Effects of a single exposure to carbon disulphide on the rate of urea production and on plasma free fatty acid and glucose concentrations in the rat

V. J. CUNNINGHAM

Medical Research Council Toxicology Unit, Biochemical Mechanisms Section,
Medical Research Council Laboratories, Carshalton, Surrey, SM5 4EF

Cunningham, V. J. (1975). *British Journal of Industrial Medicine*, 32, 140-146. **Effects of a single exposure to carbon disulphide on the rate of urea production and on plasma free fatty acid and glucose concentrations in the rat.** The concentration of plasma free fatty acids in rats was significantly increased after a short period of exposure to inhalation of carbon disulphide (4 h, 2 mg/l). In contrast, after a longer period of exposure (15 h overnight, 2 mg/l) the concentration of plasma free fatty acid was significantly decreased despite a small hypoglycaemia. At the same time plasma urea concentration was significantly higher in CS₂-treated rats. The total esterified fatty acid content of plasma was lower after exposure, but there was no change in plasma glycerol. Following an intragastric water load, no differences were observed in urine flow rate nor in renal clearances of urea and inulin between control and treated rats. It is concluded that the rate of urea production is significantly increased during acute CS₂-intoxication, and it is suggested that two factors contribute to this effect: first, an increased breakdown of proteins with which CS₂ or its metabolic products have reacted; and secondly an increased rate of utilization of plasma glucose associated with increased gluconeogenesis from amino acid precursors. It is further suggested that the stress effects of CS₂ dominate in the short term before being overcome by a diminished sympathetic response. When rats were exposed to CS₂ overnight without free access to water, the great vessel haematocrit was significantly lower than in corresponding controls. This was shown to be accounted for by differences in plasma volume. No such difference was observed when rats had free access to water during exposure. These effects probably reflect differing rates of water loss under mildly dehydrating conditions, but a direct effect of CS₂ on the cardiovascular system is not excluded.

Carbon disulphide intoxication in man is associated with a wide variety of symptoms, ranging from headache and loss of appetite following acute exposure to an increased number of deaths due to heart disease with chronic exposure (Tiller, Schilling, and Morris, 1968; Hernberg *et al*, 1970; Brieger, 1961). Although the threshold limit value for CS₂ is at present 60 µg/l, acute exposure to levels of CS₂ as high as 6 mg/l may occur if precautions are not

taken at various stages in the manufacture of viscose rayon (Rosensteel, Shama, and Fleisch, 1973). The work presented in this paper was undertaken as part of a study on the effects of acute intoxication in the rat.

During the course of this study an abnormal relationship between free fatty acid (FFA) and glucose concentrations in plasma after CS₂ exposure was found and was associated with an increased

concentration of plasma urea. It is the purpose of this paper to consider the possible relationship between these effects. In addition, routine measurements of haematocrits prompted a study of plasma volume after exposure to CS₂. It was thought originally that the effects of CS₂ on plasma volume and on plasma urea might be directly related in terms of an impairment of renal function. Evidence is presented that this is not the case. The changes in plasma volume appear to reflect a differing response to the mildly dehydrating conditions involved in the routine of exposure. The lines upon which further research will proceed are indicated, and suggestions for simple chemical observations on men acutely exposed to CS₂ are put forward.

Methods

Treatment of animals

Male albino rats of the Porton strain were fed on MRC diet 41B and weighed 200–250 g in the fed state. Food was removed at 09.30 h prior to rats being exposed either from 10.00–14.00 h or overnight (18.30–09.30 h) to 2 mg carbon disulphide per litre of air in a vertical constant flow exposure chamber as described by Magos *et al.* (1970). Control rats were kept in similar chambers without CS₂.

Plasma sampling

Rats were decapitated either within one hour of their removal from the chambers or at the end of the renal clearance experiments described below. In the former case, but not the latter, rats were anaesthetized with ether before decapitation. Blood was collected in polypropylene centrifuge tubes containing a small amount of powdered heparin. Plasma was separated by centrifugation at 10 000 g for 10 minutes.

