Blood concentration of carbon disulphide in "normal" subjects and in alcoholic subjects treated with disulfiram

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Abstract

Assay of free and acid labile carbon disulphide (free and total CS_2 respectively) in human blood was performed by gas chromatography/spectrometry. The method used a large dynamic head space volume and a "cryogenic trap". Blood CS_2 concentration was measured in 42 subjects not occupationally exposed to CS_2 (group A) and in 11 alcoholic subjects (group B) treated with disulfiram. Free CS_2 concentration showed a mean value of 261 ng/l in the 42 subjects in group A and 9482 ng/l in eight subjects of group B. Total CS_2 concentration was 897 ng/l and 40 084 ng/l in groups A and B respectively. Differences between the groups were highly significant for concentrations of both free and total CS_2. Total CS_2 concentration was about four times as high as free CS_2 concentration in both groups. A significant correlation was found between free and total CS_2 concentration both in group A and in group B. In the alcoholic subjects (group B), blood concentrations of both free and total CS_2 were related to time of sampling after treatment with disulfiram.

Carbon disulphide (CS_2) is a natural, ubiquitous polluting agent derived from various natural sources such as volcanic activity and combustion of organic substances, from biological (particularly microbial) processes, and from industrial activity.[1-5] It is widely known in occupational medicine because of the risk of serious toxic effects commonly identified in the past in the rubber, match, and viscose industries. This risk is now confined mainly to viscose production and to the exposure to CS_2 deriving from the production and use of dithiocarbamates.[5] The difficulty of accurate assay of CS_2 in biological fluids from subjects occupationally exposed to the substance[6-8] is due not only to the analytical methods, but also to the chemical properties of CS_2, which render it highly reactive with biological substrates. Research both in vitro and in vivo has shown that CS_2 in blood and biological substrates is in part free and in part subject to acid labile binding with blood proteins and, in general, with nucleophilic substances containing $-SH$, $-NH$, and $-OH$ groups.[9]

This paper describes a method of assaying blood CS_2 in subjects not occupationally exposed to CS_2 and in subjects treated with the dithiocarbamate disulfiram (Antabuse).

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Materials and methods

Carbon disulphide was assayed in the blood of 53 subjects subdivided into 42 "normal" subjects not occupationally exposed to CS_2 and 11 alcoholic subjects treated with disulfiram (Antabuse).

As occurs with dithiocarbamates, disulfiram metabolism gives rise to CS_2 production. Disulfiram was administered orally at a dosage of 200 mg/day for 19–38 days to eight of the 11 alcoholic subjects. Blood was sampled in these eight subjects between two and 72 hours from the last administration of disulfiram. One single dose of 200 mg of disulfiram was administered orally to the other three alcoholic subjects whose blood was sampled 0, 1, 2, 4, 6, 12, 24, and 48 hours after the administration of disulfiram. Informed consent was obtained from all alcoholic subjects.

TREATMENT AND STORAGE OF BLOOD SAMPLES

Blood samples (5–10 ml), taken with a glass syringe (disposable syringes were not used to avoid release of CS_2 from the rubber of the plunger), were placed in glass tubes containing two drops of 10% ethylenediaminetetracetate (EDTA) solution. The glass tube, filled to capacity to avoid any residual air

bubbles between the blood surface and the tube cap, was closed with a pierceable screw on cap fitted with a teflon rubber ring seal (pyrex or sovirel tubes were adapted by us for this purpose). After stirring briefly to favour the anticoagulant effect of EDTA, the blood samples were stored at 4°C until use.

DETERMINATION OF FREE AND BOUND CARBON DISULPHIDE (FREE CS_2 AND TOTAL CS_2)

Free CS_2

After shaking, 3 ml of blood were drawn off through the cap of the tube into a glass syringe and injected into a 70 ml glass bottle (Duran, Schott) closed with a pierceable screw on cap fitted with a teflon rubber ring seal (2·5 mm thick) arranged by us. The bottle and blood was shaken in a rotating agitator at room temperature for one hour.

