

STRUCTURAL DISTURBANCES
IN ERYTHROCYTES IN WORKERS EXPOSED
TO CARBON DISULPHIDE (CS₂)

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The symptoms which occur in individuals exposed to CS₂ are due to slight concentrations of CS₂ within the span of a few years. After exposure of 10 years or more disturbances may occur in the central and peripheral nervous systems and in circulation. This multifaceted effect of CS₂ on different systems explains the theories about the mechanism of its action in man. Very important is the role of CS₂ in lipid metabolism and its effects on blood vessels.

Attinger put forward the hypothesis that the effect of CS₂ on blood vessels is greater than on nerves (1). Scandinavian authors were of the opinion that the disease of coronary arteries was definitely related to chronic exposure to small concentrations of CS₂ (2). It was also suggested that the early occurrence of atherosclerosis was due to a disturbance in the metabolism of lipids.

In the serum of individuals exposed to CS₂ changes in the levels of cholesterol, total lipids and phospholipids were observed. At first there was an increase in the phospholipid level, but after a time the phospholipid level decreased while cholesterol and β -lipoproteins increased. The dissociation in the process of lipolysis led to an increase in the total lipids and β -lipoproteins causing damage in the endothelium of the vessels and producing atherosclerotic-like changes.

Recently, the CS₂ effects on erythrocyte metabolism have gained much attention. The erythrocyte, which has direct contact with CS₂ in the

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pulmonary circulation has become a constant and easy model on which tests can be performed to identify the chronic effects of CS₂. Experiments on laboratory animals have revealed hypochromic and normocytic anaemia, eosinopenia, monocytosis and an increase in reticuloendothelial cells. In these cases, there is also a decrease in the number of erythrocytes, haemoglobin and haematocrit, but no changes in the mean haemoglobin concentration, mean erythrocyte volume, and mean haemoglobin weight. It was suggested that there existed a simultaneous erythrocyte failure along with the above findings, and that CS₂ played a toxic role in erythrocyte haemolysis.

Mass incidence of anaemia linked to a greater sensitivity to CS₂ was observed in a group of subjects chronically exposed to CS₂. In comparison with other toxic agents e. g. benzene, CS₂ rarely produced changes which might cause anaemia. It was concluded, therefore, that the changes due to CS₂ occurred mainly in the peripheral blood system. Since the mechanism of action of CS₂ is not fully understood, the experiments were carried out in order to study the effects of CS₂ on erythrocyte lipid metabolism.

SUBJECTS AND METHODS

A group of 35 male workers aged between 25 and 55 years (mean age 43 years) who worked in chronic exposure to a concentration of 20-40 mg/m³ CS₂ were examined. The CS₂ concentrations were determined at the workplace. Most of the workers were employed between 5 and 20 years. The control group consisted of 18 workers from the same factory, with similar work conditions but with no CS₂ exposure. The age of the control group was between 25 and 53 years. All of the employees were subjected to thorough physical examinations with special attention to the systems most often damaged by CS₂. None of the employees, however, had symptoms of intoxication. In all the patients the complete blood count, serum enzyme activities and serum lipid levels were determined. Neither anaemia nor increased transaminase levels or hyperlipidemia were found.

Erythrocytes were prepared by the sedimentation method, by addition of an isotonic solution of 1% polyvinyl alcohol at a temperature of 4° C. After 20 minutes the erythrocytes were washed three times in 0.9% solution NaCl. From this erythrocyte mass the levels of haemoglobin and cell count were determined.

The lipids from erythrocytes were extracted by the Farquahar method (3). The amount of three millilitres of methanol was used for the extraction of 1 ml of erythrocytes for 10 minutes. After shaking with 6 ml of chloroform, 4.5 ml of 0.58% NaCl was added and left to stand overnight at a temperature of 4° C. The extract was filtered through a Buchner funnel in vacuum and 1/5 volume of 0.68% NaCl was added.

After filtration and ultracentrifugation, the water phase was cast off. The chloroform extract was washed twice with a mixture containing chloroform, methanol, 0.58% NaCl in a ratio of 3:47:48. The surface layer was extracted while the lower layer was steamed off in a measured test tube with nitrogen. After steaming, chloroform was supplemented to a volume of 2 ml. The resulting extract was then used to determine lipids.

Total lipids in erythrocytes were determined according to *Zolner* with the Boehringer test set (4). The amount of lipids was calculated and compared with model solutions. The lipid values acquired were found to differ 10—30% from those obtained by other methods.

