

Relevancy of bivalent sulphur excretion to carbon disulphide exposure in different metabolic conditions

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Magos, L. (1972). *Brit. J. industr. Med.*, 29, 90-94. **Relevancy of bivalent sulphur excretion to carbon disulphide exposure in different metabolic conditions.** A quantitative spectrophotometric method for the estimation of urinary bivalent sulphur by its catalytic effect on the iodine-azide reaction has been developed for rats exposed to carbon disulphide (CS₂). Urinary CS₂ was also determined. Exposure to CS₂ increased the excretion of bivalent sulphur in both fed and starved animals, but the increase in starved animals was significantly less than in fed animals. Starvation also decreased the urinary excretion of CS₂. Phenobarbitone pretreatment, which induced liver damage in animals subsequently exposed to CS₂, had no effect on either bivalent sulphur or CS₂ excretion. Three dithiocarbamate fungicides, Thiram, Ziram, and Zineb, given by mouth increased the excretion of bivalent sulphur in the following order: Zineb < Ziram < Thiram.

After 250 mg/kg sodium diethyldithiocarbamate (DDC) given intraperitoneally the same amount of CS₂ was excreted in the urine as after 4 hours' exposure to 2.0 mg/l CS₂, but the bivalent sulphur excretion was six times higher. Starvation did not decrease the excretion of CS₂ or bivalent sulphur after DDC.

The metabolic relationship between carbon disulphide (CS₂), dialkyldithiocarbamate, and tetraalkylthiuramdisulphide is well established. Because there is an increase in the serum concentration of thiol groups as a result of in vitro reaction with CS₂ or the exposure of guinea-pigs to CS₂ (Souček and Madlo, 1955; 1956), it was proposed by Souček (1957) that the first step in the metabolic pathway of CS₂ is its reaction with amino acids and proteins resulting in the formation of dithiocarbamate type compounds. On the other hand, Strömme (1965) has shown that one of the metabolic products of diethyldithiocarbamate and disulfiram (tetraethylthiuram disulphide) is CS₂. Within 4 hours after the administration of 25 mg diethyldithiocarbamates to the rat, about 10% of the sulphur administered was recovered in the exhaled air as

CS₂ (Strömme, 1965). After inhalation of CS₂ 8 to 30% of the body burden is exhaled and less than 1% is excreted in the urine of persons exposed to CS₂ (Teisinger and Souček, 1949; Harashima and Masuda, 1962). The low level of urinary excretion indicates that CS₂ is either exhaled or nearly completely metabolized. One of the major metabolites of CS₂, diethyldithiocarbamate, and disulfiram is sulphate (McKee *et al.*, 1943; Strittmatter, Peters, and McKee, 1950; Strömme, 1965). The second group of major metabolites constitutes compounds which retained sulphur in the bivalent form. Their excretion is the best indicator of the level of CS₂ exposure when assayed by using their catalytic effect on the reduction of iodine by azide (Vasak, Venacek, Kimmelova, 1963; Djurić, Surdicki, and Berkes, 1965; Jakubowski and Piotrowski, 1967).

It seems reasonable to suppose that bivalent sulphur excretion after exposure to CS₂ is related to the detoxification of CS₂. It has been reported that, of a group of workers with the same exposure, only those who exhibited a delayed excretion pattern of bivalent sulphur developed symptoms after some months of exposure (Graovac-Leposović, Djurić, Pavlović, and Jovičić, 1967).

As it is difficult to measure exactly human exposure to CS₂ or to control those factors which might influence bivalent sulphur excretion, animal experiments offered a better way to investigate factors which might alter the metabolism or the toxicity of CS₂. It was found by Bond, Butler, De Matteis, and Barnes (1969) that CS₂ showed hepatotoxic activity in rats pretreated with phenobarbitone, and Magos and Butler (1971) observed that 24 hours' starvation before exposure made rats treated with phenobarbitone more sensitive to the hepatotoxic effect of CS₂. The experiments to be described tried to relate CS₂ exposure and bivalent sulphur excretion in rats and also to investigate the effect of starvation and phenobarbitone treatment on the urinary excretion of bivalent sulphur. A sensitive spectrophotometric method has been developed to detect small differences in the catalytic effect of urine on the reduction of iodine by azide. The applicability of the method after the intraperitoneal injection of diethyldithiocarbamate and the oral administration of three different dithiocarbamate fungicides was also tested.

