Acta Medica Okayama

Volume 24, *Issue* 3 1970 *Article* 5

JUNE 1970

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Studies on hepatotoxicity induced by chlorinated hydrocarbons. II. Lipid metabolism and absorption spectrum of microsomal lipid in the mice exposed to 1, 1, 2, 2-tetrachloroethane*

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Abstract

Female Cb mice weighing 20-23 g were exposed to 800 ppm (in average) of 1, I, 2, 2.tetrachloroethane for 3 hours. Both triglyceride and phospholipid in the liver and plasma were determined at varying times after the exposure. On the other hand, there were observed the ultraviolet absorption spectra of microwmallipids in the liver at 90 minutes after the 1, 1,2, 2-tetrachloroethane or the carbon tetrachloride exposure. The results thus obtained are summarized as follows: 1. The increase of hepatic triglyceride contents attained the maximum level in the period between 20 and 25 hours after the exposure and declined to the initial levels at 90 hours later. 2. The plasma triglyceride levels decreased until 25 hours after the exposure, then tended to increase significantly and were much higher than the control levels in the period between 70 and 90 hours later. 3. Both liver and plasma phospholipid levels decreased gradually up to 25 hours after the exposure, then slowly recovered with almost the same rate of increase. 4. It was suggested that the inhalation of the above vapors induced a little change in microsomal lipids in the liver.

*PMID: 4249888 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **24**, 315—322 (1970)

STUDIES ON HEPATOTOXICITY INDUCED BY CHLORINATED HYDROCARBONS II. LIPID METABOLISM AND ABSORPTION SPECTRUM OF MICROSOMAL LIPID IN THE MICE EXPOSED TO 1, 1, 2, 2-TETRACHLOROETHANE

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Received for publication, Feb. 27, 1970

The chlorinated hydrocarbons are widely used as a detergent agent in industry, but it is said that a long term inhalation of these vapors will induce liver disturbances. Especially, in the animal experiments, it has been shown by some investigators that chlorinated hydrocarbon vapors induce fatty liver (1—4). Up to date, however, there has not been any study on the restitution process of fatty liver, that is, the problem how the fatty liver once induced by the chlorinated hydrocarbon vapors diminishes with lapse of time after the exposure. Therefore, the studies on the restitution process of fatty liver would be valuable in the field of industrial hygiene. On the other hand, RECKNAGEL *et al.* (5) have advanced the lipoperoxidation hypothesis to explain the causative factor of carbon tetrachloride-fatty liver.

Previously (3), the author demonstrated that the inhalation of 1, 1, 2, 2-tetrachloroethane induced fatty liver. The present study was conducted in order to investigate the restitution process of fatty liver, especially centering around lipid metabolism in the liver and plasma of mice exposed to 1, 1, 2, 2-tetrachloroethane. Spectrophotometric observations were also carried out on the microsomal lipid in the liver of mice at the onset of fatty liver after the 1, 1, 2, 2-tetrachloroethane inhalation, and the result was compared to that of the carbon tetrachloride inhalation.

MATERIALS AND METHODS

Animals: Female Cb mice weighing 20—23 g were used. They were fed on solid feed of Oriental Yeast Company and water was given *ad libitum* until they were sacrificed.

Exposure: The mice were placed in the exposure chamber (3) supplied with the constant current of air via a vaporizing unit, and were exposed to 800 ppm

(in average) of 1, 1, 2, 2-tetrachloroethane for 3 hours. The gas concentration was measured by gas chromatography once every 30 minutes during the exposure. During exposure the mice received neither food nor water.

Preparation of mouse liver and plasma: After the mice were lightly anesthetized with ethyl ether at varying times after the exposure, the blood was withdrawn into heparinized capillary tube by orbital bleeding technic and transferred into glass centrifuge tube. Then the mice were sacrificed by decapitation and the liver was excised and weighed for lipid analyses.

Determination of liver and plasma triglycerides: Liver triglyceride was measured by the modification of BUTLER, *et al.* (6) and plasma triglyceride by the method of VAN HANDEL (7).

Determination of liver and plasma phospholipids: Lipids were extracted from liver and plasma by the procedure of FOLCH *et al.* (8). An aliquot of chloroform-methanol extract containing phospholipids was placed into the 10 ml-glass stoppered tube and the solvent was evaporated in the water bath. The phospholipids were digested in the glass tube with perchloric acid by means of broiling over a small open fire for about 3 minutes, and phosphorus was determined by the method of FISKE and SUBBAROW (9). The weight of phospholipids was calculated by multiplying the weight of phosphorus by 25.

