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Exposure to methylene chloride

Content in subcutaneous adipose tissue

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ENGSTRÖM, J. and BJURSTRÖM, R. Methylene chloride exposure: Content in subcutaneous adipose tissue. *Scand. j. work environ. & health* 3 (1977) 215—224. The fat content of the body was calculated in 12 healthy male subjects aged 21 to 35 years by means of hydrostatic weighing and anthropometric estimation of skeletal weight. The subjects were exposed to a concentration of 2,600 mg of methylene chloride per cubic meter of inspired air (750 ppm) for 1 h while performing work at an intensity of 50 W on a bicycle ergometer. The uptake in the organism was measured continuously with the Douglas bag technique. The amount of methylene chloride absorbed correlated highly with degree of obesity and body weight. Needle biopsy specimens of subcutaneous adipose tissue were taken from the buttocks before exposure and 0, 1, 2, 3 and 4 h after exposure. The mean yield of tissue from the 72 biopsies was 25 mg. The concentration of methylene chloride in the adipose tissue was determined by gas chromatography, using a headspace method. The mean concentration was 10.2 mg/kg 1 h after exposure and 8.4 mg/kg after 4 h. There was a wide distribution around the mean values. In the six slim subjects the concentration in the adipose tissue during the 4 h after exposure was on an average twice that of the six more obese subjects. On the other hand, in spite of lower concentrations, the obese subjects had a greater calculated amount of methylene chloride in the total fat depots of the body. Two subjects were studied about 22 h after exposure, the concentration in subcutaneous adipose tissue being 1.6 and 1.7 mg/kg, respectively, at that time.

Key words: adipose tissue, alveolar air concentration, body fat concentration, exercise, exposure, man, methylene chloride.

The uptake of organic solvents in the body has been shown to vary with the degree of physical work load (1, 12, 19). Inter-individual constitutional differences with respect to uptake, distribution, elimination and the risk of harmful effects have been discussed (7, 16, 17). Adipose tissue is of major interest in this context since solvents display their greatest affinity for this type of tissue. The magnitude of fat

stores may conceivably be significant to the concentration in sensitive organs, such as bone marrow, the liver and the nervous system. Adipose tissue has been studied in great detail in experimental animals. No reports are available on the concentration of solvents in the adipose tissue of man at work or after experimental exposure.

A major objective of the present study was to expose subjects to methylene chloride under standardized conditions, including light physical exercise, and to devise a method, using needle biopsies and gas chromatography, for determining the concentration in subcutaneous adipose tissue. Another objective was to attempt to assess the significance of the magnitude

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of fat stores to uptake and distribution in the organism. An ultimate objective is to refine the method for assaying the solvent content of adipose tissue so that the method can be used on people engaged in industrial work.

The methylene chloride content of fat specimens was determined with basically the same head space technique as in the blood assays described in previously published reports from this laboratory (1, 2). In this technique the tissue sample is placed in a bottle, which is sealed. A sample is then extracted from the gas phase through a membrane in the seal, after attainment of equilibrium between adipose tissue and air. The partition coefficient is the ratio between the solvent concentration in the tissue and the gas phase in equilibrium. Thus solvents displaying a great affinity for adipose tissue have a high adipose tissue/air partition coefficient.

Several initial experiments were conducted with toluene, a solvent with a high partition coefficient. A high coefficient means that the gas phase concentration is low. Assaying samples from the gas phase then presents difficulties. Moreover, in these pilot studies we utilized Menghini needles for the biopsies. However, the extraction was unsatisfactory. Therefore, in the main part of the study we used a substance with a relatively small partition coefficient, i.e., methylene chloride, and switched to muscle biopsy needles in order to obtain better specimen extraction.

