L-Methionine DL-sulphoximine and acute ammonia toxicity

In 1964, Warren & Schenker reported that mice, injected with L-methionine DL-sulphoximine (MSO) were protected against the deleterious effects of acute ammonia intoxication although MSO by itself caused the brain ammonia concentration to rise (Warren & Schenker, 1964; Folbergerova, Passoneau & others, 1969; Hindfelt, 1973). MSO is a convulsant agent (Folbergerova, 1964; Folbergerova & others, 1969) that passes the blood brain barrier (Gershoff, 1956) and irreversibly inactivates glutamine synthetase (Pace & McDermott, 1952; Rao & Meister, 1972). Since glutamine is the non-toxic end-product of ammonia metabolism in the brain (Weil-Malherbe, 1962) MSO might be expected to be deleterious in ammonia intoxication. It was thought at that time that an accelerated glutamine formation might interfere with the oxidative metabolism (Fig. 1) by draining α-ketoglutarate from the TCA cycle (Bessman & Bessman, 1955) by oxidation of NADH (NADP) (Worcel & Erecinska, 1962) and by consuming ATP in the amidation of glutamate (Weil-Malherbe, 1962). Accordingly, Warren & Schenker (1964) postulated that the apparent protective effect of MSO was related to its interference with ammonia detoxication, decreasing the energy demands of the tissue. However, a recent study failed to show any protective effect of MSO upon the energy state of the brain in acute ammonia intoxication (Hindfelt, 1973).

In the original report on MSO and ammonia intoxication (Warren & Schenker, 1964) it was shown that the acute mortality of mice given ammonium chloride (i.v.) was decreased with MSO pretreatment, but behavioural differences compared to unpretreated controls were not commented upon. We have re-examined the situation *vis á vis* the rather unexpected findings of Warren & Schenker (1964) and sought for differences in toxic manifestations. Furthermore, it seemed imperative to rule out any non-specific physiological effects of MSO by monitoring the respiration, circulation and acid-base homeostasis of the animals.

Methods. Male Wistar rats, 250–400 g, were atropinized (0·20 mg kg^{-1}, i.p.), anaesthetized with ether and the tail artery cannulated. Every second animal was injected during the operation, with L-methionine DL-sulphoximine (Sigma), (150 mg kg^{-1}, i.p., dissolved in 1 ml of isotonic saline). Postoperatively the animals were placed in restraining cages and allowed to recover. Two hours after the cannulation an arterial baseline sample was drawn for ammonia determination and gas analyses (Po_2, Pco_2, pH—Eschweiler & Co, Kiel, Radiometer, Copenhagen). Arterial blood (200 μl) for ammonia analysis was frozen directly in liquid nitrogen and subsequently analysed by a flurometric method (Folbergerova & others 1969).

The animals were then injected via the catheter with ammonium chloride, 360 mg kg^{-1} (36 mg ml^{-1} isotonic saline). This is the LD50 dose for mice reported by Warren & Schenker (1964). The injection was made continuously over 2 min. Arterial samples were drawn at 5, 15 and 30 min after the injection. Mean arterial blood pressure was continuously monitored via the tail artery catheter and the rectal temperature measured (range 35·5–38·0°). There was no difference in body temperature between MSO-injected and uninjected rats.

Immediately after the injection of ammonium chloride the aminals exhibited generalized myoclonus, forced irregular ventilation and intense startle responses to

$$\alpha\text{-Ketoglutarate} + NH_3 + NADH + H^+ \overset{(GDH)}{\rightleftharpoons} \text{glutamate} + NAD^+$$

$$\text{glutamate} + NH_3 + ATP \xrightarrow{(GS)} \text{glutamine} + ADP + P$$

FIG. 1. The major metabolic pathway of ammonia in cerebral tissue. GDH: glutamic acid dehydrogenase. GS: glutamine synthetase.

Table 1. *The arterial acid-base state.* Gas tensions expressed in torr, base excess (BE) in mequiv litre^{-1} plasma.

	Before injection				5 min after injection			
	Po_2	Pco_2	pH	BE	Po_2	Pco_2	pH	BE
No MSO	74·0 ±3·0	40·4 ±2·2	7·40 ±0·01	−0·3 ±1·1	76·5 ±8·1	42·7 ±6·7	7·07 ±0·09	−15·4 ±2·1
MSO	86·1 ±1·5	37·3 ±1·8	7·42 ±0·02	0·2 ±2·4	92·6 ±4·3	40·0 ±3·0	7·22 ±0·04	−9·7 ±1·1

tactile and acoustic stimulation. Tonic-clonic convulsions, always ending fatally, followed within a few minutes in most of the unpretreated rats (onset 6·2 ± 1·8 min s.e.m. after injection) but were delayed in the MSO-pretreated (onset 11·0 ± 2·4 min s.e.m., $P<·05$). All unpretreated rats died (10/10) while 4 out of 10 of the MSO injected animals survived. None of the surviving animals exhibited tonic convulsions but otherwise the toxic manifestations were the same.