Methods

Male albino rats of Porton Wistar strain were exposed for 4 hours to 1.0, 2.0 or 4.0 mg/l CS₂ in a vertical type constant flow exposure chamber (Magos, Emery, Lock, and Firmager, 1970). Phenobarbitone sodium (BDH Chemicals Ltd) was given intraperitoneally in a dose of 80 mg/kg and 50 mg/kg 24 and 18 hours respectively before exposure. In the starved group food was withdrawn 24 hours before exposure. The average weight of the starved group was 215.44 ± 8.83 g (S.D.) 24 hours before exposure and 196.41 ± 6.08 g just before exposure. The weights of the fed group were 211.35 ± 88 and 218.77 ± 8.4 g respectively. No food was given to the animals either during exposure or in the ensuing 17 hours when they were kept, two to a cage, in metabolism cages. Urine was collected in flasks immersed in cardice-acetone mixture. After the collection period animals were removed and 10 ml distilled water was spread drop by drop on the inner slope of the metabolic cage to wash it down. This increased urine volumes by approximately 9.0 to 9.5 ml.

Sodium diethyldithiocarbamate (DDC—Hopkin and Williams, Chadwell Heath, Essex) in aqueous solution was given just before the urine collection period intraperitoneally in a volume of 14.3 ml/kg, Tetramethylthiuramdisulphide (Thiram) 48.1 mg/kg, zinc dimethyldithiocarbamate (Ziram) 61.2 mg/kg, and zinc ethylenbisdithiocarbamate (Zineb), 55.2 mg/kg, all technical

grade suspended in water, were given through a stomach canule in a volume of 20 ml/kg to give an approximately 0.2 mmol/kg dose. The timing of the food withdrawal followed the same schedule as in the CS₂ experiments.

Estimation of CS₂ in urine

Urines were thawed within 1 hour after the end of collection and stored at 4°C and examined 3 to 4 hours later. Identical 30-ml impingers were used for both the urine sample and the diethylamine reagent (Viles, 1940). The two impingers were connected by quickfit joints. The inlet of the sample impinger was connected by polyvinyl tubing through a valve with a nitrogen cylinder, and the outlet of the sample impinger was connected with a 50 to 500 ml/min rotameter. Urine, 20 ml, was added to the sample impinger immersed in a 55°C water bath, and 7 ml reagent was pipetted into the absorber impinger immersed in melting ice. When less than 20 ml of urine was available, the volume was made up to 20 ml with distilled water. After adjusting the airflow to 100 ml/min, the polyvinyl tubing fitted into the inlet of the sample impinger was removed for a few seconds and 2 ml glacial acetic acid was injected with a syringe into the inlet tube. After 30 min bubbling the apparatus was dismantled and the optical density of the absorber was read against reagent blank at 430 nm in a 2-cm cell in a Unicam spectrophotometer. No attempt was made to distinguish between free and bound CS₂, as the proportion of CS₂ released without acidification depends on the pH of the urine and the bubbling time.

Estimation of bivalent sulphur in urine

The method of Strickland, Mack, and Childs (1960) for cystine was modified for the urinary sulphur metabolites of CS₂. The iodine-azide reagent was prepared from stock 2M sodium azide and from stock KI₃, containing 0.065 M I₂ and 0.130 M KI. Fifty millilitres of the azide solution and 20 ml of the KI₃ solution were mixed, the pH was adjusted to 5.2 by adding 5 N HCl, and diluted to 110 ml with distilled water. Of the chilled reagent 5 ml was pipetted into 50-ml glass-stoppered flasks immersed in melting ice. The overnight urine of two animals was made up to 100 ml (or more if high activity made it necessary). From the diluted urine 1 ml was added to the reagent, and by rotation complete mixing was achieved. Flasks were loosely closed with glass stoppers used upside down. Blanks were incubated with 1 ml of distilled water. After 30 min incubation 50 ml distilled water was added to both blanks and samples, and immediately after that 5 ml of the diluted sample was pipetted into 5 ml carbon tetrachloride. After shaking for 30 seconds, time was allowed for the phases to separate. The carbon tetrachloride phase was transferred to a 1 cm cell and the absorbancy was read against water-saturated carbon tetrachloride at 525 nm. The solution obeys Beer's law and from the difference between blanks and samples the amount of iodine reduced to iodide by the total urine per kg of body weight was calculated. Using diethyldithiocarbamate instead of urine, 1 μmol DDC reduced 120 μmol I₂.

All the urinary excretion values of CS₂-exposed rats were related to the body weight taken 24 hours before exposure. In the case of DDC or fungicides the dose and the excretions were calculated on the basis of weight at the time of injection.

Results

Figure 1 shows that the excretion of bivalent sulphur increased significantly in fed and starved rats when exposure was increased from 1.0 mg/l up to 4.0 mg/l. However, in the fed rats the increase in excretion after exposure to 1.0 mg/l compared to unexposed rats was much greater than in the starved rats, although thereafter the same increment in exposure caused the same increment in bivalent sulphur excretion in both fed and starved animals. The difference in the excretion of bivalent sulphur by fed and starved rats was significant at every level of exposure.

