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Biochemical Pharmacology, Vol. 22, pp. 129–132. Pergamon Press, 1973. Printed in Great Britain.

Effect of chronic exposure to carbon disulphide upon some components of the electron transport system in rat liver microsomes

(*Received* 13 *April* 1972; *accepted* 31 *July* 1972)

A SINGLE oral dose[1] or short inhalation[2,3] of carbon disulphide leads to the inhibition of oxidative metabolism of foreign compounds in rat liver microsomes. Bond and De Matteis[1] proved that these changes are accompanied by the decrease of the microsomal level of cytochrome P-450. This, however, was not confirmed by the recent data reported by Freundt and Schauenburg.[4] The present studies were concentrated on the electron transport system in rat liver microsomes in the early stage of the chronic CS_2 intoxication.

The studies were carried out in two separate laboratories within 10-month intervals. The first experiment had been performed in January and February 1971 in the Institute of Industrial Hygiene and Occupational Diseases, Prague (Experiment 1) while the second one was carried out in November and December of the same year in the Institute of Occupational Medicine in Łódź (Experiment 2). In the first experiment male albino rats (SPF) were exposed in a toxicological chamber to CS_2 vapours at a concentration of 1·2 mg/l of air for 6 hr daily, 5 days a week. In the second experiment male albino rats (Wistar) were exposed to CS_2 at a concentration of approximately 1·5 mg/l of air for 5 hr daily, 6 days a week. The control animals were kept over the same time period without exposure. In both experiments rats were sacrificed in the 6th and 7th week of the experiment, immediately after termination of the daily exposure. After decapitation, the liver was immediately removed and washed with cold 1·15% KCl, blotted and weighed. Liver microsomes were isolated from homogenate prepared in cold 1·15% (w/v) KCl in the way described by Bond and De Matteis.[1] Following the last centrifuging at 78,000 *g*, the microsomal pellet was suspended in 0·02 M phosphate buffer pH 7·5.

The amount of cytochrome b_5 was determined from the difference spectra of the microsomal suspensions (2·5 mg protein/ml) in NADH reduced and oxidized state. An extinction coefficient of 185 mM^{-1} cm^{-1} for the difference in absorbance at 424 and 409 nm[5] was used. The amount of cytochrome P-450 was determined from the difference spectra between the microsomal suspension (1·25 mg protein/ml) reduced with $Na_2S_2O_4$ and subsequently treated with carbon monoxide and the $Na_2S_2O_4$-reduced control: an extinction coefficient of 91 mM^{-1} cm^{-1} for the difference in absorbancy at 450 and 490 nm[6] was used. All spectra were recorded with a Unicam SP 800 spectrophotometer with SP 850 Scale Expansion Accessory and slave recorder EZ 3 (manufacturer: Laboratorní Přístroje, National Enterprise, Praha).

The NADPH cytochrome *c* and NADPH ferricyanide reductase activity was measured according to the method of Williams and Kamin,[7] and the NADH cytochrome *c* and NADH ferricyanide reductase activity was measured according to the method of Jones and Wakil.[8] An extinction coefficient of 1·02 mM^{-1} cm^{-1} at 420 nm for ferricyanide[9] and of 21·1 mM^{-1} cm^{-1} for the difference in the reduced and the oxidized cytochrome *c* at 550 nm[10] were used in calculations.

Proteins were determined by the method of Lowry *et al.*[11] using bovine serum albumin, fraction V as standard.

