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by [Carlsson A](#), [Hultengren M](#)

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Exposure to methylene chloride

III. Metabolism of ^{14}C -labelled methylene chloride in rat

by ANDERS CARLSSON, M.B., and MAY HULTENGREN, engineer¹

CARLSSON, A. and HULTENGREN, M. Exposure to methylene chloride: III. Metabolism of ^{14}C -labelled methylene chloride in rat. *Scand. j. work environ. & health* 1 (1975) 104—108. The accumulation of methylene chloride and its metabolites in different organs and tissues was studied in an experimental series comprising 10 rats. Each rat was exposed for 1 h to radioactive methylene chloride (^{14}C) in a concentration amounting to 1,935 mg/m³ in inspiratory air. Radioactive carbon atoms were found in the isolated carbon monoxide after exposure. There was also a close correlation between the activity of carbon monoxide extracted from the blood sample and the amount of carboxyhemoglobin (COHb) in the specimen. The largest concentration of methylene chloride and its metabolites per gram of tissue was found in white adipose tissue. This concentration had declined by more than 90 % 2 h after exposure, whereas the concentration in the liver declined by about 25 % during the same period. The amount accumulated in the brain displayed a decline of about 75 % 2 h after exposure. Thus the examination showed that the increased concentration of COHb in the blood during exposure to methylene chloride is due to the metabolism of methylene chloride into carbon monoxide.

Key words: methylene chloride, animal exposure, metabolism, radioactive atoms, carbon monoxide, tissue concentration.

The inhalation of methylene chloride (CH_2Cl_2) has been found to result in the formation of carbon monoxide in man (1, 4, 7). Endogenous carbon monoxide is normally formed in the human body during the breakdown of hemoglobin (5, 8). An increase in this breakdown or some other change during exposure to methylene chloride may be an explanation for the rise in carboxyhemoglobin (COHb) levels (3). Another possibility is that the methylene chloride taken up by the body is metabolized into, e.g., carbon monoxide.

These issues and the accumulation of methylene chloride and its metabolites in certain organs and tissues of the rat were studied in the present investigation.

EXPERIMENTAL DESIGN

Ten male rats (Sprague-Dawley), each weighing about 200 g, were used for the experiment (Anticimex, Sollentuna, Sweden).

The air mixture used in exposure was prepared by injecting, with a microliter syringe (Hamilton-Boyd, Switzerland), a given quantity of radioactive methylene chloride (New England Nuclear, Mass., U.S.A.) into a bag (specially made of polyester-laminated aluminium foil) containing a known quantity of air. The radioactive methylene chloride had a specific activity of 0.45 mCi/mmol.

¹ Work Physiology and Technology Divisions, Department of Occupational Medicine, National Board of Occupational Safety and Health, Stockholm, Sweden.

Reprint requests to: Mr. Anders Carlsson, Kungl. Arbetarskyddsstyrelsen, Arbetsmedicinska avdelningen, Fack, 100 26 Stockholm 34, Sweden.

The rats were placed in a cage (150 mm inner diameter, Kebo-Grave, Sweden), which was well-sealed. The air mixture flowed from the aforementioned bag into the cage via a rotameter (model 1350 V, Max Sievert, Vällingby, Sweden). The surplus air mixture was removed through a tube to which a water suction unit was connected. The entire device was placed in a fume cupboard. The rate of flow, which was adjusted with a tube clip, was set at 20 l/h. Pretrials showed that this rate supplied the rat with adequate quantities of oxygen and guaranteed a constant cage temperature of about 23°C.

After 1 h of exposure to about 1,935 mg/m³ of methylene chloride in the inspiratory air the rats were killed by means of luxation of the cervical spine. The thorax was then opened by direct heart puncture, and blood samples were taken which varied from 0.5 to 1.6 ml. Approximately equal amounts of this blood were then transferred to two injection bottles. Samples were also taken for determining hemoglobin concentration (cyanmethemoglobin method with spectrophotometric reading). Specimens were taken from the liver, kidneys, adrenals, white adipose tissue, cerebrum, and cerebellum. These specimens were then assayed for their radioactive content of methylene chloride and metabolites.

