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# CORRELATION OF CARBON DISULPHIDE EXPOSURE LEVELS WITH TISSUE LEVELS AND SOME BIOCHEMICAL INDICES IN MATERNAL AND FOETAL ORGANISM

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**ABSTRACT**

A statistically significant correlation was found between the concentration of inhaled $CS_2$ and its tissue levels in maternal and foetal organism after exposure of pregnant albino rats to $CS_2$ concentrations of 50, 100 and 200 mg/m³ throughout the whole period of gestation. The highest correlation was found between $CS_2$ exposure level and $CS_2$ concentration in total foetus ($r = 0.80$), followed by maternal liver ($r = 0.75$) placenta ($r = 0.73$) and maternal blood ($r = 0.63$). The inhibition of oxygen consumption in the placenta, maternal liver and kidney proved to be closely correlated to the applied $CS_2$ concentrations, correlation coefficient being 0.57, 0.78 and 0.81 respectively. The same correlation ($r = 0.79$) was found between the level of free fatty acids in maternal liver and $CS_2$ exposure level, but no correlation existed between the same biochemical index in foetal liver and $CS_2$.

An increase of the above mentioned correlations has been observed after a repeated exposure of the progeny ($F_1$) of the originally treated mothers. The inhibition of oxygen consumption in the placenta and of DNA and RNA synthesis in the liver of $F_1$ generation was more pronounced and the correlations with the applied $CS_2$ levels were stronger in comparison with the previous generation.

Carbon disulphide toxicity has been extensively studied in adults, both in experimental and occupational exposure, but the data about its effect on pregnancy and generation are relatively scarce. This aspect of $CS_2$ toxicity is undoubtedly important because the biological effect of the agent is expected to be different in maternal organism, burdened by developing embryo and in functionally and morphologically immature foetus.

In order to investigate the relationship between $CS_2$ exposure levels and some biochemical changes in maternal and foetal organism, we carried out a series of experiments with $CS_2$ in the concentration range 50–200 mg/m³, applied to albino rats during gestation and studied two subsequent generations ($F_1$ and $F_2$).

## MATERIALS AND METHODS

Pregnant albino rats were exposed throughout gestation (21 days) to $CS_2$ vapours in concentrations of 50, 100 and 200 mg/m$^3$, 6 hours per day. From each dose group, some pregnant rats were killed at term and some were allowed to litter. The $F_1$ generation was postnatally studied. When mature, the progeny of each group were randomly paired for breeding. Fifty per cent of the pregnant $F_1$ females were continued on the $CS_2$ exposure appropriate to the test group, throughout gestation, while the others received no further $CS_2$ treatment. Caesarean section was performed at term and $F_2$ foetuses were examined.

The following biochemical indices were studied in $F_0$ mothers (at term), in $F_1$ progeny (at birth and after reaching sexual maturity, males and females separately) and in $F_2$ progeny (at birth): carbon disulphide tissue and blood level[1,9,11]; oxygen consumption of tissue homogenates, manometrically[3]; DNA and RNA in liver homogenates[2,14]; free fatty acids in liver homogenates[7]; triglycerides in liver homogenates[13] and phospholipids in liver homogenates[8].

Totally, 1 428 tests were performed and 241 animals were examined: 110 pregnant $F_0$ and $F_1$ females, 90 $F_1$ and $F_2$ foetuses and 41 adult males. The regression and correlation analyses were used for statistical evaluation of the data[6].

## RESULTS AND DISCUSSION

The data of the correlation analysis of $CS_2$ exposure and tissue levels are summarized in Table 1. The highest correlation was observed between $CS_2$ exposure levels and $CS_2$ content of the whole foetus ($r = 0.80$), although the absolute concentrations of the agent in the foetal tissue were found to be lower than in the maternal. A good correlation was found between $CS_2$ level in the maternal liver and placenta and the exposure concentration ($r = 0.75$ and 0.73, respectively). No significant correlation was observed between $CS_2$ exposure level and $CS_2$ level in the maternal blood, although $CS_2$ blood concentrations were higher than the tissue concentration. Such an uneven blood and tissue distribution of $CS_2$, attributable to the lipophylic properties of the agent and to the different metabolizing capacities of different organs, has been reported by other investigators[4,12].

