Exposure to methylene chloride. I Its concentration in alveolar air and blood during rest and exercise and its metabolism.

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Exposure to methylene chloride

I. Its concentration in alveolar air and blood during rest and exercise and its metabolism

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ÅSTRAND, I., ÖVРUM, P. and CARLSSON, A. Exposure to methylene chloride: I. Its concentration in alveolar air and blood during rest and exercise and its metabolism. Scand. j. work environ. & health 1 (1975) 78—94. Fourteen subjects were exposed to about 870 and 1,740 mg/m³ of methylene chloride in the air during rest and physical exercise on a bicycle ergometer. The duration of each exposure period was 30 min. Each subject was exposed during four periods. The concentration of methylene chloride in the alveolar air increased in the beginning but had a tendency to level off at the end of each period. There was a high correlation between the alveolar and arterial concentration of methylene chloride. The uptake of methylene chloride was about 55% of the supplied amount at rest, about 40% at a work load of 50 W, and about 30 and 35% at 100 and 150 W, respectively. The concentration of carboxyhemoglobin (COHb) increased both during and after exposure. With exposure to 1,740 mg/m³ a concentration of COHb in the blood of about 0.85 g/100 ml was reached. This value corresponds to about 5.5% COHb.

Key words: exposure, methylene concentration, arterial concentration, venous concentration, rest, work, carboxyhemoglobin, uptake.

Methylene chloride (CH₂Cl₂) is a solvent in relatively widespread use. About 2,300 tons are sold in Sweden annually. It is used as a solvent for dyes in the dyeing industry, as a dissolver of cellulose esters in the textile industry, for the extraction of fats in the food industry, as a refrigerant in air conditioning units, as a degreasing agent in the engineering industry, etc. Thus a great many workers come in contact with the substance in their work.

Methylene chloride has previously been the subject of laboratory investigations. Riley et al. (21) and Di Vincenzo et al. (10 11) exposed subjects to methylene chloride in inspiratory air. They found, among other things, that the concentration of methylene chloride in alveolar air rose very rapidly during the first minutes of exposure; the concentration increased more slowly during the subsequent 40—50 min. A further decline in the rate of concentration increase was also noted in the following hour. The concentration in venous blood rose more slowly and tended to stop increasing after about 2 h of exposure.

These authors had exposed subjects to concentrations near the present (December 1974) Swedish hygienic limit value, i.e., 500 ppm, which corresponds to 1,740 mg/m³ at 25°C. But von Oettingen (17) used very heavy exposure (up to 40,000 ppm) and found that blood concentration remained stable near a given level for about 1.5—2 h during anesthetic experiments with animals.

A similar pattern for the concentration in alveolar air and blood was found by Astrand et al. in studies of toluene (1),...
methylchloroform (4), and aliphatic components in white spirit (2). The pattern was explained in the study of white spirit by the relatively poor solubility of the solvent in blood. Methylene chloride also appears to dissolve poorly in blood. Therefore, it should be comparatively innocuous if the toxicity is low.

Stewart & Dodd (23) found that a solution of methylene chloride is absorbed through the skin. However, skin is damaged relatively quickly in this type of exposure and results in pain. Thus this kind of exposure is probably insignificant in ordinary industrial contexts, since pain would automatically serve to warn against further exposure.

The metabolism of methylene chloride was largely unknown prior to 1972. Most of the absorbed quantity was recovered unaltered in pulmonary air, and a small amount was found in the urine (11, 15). In 1972, however, Stewart et al. (24, 25) found that the carboxyhemoglobin (COHb) concentration in blood increased in subjects exposed for 1—2 h to 200—1,000 ppm of methylene chloride in inspiratory air. The authors assumed that the carbon monoxide was a product of the metabolism of methylene chloride. In 1973 Fodor et al. (13) anticipated that COHb levels should be elevated in subjects exposed both to methylene chloride and to diiodomethane, dibromomethane, or tribromomethane. The finding of carbon monoxide in the blood in conjunction with exposure means that the toxicity of at least some of the halogenated hydrocarbons must be reassessed.

Some mortalities have been reported in conjunction with very heavy, acute exposure to methylene chloride (6, 9). However, no incident of lasting harm has ever been described for persons subjected to small, acute amounts of methylene chloride. Damage to the liver and central nervous system has been reported following protracted exposure during occupational work (14, 16, 28). Damage of this type has also been reported in conjunction with the exposure of animals (15, 27).

In the present investigation subjects were exposed to methylene chloride both at rest and during exercise in the same manner as in previous studies made at the National Board of Occupational Safety and Health (1, 2, 3, 4).

SUBJECTS

Fourteen men 19 to 29 years of age served as subjects. Twelve were students. The subjects were given a careful clinical examination. The function of their respiratory and circulatory organs was tested both at rest and during exercise. The same methods were used in the medical examination as in previous studies of other solvents (1, 2, 3, 4).