EXPERIMENTAL DESIGN

Twelve male subjects from 21 to 35 years of age received the same examinations, prior to solvent exposure, as in the previously reported methylene chloride study from this laboratory (3). The subjects reported no history of disease, nor did they display any clinical symptoms of ongoing disease. Five of the subjects were smokers. None of the 12 displayed any symptoms of alcohol abuse. Hydrostatic weighing, estimation of skeletal weight and estimation of body fat weight according to von Döbeln (10, 11) also preceded the exposure experiments. The amount of adi-

pose tissue was calculated on the basis of these values and the assumption that the average fat content of adipose tissue is 62 % (15). Subjects were instructed not to drink any alcohol the day before the solvent exposure and not to eat or smoke on the morning before exposure.

The methods employed in the exposure experiments were generally the same as in the previous study of methylene chloride, except for the assays of methylene chloride in adipose tissue (3). Thus a catheter was introduced into a cubital vein in 10 of the 12 subjects. After intracutaneous anesthesia using a 1 % lidocaine solution (Xylocain, Astra, Sweden), a subcutaneous adipose tissue specimen was punched from the superior lateral quadrant of the gluteal region with a Bergström muscle biopsy needle with a diameter of 4 mm (4). No anesthesia of the hypodermis was necessary, since biopsy elicits no pain response there. The object of this biopsy prior to exposure was to preclude any factor of adipose tissue which might interfere with the methylene chloride assays.

Subjects were exposed for 1 h through a breathing valve to an inspiratory air concentration of about 2,600 mg of methylene chloride per cubic meter (750 ppm) during light exercise (50 W) on a bicycle ergometer. The air mixture was prepared in the same manner as in previous methylene chloride experiments (3).

Alveolar air samples for methylene chloride assay were taken from the breathing valve with a glass syringe during exposure and in a glass tube after exposure. For methylene chloride assay, venous blood samples (about 0.5 g) were taken from the catheters straight into 25-ml glass bottles. The ECG was recorded continuously.

The volume of expiratory air was continuously measured in bags (specially made of polyester-laminated aluminium foil) during exposure, and the methylene chloride concentration was determined. The volume of inspiratory air was estimated to be the same as the volume of expiratory air (< 1 % error), and uptake in the organism was calculated as the difference between the total amount of methylene chloride in inspiratory and expiratory air.

In all cases, adipose tissue specimens were taken within a few minutes after concluded exposure and 1, 2, 3, and 4 h later with a deviation of no more than 10 min from the exact hour, counted from the end of exposure. Including the specimen prior to exposure, a total of three adipose tissue specimens were taken from each buttock. Attempts were made to locate the biopsy channels as far from one another as possible to avoid taking specimens from tissue with deranged blood circulation. Specimens were obtained in all biopsies, and no complications in the form of hemorrhage or infection were recorded. Specimens were transferred as quickly as possible to 5-ml bottles with a membrane and valve. Blank bottles were exposed as long as the sample bottles to the air in the room in which the specimens were taken. The methylene chloride concentration in the alveolar air and subcutaneous adipose tissue of two subjects was also determined about 22 h after the termination of exposure. After exposure, the experimental conditions were less strictly standardized with respect to physical activity, food intake and smoking.

ANALYTICAL METHODS

A gas indicator (Hydrocarbon analyzer, model 116, Scott Research Lab. Inc., Plumsteadville, Pa., U.S.A.) was used to make continuous records of the concentration in inspiratory air.

The concentration in expiratory air was determined with a gas chromatograph (model F 30, FID, Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, England). The gas chromatograph was fitted with a stainless steel column (2.0 m long with an inner diameter of 2.2 mm). The stationary phase consisted of 3 % Fraktonitril III on Chromosorb G, 80—100 mesh. The temperature of the column, injection block, and detector was 100°C. Nitrogen was used as the carrier gas at a flow rate of 30 ml/min. The sample quantity, 1.0 ml of air, was injected with the sampling valve.