No consistent shifts were noted in the acid base state that would predict a protective effect of MSO (Table 1). The differences observed at 5 min after the ammonia injection reflect the more frequent occurrence of tonic-clonic convulsions with the associated release of lactic acid from muscle in the unpretreated group. There were no significant differences in the arterial ammonia concentrations between the two groups as mol kg^{-1} whole blood (means ± s.e.m.): no MSO before ammonia injection 0·037 ± 0·010, 5 min after injection 17·91 ± 5·59 (n = 10); MSO + treated before ammonia injection 0·084 ± 0·19, 5 min after injection 16·74 ± 4·23 (n = 10). The extreme concentration peaks were reached immediately after the injection and at 5 min the ammonia concentrations were 200–400 times the baseline values. In the surviving MSO-pretreated animals, the ammonia concentration at 30 min was 4·4–5·4 mmol kg^{-1}. It is true that convulsive activity is accompanied by release of ammonia. However, this minor difference between the two groups is insignificant in comparison with the amount of ammonia injected. Thus, the blood ammonia concentrations at 5 min are about 5 times higher than those observed with the LD50 dose. The survival of a single animal under these circumstances is remarkable.

The acute hyperammonaemia did not adversely affect the systemic circulation. In all experiments there was a continuous rise in mean arterial blood pressure, reaching its peak at the onset of tonic convulsions after which circulatory failure was immediate.

No published study has confirmed the observations made by Warren & Schenker (1964). This report supports their findings and it seems reasonable to conclude that MSO is protective against acute ammonia intoxication. The protective effect is not due to any specific influences upon respiration, circulation, acid-base homeostasis or toxic manifestations. The beneficial effect of MSO is its ability to suppress ammonia induced tonic convulsions.

It is also noteworthy that excessive acute hyperammonaemia is compatible with unchanged or even increased mean arterial blood pressure since ammonia has been claimed to cause severe hypotension due to bradycardia and peripheral vasodilation (Karr & Hendricks, 1949). In the present experiments atropine prevents marked bradycardia from developing. Thus, the present findings suggest that circulatory failure due to hyperammonaemia is to some extent secondary to a negative chronotropic effect upon the myocardium.

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Estimation of biological availability after oral drug administration when the drug is eliminated by urinary excretion and metabolism

Most drugs undergo "first-pass" metabolism in the liver after oral administration and the reduction of the total area below the blood concentration-time curve, following oral drug administration, compared with that obtained after intravenous drug administration cannot be regarded as a correct estimate of biological availability.

Pharmacokinetic models (see Fig. 1) that account for the "first-pass" effect require orally administered drug to be absorbed into a peripheral compartment from which drug elimination occurs, whereas intravenously given drug is absorbed directly into the central compartment (Gibaldi, Boyes & Feldmann, 1971; Vaughan & Beckett, 1974). In these pharmacokinetic models the vascular site being sampled is regarded as an integral part of the central compartment and the liver as an integral part of the peripheral compartment.

Gibaldi & others (1971) have derived an expression (eqn 1) by which the fraction of an orally administered dose absorbed into the hepatic portal system and the extent of "first-pass" metabolism can be calculated.

$$\frac{\text{Area}^{\text{oral}}}{\text{Area}^{\text{iv}}} = \frac{\text{F (flow rate)}}{\text{flow rate} + \text{F dose/area}^{\text{oral}}} \qquad (1)$$

In equation 1, flow rate is the hepatic blood flow, $\text{area}^{\text{oral}}$ and area^{iv} are the total areas below the blood level-time curve obtained after oral and intravenous drug administration respectively, dose is the oral drug dose and F is the fraction of the oral dose absorbed. When applying equation 1 the mean hepatic blood flow of 1·53 litre min^{-1} (Bradley, Ingelfinger & Bradley, 1952) is used.

Equation 1 has been extensively used in biopharmaceutical studies (Gibaldi & others, 1971; Perrier, Gibaldi & Boyes, 1973; Johnsson, Norrby & Solvell, 1967; Boyes, Scott & others, 1971; Cohen, Bakke & Davies, 1974).