All the subjects were healthy at the time of the examination, and none of them had ever suffered from any disease having a detrimental effect on respiratory or circulatory organs. Values for hemoglobin concentration, hematocrit, and erythrocyte sedimentation rate were within normal limits for all subjects. No one had albumin, blood, or reducible substances in their urine. Results from the lung function tests were also normal. Five subjects were smokers. They were asked not to smoke in the 12 h prior to the exposure experiment. Results from measurements made during the medical examination are listed in table 1.

Table 2 reports the mean values of measurements taken from the subjects during exercise on a bicycle ergometer without exposure. All the values from submaximal and maximal exercise were normal. Thus the subjects of this investigation responded normally to physical exercise and displayed a normal physical work capacity. There was no significant differences between mean values in tables 1 and 2 for subjects in this study and for subjects in studies previously performed (1, 2, 3, 4). Therefore, group differences in reactions to different solvents cannot be ascribed to physiological differences between subjects.

Occasional ectopic beats were recorded in the resting electrocardiogram (ECG) of one subject. Another subject displayed slight ST depression with segmental changes and flattening of the T wave during exercise without any other signs of the heart being affected. There were no other significant ECG changes.

EXPERIMENTAL DESIGN

Subjects were exposed during rest and exercise to methylene chloride concentrations in inspiratory air which were close
To, or about half of, the threshold limit value (TLV). The Swedish TLV for methylene chloride is 500 ppm, corresponding to about 1,740 mg/m$^3$ at 25°C. As of January 1, 1975, however, the Swedish TLV will be 100 ppm (350 mg/m$^3$). All air concentrations are henceforth reported in mg/m$^3$ values at 25°C unless otherwise specified. The air mixture was prepared in a manner similar to that described in previous studies (fig. 1).

The concentration of methylene chloride in inspiratory air was continuously followed with a gas indicator (Hydrocarbon analyzer, Model 116, Scott Research Lab. Inc., Plumsteadville, Pa., U.S.A.). Any given methylene chloride concentration in air was prepared with accuracy sufficient to produce 1,690 to 1,790 mg/m$^3$ when 1,740 mg/m$^3$ was the objective, and 820 to 920 mg/m$^3$ when the objective was 870 mg/m$^3$.

The experiments were performed along the same general lines as in previous studies of white spirit and styrene (2, 3). At the start arterial and venous catheters were introduced into a brachial artery and a medial cubital vein. The subject was then exposed. Each period of exposure lasted 30 min, and each subject was exposed on the same occasion in four consecutive periods, i.e., a total of 2 h each.

The subjects were exposed according to the following three alternatives (fig. 2). Series I: Five subjects were exposed to both about 870 and about 1,740 mg/m$^3$ of methylene chloride in inspiratory air during rest (30 + 30 min) and during exercise (30 + 30 min) at an intensity of about 50 W (300 kpm/min). Series II: Four subjects were exposed to about 870 mg/m$^3$ of methylene chloride in inspiratory air during rest (30 min) and during exercise (30 + 30 + 30 min) at an intensity of about 50 W (300 kpm/min). Series III: Five subjects were exposed to about 1,740 mg/m$^3$ of methylene chloride in inspiratory air during rest (30 min) and during exercise (30 + 30 + 30 min) at intensities of about 50 W (300 kpm/min), 100 W (600 kpm/min), and 150 W (900 kpm/min).

Fig. 3 shows the times at which the measurements were made and the tests taken. Alveolar air samples for methylene chloride assay were taken from the breathing valve with the use of a glass syringe during exposure and in glass tubes after exposure. Arterial and venous blood samples (approx. 0.5 g) were taken from the catheters and collected in 25-ml glass bottles for the analyses of methylene chloride and collected in 10-ml glass bottles.

**Table 1.** Body height, body weight and respiratory data, taken at rest, from 14 male subjects 19 to 29 years of age. Mean values and the standard errors of the mean are given. (FEV$_{\%}$ = forced expiratory volume for 1 second as the percentage of forced expiratory vital capacity; MVV$_t$ = maximal voluntary ventilation at an optional rate)

| Body height cm | Body weight kg | Vital capacity l | Residual volume l | FEV % | MVV$_t$ l/min |
|----------------|----------------|------------------|-------------------|-------|---------------|
| 183 ± 2        | 73.1 ± 2.1     | 6.2 ± 0.2        | 1.8 ± 0.1         | 84 ± 2| 201 ± 8       |