DYNAMIC HEAD SPACE (STRIPPING)

By passing two needles through the cap, the bottle was connected to the gas chromatography carrier circuit; the incoming needle passed just over but did not touch the surface of the blood sample, and the outgoing needle barely protruded beyond the inner surface of the screw cap. Connection between the glass container and the carrier circuit was established or interrupted by means of a valve. The carrier (flow rate about 20 ml/min) was allowed to enter the glass container holding the sample, for exactly six minutes. Outgoing carrier entered a cryogenic trap, downstream from which (and before the gas chromatography column) was a splitter valve, to be left open throughout the stripping process.

Sample concentration—Carrier entering the container mixed with the volatile products that were released into the available head space and transported them to the cryogenic trap, made up of a 10 cm stainless steel tube with an internal diameter of 1 mm and a U bend containing 3 mg of Tenax in its central portion. Throughout the stripping process (six minutes), the trap remained immersed in an ethylene glycol bath at −20°C. When stripping was complete, with the splitter valve closed, the cryogenic trap was removed from the bath and heated for 2·5 seconds to 200–300°C. Closure of the stripper (when stripping was finished and before heating the cryogenic trap) re-established the normal carrier flow rate of 0·7–1·0 ml/min within the gas chromatography column. Heating the trap allowed the products adsorbed by the Tenax to be released into the gas chromatography column for analysis and identification. Apart from the cryogenic trap, all the tubing, connections, and valves were maintained at 80°C to avoid condensation of solvent on the tube walls.

TOTAL CS_2

One ml of blood was drawn off in a glass syringe through the pierceable cap of the tube (after shaking) and injected into a 70 ml glass container (identical to that described above) containing 2 ml 1% hydrochloric acid solution in water. The sample was then placed in an oven at 100°C for one hour and, after cooling, the same procedure as for free CS_2 was performed. Acid labile CS_2 was freed by acid hydrolysis and was determined, together with the free CS_2 blood concentration, as total (free plus bound) CS_2. The real acid labile CS_2 can be obtained by subtracting free (assayed separately) from total CS_2.

GAS CHROMATOGRAPHY MASS SPECTROMETRY ANALYSIS

A Hewlett Packard 5890 gas chromatograph with a silica capillary column of cross linked 5% phenyl methyl silicone (0·17 μm thick, 50 m long, 0·32 mm internal diameter) was used. The initial column temperature was maintained constant for five minutes and then programmed from 35°C to 100°C at 40°C/min. The carrier was purified helium with a flow rate of 0·8 ml/min. A Hewlett Packard 5970B selective detector (quadruple) was used for identification. Identification of CS_2 was obtained from the gas chromatography retention time, comparison of m/z 76 and 78 ions, and identification of their isotopic ratio (fig 1).

Standards and calibration

Blood samples (20–30 ml) from "normal" people were drawn daily from the blood bank for each repetition of four to six points of the calibration curve. Before use the blood sample was stripped with a flow of helium and it was rejected if its background concentration of CS_2 was more than twice that of double distilled water. The non-eliminable background value was subtracted from those of the standards. Calibration curves were prepared by adding scaled quantities of CS_2.

Figure 2 shows the linearity of the calibration curve for the scaled concentrations from 390 ng/l to 22 μg/l. One or more points on the line were repeated daily, together with the assay of the samples to be evaluated. The detection threshold for CS_2 was 25 ng/l and the coefficient of variation for all points was lower than 20%. Standards of CS_2 were prepared in H_2O and homogenised by ultrasound treatment. To prepare the calibration curve, standards were injected into the head space of the glass container (70 ml) containing 3 ml of blood.

STATISTICAL ANALYSES

Non-parametric (Kruskal-Wallis test (H) and Wilcoxon-Mann-Whitney test (Z)) and parametric (Student's t test (t) and analysis of variance (F)) tests were used to compare blood concentrations of CS_2 between the individual groups. Spearman rank correlation coefficients (r_s) and regression analyses were used for the correlations between free and total CS_2