The concentration of methylene chloride in alveolar air was determined with a gas chromatograph (model F 11, FID, Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire,

England). Five-milliliter samples of alveolar air were taken from the breathing valve at the end of the expiration phase and 0.5 ml of the sample was injected into the gas chromatograph, which was fitted with a stainless steel column (0.8 m long with an inner diameter of 2.2 mm). The stationary phase consisted of 1 % Squalan on Chromosorb G, 60—80 mesh, and the column temperature was 50°C. The carrier gas was nitrogen at a flow rate of 22 ml/min.

From the chromatogram the methylene chloride content in air was calculated with the aid of standard air samples with known methylene chloride contents.

The methylene chloride content of the blood was determined with the head space method according to Åstrand et al. (2). The gas chromatograph (model F 11, FID, Perkin-Elmer) was fitted with a stainless steel column (2.0 m long with an inner diameter of 2.2 mm) packed with 3 % Fraktonitril III on Chromosorb G, 80—100 mesh. The column temperature was 75°C and the temperature of the injection block was 200°C. Nitrogen was the carrier gas at a flow rate of 30 ml/min.

The biopsy specimens of adipose tissue were placed in 5-ml glass bottles containing 100 μ l of 5M HCl. The purpose of the hydrochloric acid was to break down the adipose tissue by means of acid hydrolysis. The size of the specimen was determined by weighing. The bottles were sealed with a special teflon stopper and then incubated in a water bath at 60° C for 1 h with agitation at regular intervals. After 1 h a 0.5-ml sample of the headspace phase was taken with a gas-tight syringe and analyzed with a gas chromatograph (model 511, FID, Analytical Instruments Development Inc., Avondale, Pa., U.S.A.). The stainless steel column (2.0 m long with an inner diameter of 2.2 mm) was packed with 3 % Fraktonitril III on Chromosorb G, 80—100 mesh. The column temperature was 75°C, and the temperature of the injection block and detector was 85°C. Nitrogen was the carrier gas and the flow rate was 25 ml/min.

In order for the concentration of methylene chloride in adipose tissue to be calculated, the partition coefficient between adipose tissue and air was determined by means of in vitro experiments with human

Table 1. Body height, body weight, estimated weight of adipose tissue and the degree of obesity, expressed as estimated fat weight in per cent of body weight, of 12 subjects 21—35 years of age. Mean values and standard errors (SE) of the means are also given for the group.

Subject	Body height (cm)	Body weight (kg)	Estimated weight of adipose tissue (kg)	Estimated fat (% of body weight)
1	174	73.6	2.7	2.3
2	172	69.4	5.0	4.5
3	195	79.9	6.5	5.0
4	186	69.4	9.4	8.4
5	183	68.4	14.0	12.7
6	171	70.8	15.5	13.6
7	189	83.6	24.7	18.3
8	179	77.9	23.2	18.5
9	170	68.5	24.8	22.5
10	181	102.4	42.1	25.5
11	193	124.4	59.3	29.6
12	195	133.9	78.2	36.2
Mean $\pm$ SE	182 $\pm$ 3	85.2 $\pm$ 6.6	25.5 $\pm$ 6.7	16.4 $\pm$ 3.1

adipose tissue. Twenty-five specimens, five from each of five persons, were used. The specimens were weighed and placed in 5-ml glass bottles containing 100 μ l of 5M HCl. The bottles were sealed, and a needle was inserted through the seal. A water-operated suction device was attached to the needle, and a negative pressure was created in the bottle so that 1 ml of methylene chloride gas of a concentration of 50 ppm could be drawn in. Atmospheric pressure was obtained when air was passed into the bottle through an activated charcoal filter. After the addition of methylene chloride gas, the bottles were incubated in a water bath at 60°C for 1—2 h. The gas chromatography analysis was then performed in the manner already described.

When 1 ml of the methylene chloride gas was introduced into empty bottles, a mean concentration of 9.0 ppm (SD $\pm$ 0.5) was obtained.