Table 1 shows that phenobarbitone pretreatment had no effect on the excretion of bivalent sulphur in either fed or starved animals. Urinary CS₂ excretion was also influenced by starvation, but not by phenobarbitone (Table 2). Starvation resulted in an approximately 50% decrease in the excretion of CS₂. Actually in starved animals exposed to 2.0 mg/l CS₂, the CS₂ excretion was the same as in fed animals exposed to 1.0 mg/l CS₂.

Figure 2 shows that animals treated with 250 mg/kg DDC excreted as much CS₂ as rats exposed to 2.0 mg/l CS₂, but the urine of the former group of animals was six times more efficient in catalysing the reduction of iodine by azide than the urine of the latter. Figure 2 also shows that neither the ex-

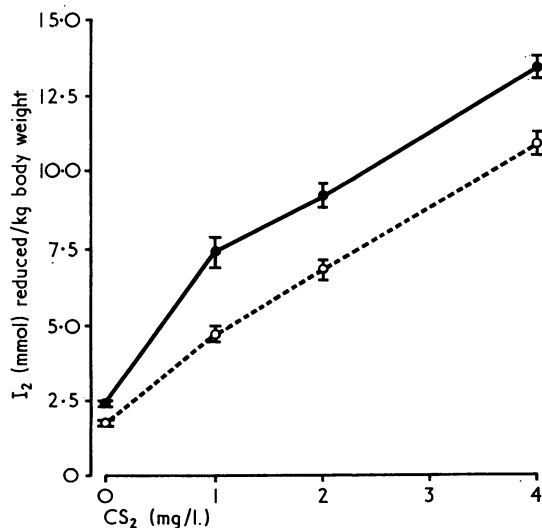


FIG. 1. Effect of 4 hours' exposure to CS₂ on urinary excretion of bivalent sulphur in fed (straight line) and starved (broken line) male rats. Starving started 24 hours before exposure; urine was collected in the first 17 hours after exposure. Bivalent sulphur was estimated from the catalytic effect of urine on the reduction of iodine by azide. The vertical lines mark the standard errors of means.

TABLE 1

EFFECTS OF STARVATION AND PHENOBARBITONE PRE-TREATMENT ON BIVALENT SULPHUR EXCRETION IN RATS EXPOSED TO CS₂

Phenobarbitone treatment	4 h. exposure to 2 mg/l CS ₂	mmol I ₂ reduced by total urine/kg of body weight	
		Fed	Starved
-	-	2.44 (0.11) N=8	1.81 (0.05) ¹ N=8
+	-	2.61 (0.14) N=8	1.93 (0.10) ¹ N=8
-	+	9.38 (0.33) N=16	6.91 (0.30) ¹ N=16
+	+	9.46 (0.36) N=18	7.50 (0.27) ¹ N=18

¹Significant difference between fed and starved is $P < 0.005$. There is no significant difference between phenobarbitone-treated and untreated rats.

Starvation started 24 hours before exposure; phenobarbitone sodium was given intraperitoneally 24 and 18 hours before exposure in doses of 80 and 50 mg/kg respectively. Urine was collected in the first 17 hours after exposure. Numbers in parentheses are the standard errors of means.

cretion of CS₂ nor bivalent sulphur was decreased by starvation after the administration of diethyl-dithiocarbamate. Actually in starved rats at the two higher doses, the excretion values were higher than

TABLE 2

EFFECT OF STARVATION AND PHENOBARBITONE PRE-TREATMENT ON URINARY EXCRETION OF CS₂

Level of CS ₂ exposure (mg/l) (4 h)	Phenobarbitone pretreatment	Urinary excretion of CS ₂ (μmol/kg)	
		Fed	Starved
1.0	-	0.50 (0.05) N=8	-
2.0	-	0.89 (0.07) N=16	0.45 (0.07) ¹ N=16
2.0	+	0.87 (0.09) N=8	0.37 (0.07) ¹ N=8
4.0	-	1.36 (0.16) N=8	-

¹Significant difference between fed and starved animals is $P < 0.005$. There is no statistically significant difference between phenobarbitone-treated and untreated rats.

Starvation started 24 hours before exposure; phenobarbitone sodium was given intraperitoneally 24 and 18 hours before exposure in doses of 80 and 50 mg/kg respectively. Urine was collected in the first 17 hours after exposure. Numbers in parentheses are the standard errors.