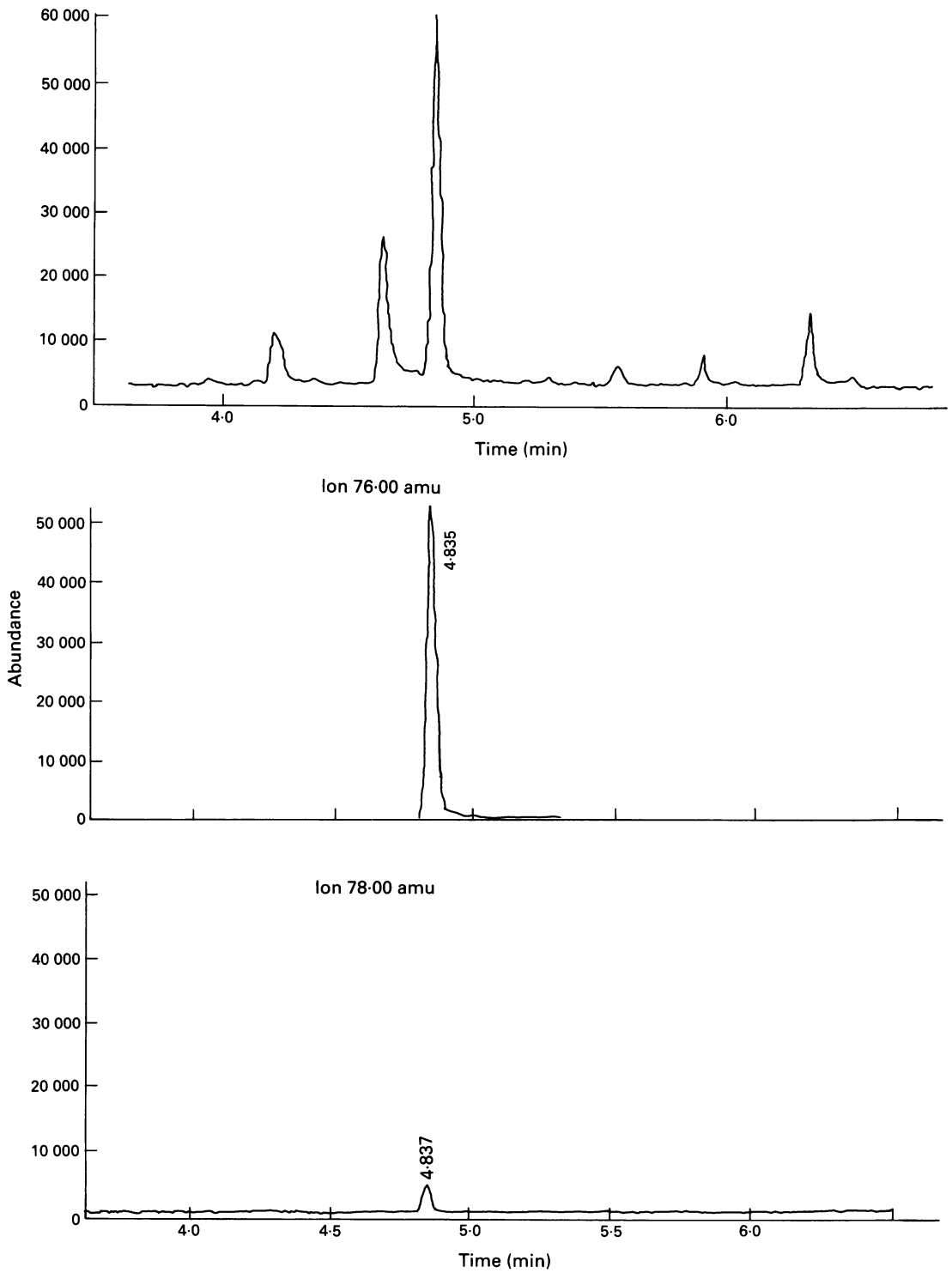

Figure 1 Gas chromatography/mass spectroscopic analysis of blood with identification of CS_2 by retention time (4·83 minutes), and ions 76 and 78 amu.

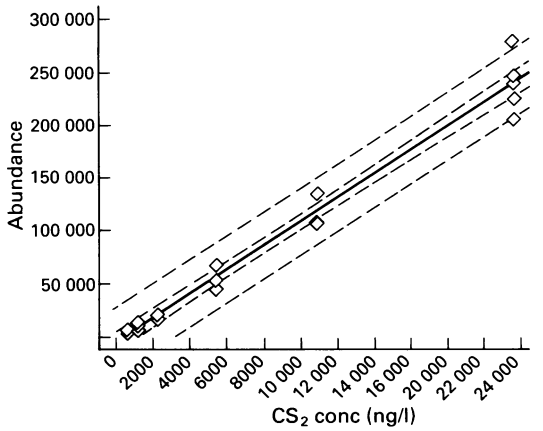

Figure 2 Calibration curve of CS_2 (correlation coefficient = 0·99).

concentration. A test was considered statistically significant with $p < 0·05$.