The concentration of methylene chloride in the air did not change significantly when the methylene chloride was added to bottles containing the hydrochloric acid solution. The introduction of the solvent to bottles containing human adipose tissue and a hydrochloric acid solution produced a methylene chloride concentration in the air, after equilibrium between the air and tissue samples, of the same magnitude as in the assay of biopsy specimens obtained after exposure.

Table 2. Pulmonary function values of 12 male subjects, 21—35 years of age, at rest. Mean values and standard errors of the means are given. (FEV % = forced expiratory volume for 1 s as the percentage of vital capacity; MVV_f = max voluntary ventilation at full rate)

Vital capacity (l)	Residual volume (l)	FEV %	MVV _f (l/min)
5.7 $\pm$ 0.2	1.6 $\pm$ 0.1	80.7 $\pm$ 1.6	184 $\pm$ 11

RESULTS

Body height, body weight and estimated adipose tissue weight are shown in table 1, in which the degree of obesity, expressed as estimated amount of body fat as percentage of the body weight, is also listed. On the average, there was no difference in the height of the subjects in the present study and the subjects in the previous methylene chloride study. However, the mean body weight was 12 kg greater than in the previous study.

All the results obtained from pulmonary function studies were within normal variations. The mean values and standard errors of the means are shown in table 2. Submaximal and maximal exercise on the bicycle ergometer yielded ordinary results with regard to the age of the subjects (table 3).

Table 3. Means and standard errors of the means of the 12 subjects' results from an exercise test on a bicycle ergometer without exposure to methylene chloride. ($\dot{V}_E$ = pulmonary ventilation, $\dot{V}O_2$ = oxygen uptake)

Exercise intensity (W)	Heart rate (beats/min)	$\dot{V}_E$ BTPS (l/min)	$\dot{V}O_2$ STPD (l/min)	Blood lactate (mmol/l)
50	93 $\pm$ 3	25.5 $\pm$ 1.0	1.04 $\pm$ 0.02	2.3 $\pm$ 0.2
100	113 $\pm$ 4	38.5 $\pm$ 2.0	1.60 $\pm$ 0.02	2.3 $\pm$ 0.2
150	137 $\pm$ 6	55.4 $\pm$ 2.9	2.20 $\pm$ 0.03	4.3 $\pm$ 0.5
Maximum	188 $\pm$ 4	141.2 $\pm$ 7.1	3.64 $\pm$ 0.15	13.8 $\pm$ 0.8

The amount of methylene chloride taken up in the organism could be calculated by means of continuous measurement of the amount of methylene chloride in inspiratory and expiratory air during exposure (table 4). The magnitude of uptake could be correlated to the degree of obesity ($r = 0.81$, fig. 1). The correlation to the absolute amount of body fat displayed a larger coefficient ($r = 0.89$) in the linear regression analysis. However, the amount of solvent uptake proved to display the greatest correlation to body weight ($r = 0.93$, fig. 2).

The subjects were subsequently divided into groups I (the slimmest) and II (subjects with a larger amount of body fat per kilogram of body weight). Mean values for height, body weight, estimated amount of body fat as percentage of body weight, solvent uptake and alveolar air and venous blood concentrations of methylene chloride

Table 4. The amount of methylene chloride in milligrams in the inspiratory air and of the amount taken up during 1 h of exposure to approximately 2,600 mg/m³ during the exercise of 12 subjects. Mean values and standard errors (SE) of the means are also given.