Assay techniques

Free fatty acids were extracted from samples (1 ml) of plasma into heptane by the method of Dole and Meinertz (1960) as modified by Trout, Estes, and Friedberg (1960) and titrated as described by Cunningham (1973). Glucose (hexokinase method), urea, and glycerol concentrations in plasma were measured using assay kits supplied by the Boehringer Corporation (London) Ltd. For assay of glycerol, samples (0.5 ml) of plasma were first deproteinized with an equal volume of trichloroacetic acid (10% w/v) and the latter was removed by ether extraction (Garland and Randle, 1962). Total esterified fatty acids were determined in samples (0.3 ml) of plasma or in extracts of liver (see below) by the method of Stern and Shapiro (1953) using Tripalmitin (Sigma Chemical Co.) as a standard. Samples (0.5 g) of liver were excised and homogenized in ethanol/diethyl ether (50 ml, 3/1 v/v). The extract was boiled and filtered and the residue was washed twice with hot ethanol/ether (5 ml). Aliquots of the filtrate were taken for analysis of total esterified fatty acid.

Plasma and red cell volumes

¹²⁵I-labelled human serum albumin and sodium chromate (⁵¹Cr) solution were obtained from The Radiochemical

Centre, Amersham, Bucks. Donor rats received labelled albumin (4 μ Ci in 0.2 ml saline (0.9% w/v)) via a tail vein on the day preceding an experiment to screen for any component of the label that might be removed rapidly from the plasma (Dewey, 1959). Blood was collected into heparin by aortic cannulation under ether anaesthesia shortly before experiments, and ¹²⁵I-labelled plasma was separated by centrifugation. ⁵¹Cr-tagged red cells were prepared essentially as described by Gray and Sterling (1950), as modified by Chaplin (1954) and Huggins, Smith, and Deavers (1966), and resuspended to a haematocrit of about 40% in ¹²⁵I-labelled plasma. Rats received about 0.1 ml doubly labelled blood via a tail vein from a weighed syringe, and after 15 minutes they were decapitated and blood was collected in heparin. Samples of collected blood and of injected material were analysed for ¹²⁵I and ⁵¹Cr in a Panax Gamma 160 counter with suitable correction for overlap of ⁵¹Cr counts in the ¹²⁵I channel. Haematocrits were determined in duplicate and corrected for entrained plasma (Heath, 1973). Plasma and red cell volumes were calculated from the dilution of injected material after correction for the volume of the injected material and for loss of plasma label from the circulation (Dewey, 1959).

Renal clearance experiments

These experiments were based on the format of Dicker and Heller (1945). Within one hour of removal from the inhalation chambers, rats received tepid water intra-gastrically (5% body weight) and at the same time a subcutaneous injection of inulin (5% w/v) in physiological saline (0.5 ml per 100 g body wt) containing about 1 μ Ci/ml (hydroxymethyl-¹⁴C)-inulin (Radiochemical Centre, Amersham, Bucks). One hour after the subcutaneous injection rats were made to urinate by gentle suprapubic pressure, this urine being discarded. Rats were then placed in individual metabolic cages which allowed collection of urine (Sharratt, 1970). After a measured period of about 30 minutes rats were made to urinate in the cages, blood samples then being collected after decapitation, and the concentrations of urea and total radioactivity were determined in plasma. Samples of urine were taken for assay of urea and radioactivity. The cages were rinsed with water, the total collected volume of urine plus rinses was recorded, and samples were taken for assay of total radioactivity excreted.

Samples of plasma (0.1 ml), urine (0.5 ml), and rinses (1.0 ml) were added to 10 ml of Instagel scintillation mixture (Packard Instrument Co. Inc.) and ¹⁴C-dpm counted using a Philips Liquid Scintillation Analyser PW 4510/01. Efficiency of counting was monitored with an external standard.

Clearance rates of urea (C_{urea}) and of inulin (C_{in}) are expressed as millilitres plasma cleared per minute per 100 g body weight at the time of the experiment. The calculation of the rates from the above information assumes that the plasma concentrations of urea and of (hydroxymethyl-¹⁴C) inulin were constant throughout the period of urine collection. This appeared to be the case for urea (see Results section) but was not established for (hydroxymethyl-¹⁴C) inulin. The resulting over-estimation of C_{in} is however likely to be small, would apply to both groups of rats considered, and would not affect the conclusions reached.

