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A NEW METHOD FOR THE ESTIMATION OF EXPOSURE TO CARBON DISULPHIDE

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SUMMARY

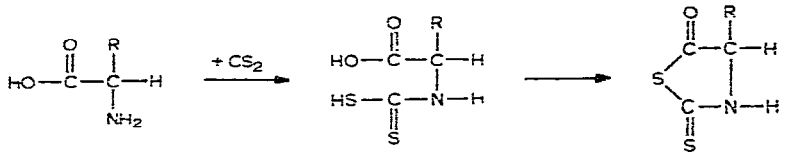
A gas chromatographic method for the analysis of metabolites resulting from exposure to carbon disulphide has been developed. The determination is based on the re-conversion of the metabolites into carbon disulphide in acidic medium, and subsequent analysis for this compound in the headspace of the reaction solution.

Different types of columns and selective detectors were tested and the influence of the reaction conditions on the recovery was investigated. Recoveries for the most important metabolites were 90% from water, 100% from urine and 10–60% from blood. Experiments *in vivo* with rabbits gave a reasonable correlation between the amount of carbon disulphide injected and the analytical results.

INTRODUCTION

Carbon disulphide is used as a solvent and a reactant in the viscose industry. Workers in this industry are often exposed for long periods to the vapour of this compound (b.p. 46°) and toxic effects of long-term exposure such as subjective complaints, disturbances of the central nervous system, damage to the eyes and cardiovascular disease occur.

Up to about 70% of the carbon disulphide retained in the lungs is metabolized[1]. Via the lungs, 8–12% will be expired, while via the urine, metabolites of carbon disulphide are excreted mainly as organic substances and partly as inorganic sulphate. Souček and Madlo[2], Cohen *et al.*[3] and Djurić *et al.*[4] reported the binding of carbon disulphide to amino acids after inspiration. The compounds formed are dithiocarbamates and thiothiazolidone:



Pergal *et al.*[5] found a tautomer of thiathiozolidone (mercaptothiazolidone) and a dithiocarbamate in the urine of exposed workers.

The most commonly used method for the detection of carbon disulphide is the kinetic iodine–azide test in urine according to Yoshida[6], further developed by Vašák and co-workers[7,8]. This method is based on the measurement of the decolorization of iodine, which is catalyzed by metabolites of carbon disulphide:

$$2\,NaN_3 + I_2 \rightarrow 2N_2 + 2NaI$$

The disadvantages of this method are its poor sensitivity (unreliable for concentrations corresponding to exposures below the MAC level of 60 mg/m^3) and its inapplicability to low creatinine (< 1.25 g/l) urines and to blood.

Maurice and Mulder[9] determined carbon disulphide in urine and blood as the xanthogenate by spectrophotometry. This method also has a high detection limit (15 μg/l in urine) and is laborious. Bighi[10] determined dithiocarbamate, a possible metabolite resulting from exposure to carbon disulphide, in water by degradation with concentrated sulphuric acid. After condensation, the resulting carbon disulphide was measured by gas chromatography. This method is very laborious and unsuitable for biological matrices as other substances are co-distilled. The detection limit is a few micrograms.

McLeod and McCully[11] analysed food crops purposely spiked with dithiocarbamate fungicides. They degraded these compounds with acids and analyzed the headspace of the reaction mixture by gas chromatography. As the metabolites of carbon disulphide are chemically similar to these compounds and as concentrations of a few parts per million could be determined, their method was chosen as the basis of the analysis of carbon disulphide metabolites in body fluids.

EXPERIMENTAL

Equipment

The gas chromatograph was a Varian Aerograph 1520 B with an electron-capture detector (ECD) (Varian) and a flame photometric detector (FPD) (custom made).

The FPD burner was constructed according to Brodey and Chaney[12] and equipped with a heat filter with 70% transmission range 350–600 nm (Spindler and Hoyer); two interference filters with maximal transmission at 394 nm and half-height widths of 9.5 and 2.5 nm (Baltzer) were used, together with a low dark current photomultiplier (EMI 9524 S). A pre-amplifier (Analog Devices 43 K) and an Oltronix A 2.5–10 HR power supply were used.

Chemicals

All chemicals were of analytical-reagent grade from different sources, except

for sodium dimethyldithiocarbamate (Fluka, Buchs, Switzerland, pure grade) and 2-thiothiazolidone-5, which was synthesized[19].