TABLE 1
Correlation of $CS_2$ exposure levels with $CS_2$ tissue levels in maternal organs and foetuses.

| Organs | Correlation coefficient | Regression equation | Level of significance |
|---|---|---|---|
| Liver | 0.75 | $47.07 + 0.108X$ | $p < 0.001$ |
| Placenta | 0.73 | $5.93 + 0.072X$ | $p < 0.001$ |
| Blood | 0.63 | $50.53 + 0.119X$ | $p > 0.05$ |
| Foetus | 0.80 | $2.40 + 0.053X$ | $p < 0.001$ |

$X = CS_2$ exposure levels

Oxygen consumption of tissue homogenates has been reported as a not very sensitive test for detecting $CS_2$ intoxication in adult male rats[5,10]. In our experiments, however, it proved to be significantly inhibited in maternal organs, the degree of inhibition in the placenta, kidney and liver being closely correlated to the exposure levels (Table 2). The correlation (except that of the placenta) disappeared in the pregnant females of the next generation ($F_1$) which were not subjected to further $CS_2$ exposure, evidently due to the absence of a direct contact with the agent. However, it was present in $F_1$ females continued on $CS_2$ exposure during pregnancy. In the latter case, the inhibition of oxygen consumption in the placenta and its correlation to the dose level were more pronounced than in the originally treated $F_0$ generation. Inhibition of oxygen consumption in the placenta was detected even in the untreated $F_1$ females. This test proved to be a sensitive indicator of $CS_2$ effect on maternal organism.

TABLE 2
Correlation of oxygen consumption ($\triangle O_2$ µl/10 min/100 mg) of tissue homogenates with $CS_2$ exposure levels.

| Group | Oxygen consumption in | Correlation coefficient | Regresion equation* | Level of significance |
|---|---|---|---|---|
| $F_0$ females | placenta | −0.57 | 30.30 − 0.085X | $p < 0.001$ |
| | liver | −0.78 | 61.23 − 0.216X | $p < 0.001$ |
| | kidney | −0.81 | 66.21 − 0.20X | $p < 0.001$ |
| $F_1$ females | placenta | −0.47 | 28.10 − 0.058X | $p < 0.05$ |
| | liver | 0.20 | 58.90 + 0.062X | $p > 0.05$ |
| | kidney | 0.04 | 67.73 + 0.007X | $p > 0.05$ |
| $F_1$ females continued on $CS_2$ exposure | placenta | −0.76 | 28.98 − 0.11X | $p < 0.001$ |
| | liver | 0.13 | 55.58 + 0.045X | $p > 0.05$ |
| | kidney | −0.40 | 69.81 − 0.085X | $p < 0.05$ |
| $F_1$ males | liver | 0.26 | 45.30 + 0.033X | $p > 0.05$ |
| | kidney | 0.61 | 54.59 + 0.109X | $p < 0.05$ |
| $F_0$ males | liver | 0.52 | 75.21 + 0.122X | $p < 0.05$ |
| | kidney | 0.05 | 82.35 + 0.0014X | $p > 0.05$ |

X = $CS_2$ exposure levels

Quite a different effect was observed in similarly treated adult $F_0$ and $F_1$ males. Instead of inhibition, a dose-related activation of oxygen consumption in the liver ($F_1$) and kidney ($F_1$) was found, in accordance with the data of other authors[5,10].

No detectable changes of oxygen consumption were found in the foetal liver ($F_1$ and $F_2$), probably because of the role of the placental barrier and the fact that the foetal liver is adapted to functioning in conditions of relative oxygen deficiency in the uterus (Table 3).

TABLE 3
Correlation of oxygen consumption ($\triangle O_2$ µl/10 min/100 mg) in foetal liver with $CS_2$ exposure levels.

| Group | Correlation coefficient | Regression equation | Level of significance |
|---|---|---|---|
| $F_1$ foetuses | 0.23 | 40.02 + 0.03X | $p > 0.05$ |
| $F_2$ foetuses not treated prenatally | −0.04 | 35.60 − 0.008X | $p > 0.05$ |
| $F_2$ foetuses treated prenatally | 0.11 | 36.39 + 0.02X | $p > 0.05$ |

X = $CS_2$ exposure levels

After chronic $CS_2$ exposure elevation of DNA and RNA has been reported in liver cells[16]. Our data confirm this observation (Table 4). A statistically significant dose-dependent elevation of DNA in the liver was found in $F_1$ females continued on $CS_2$ exposure during gestation. In their foetuses however, a dose--related reduction of DNA liver level was observed (Table 5). These findings suggest that in the maternal organism, after repeated $CS_2$ exposure, a certain process of adaptation, demonstrated by a stimulation of protein synthesis takes place, while the functionally immature foetal liver is not able to respond in a similar way.

Lipid metabolism after $CS_2$ exposure has been extensively studied but the findings have often been controversial, depending either on the species, age and sex differences, or on the dose level and the mode of application. Among the indices of lipid metabolism used in our study, the most sensitive one proved to

TABLE 4
Correlation of RNA and DNA levels in the maternal liver with $CS_2$ exposure levels.

| Group | Index | Correlation coefficient | Regression equation | Level of significance |
|---|---|---|---|---|
| $F_0$ mothers | RNA | 0.37 | 812.55 + 0.522X | $p > 0.05$ |
| | DNA | 0.20 | 204.10 + 0.089X | $p > 0.05$ |
| $F_1$ females | RNA | 0.20 | 751.93 + 0.25X | $p > 0.05$ |
| | DNA | 0.10 | 184.92 + 0.03X | $p > 0.05$ |
| $F_1$ females continued on $CS_2$ exposure | RNA | 0.135 | 699.44 + 0.21X | $p > 0.05$ |
| | DNA | 0.67 | 162.45 + 0.447X | $p < 0.01$ |