Five to seven weeks exposure of rats to carbon disulphide at a concentration of 1·2–1·5 mg/l did not result in any symptoms of chronic poisoning. The body weight of the exposed animals was also unchanged as compared with that of controls. However, the exposure resulted in an increase of the liver weight by approximately 14 per cent as compared with the control (Table 1). This phenomenon

TABLE 1. LIVER WEIGHT AND MICROSOMAL PROTEIN IN THE LIVER OF RATS EXPOSED TO CS_2

	Liver	Microsomal protein
Group	g/100 body wt	mg/total liver per 100 g body wt
CS_2	3·54 ± 0·07*(13)	49·9 ± 1·6†(10)
Control	3·11 ± 0·05(16)	43·1 ± 1·8(12)

Values given are the means ± S.E.M. of the number of observations in parentheses. Difference statistically significant in relation to control:
*$P < 0.001$,
†$P < 0.02$.

had been already observed in the case of a single exposure to carbon disulphide.[1,12] The total liver microsomal proteins increased parallely to the liver weight (about 16 per cent, Table 1), due to which no changes in its concentration can be noticed.

Table 2 presents the level of cytochromes P-450 and b_5 as well as the activity of NADPH cytochrome *c* and NADPH ferricyanide reductases and NADH cytochrome *c* and NADH ferricyanide reductases in the liver microsomes following the CS_2 exposure. The normal activities of NADPH and NADH cytochrome *c* reductases varied significantly in rats from both laboratories. Therefore the results of both experiments have been presented separately.

The level of cytochrome P-450 calculated in relation to the proteins was decreased by 47 per cent. A slight tendency of the cytochrome b_5 level to increase was found. The changes observed with respect to the cytochrome P-450 were similar to these found by Bond and De Matteis[1] in single exposure to CS_2.

Although the level of the microsomal cytochrome P-450 of rats exposed to CS_2 was significantly lowered, the activity of NADPH cytochrome *c* reductase showed a tendency to increase and that of NADH cytochrome *c* reductase was elevated by approximately 60 per cent. The activity of NADPH and NADH ferricyanide reductases remained unchanged.

The mechanism by which carbon disulphide influences the microsomal respiratory chain has not been elucidated so far. As shown in this report the exposure to carbon disulphide results in changes of only some components of microsomal electron transport system (cytochrome P-450, NADH cytochrome *c* reductase) and these changes are of opposite direction. This fact suggest a more specific effect than the simple inhibition of the protein synthesis which had been observed soon after administration of a single dose of CS_2.[1] It is worthwhile to notice that the CS_2 exposure results in the increase of the activity of lipid dependent[8] NADH cytochrome *c* reductase while NADH ferricyanide reductase, a lipid independent enzyme[8] is not subject to any changes. The level of phosphatydylocholine and lisophosphatydylocholine-phospholipids, which are responsible for the activity of NADH cytochrome *c* reductase[8] show the increasing tendency in microsomes of the rats exposed to CS_2[4] and possibly both phenomena are mutually dependent on each other.

Changes in liver weight, microsomal protein content and electron transport components in the microsomes are most pronounced in the rats killed immediately after termination of the daily exposure. Values similar to those of the control were found for 2 rats killed 3 days later. This may suggest that the changes observed in this experiment as well as those after single CS_2 dose administration are easily reversible. At present it is difficult to decide whether these changes were due to chronic or only to the current exposure to CS_2.

Acknowledgement—The author feels greatly indebted to the Director of the Institute of Hygiene and Epidemiology in Prague, Prof. Dr. J. Teisinger, for his kind interest in this study, and to Dr. V. Kujalová, Chief of Biochemical Laboratory, for assistance and helpful comments. Thanks are due also to Prof. Dr. J. Koštíř and Dr. Z. Šípal from the Biochemical Department of the Charles University for enabling determination of the microsomal cytochromes in their laboratories.