RESULTS AND DISCUSSION

Detectors and columns

First the two detectors were optimized. Fig. 1 shows the dependence of the signal-to-noise ratio of the FPD on the photomultiplier voltage. In contrast to the finding of Brodey and Chaney[12], there is little influence provided that the voltage is above 500 V, which was therefore maintained at 600 V during the remainder of the experiments.

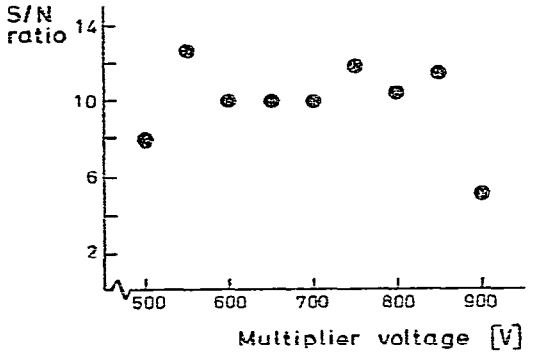


Fig. 1. Signal-to-noise ratio as a function of the multiplier voltage of the FPD; 5 ng of CS_2 was used as a signal. Column: 15% SE-30 on Chromosorb G, 80–100 mesh, AW DMCS, 1.6 m × 2.1 mm I.D., glass.

Table I shows the influence of gas flow ratios on the signal-to-noise ratio. Air from a compressor was preferred to oxygen during the remainder of the work because of lower noise obtained and for convenience.

Table II shows the contributions of various units to the noise. Clearly the flame itself contributes the major portion of the total noise. At a multiplier voltage of 600 V, the flame noise is lower than that reported by Stevens *et al.*[13].

The ECD was operated at 120°. Fig. 2 shows the dependence of the standing

TABLE I

SIGNAL-TO-NOISE RATIO AS A FUNCTION OF THE GAS FLOWS AT THE FPD

Signal: 5 ng CS_2. Column: 1.6 m × 2.1 mm I.D., 15% SE-30 on Chromosorb G, 80–100 mesh.

Recommendation of gas flow-rates	*Flow-rate (ml/min)*				*S/N ratio*
	H_2	O_2	*Air*	N_2	
Melpar Manual*	150	20	30	100	16
Stevens *et al.*[13]	80	16	0	100	18
Stevens *et al.*[13]	80	16	0	20	28
This work	80	0	35	100	27
This work	80	0	35	20	16

* Micro Tek, Austin, U.S.A.

TABLE II

CONTRIBUTIONS OF NOISE AT THE FPD

Noise in 10^{-12} A.

Source	*Recorder*	*Amplifier*	*Multiplier dark current*		*Multiplier dark shot*		*Flame*	
			600 V	*750 V*	*600 V*	*750 V*	*600 V*	*750 V*
Brodey and Chaney[12]	—	—	—	2000	—	—	—	150
Stevens *et al.*[13]	—	—	—	—	—	—	—	20
This work*	<1	1.5	40	140	2	6.8	(1) 7 (2) 2.7	(1) 45 (2) 16

* Bandwidth of half maximum transmittance of interference filter: (1) 9.5 nm; (2) 2.5 nm.

current, which is of decisive importance for the detection limit, on the gas flow. The implications with regard to the simultaneous optimization of the flow for the separation and detection will be discussed below.

The FPD was used in combination with the graphitized carbon black column proposed by Bruner *et al.*[14] for the separation of sulphur dioxide, hydrogen sulphide and methyl sulphide, but modified in such a way that a coating of 0.3% SE-30 was used instead of Dexsil and the support was silanized before use. These modifications were applied in order to obtain a better comparison with the experiments with the ECD.

The minimum plate height with this column, obtained at a mean linear gas velocity $<v>$ of 85 mm/sec, was 2.1 mm. With a linear velocity of 300 mm/sec, the plate height increased to about 6 mm. The use of this high gas velocity is advantageous in this type of analysis, despite of the large plate height. The response of the FPD varies