Results

Free fatty acid and glucose concentrations in plasma after acute exposure of rats to CS₂

In rats exposed to CS₂ (2 mg/l) from 10.00–14.00 h, a significant increase in the concentration of FFA in plasma relative to that of controls was observed (Table 1). In contrast, after a longer period of exposure (2 mg/l, 18.30–09.30 h overnight) there was a significant decrease (Table 1). Rats were exposed overnight without access to food and with or without access to water. In each case a significantly lower plasma FFA concentration was observed in

CS₂-treated rats despite a small but significant hypoglycaemia (Table 2). Tarkowski and Cremer (1972) also report hypoglycaemia after 15 hours' exposure. Glycerol concentrations in plasma were measured as an indication of the rate of lipolysis in adipose tissue, but there were no significant differences in this parameter (Table 2).

The esterified fatty acid content of plasma was significantly lower in treated rats (Table 2), although there was no indication of fat accumulation in liver. The esterified fatty acid content of liver in eight CS₂-treated rats was 133 ± 8 μ equiv/g wet weight (means $\pm$ SEM) and in eight controls, 142 ± 10

TABLE 1
FREE FATTY ACID CONCENTRATIONS IN PLASMA OF RATS ACUTELY EXPOSED TO CS₂

Treatment	Plasma FFA (μ equiv/ml)	
	Control	CS ₂ -treated
2 mg CS ₂ /l 10.00 h–14.00 h Rats in postabsorptive state	0.481 ± 0.024 (24)	0.600 ± 0.038 (24) P < 0.02
2 mg CS ₂ /l 18.30 h–09.30 h 24 h starved rats NO ACCESS to water during exposure	0.808 ± 0.026 (37)	0.654 ± 0.019 (39) P < 0.001
2 mg CS ₂ /l 18.30 h–09.30 h 24 h starved rats FREE ACCESS to water	0.821 ± 0.027 (16)	0.673 ± 0.021 (16) P < 0.001

Results are given as mean $\pm$ SEM (number of rats). The significance of differences between means was assessed by Student's *t* test.

TABLE 2
OVERNIGHT EXPOSURE OF RATS TO CS₂ (2 mg/l, 15 h)

Condition	Concentrations in plasma				Haematocrit
	Glucose (mM)	Glycerol (mM)	Esterified fatty acid (μ equiv/ml)	Urea (mM)	
Without free access to water during exposure					
Control	6.97 ± 0.17 (24)	0.166 ± 0.010 (16)	5.72 ± 0.17 (28)	8.72 ± 0.49 (6)	46.5 ± 0.4 (23)
CS ₂ treated	5.83 ± 0.18 (24)	0.169 ± 0.011 (16)	4.30 ± 0.10 (30)	11.2 ± 0.8 (6)	41.9 ± 0.3 (23)
P	< 0.001	NS	< 0.001	< 0.05	< 0.001
With free access to water during exposure					
Control	7.00 ± 0.18 (16)	0.207 ± 0.008 (16)	5.76 ± 0.18 (15)	5.63 ± 0.20 (6)	—
CS ₂ treated	6.26 ± 0.18 (16)	0.187 ± 0.007 (16)	4.07 ± 0.23 (15)	9.11 ± 0.36 (6)	—
P	< 0.01	NS	< 0.001	< 0.001	—

All rats had been starved for 24 h. Results are expressed as mean $\pm$ SEM (number of rats). The significance of differences between means was assessed by Student's *t* test.

$\mu\text{equiv/g}$ wet weight. In addition, it was observed that CS_2 -treated rats had significantly higher plasma urea concentrations, the increase relative to controls being greater in rats given free access to water (Table 2).

Plasma and red cell volumes

Lower haematocrit values were consistently observed in rats exposed to CS_2 when there was no free access to water during exposure (Table 2). Because changes in plasma volume might affect the concentration of plasma constituents, and because this effect might reflect a direct action of CS_2 on the cardiovascular system, an analysis of the factors affecting the observed haematocrit was carried out after simultaneous intravenous injection of ^{125}I -labelled albumin and ^{51}Cr -tagged red cells (Table 3).

When control rats not allowed access to water are compared with control rats allowed access to water, a small but significant decrease in plasma volume, expressed as % body weight at the time of determination, is seen to account for an increased great vessel haematocrit. This effect is consistent

with reports that the plasma volume in rats reduces proportionately more than body weight under dehydrating conditions (Kutscher, 1968; Horowitz and Borut, 1970; Mogharabi and Haines, 1973). No significant differences were observed in total red cell volume or in F_{cells} (the ratio between the true whole blood haematocrit and the observed great vessel haematocrit (Gregersen and Rawson, 1959)). Thus the observed changes in haematocrit are attributable solely to changes in plasma volume.

