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CARBON DISULPHIDE EFFECT ON THE MATERNAL ORGANISM DURING GESTATION

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ABSTRACT

A concentration range of 2000, 1000, 200, 100 and 50 mg/m³ of carbon disulphide was applied to pregnant albino rats during gestation. Maternal weight gain and clinical condition were checked daily; indices of lipid and energy metabolism, DNA and RNA liver levels and liver and placenta morphology were studied at the term of pregnancy. Caesarian section was performed at term, and the number of live foetuses, resorption sites, corpora lutea and foetal weight were registered as well as carbon disulphide distribution in the maternal organs, placenta and foetus. It was found that CS_2 crosses the placental barrier, the ratio of its concentration in the maternal blood and foetus being from 5 : 1 to 16 : 1 depending on the applied CS_2 level. Signs of overt intoxication were present at 2000, 1000 and 200 mg/m³, demonstrated by reduced weight gain, tremor, bloody nasal secretion, vaginal bleeding and marked morphological changes in the liver and placenta. A considerable increase of preimplantation lethality (causing up to 100% loss of progeny in some females treated with 2000 mg/m³) and reduction of foetal weight at birth were found in the same test groups. Changes in lipid metabolism, inhibition of the oxygen consumption of liver, kidney and placenta, and an increase of RNA in the liver were found. These changes were more pronounced in pregnant animals in comparison with similarly treated non-pregnant females, which testifies to the fact that pregnancy enhances the toxic effect of CS_2.

At 50 mg/m³ CS_2 no apparent or subtle changes in the maternal organism were found. However, the progeny of this test group was affected adversely by the noxious agent, a fact that points to the specific effect of carbon disulphide on foetal development.

Carbon disulphide has been reported to interfere with the normal course and outcome of pregnancy. An increase of spontaneous abortions (predominantly in the first trimester of gestation), of late toxicosis of pregnancy and of premature births has been observed in women engaged in artificial fibre production[2,4,9].

The present study was designed to evaluate the effect of experimental multi-leveled CS_2 inhalation exposure on the maternal organism; to elucidate the type and magnitude of the induced deviations; to find safe exposure levels for mother and progeny; and to answer the question whether CS_2 is capable of impairing the progeny in dose levels which do not affect the mother, i.e. whether CS_2 possesses a specific embryotropic effect.

MATERIALS AND METHODS

CS_2 in a concentration range of 2000, 1000, 200, 100 and 50 mg/m³ was applied to pregnant albino rats (initial body weight 155–178 g) during gestation (21 days). The experimental groups consisted of 18 animals each. The first two experimental groups (1000 and 2000 mg/m³) were treated 4 hours per day, and the rest – 6 hours per day. CS_2 was determined daily in the experimental chambers[8]. Maternal weight gain and clinical condition were checked daily: oxygen consumption in the liver, kidney and placenta tissue preparations[7], certain indices of lipid metabolism in liver extracts (free fatty acids[3], triglycerides[14], phospholipids[11], cholesterol[6]), GOT, alcaline phosphatase and lactate dehydrogenase activity in the liver (Boehringer tests) and liver and placenta morphology were studied at the term of pregnancy. The indices of lipid and energy metabolism were followed in similarly treated non-pregnant females as well, in order to evaluate the effect of pregnancy on intoxication with CS_2. Caesarian section was performed at term and the number of live foetuses, resorption sites, corpora lutea, foetal weight and malformations were registered, as well as carbon disulphide distribution in the maternal organs, placenta and foetus[1,12].

RESULTS

Clinical signs of overt intoxication demonstrated by apathy, drowsiness or hyperexcitement, unbalanced gait, ruffled fur, tremor, bloody nasal and vaginal discharge, and diminishing weight gain were observed at 2000 mg/m³. No visible signs of intoxication were registered at the other dose levels, except a reduction of weight gain at 1000 and 200 mg/m³ (Table 1). Marked

TABLE 1
Maternal weight gain during gestation in rats exposed to various concentrations of CS_2.

	CS_2 exposure level (mg/m³)					
	0 (Control)	2000	1000	200	100	50
Mean weight gain per group (g)	85.6 ± 4.5	28.0 ± 8.9	55.0 ± 8.5	62.2 ± 6.3	68.1 ± 6.4	82.1 ± 5.8
Level of significance		$p < 0.01$	$p < 0.05$	$p < 0.05$		

morphological changes in the liver (parenchymatous dystrophy, reduced glycogen content) and placenta (necrotic changes accompanied by an acute inflammatory reaction) were found in the same test groups. Lower concentrations did not cause any considerable pathomorphological deviations. At 100 mg/m³ a mild parenchymatous dystrophy of the central hepatocytes was noted, while at 50 mg/m³ no detectable morphological changes were found.