TABLE 5
Correlation of RNA and DNA levels in the foetal liver with $CS_2$ exposure levels.

| Group | Index | Correlation coefficient | Regression equation | Level of significance |
|---|---|---|---|---|
| $F_1$ foetuses | RNA | 0.055 | 742.48 + 0.07X | $p > 0.05$ |
| | DNA | −0.36 | 362.30 − 0.34X | $p > 0.05$ |
| $F_2$ foetuses not treated prenatally | RNA | 0.05 | 691.45 + 0.08X | $p > 0.05$ |
| | DNA | −0.34 | 314.93 − 0.56X | $p > 0.05$ |
| $F_2$ foetuses treated prenatally | RNA | −0.46 | 647.19 − 0.298X | $p < 0.05$ |
| | DNA | −0.52 | 299.04 − 0.51X | $p < 0.05$ |

TABLE 6
Correlation of some indices of lipid metabolism in the liver of pregnant albino rats and their generation with $CS_2$ exposure levels.

| Group | Index | Correlation coefficient | Regression equation | Level of significance |
|---|---|---|---|---|
| $F_0$ mothers | free fatty acids | 0.79 | 9.21 + 0.026X | $p < 0.001$ |
| | triglycerides | −0.29 | 2013.4 − 3.08X | $p > 0.05$ |
| | phospholipids | −0.21 | 1884.9 − 0.065X | $p > 0.05$ |
| $F_1$ females | free fatty acids | 0.35 | 9.00 + 0.116X | $p > 0.05$ |
| | triglycerides | −0.53 | 1878.6 − 7.06X | $p < 0.01$ |
| | phospholipids | −0.46 | 1109.2 − 1.61X | $p > 0.05$ |
| $F_1$ females continued on $CS_2$ exposure | free fatty acids | 0.785 | 9.28 + 0.025X | $p < 0.001$ |
| | triglycerides | −0.57 | 1802.2 − 8.10X | $p < 0.01$ |
| | phospholipids | −0.56 | 1140.3 − 1.87X | $p < 0.01$ |
| $F_1$ males | free fatty acids | 0.25 | 10.58 + 0.0044X | $p > 0.05$ |
| | triglycerides | 0.17 | 883.5 + 0.39X | $p > 0.05$ |
| | phospholipids | 0.15 | 883.7 + 0.22X | $p > 0.05$ |

be the level of free fatty acids in the liver. Significant elevation of free fatty acids, correlated to the exposure concentration ($r = 0.79$) was found in the liver of $F_0$ females at term (Table 6). This effect was not present in the next generation ($F_1$) tested at birth and when mature, but reappeared when $CS_2$ exposure was extended to $F_1$ females during gestation. It seems, therefore, that the observed effect is a result of the direct effect of $CS_2$ on the organism. The elevation of free fatty acids indicates a disturbance in lipid metabolism, caused either by the binding of pyridoxalophosphate by $CS_2$[15], or by an impaired esterification.

The same mechanism is probably responsible for the observed tendency to reduction of triglycerides and phospholipids in $F_0$ maternal liver, detectable even in $F_1$ females and growing stronger in $F_1$ females continued on $CS_2$ exposure.

No correlation between the indices of lipid metabolism and the exposure levels was found in $F_0$ and $F_1$ males, which is an additional proof that the female organism burdened by pregnancy is more susceptible to the noxious effect of $CS_2$.

## CONCLUSION

A statistically significant correlation was found between $CS_2$ exposure levels and tissue levels in the maternal liver, placenta and foetus.

The inhibition of oxygen consumption in maternal organs was found to be closely correlated to the exposure levels, whereas in similarly treated male animals such correlation was not observed. The inhibition of oxygen consumption was most pronounced in the placenta. The placental effect persisted in the females of the next ($F_1$) generation and augmented after the repeated exposure of $F_1$ during gestation. This test could be recommended as a sensitive indicator of $CS_2$ effect on maternal organism and progeny.

A statistically significant correlation between the indices of lipid metabolism and $CS_2$ exposure levels was found in $F_0$ and $F_1$ pregnant females, but it was absent in the similarly treated $F_0$ and $F_1$ males. The correlation grew stronger in $F_1$ females continued on $CS_2$ exposure. The indices of lipid metabolism, and especially the level of free fatty acids proved to be adequate in evaluating the effect of $CS_2$ in pregnancy and progeny.

A correlation between $CS_2$ exposure levels and the levels of DNA and RNA in the liver appeared only after a repeated exposure of $F_1$ generation during gestation. The changes observed in $F_1$ mothers and $F_2$ foetuses were not uniform; they testified to a stimulation of DNA synthesis in the mothers and to an inhibition in the foetuses.

In comparison to the other tests used in our study, DNA and RNA liver level proved to be less sensitive for detection of pre- and postnatal effects of $CS_2$.

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