Isolation of microsome in the liver: Fundamentally, this experiment was performed by a modification of the RECKNAGEL *et al.* procedure (10). Twelve mice were divided into two groups. One group was employed for the exposure of 1, 1, 2, 2-tetrachloroethane and the other group for that of carbon tetrachloride. The mice were exposed to 800 ppm (in average) of the agents for 3 hours. At 90 minutes after exposure, the mice were killed by decapitation, the liver was immediately excised and chilled in the ice-cold sucrose-EDTA medium. About 2.8 g of liver gathered from 3 mice was homogenized in 18 ml of ice-cold 0.3 M sucrose, 0.003 M EDTA medium. The homogenate was centrifuged for 15 minutes at 8,000 g in the RP 40 rotor of the Hitachi 55PA ultracentrifuge. The supernatant fraction was then centrifuged for 60 minutes at 94,000 g in the same rotor.

Microsomal lipid extraction and spectrophotometric analysis: The microsome fraction thus obtained was transferred with 5 ml of methanol into a 50 ml-glass stoppered cylinder, 10 ml of chloroform and 15 ml of chloroform-methanol (2 : 1) were added. The mixture was shaken gently for 5 minutes and allowed to stand overnight in a cold room. The mixture was filtered and 10 ml of water was added to the filtrate. The two-phase system thus obtained was shaken gently and centrifuged. The upper phase was removed. The lower chloroform phase was evaporated under a stream of nitrogen at 35—40°C and dried. The total lipid thus extracted was dissolved in cyclohexane to a concentration of 0.5 mg per ml. Optical densities were determined against a cyclohexane blank over the range from 220 to 230 mμ, in Hitachi 139 ultraviolet spectrophotometer in cells with 1-cm light path.

RESULTS

Changes in hepatic lipids: The amounts of triglyceride and phospholipid

in the liver of mice at appropriate intervals after 1, 1, 2, 2-tetrachloroethane inhalation are shown in Table 1. Triglyceride contents remarkably increased with lapse of time after the exposure, the concentration reaching the maximum level in the period between 20 and 25 hours later ($P<0.001$). Then, the triglyceride contents gradually decreased and almost recovered to the initial level at 90 hours later. On the contrary, phospholipid contents gradually decreased with lapse of time after the exposure and reached the minimum level at 25 hours later ($P<0.001$). Then, they increased slowly and almost recovered to the control level at 90 hours later.

TABLE 1. TRIGLYCERIDE AND PHOSPHOLIPID CONTENTS IN THE LIVER OF MICE AT VARIOUS TIMES AFTER EXPOSURE TO 800 PPM OF 1, 1, 2, 2-TETRACHOROETHANE FOR 3 HOURS. EACH VALUE REPRESENTS THE MEAN ± STANDARD DEVIATION.

Time after exposure (hour)	Number of mice	Liver lipids mg/g (w. w.)	
		Triglycerides	Phospholipids
Control	8	8.4 ± 1.0	23.2 ± 2.3
5	5	21.0 ± 3.4	22.5 ± 1.8
20	5	50.4 ± 8.7	18.3 ± 2.7
25	5	50.0 ± 9.5	16.7 ± 2.2
30	5	40.6 ± 6.9	18.3 ± 1.8
45	5	26.4 ± 5.6	18.0 ± 1.9
70	5	15.0 ± 3.2	21.6 ± 2.9
90	5	9.1 ± 1.5	22.1 ± 1.9

Changes in plasma lipids: The amounts of triglyceride and phospholipid in the plasma of mice at varying times after 1, 1, 2, 2-tetrachloroethane exposure are shown in Table 2. Both triglyceride and phospholipid concentrations in the plasma decreased after the exposure, and continued to decrease until 25 hours later ($P<0.001$). With lapse of time after that, the plasma phospholipid levels gradually rose and recovered to the control

TABLE 2. TRIGLYCERIDE AND PHOSPHOLIPID CONTENTS IN THE PLASMA OF MICE AT VARIOUS TIMES AFTER EXPOSURE TO 800 PPM OF 1, 1, 2, 2-TETRACHLOROETHANE FOR 3 HOURS. EACH VALUE REPRESENTS THE MEAN ± STANDARD DEVIATION.