**Table 2.** Results from the exercise test on a bicycle ergometer without exposure to methylene chloride. Mean values and the standard error of the means are given. ($V_E$ = expiratory volume; $V_O_2$ = oxygen uptake per unit of time)

| Exercise intensity W | No. of subjects | Heart rate beats/min | $V_E$ BTPS l/min | $V_O_2$ STPD l/min | Blood lactate mmol/l |
|----------------------|-----------------|----------------------|------------------|--------------------|---------------------|
| 50                   | 14              | 102 ± 3              | 27.0 ± 1.2       | 1.07 ± 0.03        | 2.3 ± 0.2           |
| 100                  | 14              | 123 ± 4              | 39.3 ± 1.2       | 1.61 ± 0.02        | 2.6 ± 0.2           |
| 150                  | 14              | 151 ± 5              | 55.9 ± 2.2       | 2.25 ± 0.04        | 5.1 ± 0.6           |
| max. work            | 14              | 192 ± 3              | 136.3 ± 5.9      | 3.72 ± 0.16        | 14.7 ± 0.3          |

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The air mixture was produced as follows: Compressed air was led through a charcoal filter (A) to rotameters (B and C) connected in parallel; both rotameters were fitted with valves to regulate the airflow. Air passed from one rotameter (C) into a wash bottle (D) containing methylene chloride. In the bottle (D) there was a tube (E) containing a capillary tube (F). The methylene chloride was ascending in the tube (E), and the air passed through the capillary tube (F). Air and the methylene chloride mixture were then fed to a closed vessel (G) in which mixing took place. Thereafter, the air mixture was conducted to the base of a cylinder (H) from which inspiratory air was sucked into the breathing valve via a metal tube (I). Excess gas was removed through a chimney (K). The device was placed in a fume cupboard, and excess gas was exhausted.

Fig. 1. The air mixture was produced as follows: Compressed air was led through a charcoal filter (A) to rotameters (B and C) connected in parallel; both rotameters were fitted with valves to regulate the airflow. Air passed from one rotameter (C) into a wash bottle (D) containing methylene chloride. In the bottle (D) there was a tube (E) containing a capillary tube (F). The methylene chloride was ascending in the tube (E), and the air passed through the capillary tube (F). Air and the methylene chloride mixture were then fed to a closed vessel (G) in which mixing took place. Thereafter, the air mixture was conducted to the base of a cylinder (H) from which inspiratory air was sucked into the breathing valve via a metal tube (I). Excess gas was removed through a chimney (K). The supply of air containing methylene chloride was delivered to the cylinder (H) at a rate of 60 to 100 l/min and was never less than a subject's pulmonary ventilation. The device was placed in a fume cupboard, and excess gas was exhausted.

for COHb assay. Preceding studies on solvents provide the details of these techniques (1, 2, 3, 4).

A mean value for the concentration of methylene chloride in alveolar air was calculated for each subject and each period on the basis of the final three determinations. The concentration of methylene chloride in arterial and venous blood was calculated as the mean value for each subject on the basis of the final two determinations in each period. These concentrations tended to be at a given level at the end of each period. However, the COHb concentration increased continuously. Thus the highest COHb value (generally the final value) was selected for each subject and period.

The concentration of methylene chloride in alveolar air and blood and the concentration of COHb in blood were followed for another 2 h after the conclusion of the four periods of exposure. The exact times for samplings are shown in fig. 3 and in the figures describing the results. The final value in each 30-min period for each subject was used in calculating the mean values for the respective concentrations 30, 60, 90, and 120 min after concluded exposure.

The ECG was recorded continuously during exposure, and heart rate was determined every other minute during these recordings. The mean value for the three final determinations during each exposure period was used. Blood samples for lactic acid assay were taken at the end of each period.

The volume of expiratory air was continuously measured in bags (specially made of polyester-laminated aluminium foil) throughout the entire exposure, i.e., for 2 h, and the methylene chloride content of expiratory air was determined. The figures describing results show how the expired air was fractionated in 20 to 30 different bags in the three types of exposure experiments. The volume of inspiratory air was estimated to be the same as the volume of expiratory air, and the uptake of methylene chloride in the organism was calculated as the difference between the total amounts in inspiratory and expiratory air. The alveolar ventilation per minute was calculated for the latter half of each period, i.e., for about 15 min.

The oxygen content of expiratory air was analyzed, and oxygen uptake was calculated for the latter half of each exposure period at rest and for the final 5—10 min of exercise. The mean value for the number of determinations (bags) was used.

These latter measurements, as well as the determination of blood lactic acid concentration, were made in order to facilitate the assessment of exercise severity for each subject.