The CS_2 tissue level was higher in the maternal liver than in the placenta (Table 2). It was noted that at 1000 and 2000 mg/m³ CS_2 tissue concentrations were nearly identical, despite the two-fold difference of the applied dose, which indicates the limit of tissue metabolizing and detoxifying capacities. At the lower exposure levels, the CS_2 content of the liver and placenta sharply decreased, proportionally to the applied dose. The CS_2 content of the total foetus was considerably lower in comparison with the maternal organs, the ratio between

TABLE 2
Distribution of CS_2 in maternal organs and foetus (mean ± S.E.).

Exposure level (mg/m³)	CS_2 (μg/100 g) in:			
	maternal blood	maternal liver	placenta	foetus
2000	not estimated	269.5 ± 45.5	91.0 ± 21.7	103.0 ± 31.1
1000	not estimated	240.7 ± 41.3	86.5 ± 12.9	67.0 ± 12.8
200	73.3 ± 18.9	50.0 ± 7.5	25.5 ± 7.6	13.4 ± 8.4
100	32.8 ± 7.6	26.6 ± 9.9	not detectable	2.4 ± 0.4

CS_2 level in the foetus and maternal liver varying from 1 : 2 (at 2000 mg/m³) to 1 : 13 (at 100 mg/m³). A similar relationship was found between the CS_2 foetal level and the maternal blood level (1 : 5 at 200 mg/m³ and 1 : 16 at 100 mg/m³). This means that with increased exposure concentration the efficiency of the placental barrier decreased, due probably to the morphological and functional impairment of the placenta.

Biochemical testing was carried out at the lower dose levels: 200, 100 and 50 mg/m³ (Table 3). A reduction in the activity of some metal-activated enzymes (alkaline phosphatase and lactate dehydrogenase) in liver homogenates, statistically significant at 200 mg/m³ was found, which may be a result of the binding of metal ions by the intermediate products of the CS_2 metabolism[10]. An increase of GOT activity was observed at 100 and 200 mg/m³ (dose levels causing a pathomorphologically proven hepatic lesion).

Oxygen consumption was found to be significantly inhibited in the maternal liver, kidney and placenta at 200 mg/m³. The same effect, although statistically unsignificant, was noted at 100 mg/m³. In similarly treated non-pregnant females, the oxygen consumption of the liver was inhibited to a slighter degree (Table 4), which points out the higher vulnerability of the pregnant organism. Two kinds of statistical comparisons were performed: dose-groups were compared with the corresponding control groups, separately for pregnant and non-pregnant females and pregnant controls were compared with the non-pregnant controls.

TABLE 3
Biochemical indices in liver homogenates of pregnant albino rats treated with CS_2 throughout gestation (mean ± S.E.).

Index	CS_2 exposure level (mg/m³)			
	0 (Control)	50	100	200
Lactate dehydrogenase (mU/mg protein)	9845 ± 709	8278 ± 923	8050 ± 813	7555 ± 513*
Alkaline phosphatase (mU/g protein)	955 ± 86.1	813 ± 133.4	744 ± 79.2	549 ± 113*
GOT (mU/mg protein)	320 ± 49.8	479 ± 51.1	501 ± 29.2*	620 ± 68.2*
Oxygen consumption (μl/10 min/100 mg dry weight) in:				
liver	65.1 ± 8.1	45.1 ± 6.8	62.5 ± 7.9	25.8 ± 6.7*
kidney	69.4 ± 5.7	50.9 ± 6.6	48.7 ± 7.3	25.8 ± 2.4*
placenta	28.6 ± 3.7	33.0 ± 7.3	18.6 ± 2.7	12.8 ± 1.5*
RNA (mg/100 g)	807.0 ± 38.9	900 ± 50.6	820 ± 38.3	930 ± 70.0
DNA (mg/100 g)	200 ± 0.0	220 ± 20.0	200.0 ± 0.0	225 ± 25.0
Free fatty acids (mg/100 ml)	9.5 ± 0.97	10.5 ± 0.76	11.7 ± 0.45	14.7 ± 0.73*
Triglycerides (mg/100 ml)	1532 ± 743.5	1375 ± 226.3	1580 ± 70.7	1580 ± 158.1
Phospholipids (mg/100 ml)	1126 ± 139.3	1070 ± 121.2	1067 ± 39.4	1100 ± 70.7
Cholesterol (mg/100 ml)	111 ± 7.0	145 ± 26.5	99 ± 1.9	132 ± 6.2