Time after exposure (hour)	Number of mice	Plasma lipids mg/ml	
		Triglycerides	Phospholipids
Control	8	0.98 ± 0.13	0.84 ± 0.09
5	5	0.62 ± 0.12	0.64 ± 0.06
20	5	0.43 ± 0.10	0.58 ± 0.09
25	5	0.39 ± 0.06	0.54 ± 0.08
30	5	0.52 ± 0.11	0.69 ± 0.10
45	5	0.67 ± 0.14	0.71 ± 0.09
70	5	1.63 ± 0.24	0.87 ± 0.08
90	5	1.37 ± 0.31	0.82 ± 0.10

level at 90 hours after the exposure, while the plasma triglyceride concentrations subsequently recovered and were much higher than the control level in the period between 70 and 90 hours after the exposure ($P<0.001$).

Changes in hepatic and plasma triglycerides : As can be seen in Fig. 1, liver triglyceride levels rapidly rose during 20 hours after the exposure. The average rate of increase up to 20 hours later was approximately 1.8 mg/g liver w. w. per hour. The concentration of hepatic triglycerides attained the maximum level in the period between 20 and 25 hours after the exposure, and then they decreased slowly and almost recovered to the control level at 90 hours later. The half life of hepatic triglycerides concomitant with restitution was about 25 hours. On the cotrary, the plasma triglyceride levels decreased significantly after the exposure, reaching the minimum level at 25 hours later and then showing a tendency of recovery. However, the concentration of plasma triglyceride was more than that of the controls following a decrease in hepatic triglyceride levels, especially in the period between 70 and 90 hours after the exposure.

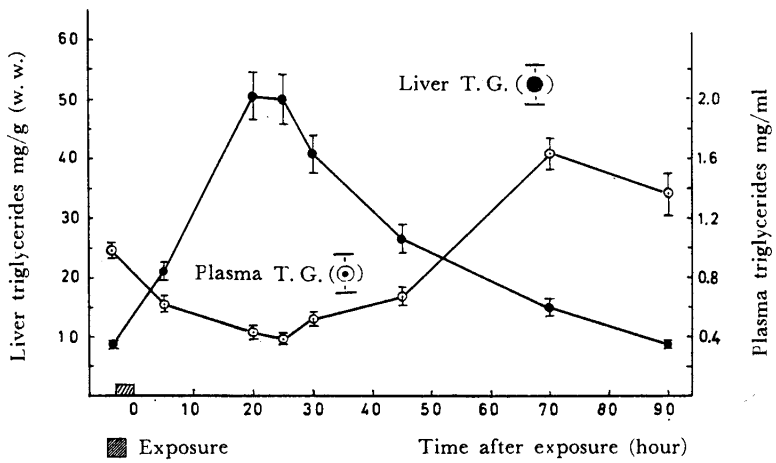

Fig. 1 Changes in triglyceride levels in the liver and plasma of mice at appropriate intervals after exposure to 800 ppm of 1, 1, 2, 2-tetrachloroethane for 3 hours. Each point represents the mean ± SEM.

Changes in hepatic and plasma phospholipids : As shown in Fig. 2, both liver and plasma phospholipid levels were significantly reduced until 25 hours after the exposure, then recovered gradually with lapse of time. There was almost a parallel relationship between the change in the hepatic phospholipid levels and that in the plasma phospholipid levels.

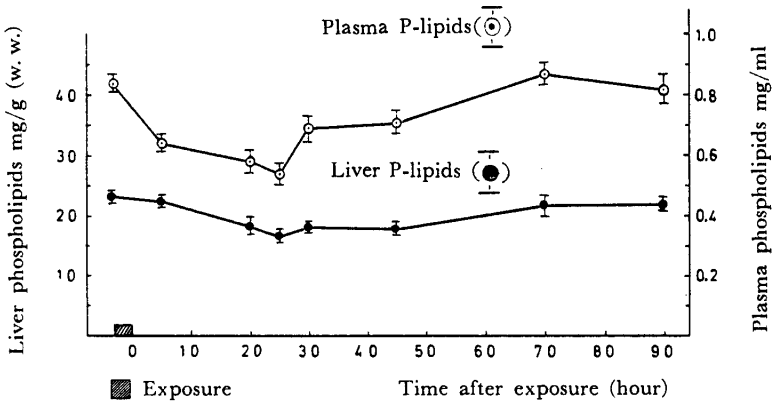

Fig. 2 Changes in phospholipid levels in the liver and plasma of mice at appropriate intervals after exposure to 800 ppm of 1, 1, 2, 2-tetrachloroethane for 3 hours. Each point represents the mean ± SEM.