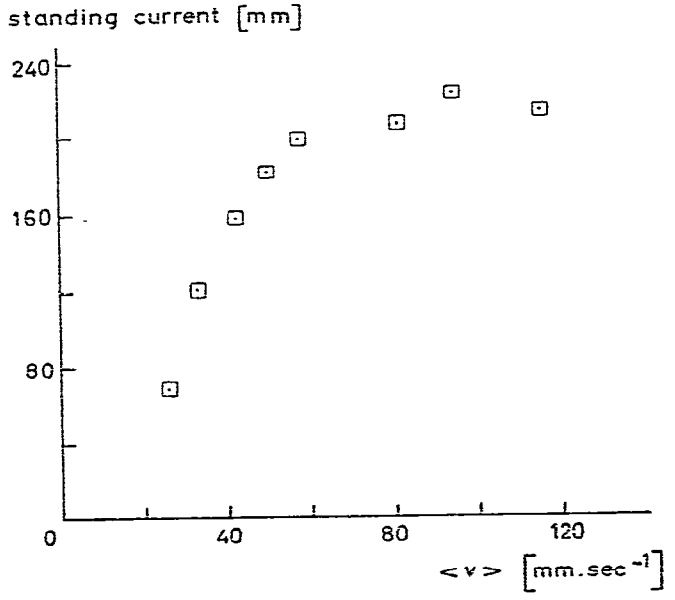


Fig. 2. Standing current of the ECD as function of linear gas flow-rate.

quadratically with the mass flow of sulphur and an increase in the gas velocity therefore results in a quadratic increase in the response if the plate number is constant. This is only partly counteracted by an increase in the plate height.

The high gas velocity used in most experiments made it possible to work at such a low temperature (50°) that a capacity ratio of 18 was obtained. This is advantageous, as in biological samples high peaks with substantial tailing and low retardation occur (see Fig. 7) and at higher temperatures these peaks would interfere with the carbon disulphide peaks, even at high plate numbers.

As the detection limit of the ECD is much better than that of the FPD, considerable effort was spent on obtaining a good column for use in combination with this detector. Table III lists the three stationary phase systems used and the results obtained. The best stationary phase was SE-30, while Triton X-100, as used by Ronkainen *et al.*[15] for the separation of hydrogen sulphide and methanethiol, was not suitable for the separation of carbon disulphide from interfering peaks. Also at variance with the findings of these authors, our results with glass column were better than those with stainless steel or fluorinated ethylene-propylene (FEP). No significant influence on the plate height was observed when the solid support and liquid loading were varied.

TABLE III

EFFECT OF STATIONARY PHASE ON MINIMUM PLATE HEIGHT (H) FOR CS_2

Temperature: 60°. Columns: glass, 1.60 m × 2.1 mm I.D. Carrier gas: nitrogen.

Stationary phase	*Support*	*Minimum H*
SE-30 (5-15%)	Chromosorb W	1.3
Triton X-305 (5%)	Aeropak	2.5
Poly-*m*-phenyl ether (5%)	Chromosorb W	Tailing

Final analytical measurements with the ECD were carried out with a column of length 1.5 m and I.D. 2.4 mm, loaded with 15% SE-30 on Chromosorb G or Aeropak, 80–100 mesh, AW DMCS. The minimum plate height (H) was 1.2 mm and the capacity ratio (k') was 4. Fig. 3 gives an example of an H *versus* $<v>$ graph for carbon disulphide.

Calibration (carbon disulphide releasing reaction and recovery from water)

The necessary working range was estimated as follows. A volume of 10 m^3 breathed in during 1 day corresponds to 600 mg of carbon disulphide if the MAC level of 60 mg/m^3 (U.S.A., G.F.R.) is present. When metabolization is estimated at 50% and the daily excretion in urine at 1%, an amount of 2.4 mg can be expected in a daily amount of urine of 1.3 l. Thus a concentration of 2 mg/ml should be easily detectable. The working range used was 30–300 μg/l. For making the calibration in this range, 1 ml of carbon disulphide was dissolved in 100 ml of *n*-hexane (solution A). Then 1 ml of solution A was dissolved in 100 ml of *n*-hexane (solution B), and solution B was kept in a refrigerator at 4°.

A 5–40-μl amount of solution B was injected into the closed headspace vessel through the septum for the SE-30–ECD combination, while 50–300 μl were injected for the carbon black–FPD combination. The vessel was closed with a silicone rubber