*Statistically significant ($p < 0.05$)

A 54% increase of free fatty acids was found in liver homogenates at 200 mg/m³, while in the non-pregnant females, at the same dose level, no deviations from the control values were observed. An elevation (statistically insignificant) of cholesterol was noted at 200 mg/m³ both in pregnant and non-pregnant females. A reduction of phospholipids was noted at 200 mg/m³ in the non--pregnant females and in the pregnant control animals, compared to the non--pregnant controls. Evidently, the effect of CS_2 on lipid metabolism is enhanced by the pregnancy itself, as can be seen when the control values of pregnant and non-pregnant females are compared. In the untreated pregnant animals at the end of gestation, the free fatty acids and cholesterol in the liver were significantly elevated in comparison to the non-pregnant control females (Table 4).

No significant changes in DNA and RNA liver level were found either in the pregnant or the non-pregnant females.

The two-generation study of pre- and postnatal development of the progeny[13] revealed signs of dose-related embryotoxic and teratogenic effect in all

TABLE 4
Comparative data of some indices of lipid and energy metabolism in pregnant and non-pregnant females (mean ± S.E.).

Index	Pregnant or non-pregnant	CS_2 exposure level (mg/m^3)		
		0 Control	50	200
Oxygen consumption (μlO_2/10 min/100 mg liver dry weight)	pregnant	65.1 ± 8.1	45.1 ± 6.8	25.8 ± 6.7*
	non-pregnant	48.2 ± 2.8	40.4 ± 2.2	26.8 ± 2.3*
Free fatty acids (mg/100 ml)	pregnant	9.5 ± 0.9*	10.5 ± 0.7	14.7 ± 0.7*
	non-pregnant	6.7 ± 0.4	5.7 ± 0.2	7.3 ± 0.8
Triglycerides (mg/100 ml)	pregnant	1532 ± 743	1375 ± 226.3	1580 ± 158
	non-pregnant	1102 ± 97.5	1078 ± 187.6	1462 ± 190
Phospholipids (mg/100 ml)	pregnant	1126 ± 139*	1070 ± 121.2	1100 ± 71
	non-pregnant	1845 ± 169	2075 ± 260.6	1280 ± 88.5*
Cholesterol (mg/100 ml)	pregnant	111.4 ± 7.0*	145 ± 26.4	132 ± 6.2
	non-pregnant	81.4 ± 6.3	105 ± 7.7	101 ± 5.7

*Statistically significant ($p < 0.05$). Two kinds of statistical comparisons were performed: dose-groups were compared with the corresponding control groups, separately for pregnant and non-pregnant females and pregnant controls were compared with the non-pregnant controls.

exposure groups except that of 50 mg/m^3. An increase in embryonic lethality, reduction of foetal weight, and various malformations such as hydrocephalus, club foot, generalized oedema, hypognathia, retarded postnatal development, etc. were noted.

At 50 mg/m^3 no apparent or subtle changes in the maternal organism were observed. However, the progeny of this test group, although morphologically and biochemically normal, exhibited certain statistically significant behavioral deviations when studied postnatally[5]. These findings point out the specific effect of CS_2 on the postnatal development of the progeny.

DISCUSSION

Carbon disulphide crosses the placental barrier. With increased exposure concentration, the efficiency of this barrier diminishes due to the morphological and functional impairment of the placenta. CS_2 affects the developing embryo, both directly and indirectly, by inducing morphological and functional changes in the maternal organism, causing increased embryonic lethality, reduction of foetal weight, various malformations such as hydrocephalus, club foot, generalized oedema, hypognathia, etc., retarded postnatal development and behavioral changes.

The effect of CS_2 on the maternal organism is dose-dependent and detectable at all exposure levels except at 50 mg/m³. Sensitive biochemical indices for detecting the adverse effect on mothers in our study proved to be alkaline phosphatase, GOT, the oxygen consumption of liver, kidney and the placenta, and the level of free fatty acids in liver homogenates.

Pregnant females are more sensitive to the toxic effect of CS_2 than to non-pregnant ones. It appears that pregnancy enhances the toxic effect of the noxious agent on lipid metabolism, producing even in control conditions deviations similar to those effected by CS_2.

Carbon disulphide exerts a specific effect on the postnatal development of the generation. Signs of this effect emerge at 50 mg/m³, a dose level which does not affect the mother.

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