In contrast, no such effect on plasma volume was seen in the case of CS_2 -treated rats (Table 3) and there were also no changes in total red cell volume or in F_{cells} . The difference in haematocrit between control and CS_2 -treated rats when there was no free access to water (Table 2) may therefore stem from the differing response of the rats to conditions of mild dehydration. Either the CS_2 -treated rats did not dehydrate to the same extent as control rats or they had an abnormal water distribution.

Renal function tests

Two observations prompted the renal function tests

TABLE 3

PLASMA AND RED CELL VOLUMES IN RATS EXPOSED TO CS_2 (2 mg/l) OVERNIGHT WITH AND WITHOUT FREE ACCESS TO WATER

		Free access to water	No access to water	
Control	Plasma volume	4.37 $\pm$ 0.08 (8)	4.10 $\pm$ 0.08 (6)	P < 0.05
	Red cell volume	2.31 $\pm$ 0.04	2.37 $\pm$ 0.04	NS
	Haematocrit	42.6 $\pm$ 0.6	44.4 $\pm$ 0.5	P < 0.05
	F cells	0.814 $\pm$ 0.008	0.826 $\pm$ 0.008	NS
CS_2 -treated	Plasma volume	4.46 $\pm$ 0.7 (9)	4.38 $\pm$ 0.06 (7)	NS
	Red cell volume	2.29 $\pm$ 0.05	2.30 $\pm$ 0.03	NS
	Haematocrit	42.2 $\pm$ 0.4	42.3 $\pm$ 0.3	NS
	F cells	0.827 $\pm$ 0.013	0.839 $\pm$ 0.010	NS

Volumes are expressed as ml/100 g body weight at time of determination. Results are expressed as mean $\pm$ SEM with number of rats in parentheses.

TABLE 4

INULIN AND UREA CLEARANCES IN CS_2 TREATED AND CONTROL RATS

	Plasma urea concentration (mM)	Rate of urea excretion ($\mu\text{moles/min/100 g body wt}$)	C_{urea}	C_{in} (ml/min/100 g body wt)	Urine flow rate
CS_2	10.13 $\pm$ 0.46	4.14 $\pm$ 0.41	0.409 $\pm$ 0.036	0.851 $\pm$ 0.052	0.052 $\pm$ 0.006
Control	6.83 $\pm$ 0.32	2.76 $\pm$ 0.36	0.404 $\pm$ 0.049	0.909 $\pm$ 0.060	0.044 $\pm$ 0.004
	P < 0.001	P < 0.05	NS	NS	NS

All rats were starved for 24 h but had free access to water during exposure. After exposure in the presence or absence of CS_2 (2 mg/l, 15 h) rats received a subcutaneous injection of labelled inulin and an intragastric water load. After 1 h, urine was collected for a period of about 30 min. Inulin clearance and urine flow rate were measured in nine rats in each group, and urea clearance in six of these nine rats.

(Table 4): first, a significantly higher plasma urea concentration in treated rats (Table 2), and, second, a possible change in fluid balance. In rats exposed to CS₂ (2 mg/l) overnight with free access to water, and in corresponding controls, inulin clearance and urine flow rate were the same after an intragastric water load (Table 4). The difference in plasma urea concentration between the two groups at the end of the clearance experiments (Table 4) was similar to that observed in separate experiments immediately after exposure (Table 2), and plasma urea concentrations were therefore assumed to be constant while renal clearance was measured. This difference in concentration between groups was accompanied by a corresponding difference in the rate of urea excretion, i.e., plasma urea clearance was the same in both groups (Table 4).

Discussion

The renal function tests carried out on rats following acute CS₂-intoxication indicate that the raised plasma urea is not due to any renal malfunction leading to a decreased elimination of urea but to an increased rate of urea production in the animal. Although the possibility of a reversible change during exposure is not excluded, it is clear that there is no direct relationship in terms of renal function between the raised plasma urea and the effects of CS₂ on plasma volume. After a period of overnight exposure to CS₂ the rats showed signs of sedation, and it is possible that the evaporative loss of water may have been affected by differences in physical activity and pulmonary ventilation during exposure. No significant differences in weight loss during exposure were observed (results not shown), but it is emphasized that the conditions of dehydration were mild and that plasma volume in control rats decreased proportionately more than body weight. It is therefore not possible to distinguish, on the basis of these results, an effect of CS₂ on water loss from an effect on fluid distribution in the animal.