Cholesterol was calculated according to the *Zolner* method, with the Boehringer test set. The obtained cholesterol findings were 10—30% lower than the values obtained by other methods.

Total phosphorus in erythrocytes was calculated according to the *Bartlett* method (5). The phospholipid level was given in percent as total phosphorus.

The fundamental differences in the mean arithmetic values were graded according to the Student's »t« test. Statistically significant were the differences when »t« was greater than the theoretical at the 1% level of significance ($p \geq 0.01$).

RESULTS

In the workers affected by CS₂, many changes in the levels of lipids in erythrocytes were found. The mean cholesterol level was 0.4630 mg/10¹⁰ erythrocytes and it was statistically significant compared with the control group whose mean was 0.7819 (Table 1).

Table 1.
The content of cholesterol, total lipids and phospholipids (mg/10¹⁰ erythrocytes) in the control group (N = 18) and in workers exposed to CS₂ (N = 35). Means ± SD.

	Cholesterol	Total lipids	P — lipids	Cholesterol P — lipids
Control group	0.7819 ± 0.0709	3.1501 ± 0.6565	0.0638 ± 0.0083	11.8—12.8
Workers exposed to CS ₂	0.4630 ± 0.1105	2.8745 ± 0.5231	0.0573 ± 0.0091	7.3— 8.6
	p < 0,01	p = 0,02	without significance	

Total lipid level in the CS₂ affected workers was 2.8745 mg/10¹⁰ erythrocytes, which was lower than in the control group (3.1501). However, no statistically significant differences were found in the total lipid levels.

The phospholipid level was only slightly reduced and the average was 0.0573 mg/10¹⁰ erythrocytes while in the control group the average was 0.0638. Significant differences were seen in the cholesterol to phospholipids ratio. This ratio was lower in the CS₂ affected workers than in the control group: 7.3—8.6, while in the control group it was 11.8—12.8. No significant differences were found in haematocrit, haemoglobin or erythrocyte count between the control and exposed group (Table 2).

Table 2.

Haemoglobin content, haematocrit levels, and erythrocyte count in the peripheral blood of workers exposed to CS₂ and in the control group

	Haematocrit %	Haemoglobin g%	Erythrocyte count (mm ³)
Control group	44.4	14.2	4 239 044
Workers exposed to CS ₂	45.0	14.5	4 377 422

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DISCUSSION

It was substantiated that there exists a definite change in lipids in the erythrocytes of the workers exposed to slight concentrations of CS₂. The main function of erythrocytes, the transport of oxygen into tissues, is undoubtedly disturbed under the influence of CS₂. The mechanism of adaptation is incited through an anaerobic glycolysis and an increase in 2,3-diphosphoglyceric acid. Simultaneous depletion of ATP was also found. In addition, the weakening of the erythrocyte membrane took place which might be due to a decrease in membrane lipids. Cholesterol levels decreased, especially in the cholesterol to phospholipid ratio, causing fragility in the erythrocytic membrane, which became weak, as well as a defect in the process of transport. Consequently, haemolysis occurred more readily.

The association between the anaerobic glycolytic function in the erythrocyte and the erythrocyte-lipid changes seems to be fundamental in the toxic effects of CS₂ in the human body. It may be assumed that lipid defects in erythrocytes may precede the changes seen in the serum with concurrent primary organic syndromes. Starting from this assumption further experiments in this field will be undertaken.

References

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Sažetak

STRUKTURNE PROMJENE U ERITROCITIMA RADNIKA EKSPONIRANIH UGLJIKOVOM DISULFIDU

Autori su proučavali učinak malenih doza ugljikova disulfida (CS₂) na metabolizam lipida u eritrocitima ljudi. U tu su svrhu odabrali skupinu od 35 muškaraca profesionalno eksponiranih CS₂ tijekom 5 do 20 godina. Kontrolnu je skupinu sačinjavalo 18 radnika. Radnici u obje skupine bili su klinički pregledani i nađeni potpuno zdravima. Osim sveukupnih lipida u eritrocitima, autori su još određivali posebno kolesterol i fosfolipide.

Količina sveukupnih lipida bila je u eksponiranih radnika nešto niža ali ta razlika nije bila značajna u odnosu na kontrolnu skupinu. Fosfolipidi su bili također samo neznatno smanjeni u eksponiranih radnika. Statistički značajna razlika nađena je u omjeru kolesterola i fosfolipida. Taj je omjer bio u eksponiranih radnika niži.

Nađenim biokemijskim poremećenjima autori pripisuju moguću ulogu u nastajanju kasnijih kliničkih manifestacija učinaka CS₂.

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