichloride which, together with ethylene bromohydrin, is known to produce the same metabolites as EDB[4].

Fertility studies in rats with S-(β-hydroxyethyl)-cysteine (III) given orally (5 × 1 g/kg) have shown that it has no anti-spermatogenic activity. Ethylene dimethanesulphonate at either 1 × 100 mg/kg intraperitoneally or 10 × 25 mg/kg orally produces infertility in male rats by direct action on the intermediate range of spermatogenic cells (spermatids and spermatocytes), whereas ethylene dibromide shows no comparable activity. This suggests that the anti-spermatogenic and the anti-tumour activity of EDS is not due to any of its isolated metabolites but involves a mechanism of specific cellular alkylation[2].

*Zusammenfassung.* Äthylenglykol-dimethansulfonat (EDS) wird von der Ratte zu N-Acetyl-S-(β-hydroxyäthyl)-cystein (V), N-Acetyl-S-(β-hydroxyäthyl)-cystein-S-oxyd (VI), und Methansulfonsäure abgebaut. Äthylendibromid (EDB) gibt ausser V und VI, S-(β-Hydroxyäthyl)-cystein (III) und wird weiter zu Kohlendioxyd und anorganischem Sulphat via S-(β-Hydroxyäthyl)-cystein-S-oxyd (IV) abgebaut. Keine der erwähnten Stoffwechselprodukte sind gegen Tumoren oder gegen Spermatogenese wirksam.

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## Xanthine Oxidase Activity in the Brain

Brain has been reported to be devoid of xanthine oxidase (XO) activity (xanthine oxidoreductase 1, 2, 3, 2 I.U.B.)[1] and in the recent treatises of McIlwain[2] and of Friede[3] there is no reference to the presence of this enzyme in the brain. However, a low activity was found in the brain of the dog and the sheep[4]. The enzymatic activity of xanthine dehydrogenase (XD) in the brain of the mouse was found to increase up to 10 times in the animals infected with yellow fever and other virus[5], and it was reported recently that XO decreases sharply in the brain of the rat during narcosis produced by sodium barbital (Medinal)[6]. These contradictory results led us to reinvestigate the XO activity of the brain of some commonly-used animals for experimental research of the nervous system.

Wistar rats, white mice (average 20 g), adult guinea-pigs and male rabbits were decapitated and the brains rapidly taken out washed with ice-cold distilled water and blotted with Whatman No. 50 paper to remove blood clots. Brain was carefully separated from the cerebellum, weighed and maintained in the freezer until homogenized with phosphate buffer 0.025 *M*, pH 7.0 in a Potter-Elvehjem all glass homogenizer using a teflon pestle. The volumes of the homogenates were adjusted to 1:10 with the buffer and centrifuged for 15 min at 10,000 *g* and the supernatant pipetted off. Cow brains were obtained from the slaughterhouse and kept frozen until used. XO was measured spectrophotometrically as explained in the Table.

XD was estimated using Thunberg evacuated tubes containing in the side arm 0.4 ml of 0.1% of 2, 3, 5-triphenyl tetrazolium chloride (TPTC) as hydrogen acceptor and 0.052 μmoles xanthine. The formazan produced after 30 min incubation at 37 °C was extracted with petroleum ether and measured colorimetrically at 495 nm. In these conditions low values were obtained but when preincubated 10 min with 0.28 μmoles of nicotinamide adenine dinucleotide (NAD) /ml of the supernatant, as hydrogen acceptor, the reaction increased markedly. The values expressed in μg formazan produced per mg protein were 15, 19, 17.5, 10 and 9 respectively for the rat, the mouse, the guinea-pig, the rabbit and the cow. Protein was estimated spectrophotometrically using the formula of Kalckar[7].

The results are summarized in the Table. The rat and the guinea-pig showed the highest values in the brain, but

Xanthine oxidase in brain and cerebellum of some animals

| Animals | No. | XO in μmoles/mg protein | |
|---|---|---|---|
| | | Brain range | Cerebellum average |
| Mouse | 25 | 0.012–0.022 | 0.003 |
| Rat | 35 | 0.023–0.068 | 0.018 |
| Guinea-pig | 5 | 0.018–0.042 | 0.020 |
| Rabbit | 2 | 0.008–0.010 | 0.010 |
| Cow | 2 | 0.004–0.008 | 0.008 |

XO measured in 0.5 ml of the supernatant incubated with 0.05μmoles of hypoxanthine and *Tris* buffer pH 7.4 for 2 h at 37 °C and the reaction stopped by the addition of 2 ml 12% (v/v) perchloric acid. The filtrate adjusted to 4 ml with the acid. Absorbancy read at 290 nm and the results calculated in uric acid and expressed in μmoles produced/mg protein of the supernatant. 2 blanks run for each determination: one with all the reagents plus brain but not incubated and the other with all reagents except the substrate and incubated for 2 h.

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