The two most obvious changes in concentration of rat plasma constituents after CS₂ intoxication were those of urea and esterified fatty acids. These concentrations are easily determined and may be of some use in the assessment of the degree of acute CS₂-intoxication in man, although the dependence of both concentrations on a variety of factors, notably diet, would reduce their diagnostic usefulness. On inhalation CS₂ is taken up by all tissues in the body where it is found in both free and bound forms (Brieger, 1967). It may react directly with a variety of amino acid side chains (Vašák and Kopecký, 1967) and has, for example, been shown to react with plasma albumin (Brieger, 1967). The increased rate of urea production may therefore reflect, in part, the breakdown of such amino acids and

proteins with which CS₂ or its metabolic products have reacted. A further possible contributory cause of the increased rate of urea production, namely an increased demand for glucose in the whole animal, is discussed later.

If the fall in plasma concentration of esterified fatty acid after CS₂ is due to decreased secretion of lipoprotein by the liver, then two factors may be involved: (a) a lower plasma-free fatty acid concentration leading to reduced uptake of free fatty acid by the liver, and (b) decreased synthesis of the protein moiety of lipoproteins. This latter factor may be related to an increased breakdown of amino acids or to a transient inhibition of protein synthesis in the liver of CS₂-treated rats (Bond and de Matteis, 1969). However, no accumulation of esterified fatty acid in total liver samples was observed in the present work, and in the absence of more detailed study of plasma triglyceride turnover after acute CS₂-intoxication no further comment can be made.

The rise in plasma FFA concentration which occurred after four hours' exposure is in marked contrast to the fall which took place after 15 hours' exposure, despite an associated hypoglycaemia. Preliminary investigations indicate no difference in the fractional turnover rate of plasma FFA after CS₂-intoxication (Cunningham, unpublished observations), and it is therefore assumed that the fall in plasma FFA concentration is due to a decreased output of FFA from adipose tissue. The absence of any significant differences in plasma glycerol concentrations suggests that an increased re-esterification of FFA in adipose tissue rather than decreased lipolysis was occurring. Although the hypoglycaemia is small in itself, failure of the rat to respond by an expected increased output of FFA from adipose tissue is significant. Because of the role of catecholamines in the release of FFA from adipose tissue and in the supply of plasma-borne substrates to the tissues, such an abnormal response to a lowered glucose concentration is consistent with the report of Magos and Jarvis (1970) that successive exposures of rats at the level used here (2 mg/l) results in a progressive inhibition of catecholamine synthesis. Magos, Jarvis, and Butler (1974) have put forward an hypothesis relating symptoms of CS₂ intoxication to a disturbed catecholamine metabolism. However, it is emphasized that after a short exposure of four hours an increased plasma FFA concentration was observed. It is possible that during such an exposure a non-specific stressful effect dominates before being overcome by progressive inhibition of the sympathetic response.

What light do the changes observed in these experiments throw on the mode of action of CS₂? If the changes observed in plasma substrate concentrations may be attributed principally to a single central effect, two observations in the present work

suggest that the measurement of the turnover rate of plasma glucose in the whole animal may be of importance. In the normal rat, the fractional turnover rate of plasma glucose correlates positively with its concentration (Heath and Corney, 1973), such that a fall in the latter is associated with a proportionately greater fall in the absolute rate of its utilization. That this relationship might not hold when CS₂-treated rats are compared with corresponding controls is consistent with (a) a lower output of FFA from adipose tissue, probably due to an increased rate of re-esterification despite hypoglycaemia, and (b) the higher rate of urea production if this were consequent upon an increased rate of gluconeogenesis from amino acid precursors. In addition, Kürzinger and Freundt (1969) report diminution of liver glycogen in rats exposed to CS₂, suggesting an increased demand for glucose. While the observed changes might also reflect a lower overall metabolic rate in the CS₂-treated rat, the above hypothesis can be tested by the direct determination of the fractional turnover rate of plasma glucose which will form the basis for future work.

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