**ANALYTICAL METHODS**

Respiratory volumes, blood lactate concentration, and heart rate were determined according to methods described in the toluene study (1). The oxygen and carbon dioxide content of expiratory air was determined with an oxygen analyzer (Beckman model E 2) and a carbon dioxide analyzer (Beckman model LB 2), and oxygen uptake was calculated. The meth-
Fig. 2. Fourteen subjects were exposed during four periods of 30 min each: at rest and during exercise according to three different alternatives (series I, II or III). There was a pause of 20 min without exposure between rest and exercise. During the last 5 min of this pause the subject was cycling at a work load of 50 W. (Low = about 870 and high = about 1,740 mg/m$^3$ of methylene chloride in the inspiratory air)

od error for these analyses was indicated in the white spirit study (2).

Alveolar ventilation ($V_A$) was calculated on the basis of pulmonary ventilation ($V_E$), respiratory rate, and dead space ($V_E \cdot$respiratory rate $\cdot$ dead space $= V_A$). Respiratory rate was recorded by using the ECG strip chart recorder with the aid of a heat receptor located in the breathing valve. Dead space in subjects, including the valve, was set at 150 cm$^3$ for all subjects. No corrections were made for any larger dead space occurring during exercise, since differences at the ventilations measured are comparatively slight.

The concentration of methylene chloride in inspiratory and expiratory air was determined with a gas chromatograph (Model F 11, FID, Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, England) equipped with a stainless steel column (1-m long, 2.2-mm inner diameter) packed with 8 % Carbowax 400 on chromosorb W (80—100 mesh). The column temperature was 60°C. A 1-ml sample was injected.

The column temperature was 30°C. A 1-ml sample was injected.

The methylene chloride content was calculated on the basis of the chromatogram with the aid of standard air samples containing known amounts of methylene chloride.

The methylene chloride content of blood was determined with a "head space" method (4). The assay was performed by using a gas chromatograph (Model F 30, FID, Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, England) equipped with a stainless steel SCOT column (15-m long, 0.51-mm inner diameter) with Carbowax 400 as the stationary phase. The carrier gas flow was 4 ml/min, and the column temperature, 80°C. The methylene chloride content of the head space was calculated from individual blood samples and standard air samples containing known amounts of methylene chloride. The error of the method for a single determination was calculated on the basis of 10 double determinations with blood in which methylene chloride contents ranged from 2.9—3.4 mg/kg of blood and amounted to ±7.6 % of the mean value.

The COHb content in blood was determined with a method described by Övrum (18). A 0.2-ml citrate solution, as an anticoagulant, and 1 drop of octyl alcohol, as an antifoaming agent, were added to a 10-ml injection bottle. The bottle, includ-
ing a serum cap (rubber membrane), was weighed before and after the addition of 0.5—1.0 ml of blood.

The rubber membrane sealing a bottle containing a blood sample was pierced with a needle connected via a tube to a water suction unit. The needle was removed after 10—20 s, and 3.0 ml of reagent solution (2 parts 3.2 potassium ferricyanide + 8 % Saponin in water + 1 part 0.8 % lactic acid in water) was transferred with a syringe to the bottle by piercing the membrane. After removal of the needle, the bottle was shaken for 20 min in a shaker. Thereafter the membrane was pierced with a needle so as to equalize the pressure. A needle connected to a plastic or latex tube filled with water was inserted through the membrane. The other end of the tube was submerged in a water-filled beaker. With a gas-tight syringe exactly 1 ml of air was then removed from the bottle. The negative pressure in the bottle was simultaneously equalized when a corresponding volume of water was sucked into the bottle. The syringe tip was withdrawn when the influx of water ceased.

The sample in the syringe was then injected into a gas chromatograph (Model F11, with a thermal conductivity detector, Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, England) equipped with a stainless steel column (2-m long, 2.2-mm inner diameter) filled with molecular sieve 13X (60—80 mesh). Helium (30 ml/min) was used as the carrier gas, and the column temperature was 40°C. A standard gas (1 ml) with a CO content of the same magnitude as the samples was used in the evaluation of concentrations.

The following formula was used in calculating the concentration of the carboxyhemoglobin: % COHb =

$$\frac{6.82 \cdot a \cdot V_1 - (V_2 + V_3 + V_4) \cdot t_{\text{sample}} \cdot 10^3}{V_3 \cdot h \cdot t_{\text{standard}}}$$

in which \(a\) = the volume of CO per volume of air in standard gas, \(V_1\) = bottle volume (ml), \(V_2\) = the volume of reagent solution, \(V_3\) = the blood volume, \(V_4\) = the volume of citrate solution + octyl alcohol, \(h\) = hemoglobin content (g Hb/100 ml of blood), \(t_{\text{sample}}\) = the peak height for CO in the sample's gas chromatogram, \(t_{\text{standard}}\) = the peak height for CO in the chromatogram of the standard gas.

The error of the method for a single determination, calculated from 10 double determinations, was 3 % of the mean value of 4.25 % COHb.

The hemoglobin content of blood was determined before and after exposure by spectrophotometric means with the cyanmethemoglobin method.