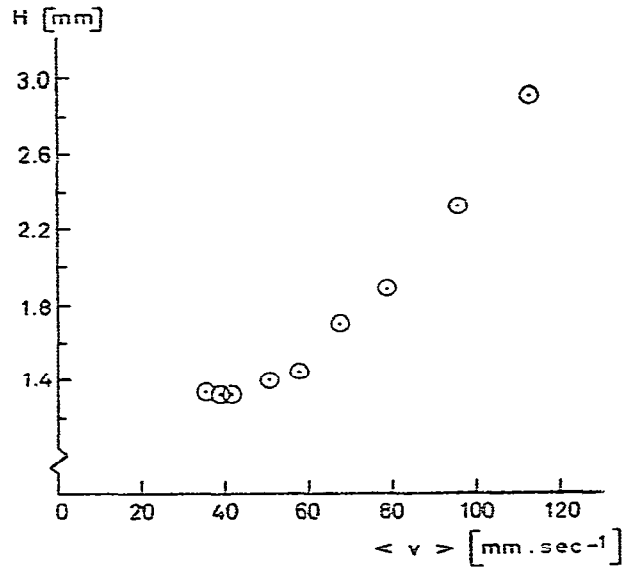


Fig. 3. *H versus* $<v>$ curve for 1.560 m × 2.1 mm I.D. 15% SE-30 on Aeropak, 80–100 mesh, AW DMCS. Sample: *ca.* 5 ng of CS_2. Temperature: 60°. $H/<v> \approx 0.21$; $k' = 3.4$.

septum, covered on the inside with a 0.1-mm thick sheet of PTFE. The vessel contained 2 ml of 5 *M* sulphuric acid plus 2 ml of distilled water and was kept on a water-bath at 60° for 15 min. The internal standard was carbon tetrachloride for SE-30–ECD and dimethyl sulphide for carbon black–FPD; these internal standards are mainly used in order to correct for the variable injection volumes.

The least-squares method was used to calculate the calibration lines as $y = a + bx$, where x is the logarithm of the amount of sulphur and y is the logarithm of the detector response (either peak height or area). Table IV shows the results for the FPD system. Along with the line parameters values for the 95% confidence interval for the estimate of the concentration[16,17] were calculated.

Table IV shows that the logarithm of the peak height gives a worse correlation than the logarithm of the peak area. However, in biological matrices there are often drifting baselines and unseparated peaks, so that one is obliged to use the peak height.

TABLE IV

RELATIVE 95% CONFIDENCE INTERVALS ($\Delta\hat{X}$%) OF THE ESTIMATE OF THE CONCENTRATION FROM A DUPLICATE SAMPLE FOR THE CARBON BLACK–FPD COMBINATION

Column: 0.3% SE-30 and 0.5% orthophosphoric acid on Carbopack HT-100, 1.6 m × 2.1 mm I.D., glass. Carrier gas: 100 ml/min nitrogen. FPD: 80 ml/min hydrogen, 35 ml/min air.

Log mass (ng)	$\Delta\hat{X}$ (%)	
	Log area	*Log height*
−0.26	43	151
0.44	5	4
0.70	5	6

TABLE V

RELATIVE 95% CONFIDENCE INTERVALS ($\Delta\hat{X}\%$) OF THE ESTIMATE OF THE CONCENTRATION FROM A DUPLICATE SAMPLE FOR THE CARBON BLACK–FPD COMBINATION

Transformed values. Conditions as in Table IV.

Mass (ng)	$\Delta\hat{X}$ (%)	
	Area	*Height*
0.76	26	49
1.32	21	39
1.82	26	48

Table V shows the confidence intervals after transformation. The limit of determination for a 95% confidence interval of 33% relative was 300 pg of carbon disulphide in water. As the upper limit of the quadratic calibration plot for sulphur is at approximately 60 ng (ref. 18), the working range for the carbon black–FPD combination is between 300 pg and 80 ng of carbon disulphide; for 1-ml headspace samples, this range corresponds to 11–510 μg/l.

For the SE-30–ECD combination, the peak height was also used as the analytical signal, for the same reasons. Table VI shows the precision obtained. The limit of determination for a 95% confidence interval of 33% relative was 12 pg; the upper limit depends on the standing current and was 1.2 ng in this instance. For a headspace sample of 1 ml, this level corresponds to 0.45–45 μg/l in water.

Irrespective of the combination used, the calibration could be carried out in one headspace vessel. After 4 h, the calibration lines had not changed.