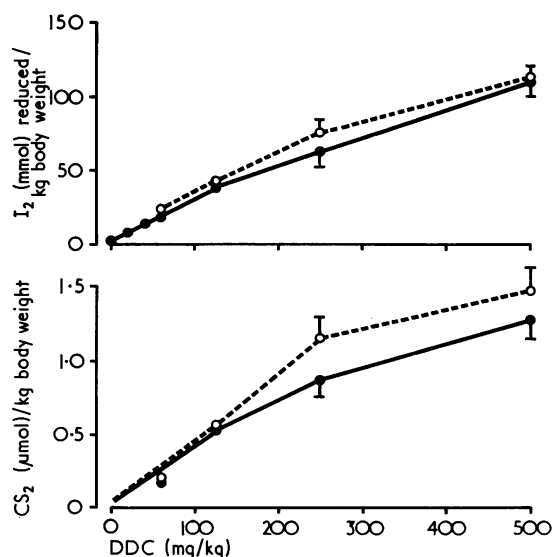


FIG. 2. Effect of sodium diethyldithiocarbamate on urinary excretion of CS₂ and bivalent sulphur in fed (straight line) and starved (broken line) rats. DDC was given intraperitoneally and urine was collected in the next 17 hours. One sample was obtained from two animals. Every point represents the mean value of three samples. Vertical bars mark the standard errors of means.

in fed animals, but the differences were not significant.

All the three dithiocarbamate fungicides increased bivalent sulphur excretion. The increase in the reduction of iodine by urine after the administration of 0.2 mmol/kg Thiram, Ziram, and Zineb was 8.95, 3.66, and 1.62 mmol I₂/kg of body weight, respectively.

Discussion

The increase in bivalent sulphur excretion is not specific for CS₂ exposure. Novak, Djurić and Fridman (1969) reported that the urine of persons treated with disulfiram was more effective in catalysing the iodine-azide reaction than the urine of control persons. The results presented here have shown that rats treated with dithiocarbamate fungicides like Thiram, Ziram, and Zineb or injected with sodium diethyldithiocarbamate also excreted significantly more bivalent sulphur than control rats. Of the three dithiocarbamate fungicides, Thiram, a compound closely related to disulfiram, was the most effective.

In spite of the close metabolic and pharmacological relationship between CS₂ and sodium diethyldithiocarbamate (Magos and Jarvis, 1970a, 1970b) the proportion of CS₂ to bivalent sulphur in the urine of rats exposed to CS₂ or treated with DDC is

not similar. Thus rats dosed with 250 mg/kg DDC excreted the same amount of CS₂ as rats exposed for 4 hours to 2.0 mg/l when the estimated uptake is approximately 0.9 mmol/kg CS₂ but they excreted six times as much bivalent sulphur. The bivalent sulphur metabolite of CS₂ cannot be dithiocarbamate-type compounds as unconjugated dithiocarbamates should give the same results with the bivalent sulphur estimation using the conversion factor of 1 μmol DDC = 120 μmol I₂ as with the carbon disulphide evolution method. Furthermore, judged from the different effects of starvation on bivalent sulphur excretion in DDC treated and CS₂ exposed rats, it is unlikely that the metabolite is a conjugated dithiocarbamate which is the main metabolic product after DDC (Strömme, 1965).

Contrary to expectations, phenobarbitone pretreatment, which renders the liver cells more sensitive to damage by CS₂, had no effect on either CS₂ or bivalent sulphur excretion, suggesting that the effect of phenobarbitone on carbon disulphide hepatotoxicity may not be mediated through altered CS₂ metabolism. However, it must be pointed out that the amounts of CS₂ and bivalent sulphur excreted after exposure to CS₂ probably reflect only a small part of the net CS₂ uptake (uptake by inhalation minus release by exhalation).

It might be expected that if less CS₂ is converted to bivalent sulphur, the more CS₂ would be excreted. However, 24 hours' starvation before exposure significantly decreased the amount of both bivalent sulphur and CS₂ excreted by the animals within 17 hours after exposure. One possible explanation of this finding is that in starved rats the cell constituents were able to bind more CS₂ without a corresponding increase in the formation of bivalent sulphur. Another explanation could be that starvation increased the metabolism of CS₂ which leads to non-bivalent sulphur end-products.

It would be interesting to know whether bivalent sulphur excreted as a result of exposure to CS₂ consists of one or more compounds produced by one or several reactions. As the straight line drawn through the bivalent sulphur excretion values of starved animals intercepts the ordinate at their normal value, it seems reasonable to suppose that one type of compound or one type of reaction is responsible for their bivalent sulphur excretion. However, in the case of fed animals this reaction appears to be supplemented by another type of compound of limited availability. This additional metabolic pathway—if it is present in men—might explain individual differences in sensitivity to the toxic effects of CS₂.

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