Subject	Amount supplied (mg)	Amount taken up (mg)	Uptake (% of the amount supplied)
1	3,238	1,034	32.0
2	2,964	1,201	40.5
3	3,874	1,152	29.7
4	3,967	1,018	25.7
5	3,534	1,211	34.3
6	3,566	1,077	30.2
7	3,712	1,393	37.5
8	3,577	1,258	35.2
9	3,542	1,032	29.1
10	4,447	1,591	35.8
11	4,740	1,672	35.3
12	4,560	1,728	37.9
Mean $\pm$ SE	3,810 $\pm$ 155	1,281 $\pm$ 74	33.6 $\pm$ 1.2

Fig. 1. Uptake of methylene chloride in the organism during exercise at an intensity of 50 W and with exposure to 2,600 mg/m³ in inspiratory air for 1 h. Uptake is related to the estimated amount of body fat expressed as the percentage of body weight. Regression line equation: $y = 961 + 19.5 x$.

Fig. 2. Uptake of methylene chloride in the organism during exercise at an intensity of 50 W and with exposure to 2,600 mg/m³ in inspiratory air for 1 h. Uptake is related to body weight. Regression line equation: $y = 388 + 10.5 x$.

Table 5. Body height, body weight, estimated body fat as percentage of body weight, total uptake of methylene chloride, and alveolar and venous blood concentration in group I (slim) and group II (with larger fat stores). Mean values and standard errors of the means are given.

Variable	Group I (subjects 1—6)	Group II (subjects 7—12)
Body height (cm)	180 ± 4	185 ± 4
Body weight (kg)	71.9 ± 1.8	98.5 ± 10.8
Estimated body fat (% of body weight)	7.8 ± 1.9	25.1 ± 2.8
Methylene chloride uptake (mg)	1,116 ± 34	1,446 ± 110
Alveolar air con- centration (mg/m ³ during final 10 min of exposure)	1,643 ± 57	1,624 ± 76
Venous blood concentration ^a (mg/kg during final 10 min of ex- posure)	15.6 ± 0.8	14.6 ± 1.0

^a For five subjects in each group.

during the final 10-min period of exposure are listed in table 5.

The subjects in group II displayed a larger total methylene chloride uptake than those in group I. However, the uptake per kilogram of body weight was not greater (15.0 mg/kg compared to 15.6 mg/kg in group I). There were no significant differences between the groups with respect to the concentration of methylene chloride in alveolar air or venous blood at the end of exposure.

The *in vitro* experiments yielded a mean value of 51.3 (SD = ± 10.7) for the partition coefficient between adipose tissue and air at 60°C. The partition coefficient was expressed as the concentration in adipose tissue (mg/kg) divided by the concentration in air (mg/l). The mean value of the weight of specimens studied amounted to 31.3 mg (range 10.7 to 92.3). There was no correlation between the partition coefficient and specimen size. The mean weight of the biopsy specimens obtained in conjunction with the exposure experiments was 24.8 mg (range 4.4 to 159.1).

The mean values for the methylene chloride concentrations in adipose tissue

during 4 h after concluded exposure are shown in fig. 3. One hour after concluded exposure the mean of the concentration was 10.2 mg/kg. Four hours after the termination of exposure, the mean value amounted to 8.4 mg/kg. There was a wide spread around the mean values. On the average, the mean values for the concentration in group I were twice as high as in group II during the 4 h after concluded exposure. There was a tendency in group I for the concentration to drop following a peak 1 h after exposure had ceased. In group II, however, the mean concentration remained relatively constant throughout the 4 h after the end of exposure. The mean values for the groups differed significantly according to Student's *t* test ($p < 0.05$). Two of the subjects were studied about 22 h after concluded exposure. The methylene chloride content in their adipose tissue then amounted to 1.6 and 1.7 mg/kg, respectively.

Linear regression analysis disclosed a declining concentration of methylene chloride in adipose tissue with an increasing

Fig. 3. Concentration of methylene chloride in the subcutaneous adipose tissue of all subjects and of the six slim (group I) and six obese (group II) subjects for 4 h after 1 h of exposure to about 2,600 mg of methylene chloride. Light exercise (50 W) was performed during exposure. The figure shows the mean values (M) and standard errors (SE) of the means.

Fig. 4. Concentration of methylene chloride in subcutaneous adipose tissue 4 h after exposure termination as related to the estimated body weight. Regression line equation: $y = 12.2 - 0.23 x$.