ANALYTICAL METHODS

The concentration of methylene chloride in inspiratory air was determined with a gas chromatograph (1), and it proved to have a mean value of 1,935 mg/m³ (SD = ± 90 mg/m³).

Blood carbon monoxide is normally bound to hemoglobin in the form of COHb. When the COHb content of blood was analyzed, the released gaseous carbon monoxide was measured. Radioactive methylene chloride was also released from the blood. After the gas (8 ml) had been collected in a 10-ml all-glass syringe (Becton, Dickinson and Co., U.S.A.), it passed five times over activated charcoal loosely packed in a 2-cm long latex tube with an inner diameter of 0.3 cm (fig. 1). The methylene chloride was absorbed, whereas the carbon monoxide passed over. The meth-

Fig. 1. Method for measuring the radioactive content of formed carbon monoxide: The gas from the blood sample consisting of carbon monoxide and methylene chloride was collected in an all-glass syringe (A). This gas mixture passed over activated charcoal (B) when the methylene chloride was absorbed. After passage over the activated charcoal the gas was analyzed for methylene chloride and carbon monoxide. Thereafter, the gas was allowed to pass over hopcalite (C), a substance which catalytically converts carbon monoxide into carbon dioxide. Another analysis was made of the carbon monoxide content of the gas. In the first method the formed carbon dioxide was dissolved in 3 ml of Carbosorb (D), into which scintillation liquid was mixed, and the radioactivity of the sample was measured. In the second method the carbon dioxide was allowed to bubble through 3 ml of 0.02 M calciumhydroxide. The precipitate obtained was removed. To this precipitate scintillation liquid was added and the radioactivity of the sample was measured.

ylene chloride content of the gas was analyzed after passage over the activated charcoal so as to check on the degree of absorption. This check was made with a gas chromatograph. After passing over the activated charcoal, the gas was checked for carbon monoxide content with a gas chromatograph. The error of the method for a single determination, calculated for 10 double determinations, was 3.0 % of the mean value of 4.25 % COHb (1).

In order to facilitate simple measurement of the radioactive content of the carbon monoxide, the carbon monoxide was converted into carbon dioxide, which is soluble in a liquid scintillation system. Thus the gas was allowed to pass five times over hopcalite (40 % CuO + 60 % Mn₂O), a substance which catalytically converts carbon monoxide into carbon dioxide. The hopcalite was also loosely packed in a latex tube. An analysis of the carbon monoxide content of the gas

was made after it passed over the hopcalite in order to check on the conversion of carbon monoxide into carbon dioxide (fig. 1).

The carbon dioxide obtained in this manner was dissolved in 3 ml of Carbosorb (Packard Instrument AB, Bandhagen, Sweden), into which 12 ml of scintillation liquid, Permafluor V (Packard Instr. AB), was mixed.

An additional blood sample was taken from each rat. This sample was processed in the same manner as the first, with the exception that the carbon dioxide formed was allowed to bubble through 0.02 M calcium hydroxide (lime water) (fig. 1). The precipitate obtained was removed and mixed with 10 ml of scintillation liquid consisting of 5.5 g of Permablend III (Packard Instr. AB) dissolved in 1 l of toluene.

The specimens taken from organs and tissues were weighed and then digested for 2–4 h at +50°C in 1 ml of Soluene-350 (Packard Instr. AB). Nine milliliters of the Permablend III scintillation liquid dissolved in toluene was then added.