RESULTS

Pulmonary ventilation and blood circulation

The subject who displayed occasional ectopic beats in his resting ECG during the medical examination also displayed occasional ectopic beats during exposure.

![Fig. 3. Times for different measurements and sampling prior to, during, and after a 30-min exposure.](image-url)
Prior to exposure two other subjects showed atrial rhythm arising alternately from two different foci. This arrhythmia persisted during exposure at rest, but disappeared during exercise. The subject who displayed ST depression in the medical examination in conjunction with exercise also displayed the same type and degree of change during exposure and exercise. In conjunction with exercise and exposure two other subjects developed ST depressions and segmental changes. They displayed no other signs of effect on the heart.

Values for alveolar ventilation, oxygen uptake, and heart rate during exposure at rest were of a normal magnitude (table 3). The values during 50 W (300 kpm/min) work failed to differ systematically from the corresponding values in the medical examination without exposure (tables 2 and 3). The subjects who were exposed during exercise at 100 and 150 W (600 and 900 kpm/min) displayed a physical work capacity which was above average for the group. This circumstance was reflected in lower than average values for heart rate and blood lactate concentration (tables 2 and 3). The slow increase in heart rate and oxygen uptake noted in the four subjects exposed during 50 W exercise (300 kpm/min) for 1.5 h was moderate and probably unrelated to exposure.

No differences were found in alveolar ventilation, oxygen uptake, and heart rate either at rest or during exercise at an intensity of 50 W (300 kpm/min) between exposure to about 870 mg/m³ or to about 1,740 mg/m³ (table 3).

At an exercise intensity of 50 W (300 kpm/min) the subjects utilized an average of 27 % of their maximal aerobic work capacity (max \( \dot{VO}_2 \)), about 45 % at 100 W (600 kpm/min), and about 64 % at 150 W (900 kpm/min). Lactic acid concentrations in blood at corresponding intensities suggested that 50 and 100 W can be regarded as relatively light work, and 150 W, as relatively heavy work (tables 2 and 3).

Alveolar air and arterial blood concentrations of methylene chloride during exposure

After 30 min of exposure at rest to about 870 mg/m³ of methylene chloride in in-
spiratory air (series I and II) the concentration in alveolar air amounted to about 250 mg/m³ or about 30 % of the concentration in inspiratory air (table 3). The corresponding arterial blood concentration was about 2.3 mg/kg. The alveolar concentration nearly doubled, rising to 480 mg/m³ and corresponding to about 55 % of the concentration in inspiratory air, whereas the arterial concentration somewhat more than doubled, rising to about 5.1 mg/kg during exercise at 50 W. The alveolar ventilation increased about threefold during the corresponding work load (fig. 4 a).

The alveolar and arterial concentrations slightly more than doubled in exposure at rest to about 1,740 mg/m³ of methylene chloride (series I and III) as compared to the values recorded in exposure to half that concentration (table 3). During work at 50 W both alveolar and arterial concentrations increased in about the same way as in exposure to the lower methylene chloride concentration. The alveolar concentration amounted to approximately 55 % of the concentration in inspiratory air, and the resting arterial concentration rose from 5.5 mg/kg of blood to 10.6 mg/kg (fig. 4 a).

In work at 100 and 150 W alveolar ventilation increased fivefold and sevenfold, respectively, when compared to resting conditions. On the other hand, alveolar concentration only increased slightly, i.e., to 1,090 and 1,220 mg/m³, respectively, corresponding to 63 and 70 %, respectively, of the concentration in inspiratory air (series III). Equivalent arterial concentrations rose to 13.4 mg/kg and 14.8 mg/kg of blood, respectively (table 3, fig. 5 a).

During the mentioned periods the increase in alveolar and arterial concentrations tended to decline towards the end, i.e., after 25—30 min of each period. However, this flattening of the curve slope became far more striking during the third and fourth periods of series II, which comprised constant exposure and exercise for 90 min at a work intensity of 50 W (table 3, fig. 6 a).

The relationship between arterial and alveolar concentrations of methylene chloride at the end of each exposure period was linear (fig. 7).

**Venous concentration and arteriovenous methylene chloride difference during exposure**

The venous concentration of methylene chloride generally followed the arterial concentration (figs 4 a, 5 a and 6 a). The arteriovenous difference, which to some extent reflects the release of methylene chloride to other organs, was about twice as great in exposure to the higher concentration as in exposure to the lower concentration at rest (table 3). This difference increased further in work at 50 and 100 W but dropped at 150 W to about the same value as at rest (table 3). Release from arterial blood per unit of time, expressed in mg/min, was naturally higher at 150 W than at 100 W, since cardiac output was then far greater. Cardiac output has been measured in previous studies (2, 3, 4). It then amounted to 5 l/min at rest, 10 l/min at 100 W, and about 18 l/min at 150 W.