The degradation of sodium dimethyldithiocarbamate and 2-thiothiazolidone-5 to carbon disulphide in water was analysed. The courses of the reactions were followed with the SE-30–ECD combination. The solutions were freshly prepared daily with double-distilled water and the concentration was checked spectrophotometrically (at wavelengths of 254 and 282 nm). Mechanical shaking was applied every 2.5 min, and a sample was taken every 5 min. The temperature of the reaction was 60°. Fig. 4 shows that 15 min after the beginning of the reaction, the maximum level of the dithiocarbamate had been reached. At room temperature, this maximum value was reached after 75 min, but the recovery was then lower. For the thiazolidone (Fig. 5), the maximum level was reached after 25 min, and in the remainder of the work a reaction time of 30 min was used. The values were stable for at least 90 min.

The recoveries are shown in Table VII. Dithiocarbamate gave a good recovery

TABLE VI

RELATIVE 95% CONFIDENCE INTERVALS ($\Delta\hat{X}\%$) FOR THE ESTIMATE OF THE CONCENTRATION FROM A DUPLICATE SAMPLE FOR THE SE-30–ECD COMBINATION

Conditions: 15% SE-30 on Aeropak, 80–100 mesh, AW DMCS, 1.6 m × 2.1 mm I.D., glass.

Mass (ng)	$\Delta\hat{X}$ (%)
0.12	32
0.25	13
0.49	6

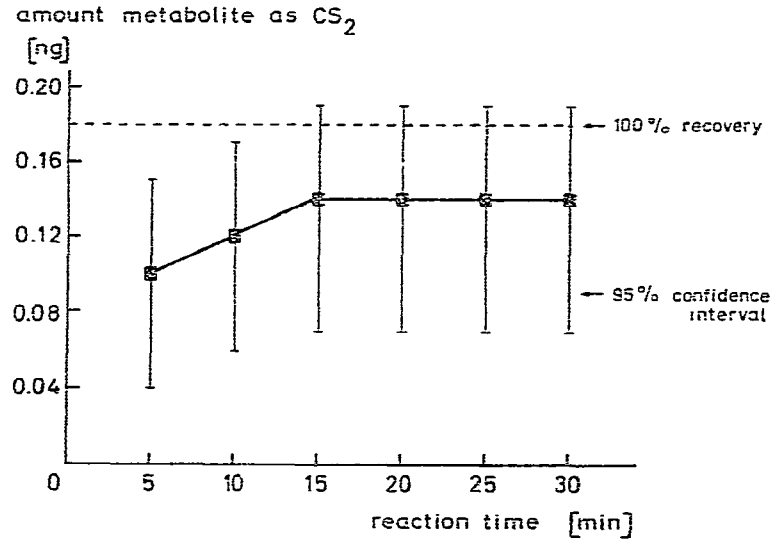


Fig. 4. Conversion *versus* reaction time for sodium dimethyldithiocarbamate. Temperature: 60°. Solution in 2.5 *M* sulphuric acid.

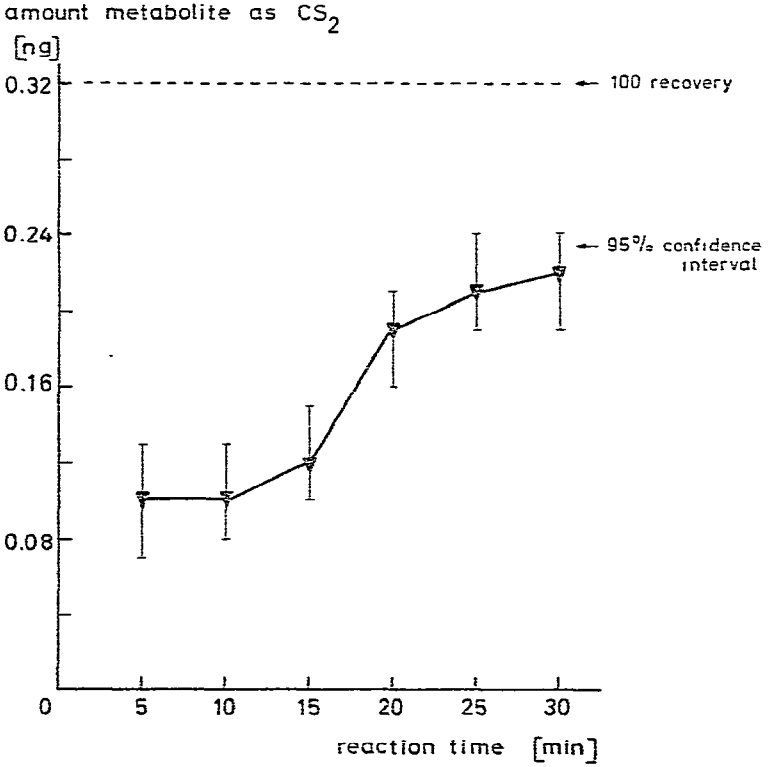


Fig. 5. Conversion *versus* reaction time for 2-thiothiazolidone-5. Conditions as in Fig. 4.