A liquid scintillation counter was used (model 2425 Tri Carb, Packard Instr. Co. Inc., Downer Grove, Ill., U.S.A.). A 100 % counting efficiency is not usually attained when the activity of soft beta particles such as ^{14}C is measured. There are many reasons for this occurrence, one being discoloration and another being the presence of substances which impede the emission of light from the solute. Such occurrences are called quenching. A series of samples were analyzed with the same known level of activity (^{14}C toluene), but with different quenching factors. The calibration curve (fig. 2) obtained in this manner made it possible to convert a specimen's counter-per-minute value into absolute activity, i.e., disintegration per minute (dpm). A standard sample with a known level of activity (^{14}C -toluene) was also used at each measurement in order to check the stability of the scintillation counter. Background counts for each liquid scintillation system were also measured. Polyethylene scintillation vials (Packard Instr. AB) were employed in the experiments. Each specimen was measured in two cycles of either 20 min or a maximum of 20,000 impulses. The value for activity was calculated as the mean value of these two determinations ($\text{SD} = \pm 0.7\%$ in 20,000 impulses).

Fig. 2. Calibration curve for measuring absolute activity. The horizontal axis stands for the external standard channels ratio (A.E.S.) and the vertical axis stands for the counting efficiency as a percentage of ^{14}C (η).

RESULTS

Radioactive measurement of carbon monoxide

The radioactive content of the carbon monoxide formed in exposure closely correlated with the amount of COHb formed. As previously mentioned, double blood samples were taken after every exposure. These samples were processed in different ways after the absorption of methylene chloride and the conversion of carbon monoxide into carbon dioxide.

The first method, in which carbon dioxide was dissolved in Carbosorb, produced a close correlation between the amount of COHb formed and the radioactive content of carbon monoxide (fig. 3) (correlation coefficient $r = +0.893$). A correction was made for the rat's COHb content prior to exposure. The mean value for the four control rats was used (0.26 %; $\text{SD} = \pm 0.02\%$).

The second method, in which the carbon dioxide was bubbled through lime water, resulted in a precipitation of calcium carbonate (6). Close correlation was also found here between the activity in the precipitate and the amount of COHb formed (correlation coefficient $r = +0.936$). Since the particles of calcium carbonate formed did not remain suspended in the scintillation liquid, but were allowed to settle, the second method did not result in a count as high as the first method.

Fig. 3. Absolute activity ($\text{dpm} \cdot 10^3$) in relation to the amount of carboxyhemoglobin ($\text{COHb g} \cdot 10^{-3}$). Every point stands for one exposed rat.

Accumulation of methylene chloride and metabolites in organs and tissues

White adipose tissue contained the highest concentration per gram of tissue immediately after the termination of exposure (fig. 4). The concentration of methylene chloride and metabolites in this tissue and in the brain diminished very rapidly. Levels in the liver, kidneys, and adrenals declined far more slowly during the 6-h follow-up after exposure.

DISCUSSION

The results show that the concentration of COHb in blood increases during exposure to methylene chloride in inspiratory air. This circumstance has been noted in a number of studies (1, 4, 7).

Kubic et al. (2) showed that carbon monoxide is formed after an intraperitoneal injection of methylene chloride into rats and that this carbon monoxide is a metabolite of methylene chloride. In the present study rats were exposed to ^{14}C -labelled methylene chloride in the inspiratory air. The increased level of carbon monoxide proved to be derived from methylene chloride. Thus the methylene chloride taken up by the rat was metabolized into products such as carbon monoxide.

Stewart et al. (7) showed that no increase in the level of urobilinogen could be demonstrated in man exposed to methylene chloride. Thus there was no increased endogenous formation of carbon monoxide due to an enhanced metabolism of hemoglobin. There should be no doubt that the increased amount of carbon monoxide formed during exposure to methylene chloride is a metabolite of the methylene chloride taken up.

Carbon monoxide is dangerous because it impedes oxygen transport. The half-life of carbon monoxide is prolonged during exposure to methylene chloride as compared to ordinary exposure to carbon monoxide. This occurrence was previously pointed out by Stewart et al. (7), and it has been confirmed in the present study.

Fig. 4. Accumulation of methylene chloride ($\mu\text{g CH}_2\text{Cl}_2/\text{g tissue}$) and its metabolites in organs and tissues during the 6-h follow-up after exposure to methylene chloride in the inspiratory air.

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