Results

Free CS_2 concentration had a mean value of 261 ng/l in the 42 normal subjects (group A) and 9482 ng/l in the eight alcoholic subjects treated for 19–38 days with disulfiram (group B). Total CS_2 concentrations were higher than those of free CS_2, with values of 897 ng/l and 40 084 ng/l in groups A and B respectively. Differences between the two groups were highly significant for both free and total CS_2 concentrations (table 1). Table 1 shows a similar total:free CS_2 ratio in the 42 normal subjects (3·9) and in the eight alcoholic subjects treated with multiple doses of disulfiram (4·3). The difference between groups was not significant. Figure 3 shows the distribution of values for the ratio among 50 subjects (groups A and B). Free CS_2 showed a significant relation with total CS_2 (table 2; fig 4), both within each group and overall. In the eight alcoholic subjects treated for 19–38 days, the times at which blood samples were taken varied from two to 72 hours after the last dose of disulfiram. These subjects showed no significant relation between the total intake of disulfiram and the blood concentration of CS_2, although blood CS_2 concentration was inversely proportional to the interval between the last administration of disulfiram and the time at which the blood sample was taken. The few data reported in figures 5 and 6 suggest an exponential reduction in free and total (free + bound) blood CS_2 concentration as the time to sampling increased. For group B, the mean blood half life of CS_2 was 11 hours for free CS_2 and seven hours for total CS_2 in the first 24 hours after last administration of disulfiram, and 23 and 17 hours respectively in the 72 hour interval.

Figure 7 shows the blood variations of free and total CS_2 in the three alcoholic subjects who were tested during the 48 hours after the administration of one single dose (200 mg) of disulfiram. Free CS_2 and total CS_2 concentration increased in blood after administration of disulfiram and reached maximum concentrations after 12 and 24 hours respectively. Moreover, after 48 hours, free and total CS_2 blood concentrations were still higher than those measured before administration of disulfiram (time zero).

Discussion

In experimental animals treated with CS_2 at much higher doses than the concentrations absorbed during occupational exposure, free and bound CS_2 are normally measured with ease in blood and other biological media by colorimetric methods.[10–12] In human subjects the monitoring of occupational exposure to CS_2 by its measurement in biological media has generally been considered unsuitable because of the poor reproducibility of the biological data.[6–8 13] Recently, Campbell *et al*[14] tried to measure free and acid labile CS_2 concentrations in the blood of workers occupationally exposed to the substance in the rayon industry. The detectable limit for CS_2

Table 1 Values for free (free CS_2), total (total CS_2) and ratio (total:free) of CS_2 concentration in blood of normal people (N) and alcoholic subjects (Alc)

	Free CS_2 (ng/l)		*Total CS_2 (ng/l)*		*Ratio Total CS_2:free CS_2*	
Group	*N*	*Alc*	*N*	*Alc*	*N*	*Alc*
No of subjects	42	8	42	8	42	8
Mean	261	9482	897	40084	3·90	4·30
Median	160	9070	627	24273	3·40	3·40
Geometric mean	195	4932	665	18484	3·40	3·70
SD	206	9025	759	40768	2·42	2·56
SE	32	3191	117	14414	0·37	0·90
Minimum	37	595	144	1448	1·30	1·60
Maximum	758	27055	3095	107130	12·70	9·60

Free CS_2; N:Alc; $F = 47·9$; $p < 0·0001$; $H = 18·6$; $p < 0·0005$; $t = 6·92$; $p < 0·0001$; $z = 4·31$; $p < 0·0005$.
Total CS_2; N:Alc; $F = 42·7$; $p < 0·0001$; $H = 18·1$; $p < 0·0005$; $t = 6·52$; $p < 0·0001$; $z = 4·25$; $p < 0·0005$.