However, it should be pointed out, as in the previous studies on solvents, that the venous blood sampled was peripheral and not central. It is conceivable that peripheral blood does not provide the same information during rest as during exercise in view of the redistribution of blood circulation which takes place in conjunction with the transition from rest to work.

**Uptake in the organism**

The organism's uptake could be determined by means of continuous measurement of the amount of methylene chloride in inspiratory and expiratory air (table 4). An example of each type of experiment is provided in figs 4 b, 5 b, and 6 b. Uptake amounted to about 130 mg in exposure to the lower concentration at rest (series I + II) in 30 min and to approximately 260 mg in exposure to the higher concentration (series I + III); both uptake levels constituted about 55 % of the amount supplied. About 270 mg was taken up in 30 min at the light exposure during exercise at an intensity of 50 W (series I + II) and about 510 mg at the heavy exposure (series I + III); both uptake levels corresponded to about 40 % of the amount supplied (table 4 and fig. 4 b).
Fig. 4a. Concentration of methylene chloride (CH₂Cl₂) in alveolar air, in arterial blood and venous blood and of carboxyhemoglobin (COHb) in blood in one subject during and after exposure. Exposure was performed with 870 and 1,740 mg/m³ of methylene chloride during rest and exercise at an intensity of 50 W (300 kpm/min). (VA = alveolar ventilation l/min)

Fig. 4b. The amount of methylene chloride supplied and taken up during the same periods presented in fig. 4a. (Same subject as in fig. 4a)
Fig. 5a. Concentration of methylene chloride (CH₂Cl₂) in alveolar air, in arterial blood and venous blood and of carboxyhemoglobin (COHb) in blood in one subject during and after exposure. Exposure was performed with 1,740 mg/m³ of methylene chloride during rest and exercise at an intensity of 50, 100 and 150 W (300, 600 and 900 kpm/min). (Vₐ = alveolar ventilation l/min)

In the series with increasing work intensity (series III) uptake amounted to 565 mg at 50 W (2nd period), 575 mg at 100 W (3rd period), and 640 mg at 150 W (4th period); these uptake levels were 44, 28 and 23 %, respectively, of the total amount supplied. Mean uptake dropped to 34 % during the third period and to 31 % during the fourth period in protracted exposure to low levels during exercise (series II) at a constant work intensity (fig. 6 b).

There was no linear relationship between uptake and the amount of methylene chloride supplied (fig. 8). The correlation between arterial blood levels and the amount of methylene chloride taken up was relatively poor (fig. 9). The correlation proved to be even poorer between venous blood concentration and the amount of methylene chloride taken up.

**Excretion of methylene chloride after exposure**

Figs. 4 a, 5 a, and 6 a show that the concentration of methylene chloride in alveolar air dropped very rapidly after the conclusion of exposure. The rate of excretion was considerably lower after 30 min (table 5). Thus most subjects had demonstrable traces of methylene chloride in their alveolar air as long as 18 h after exposure. The rate of excretion, measured as the concentration in alveolar air, arterial blood and venous blood, was not linear in a log-log system (cf. 1, 2, 3, 4).

**Metabolism of methylene chloride**

Figs. 4 a, 5 a, and 6 a show that the COHb concentration increased both during the course of exposure and after the end of
exposure. The final determinations were made 2 h after the end of exposure. However, the rate of increase declined towards the end of the 2 h. This decline suggests that peak values were achieved in most cases. [Stewart et al. (24, 25) arrived at the same results in 1972.] It was also found that the venous concentrations did not differ systematically from arterial concentrations. For technical reasons more arterial samples were taken than venous samples. Therefore, only arterial concentrations are noted in the tables.

Table 6 reports the mean value for the highest (final) COHb concentrations in each 30 min exposure period. The values are corrected for the resting values, which averaged 0.12 g/100 ml. Four of the five subjects who reported being smokers had elevated resting values prior to exposure (0.24, 0.14, 0.34 and 0.22 g/100 ml). In exposure at rest to about 870 mg/m³ of methylene chloride (series I) the concentration rose to 0.12 mg/100 ml from 0 and further to 0.26 g/100 ml in the transition to double the concentration. The COHb content was less than expected in the fourth period during exercise at an intensity of 50 W with a doubling of the uptake of methylene chloride. The COHb concentration in series II remained about the same during periods 1 and 2 as in series I. The concentration increased slowly in continued work at 50 W for an additional hour. The value was low for period 1, in which the exposure was the higher concentration, in series III. During work at 50 W the concentration increased relatively little. Only a small relative increase occurred also in the last two periods with exercise at 100 and 150 W. After the end of exposure the COHb level rose more in series III than in the other two series (table 5). All three series are illustrated in fig 10.