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TABLE 2. EFFECTS OF CARBON DISULPHIDE EXPOSURE ON THE MICROSOMAL CYTOCHROMES LEVELS AND MICROSOMAL REDUCTASES ACTIVITY IN RAT LIVER

Microsomal cytochromes and reductases	Unit	Experiment 1		Experiment 2	
		CS_2	Control	CS_2	Control
P-450	nMole/mg of microsomal protein	0·71 ± 0·05*(4)	1·35 ± 0·05(6)		
b_5	nMole/mg of microsomal protein	0·93 ± 0·02(4)	0·84 ± 0·03(6)		
NADPH cytochrome *c* reductase	nMole/mg of microsomal protein/min	119 ± 6(4)	107 ± 8(6)	58 ± 6(6)	48 ± 8(6)
NADPH ferricyanide reductase	nMole/mg of microsomal protein/min			211 ± 21(6)	205 ± 23(6)
NADH cytochrome *c* reductase	μMole/mg of microsomal protein/min	3·58 ± 0·24†(4)	2·25 ± 0·20(6)	1·09 ± 0·12‡(6)	0·69 ± 0·05(6)
NADH ferricyanide reductase	μMole/mg of microsomal protein/min			4·33 ± 0·18(6)	4·82 ± 0·42(6)

Values given are the means ± S.E.M. of the number of observations in parentheses. Difference statistically significant in relation to control:
* $P < 0·001$.
† $0·001 < P < 0·01$.
‡ $0·01 < P < 0·02$.

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Biochemical Pharmacology, Vol. 22, pp. 132–134. Pergamon Press, 1973. Printed in Great Britain.

The binding of aflatoxin B_1 with serum albumin

(*Received* 5 *June* 1972; *accepted* 31 *July* 1972)

MANY serum proteins bind small mol. wt substances for transport or other biological reactions.[1] Many of the coumarin anticoagulants are bound to serum albumin and this binding affects their pharmacokinetic properties.[2] Aflatoxin B_1 has been shown to possess haemorrhagic properties.[3,4]

It is of interest, therefore, to study the interaction of aflatoxin B_1 with serum albumin in view of the structural similarity of the toxin and synthetic coumarins[5] and also in an attempt to obtain an insight into its binding with the intracellular receptor site for anticoagulant activity. For obtaining the binding data, the method of equilibrium dialysis was employed. This method permits easy determination of protein interactions of drugs.[6]

Portions of Visking cellophane tubing of 12 cm (3 cm dia., Scientific Instrument Centre Ltd., London, U.K.) were prepared. They were cleaned by rinsing in a shaking bath (Gallenkamp, U.K.) of deionized water for at least 48 hr and were then stored in a 0·067 M sodium phosphate buffer, pH 7·4 ($I = 0{\cdot}170$) at a refrigerated temperature of 4°. Just before use, each bag was washed thoroughly with the buffer and used before it dried. For the measurement of free and bound drug, 10 ml sample of albumin was placed inside the bag and dialysed against 15 ml sample of medium containing aflatoxin B_1 (purchased from Makor Biochemicals Ltd., Jerusalem) or 4-hydroxycoumarin (purchased from Kock–Light Laboratories, Colnbrook, U.K.), in a 50 ml glass tube and covered with cotton wool. In this study we used bovine serum albumin (BSA) and human serum albumin (HSA). They were obtained from Sigma Chemical Co., U.S.A. and we considered it unnecessary to further purify the crystalline albumins. The concentration of albumin in 0·067 M sodium phosphate buffer, pH 7·4, which was used in all the experiments was 0·4 mg/ml. The solutions of the drugs were made to give a concentration 20 μM each in 0·067 M sodium phosphate buffer, pH 7·4, and diluted as required. Aflatoxin B_1 and 4-hydroxycoumarin are not readily soluble in water; therefore, they were dissolved in a minimum quantity of *N,N*-dimethylformamide and 0·01 N sodium hydroxide respectively after which each drug was diluted to volume with the buffer.

For each concentration of the drug three tubes were placed and rocked at 150 cycles/min at 25° ± 1° for 10 hr. At this time equilibration had occurred under our experimental conditions. Included in all runs were control bags which contained only buffer. Four different concentrations (5, 10, 15 and 20 μM) of each compound were used while the concentration of the protein was kept constant. The amount of free drug in equilibrium with the bound drug was estimated by measuring the concentration,