Fig. 5. Estimated total amount of methylene chloride in fat stores 4 h after exposure in relation to the estimated amount of body fat expressed in per cent of body weight. Regression line equation: $y = 9.6 + 9.5 x$.

degree of obesity. An analysis hour for hour after the termination of exposure revealed an increased negative correlation with time. Four hours after exposure had ceased the correlation coefficient amounted to -0.78 (fig. 4).

Different segments of adipose tissue probably have varying degrees of blood perfusion and therefore different solvent concentrations. If, despite this, it is assumed that the concentration of methylene chloride in the subcutaneous adipose tissue of the buttocks is representative of the concentration in the total amount of estimated adipose tissue, the total amount of methylene chloride in this tissue can be calculated. Fig. 5 shows the correlation between the degree of obesity and the

estimated total amount of methylene chloride in the adipose tissue 4 h after the termination of exposure. There is a positive correlation, and the correlation coefficient is 0.84 . Even though the subjects with a greater degree of obesity had lower concentrations of methylene chloride in adipose tissue than the slimmer subjects, the total amount of methylene chloride in their fat stores, according to this calculation, was greater. On the whole about 100 – 450 mg of a total uptake of $1,300$ mg were located in the adipose tissue.

DISCUSSION

In this study, determinations of the methylene chloride concentrations in biopsy specimens were made from samples extracted from the head space after equilibrium between the specimen and the gas phase in a sealed bottle. The total methylene chloride content in each original specimen was calculated with the aid of the partition coefficient obtained from in vitro experiments with the addition of known quantities of methylene chloride to human adipose tissue specimens. The procedure does contain an element of uncertainty, since it has not been established that the partition coefficient in the in vitro admixture is the same as in the analysis of specimens for which the solvent was supplied in vivo. In the future, therefore, we will employ a method in which the solvent is extracted from the sample by means of continuous sampling of the ambient gas phase. In the following discussion, however, we have assumed that the present method has given reliable results.

The effect of the magnitude of fat stores on the elimination of organic solvents has been discussed in different studies. Efforts have been made to determine whether or not women run a greater risk of hematological damage from benzene than men (5, 14, 18). In 1976 the American National Institute of Occupational Safety and Health was of the opinion that a greater susceptibility of women to benzene poisoning has not been documented in the literature (18). However, kinetic studies have been under-

taken which point to differences between the sexes in respect to the rate of benzene elimination. Sato et al. reported such conditions in both rats and humans, and they feel that the lower rate of excretion in women is mainly ascribable to the fact that their fat stores are larger than men's (17). However, the material with human subjects was small, and the magnitude of fat stores was not determined. Gostomzyk exposed mice with genetically attributable obesity and control mice to halothane. The obese mice, compared to the control mice, displayed a slower halothane uptake in white adipose tissue and a slower elimination from this tissue. This result was felt to be due to poorer blood perfusion and an extended intracellular diffusion distance in the white adipose tissue of the obese mice (13).

There is no information in the literature on any link between the size of fat stores and the rate of methylene chloride metabolism in man. Prior to this study we were doubtful about the possibility of analyzing methylene chloride in the adipose tissue. Elimination of this highly volatile solvent, mainly via the lungs, is supposed to be very rapid. DiVincenzo et al. (9) exposed subjects to 100 ppm and 200 ppm for 2 to 4 h and felt that "very little vapor" reached the fat stores and muscle tissue under these conditions. The half-time in blood after 2 h of exposure was 40 min, and prolongation of exposure to 4 h did not have any significant effect on the half-time. Therefore it was not felt that there was any risk of accumulation of methylene chloride in the adipose tissue of employees subjected daily to low concentrations. Furthermore DiVincenzo et al. (8) administered high intraperitoneal doses of methylene chloride to rats and noted that only 2 % of the dose could be found in the organism after 24 h, and then mainly in the liver, kidney and adrenal tissue. There was no significant storage in adipose tissue (8).

