The metabolic fate of [^{35}S]-diallyl disulphide in mice

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Summary. Diallyl disulphide (DADS) is a major constituent of garlic oil. Uptake of [^{35}S]-labelled diallyl disulphide by mouse liver is highest at 90 min after treatment. 2 h after treatment with [^{35}S]-DADS, 70% of the radioactivity is present in the liver cytosol of which 80% is metabolized to sulphate.

Garlic (*Allium sativum* Linn.) has been accredited with many therapeutic values. Among these are its use in diabetes, heart disease and atherosclerosis[1-5]. The active principles of garlic have been found to be sulphur-containing compounds[6]. One of the compounds, viz. allicin or diallyl disulphide oxide, has lipid lowering[7] and hypoglycaemic[8,9] properties. Allicin is a derivative of diallyl disulphide (DADS). DADS accounts for over 60% of the essential oil of garlic[7]. DADS has been shown to have insecticidal[10] and antifungal[11] properties. The results of our recent studies with suckling rats show that DADS can also lower the levels of plasma and liver cholesterol (unpublished results).

Although the effect of garlic oil and its components such as DADS on mammalian oxidative phosphorylation[12], protein, lipid and glycogen syntheses[13] has been studied, its uptake and biological fate in the mammalian system have not been investigated. In mammals, liver is the main site where xenobiotics are metabolized[14]. The present paper reports the method of labelling DADs and the uptake and metabolic fate of the labelled compound in mice.

Materials and methods. 75-day-old female Swiss mice weighing approximately 26 g were used in these experiments. Mice were fed with a nutritionally sufficient laboratory diet and water ad libitum.

[^{35}S]-DADS was prepared as follows: To an aqueous solution (5.0 ml) containing 360 mg of hydrated sodium sulphide 96 mg [^{35}S] sulphur (1.89×10^6 cpm/mg) was added and stirred for 1 h at 60 °C. After allowing the mixture to attain room temperature (23 °C), 380 mg allyl chloride was added slowly and stirred for 1 h. The mixture was then repeatedly extracted with ether (3×3.0 ml). Combined ether extracts were washed with brine and dried with sodium sulphate. Removal of ether yielded an oily residue. Further purification of this oily residue by vacuum distillation (b.p. 82 °C/13 mm) yielded a colourless oil (sp. act. 4.73×10^6 cpm/mmole). Analysis by GLC (OV-17, 120 °C) and high-pressure liquid chromatography (75% aqueous methanol) showed that it contained 98% diallyl disulphide and 2% diallyl trisulphide.

[^{35}S]-DADS (sp. act. 4.73×10^6 cpm/mmole) was injected i.p. at a sublethal dose of 100 mg/kg b.wt. [^{35}S]-DADS was emulsified in 0.9% NaCl using a small drop of Tween 80 before administration. Animals were sacrificed by cervical fracture at different time intervals after the injection. The liver was removed and immediately homogenized in 5 volumes of 0.25 M sucrose. Liver homogenate taken from animals 2 h after injection with the labelled DADS was centrifuged at $650 \times$ g for 10 min to sediment the nuclei. The supernatant was centrifuged at $10{,}000 \times$ g for 30 min to sediment mitochondria. The postmitochondrial supernatant was centrifuged at $105{,}000 \times$ g for 1 h in a Beckman L2-65 B ultracentrifuge to pellet the microsomes. The postmicrosomal fraction was designated as cytosol. Aliquots from the cytosol were taken for the isolation of proteins and lipids by methods described earlier[15,16].

To 5 ml of the cytosol 0.025 ml of aqueous sodium sulphate (290 mg/ml) was added. Sulphate was precipitated by the addition of 0.2 ml of aqueous barium chloride (500 mg/ml). The resultant barium sulphate suspension was centrifuged and the clear supernatant was taken for counting. The decrease in radioactivity after precipitation was taken as a measure of the amount of activity present as sulphate. The amount of unchanged [^{35}S]-DADS was estimated by extracting the supernatant with petroleum ether and measuring the radioactivity in the petroleum ether extract.

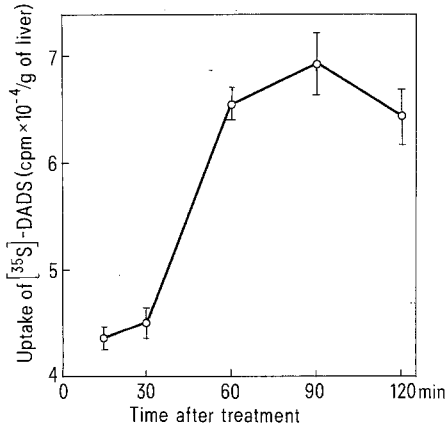
Aliquots of homogenate, nuclei, mitochondria, microsomes, cytosol and different constituents of the cytosol were taken for counting. For radioassay, 0.1 ml of sample was added to 5 ml of toluene: methanol (1:1) mixture containing 0.4% BBOT and counted in a Beckman LS-100 liquid scintillation spectrometer.