The uptake of methylene chloride was far greater during the exercise periods than during the rest periods (table 4). The arterial blood concentration was also higher during exercise than at rest. If CO is assumed to be derived from the metabolism of methylene chloride and the degree of metabolism is constant, the COHb level should increase, after a slight delay, in step with the uptake of methylene chloride. Values less than expected during the fourth period of series I and III may, however, have been due to the flushing out of
CO from the alveoli in conjunction with the increased pulmonary ventilation which occurred during exercise. Values greater than expected, especially 30 min after the end of exposure in series I and III, may analogously be due to the reduced ventilation at rest and the attendant low level of "airing."

In conjunction with exposure to the TLV, extra CO was produced in quantities corresponding to a blood COHb level of 0.7 g/ml. In addition there was a resting value of more than 0.1 g/100 ml, i.e., an aggregate of 0.85 g/100 ml. This value corresponds to about 5.5 % COHb at a normal Hb level of about 15.4 g/100 ml.

The hemoglobin content was not altered by exposure. Thus there was no accelerated breakdown of hemoglobin with attendant CO production during exposure.

The following supplementary experiment was conducted in order to ascertain whether COHb was formed in the blood. Air containing 1,740 mg/m³ of methylene chloride was admitted into a 50-ml rotating round retort containing 5 ml of heparinized blood (temp. = 37°C). After 3 h of exposure the COHb level in the blood diminished. Thus the experiment showed that CO is not formed in the blood under these experimental conditions. The amount initially found was flushed out to some extent during the course of the experiment. Thus exposure to methylene chloride probably results in the formation of CO in some other organ than the blood, e.g., in the liver.

**DISCUSSION**

As in previous studies no effect on blood circulation or respiration could be demonstrated as a result of exposure to a solvent (1, 2, 3, 4). Thus neither methylene cloro-
impairment in the present study was apparently due to the fact that the present concentrations were much lower. Thus subjects did not display more than about 4 % COHb when work was performed at the end of the fourth period.

The concentration of methylene chloride in alveolar air was relatively high at rest in relation to the degree of exposure. The concentration rose stepwise during exercise with increasing intensity. The arterial blood concentration displayed a rate of increase which declined, especially during the fourth period. Similar courses for the concentration in alveolar air and arterial blood were obtained in exposure to substances with limited solubility in blood (2, 3). However, the alveolar membrane must not impede diffusion of the substance from air into blood. Van Rees (20) recently pointed out that the rate of diffusion of a solvent through the alveolar membrane is probably never the limiting factor in the uptake in blood. Thus methylene chloride uptake in the organism primarily depends upon its solubility in blood. The resting uptake reported in the present study, i.e., about 50 % of the amount supplied to the
alveoli, which declined in the course of time both during constant exposure and during exposure with increasing work intensity, fully supports this view.

In reports on the studies of toluene (1) and methylchloroform (4) it was recommended that exposure during occupational work should be controlled by measuring the concentration in ambient air and sometimes also that in alveolar air.

Since during exposure methylene chloride shows similarities to these two solvents, both methods should also be used in conjunction with exposure to methylene chloride.

As noted in the section on metabolism, extra CO is formed during exposure to methylene chloride. A small amount of endogenous CO is always produced, as first demonstrated by Sjöstrand in 1949.
Table 5. Concentration of methylene chloride (CH₂Cl₂) in alveolar air and blood and carboxyhemoglobin (COHb) in blood after the exposure. Mean values and standard error of the means are given. The resting values before exposure were subtracted from the COHb values. (Series I: 870 and 1,740 mg/m³ rest + 50 W; series II: 870 mg/m³ rest + 50 W; series III: 1,740 mg/m³ rest + 50 + 100 + 150 W; see table 4)