Carlsson and Hultengren exposed rats for 1 h to isotope-tagged methylene chloride (1,935 mg/m³) in inspiratory air. Immediately after the termination of exposure they obtained the highest concentration of methylene chloride or its metabolites in white adipose tissue, i.e., about 80 mg/kg. The concentration dropped very

rapidly in the hours immediately following. Two hours later the concentration in white adipose tissue had declined by 90 %, and 6 h later it was lower than in the liver, adrenal tissue and kidney (6).

In comparison to DiVincenzo's material comprising human subjects, the subjects in the present study were exposed to higher concentrations of methylene chloride for a shorter time. These differences in the experimental design might be the reason for the different results obtained. However, our present results must be interpreted as suggesting that there is a risk of accumulation in adipose tissue when there is repeated exposure during a normal work week. The risk of accumulation should be greater in obese people since the mean value for such persons in the present study failed to display any tendency to decline during the 4 h following exposure, whereas the concentration did decline in the slim subjects. The two determinations of the methylene chloride concentration in adipose tissue nearly 24 h after concluded exposure also provide support for the view that elimination occurs at a relatively slow pace. One of these subjects was the most obese, whereas the other had an average amount of body fat (13.6 % of his body weight).

In experiments with human subjects exposed to benzene, Sato et al. (17) reported a varying rate in the sexes in respect to the decline in alveolar air concentration after exposure termination. Three to five hours after exposure, the concentration declined more slowly in the women, who also had a higher concentration than the male subjects 4 h after exposure. Sato et al. calculated the metabolic clearance and was unable to detect any major difference between the sexes. Therefore, the author drew the conclusion that the slower elimination of benzene in women was mainly ascribable to their larger fat stores.

Fig. 6 summarizes the assay of the methylene chloride concentration in alveolar air after the terminated exposure of the two groups of subjects in the present study. The concentration is represented on a semilogarithmic scale. The curves have about the same shape as in Sato's study of benzene. During the first 2 h after exposure, the concentrations in alveolar air tended to be lower and decline more

Fig. 6. Concentration of methylene chloride in the alveolar air of six slim (group I) and six obese (group II) subjects for 4 h after a 1-h exposure to about 2,600 mg of methylene chloride. Light physical exercise (50 W) was performed during exposure.

rapidly in the obese subjects than in the slim ones. Concentrations then dropped more slowly in the obese group. This occurrence may be interpreted as meaning that the obese subjects, when compared to the slim subjects, transfer methylene chloride to their fat pools after concluded exposure more readily and thereby eliminate smaller quantities via alveolar air.

During the later phase of elimination, the obese subjects tended to have a higher concentration in alveolar air as they released methylene chloride from this fat pool. Four hours after exposure the concentration in this group was significantly higher ($p < 0.05$) than in the other group. During the period from 1–4 h after concluded exposure, the half-time was constant (about 60 min) in the slim subjects. The curve reflects a two compartment system, in concurrence with a previous report (9). The more complicated curve configuration in obese subjects is probably largely occasioned by the larger fat stores involved. The half-time in these subjects increased during the course of time. The course suggests a slow rate of elimination in obese subjects. The aforementioned interpretations assume that there are no differences between fat and slim subjects in respect to the biotransformation of methylene chloride, a factor which was not examined in the present experiments.

One important issue is whether or not the biopsy of subcutaneous adipose tissue is a better way of demonstrating retention in the organism than the assay of alveolar air. The two subjects who underwent adipose tissue biopsy about 22 h after the termination of exposure displayed 1.6 and 1.7 mg of methylene chloride per kilogram of adipose tissue, respectively, and 2.3 and 1.5 mg/m³, respectively, in alveolar air. Future studies will reveal which parameter is the most sensitive in this respect.

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