TABLE VII

RECOVERIES OF SODIUM DIMETHYLDITHIOCARBAMATE AND 2-THIOTHIAZOLIDONE-5 IN WATER

Compound	*Concentration (mg/l)*	*Recovery (%)*
Sodium dimethyldithiocarbamate	0.3	65
	0.4	110
	0.4	90
	0.7	83
		Mean 87 ± 19
2-Thiothiazolidone-5	0.1	58
	0.4	39*
	0.7	69
	1.0	50
		Mean 49 ± 14

* Ascorbic acid added.

and the thiazolidone a moderate recovery. The addition of ascorbic acid as a reductant gave no improvement. The limit of determination for the thiocarbamate (in water, recovery 87%, 1 ml injection volume) was 1.2 μg/l, and for the thiazolidone (recovery 49%, 1 ml injection volume) 1.4 μg/l.

Determination and recovery from biological fluids

The two substances considered in the previous section were dissolved in water and added to urine. The recoveries are shown in Table VIII. The recovery of the dithiocarbamate again was good, while the thiazolidone recovery was worse than in water. Ascorbic acid or a strong reducing combination such as 1.5% of tin(II) chloride in 5 *M* hydrochloric acid gave an improvement of a factor of two. However with this reductant there were disturbing peaks in the gas chromatogram.

With the SE-30–ECD system, the maximum volume that could be injected was only 50 μl, because there was a large interfering peak just before that of carbon disulphide. The limits of determination for sodium dithiocarbamate (recovery 100%, 1 ml injection volume for FPD, 50 μl for ECD) were 1 μg/l for the FPD and 20 μg/l for the ECD, and for 2-thiathiozolidone-5 (47% recovery) 1.4 μg/l for the FPD and 48 μg/l in urine for the ECD.

The procedure used for urine was also used for blood (Table IX). The recoveries were low and dependent on concentration. The addition of ascorbic acid,

TABLE VIII

RECOVERIES OF SODIUM DIMETHYLDITHIOCARBAMATE AND 2-THIOTHIAZOLIDONE-5 IN URINE

Compound	*Concentration (mg/l)*	*Recovery (%)*
Sodium dimethyldithiocarbamate	0.5	100
2-Thiothiazolidone-5	0.4	29
	0.4	50*
	0.5	61**

* Ascorbic acid added.

** 1.5% $SnCl_2$ in 5 *M* HCl instead of 5 *M* H_2SO_4 added.

TABLE IX

RECOVERIES OF SODIUM DIMETHYLDITHIOCARBAMATE AND 2-THIOTHIAZOLIDONE-5 IN BLOOD

Compound	*Concentration (mg/l)*	*Recovery (%)*
Sodium dimethyldithiocarbamate	0.3	11
	8	61
2-Thiothiazolidone-5	0.5	7
	0.5	10*

* Ascorbic acid added.

lengthening the reaction time or the use of concentrated (instead of 5 *M*) sulphuric acid did not increase the recovery.

The limits of determination were (linear extrapolation) 10 μg/l for sodium dithiocarbamate and 10 μg/l for 2-thiathiozolidone-5.

For the determination *in vivo*, three rabbits were used. They were injected intraperitoneally with a solution of carbon disulphide in olive oil, beginning with 1 mg of carbon disulphide per kilogram body weight and subsequently 2, 4, 8, 16 and 32 mg/kg. The 24-h urine was collected, separated from the faeces and analyzed on the same day.

The urine of the untreated rabbits showed many peaks in the chromatogram that do not appear in the chromatogram of human urine. This was a problem especially with the SE-30–ECD combination as two unidentified peaks with a retention time about the same as that of carbon disulphide appeared. Even a 6-m column did not separate one of these peaks from that of carbon disulphide. As the chromatogram with the carbon black–FPD combination revealed that no carbon disulphide was present, we decided to use only this combination in further experiments.