| No. of subjects | Alveolar conc. CH₂Cl₂ mg/m³ | Arterial blood conc. CH₂Cl₂ mg/kg | Venous blood conc. CH₂Cl₂ mg/kg | COHb blood conc. g/100 ml |
|-----------------|-----------------------------|---------------------------------|-------------------------------|--------------------------|
| end of exp.     | 4                           | 1,029 ± 31                      | 11.0 ± 0.6                    | 0.48 ± 0.04              |
| after 30 min    | 4                           | 212 ± 3                         | 2.1 ± 0.4                     | 0.63 ± 0.06              |
| » 60 »          | 4                           | 117 ± 4                         | 1.1 ± 0.1                     | 0.70 ± 0.05              |
| » 90 »          | 4                           | 77 ± 3                          | 0.8 ± 0.1                     | 0.71 ± 0.05              |
| » 120 »         | 4                           | 57 ± 4                          | 0.5 ± 0.1                     | 0.71 ± 0.06              |
| » 180 »         | 4                           | 20 ± 6                          | —                             | —                        |
| » 18 h          | 4                           | 2.0 ± 0.2                       | —                             | —                        |
| end of exp.     | 4                           | 538 ± 38                        | 5.8 ± 0.4                     | 0.39 ± 0.04              |
| after 30 min    | 4                           | 148 ± 22                        | 1.6 ± 0.2                     | 0.44 ± 0.03              |
| » 60 »          | 4                           | 77 ± 9                          | 0.8 ± 0.1                     | 0.49 ± 0.04              |
| » 90 »          | 4                           | 50 ± 5                          | 0.5 ± 0.1                     | 0.48 ± 0.05              |
| » 120 »         | 4                           | 38 ± 7                          | 0.3 ± 0.1                     | 0.49 ± 0.06              |
| » 180 »         | 4                           | 21 ± 5                          | —                             | —                        |
| » 18 h          | 4                           | 2.0 ± 0.2                       | —                             | —                        |
| end of exp.     | 5                           | 1,222 ± 43                      | 14.8 ± 0.4                    | 0.34 ± 0.02              |
| after 30 min    | 5                           | 362 ± 25                        | 4.1 ± 0.3                     | 0.43 ± 0.05              |
| » 60 »          | 5                           | 229 ± 23                        | 2.6 ± 0.2                     | 0.50 ± 0.08              |
| » 90 »          | 5                           | 142 ± 11                        | 1.6 ± 0.2                     | 0.55 ± 0.09              |
| » 120 »         | 5                           | 80 ± 10                         | 1.1 ± 0.2                     | 0.62 ± 0.05              |
| » 180 »         | 5                           | 49 ± 8                         | —                             | —                        |
| » 18 h          | 5                           | 3 ± 1                          | —                             | —                        |

Table 6. Concentration of COHb in g/100 ml of blood during the four exposure periods in the three series of experiments. The resting values before exposure were subtracted. Mean values and standard error of the means are given. (Series I: 870 and 1,740 mg/m³ rest + 50 W; series II: 870 mg/m³ rest + 50 W; series III: 1,740 mg/m³ rest + 50 + 100 + 150 W; see table 4)

| Series | No. of subjects | Resting value before | Period 1       | Period 2       | Period 3       | Period 4       |
|--------|-----------------|----------------------|----------------|----------------|----------------|----------------|
| I      | 4               | 0.12 ± 0.04          | 0.12 ± 0.02    | 0.26 ± 0.03    | 0.43 ± 0.03    | 0.48 ± 0.04    |
| II     | 4               | 0.11 ± 0.04          | 0.12 ± 0.01    | 0.23 ± 0.02    | 0.26 ± 0.03    | 0.39 ± 0.04    |
| III    | 5               | 0.14 ± 0.05          | 0.10 ± 0.02    | 0.23 ± 0.03    | 0.34 ± 0.02    | 0.34 ± 0.02    |

(22). The endogenous CO formed amounts to about 18.7 μmol/h (7). Coburn (8) offers further references. If none of this production flushes out and the entire amount accumulates in 5 l of blood containing 770 g of Hb, a concentration increase equivalent to 0.08 % COHb would be obtained in 2 h. Thus endogenous production is slight when compared to the production attained in exposure to methylene chloride. Additional COHb amounting to 0.5 g/100 ml, corresponding to 3.2 % with a normal Hb content of 15.4 g/100 ml, was obtained in exposure to 870 mg/m³ of methylene chloride for 2 h.

Irrespective of its genesis CO blocks oxygen uptake in the organism. This very serious effect of CO has led to extensive discussion of the TLV for CO in air. Scientists at the National Institute for Occupational Safety and Health in Sweden feel that a reduction to 35 ppm is justified in order to prevent a COHb content exceeding 5 % (26). The U.S. value, like the
Swedish value (December 1974), is currently 50 ppm.

The COHb level, including the resting value, exceeded 5% when subjects were exposed to the TLV of methylene chloride (500 ppm or 1,740 mg/m³) for 2 h. The concentration would increase further during an 8-h work day (19). Therefore, lowering the TLV of methylene chloride to such an extent that the COHb is never able to rise to more than 5% is justified. The U.S. value was reduced to 250 ppm in 1972 (5). However, this reduction is probably inadequate. As mentioned earlier, additional carbon monoxide corresponding to 0.5 g/100 ml would be formed during exposure to 250 ppm for 2 h. With an ordinary resting value of slightly more than 0.1 g/100 ml the COHb level would then amount to about 4%. The concentration might then rise to more than 5% if exposure to this concentration continued for 8 h.

This circumstance should be sufficient justification for supplementing the aforementioned sampling of ambient and alveolar air with an additional test, viz., COHb in blood. However, it is obvious that any such blood sampling would meet with considerable practical difficulties. Therefore, it is suggested that the TLV for methylene chloride be set at a level which guarantees that the COHb content of blood will never exceed 5%.

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