Results and discussion. The uptake of [^{35}S]-DADS by the liver of mice as a function of time is shown in the figure. The rate of uptake is most rapid during the first 30 min. The specific activity (cpm/g liver) attains a peak at 90 min after injection of the labelled DADS and declines thereafter. The distribution of the radioactivity in the hepatic

Distribution of radioactivity in liver of mice 2 h after injection with [^{35}S]-diallyl disulphide

	Radioactivity (%)
Subcellular fractions:	
Nuclei	8.38
Mitochondria	13.44
Microsomes	6.45
Cytosol	71.73
Cytosol components:	
Lipid	Traces
TCA precipitate (protein)	10.02
TCA supernatant	89.01
Cytosol:	
Diallyl disulphide	8.6
Sulphates	81.4

Values represent means from 5 animals each.



The uptake of [^{35}S]-diallyl disulphide by the liver of mice as a function of time. The values are means ± SEM of 5 animals for each time interval.

subcellular fractions 2 h after [^{35}S]-DADS injection is shown in the table. The results show that the cytosol has over 70% of the total radioactivity. The distribution of the radioactivity in the other subcellular fractions is in the order mitochondria > nuclei > microsomes. Further analysis of the cytosol shows that the trichloroacetic acid (TCA) soluble fraction (supernatant) contains nearly 90% of the [^{35}S] radioactivity (table). The TCA insoluble constituent (protein) accounts for approximately 10% of the radioactivity with only traces of [^{35}S] associated with the lipids. Of the radioactivity present in the cytosol, over 80% is found as sulphates and only 8% as [^{35}S]-DADS. Sulphur-containing compounds are liable to be oxidized to sulphates by the hepatic mixed-function oxidases[17]. It is interesting to note that we have recently observed substantial increases in hepatic microsomal mixed-function oxidases within 2 h following administration of diallyl disulphide (unpublished results). It appears that the bulk of DADS is converted to sulphates before it is transferred to the cytosol fraction preparatory to elimination from the system.

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Chelate treatment in acute cadmium poisoning

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Summary. Treatment of cadmium-poisoned rats with mixed ligand chelates does not decrease the lethality of cadmium more than treatment with one chelate alone.

Cadmium (Cd) is one of the most poisonous metals in our environment. Until now, no effective treatment against intoxication with this metal has been discovered. The report by Schubert and Derr[1], which described successful therapy for acute Cd-poisoning in mice, was therefore of great interest. The work reported here was performed to determine whether such favourable results using a similar therapeutic regimen could be achieved with another animal species, namely the rat.

All experiments were carried out with male albino rats of the Heiligenberg strain (age: 7 weeks, b.wt: 180–200 g). In the 1st experiment 0.03 mmoles/kg $CdCl_2$ (= 5.5 mg/kg) were injected i.v. and the chelants administered i.p. 60 min later. In the 2nd experiment $CdCl_2$ was given i.p. at a higher dose of 0.1 mmoles/kg. The LD 50/24 h for injection i.p. had been determined previously by the staircase-method[2] to be 0.0444 ± 0.001 mmoles/kg. The chelants were also injected i.p. but this time 5 min after the Cd, according to the corrections given recently by Schubert[3]. When 2 chelants were given simultaneously they were mixed before administration. All the injected solutions were between pH 6.5 and 7.2.

In the control of the animals in the 1st experiment in addition to the liver and kidneys the Cd concentration was determined in the spleen, testes, blood, femur and muscle. The figures were at the lower limit of our detection method (< 1 to ~ 3 ppm) and we therefore did not include these values in our further determinations.

Table 1 shows that there is an equal enhancement of urinary Cd-excretion after treatment with Na-dimercaptopropanesulfonate (Dimaval, DMPS – a gift from Heyl and Co., Berlin), $CaNa_2$-EDTA (EDTA), or the combination of

Table 1. Cd content (µg per organ) in liver and kidney 24 h after i.v. injection of rats with 5.5 mg (i.e. 0.03 mmoles)/kg $CdCl_2$ and in the 24 h urine without or with chelate treatment 1 h after Cd

Chelant Primary 0.5 mmoles/kg	Secondary 2.0 mmoles/kg	Urine	Liver	Kidneys
–	–	4.69 ± 4.61	260.1 ± 1.15	16.2 ± 0.41
–	SA 1 animal only	–	214.5	12.4
–	DMPS	72.6 ± 23.2*	170.2 ± 9.5*	17.0 ± 1.1
EDTA	–	97.4 ± 24.4*	177.7 ± 7.3*	17.7 ± 1.8
EDTA	SA	No survival		
EDTA	DMPS	71.9 ± 10.1*	184.1 ± 8.9*	19.2 ± 1.5

3 (control) or 6 animals. Mean values ± SE. * Statistically significant difference from the control ($p < 0.05$).