As a measure of the detector response the logarithm of the peak height was measured rather than the logarithm of the peak area, because there were often drifting baselines (Fig. 7). Table X and Fig. 6 show that for two rabbits there is a good relationship between the amount of carbon disulphide injected and the amount of degraded carbon disulphide in urine. For one rabbit, the relationship was less clear. With the last

TABLE X

AMOUNTS OF CS_2 IN URINE OF RABBITS AFTER INTRAPERITONEAL INJECTION

Amount injected (mg/kg body weight)	*Amount of metabolite in urine as CS_2 (μg/l)**		
	Rabbit A	*Rabbit B*	*Rabbit C*
1	ND	ND	ND
2	ND	ND	ND
4	ND	110	60
4	ND	70	90
8	140	130	120
8	70	60	80
16	880	190	190
32	290	410	720

* ND = below 6 μg/l.

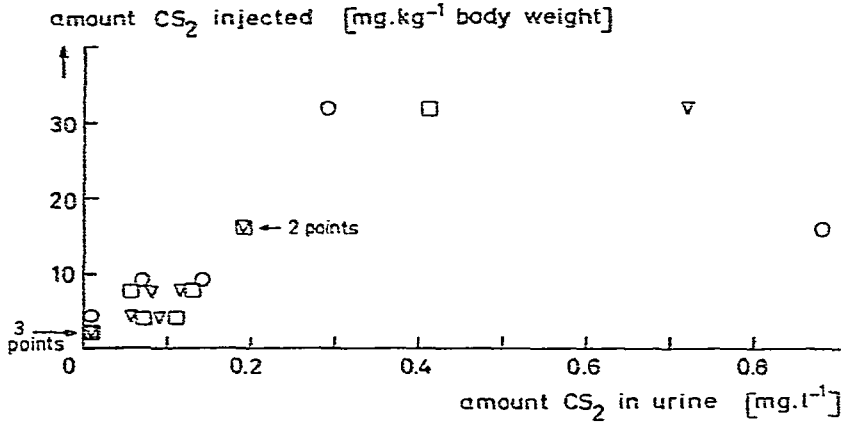


Fig. 6. Amounts of metabolite (as CS_2) in urine as a function of the amounts of CS_2 injected. ○, Rabbit A; □, rabbit B; ▽, rabbit C.

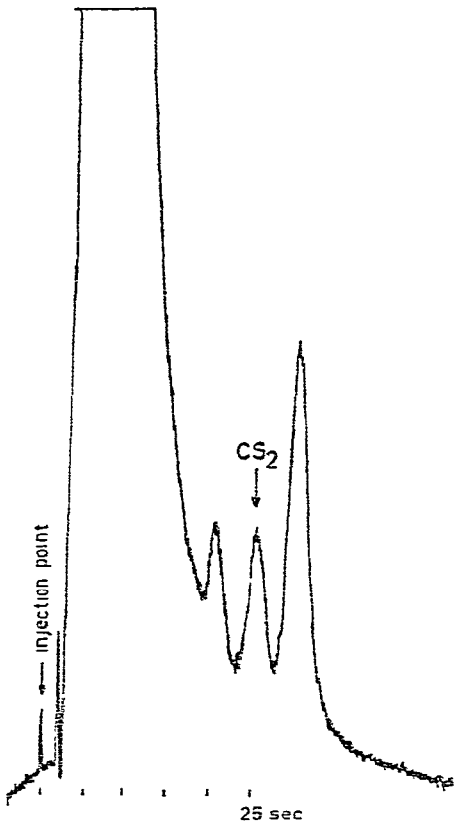


Fig. 7. Gas chromatogram of rabbit urine. Carbopack column with FPD detection. Sample size: 1.63 ng of CS_2.

injection (32 mg/kg) the amount of "free carbon disulphide" was measured by analyzing the headspace of urine without the addition of sulphuric acid and at room temperature. The amounts of "free carbon disulphide", expressed as a percentage of the total degraded carbon disulphide, were 2.7, 1.5 and 1.4% for the three rabbits

CONCLUSION

It can be concluded that the column–detector combinations described (SE-30–ECD and carbon black–FPD) are suitable for the analysis of urine for carbon disulphide and metabolites via the headspace method. The former combination has a lower detection limit and a better precision, while the latter combination shows a better selectivity. The former combination was unsuccessful in separating the carbon disulphide peak from interfering peaks in rabbit urine.

As recoveries of the most common metabolites, the dithiocarbamates, are good, the measurement of the exposure of personnel to carbon disulphide by analyzing their urine seems feasible. This will be the subject of a later paper. The method is less successful for the analysis of blood as the recoveries are low and dependent on concentration.

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