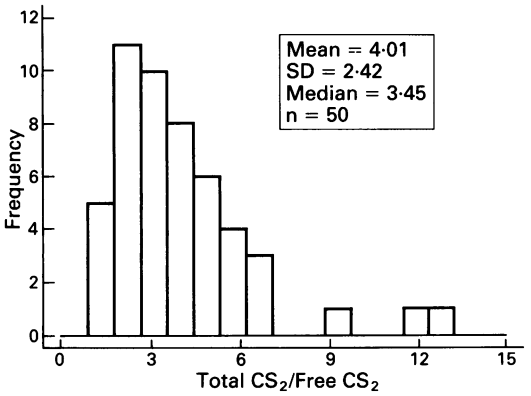

Figure 3 Distribution of the ratio values between total and free CS_2 concentrations (total CS_2:free CS_2) in the blood of 42 "normal" and eight alcoholic subjects.

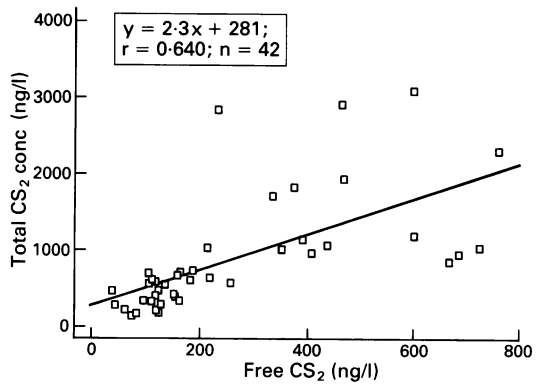

Figure 4 Correlation between free CS_2 and total CS_2 in "normal" subjects ($y = 2{\cdot}3 \times + 281$; $n = 42$; $r = 0{\cdot}640$; $p < 0{\cdot}001$; $r_s = 0{\cdot}817$; $p < 0{\cdot}00001$).

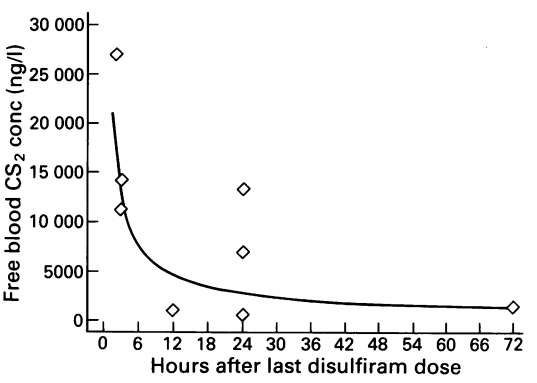

Figure 5 Correlation between free CS_2 (y) and time interval (x) after the last administration of disulfiram ($y = \log 10{\cdot}206 \times {}^{-0{\cdot}710}$; $r = -0{\cdot}640$; $n = 8$; NS).

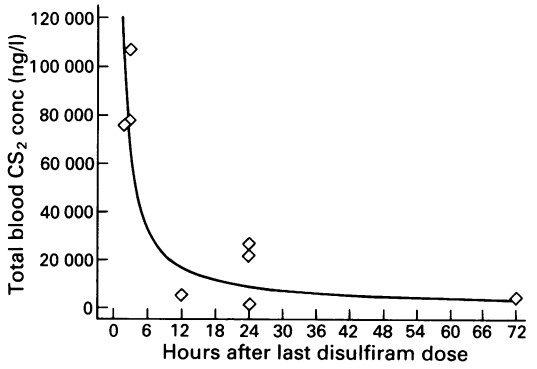

Figure 6 Correlation between total CS_2 and interval after the last administration of disulfiram ($y = \log 12{\cdot}049 \times {}^{-0{\cdot}927}$; $r = 0{\cdot}764$; $n = 8$; $p < 0{\cdot}05$).

concentration in blood was 15 μg/l. With this limit, determination of blood CS_2 concentrations in control subjects and in workers tested before shift work was impossible. Only at the end of the shift were values of blood bound CS_2 (332 (first workday) and 228 μg/l (fifth work day)) measurable. Our dynamic head space method for blood CS_2 assay has two characteristic features: (1) a large head space volume (70 ml) compared with the blood sample volume (3 ml); (2) a cryogenic trap that makes it possible to concentrate CS_2 after its aerodispersion in the large head space. The gas is first adsorbed in the cryogenic trap by the Tenax because of the low temperature, and is subsequently released when the trap is heated to 200–300°C. With the detectable limit of our method equal to 25 ng/l, it was possible to detect blood concentrations of CS_2 in "normal" people (group A) within the following range (table 1): free CS_2 from 37 ng/l to 758 ng/l and total CS_2 from 144 ng/l to 3095 ng/l.

To verify the validity of our methods, especially at maximum blood concentrations, we tested blood samples from alcoholic subjects treated with disulfiram (Antabuse). Metabolism of this compound, as for all dithiocarbamates, produces CS_2. Data in table 1 show that after the 19–38 days of treatment with disulfiram, the mean concentration of free CS_2 was 9482 ng/l (about 35 times that for normal subjects (261 ng/l)) and the mean value of total CS_2 was 40 084

Table 2 Correlations between free CS_2 (x) and total CS_2 (y)

Group A	$y = 2{\cdot}3x + 281$; $r = 0{\cdot}640$; $n = 42$; $p < 0{\cdot}001$; $r_s = 0{\cdot}817$; $p < 0{\cdot}00001$
Group B	$y = 3{\cdot}2x + 10073$; $r = 0{\cdot}701$; $n = 8$; p = NS; $r_s = 0{\cdot}762$; $p < 0{\cdot}05$
Groups A + B	$y = 3{\cdot}7x + 731$; $r = 0{\cdot}845$; $n = 50$; $p < 0{\cdot}001$; $r_s = 0{\cdot}883$; $p < 0{\cdot}00001$

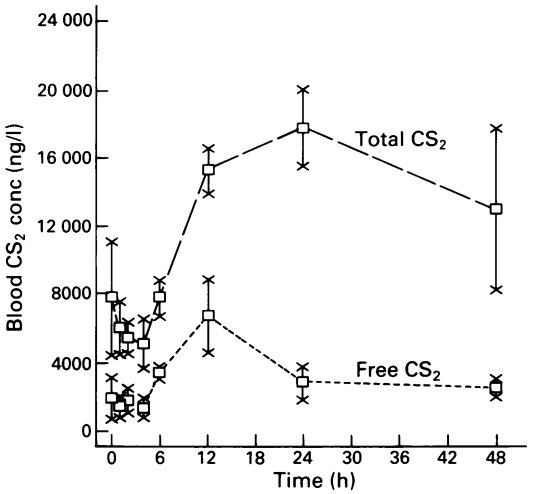

Figure 7 Variations of free and total CS_2 concentration in the blood of three subjects after one single dose (200 mg) of disulfiram.

ng/l (about 50 times that for normal subjects (897 ng/l)). Moreover, fig 7 shows that free CS_2 concentration 12 hours after one single dose of disulfiram, reached about 6500 ng/l, and the total CS_2, after 24 hours, about 18 000 ng/l. The ratio between total and free CS_2 was not related to the concentrations. Total CS_2 was about four times higher than free CS_2 in both normal (total:free CS_2 = 3·9) and alcoholic (total:free CS_2 = 4·3) subjects. A similar ratio for bound:free CS_2 (3) was also found in animals after inhalation of CS_2.[11] Free and total CS_2 concentrations correlated with each other (table 2), both in groups A and B and in the two groups considered together.

The higher blood CS_2 values found in alcoholic subjects (group B) were not significantly related to the duration of treatment (total disulfiram dosage), but were inversely proportional to the interval between the last administration of disulfiram and the time at which the blood sample was taken (figs 5 and 6). The blood half life of CS_2 in the first 24 hours after administration of disulfiram was 10·6 hours for free CS_2 and 6·9 hours for total CS_2. In the 72 hours after the end of the treatment with disulfiram, half life of CS_2 was 22·8 and 17·3 hours respectively for free and total CS_2. The half life values we found are close to those calculated in alcoholic subjects treated with Antabuse.[15] Analysis of expired air collected from these subjects, treated orally with 500 mg disulfiram, indicated a half life of free CS_2 of about 11 hours.

On the basis of our data it seems reasonable to suggest that the method presented here can be of use in evaluating occupational exposure to CS_2. Biological monitoring by assaying blood CS_2 concentrations in occupationally exposed subjects could confirm the validity of this approach. The appearance of high concentrations of CS_2 after the administration of disulfiram, moreover, suggests that our method could be suited in the biological monitoring of exposure to dithiocarbamates.

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