The absorption spectra of microsomal lipids: Figs. 3 and 4 show the ultraviolet absorption spectra of microsomal lipids in the mouse liver at 90 minutes after the 1, 1, 2, 2-tetrachloroethane and the carbon tetrachloride exposures, respectively. As shown in Figs. 3 and 4, the maximum absorption on difference spectrum was detected at 225 mμ in the case of 1, 1, 2,

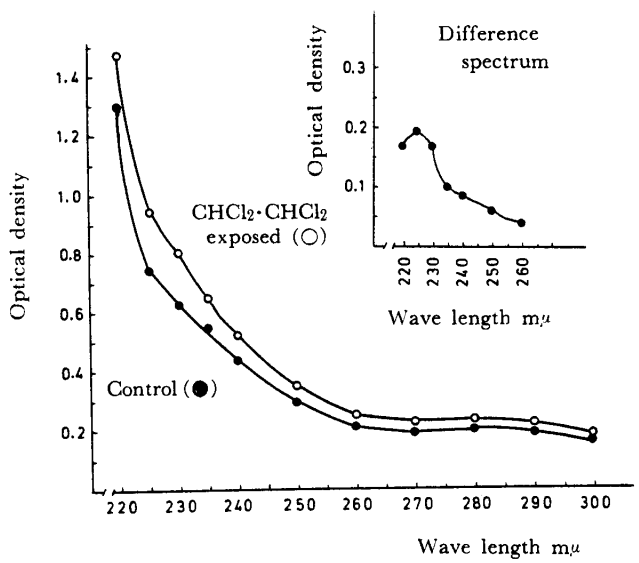

Fig. 3 Absorption spectrum of liver microsomal lipids in mice 90 minutes after exposure to 800 ppm of 1, 1, 2, 2-tetrachloroethane for 3 hours. Each point shows the mean obtained from two experiments.

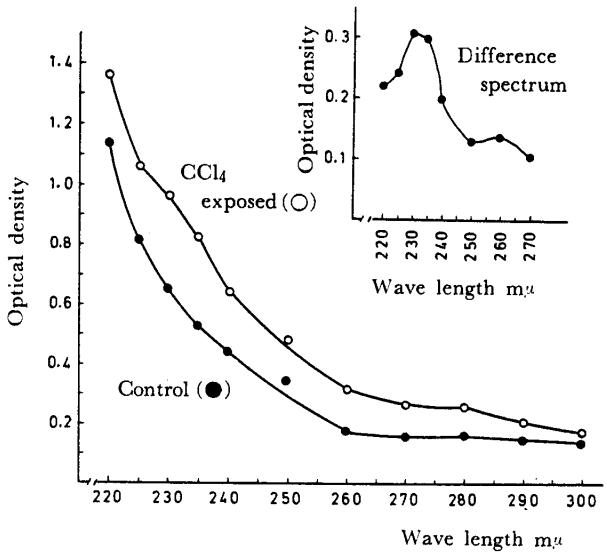

Fig. 4 Absorption spectrum of liver microsomal lipids in mice 90 minutes after exposure to 800 ppm of carbon tetrachloride for 3 hours. Each point shows the mean obtained from two experiments.

2-tetrachloroethane exposure, while it was detected at 230 mμ in the case of carbon tetrachloride exposure.

DISCUSSION

In 1965, LOMBARDI *et al.* (11) observed the changes in hepatic and serum triglycerides during onset time of intoxication after carbon tetrachloride administration to the rats, and they found that the former remained constant during the first hour of intoxication, then rose rapidly, while the latter decreased very sharply during the first hour of intoxication, then adjusted to a slower rate of decline. Moreover, they (11) demonstrated a marked inhibition of serum lipoprotein synthesis, especially that of very low density (VLD-) lipoprotein. In the same year, STERN *et al.* (12) have stated that there are marked changes in triglyceride levels, determining the lipid components in rat liver and plasma with lapse of time after carbon tetrachloride administration.

In this study on 1, 1, 2, 2-tetrachloroethane inhalation by mice, it was demonstrated that: i) judging from the increase in hepatic triglyceride levels, the development of fatty liver reached the maximum peak in the period between 20 and 25 hours after the exposure, and declined at 90 hours later (Table 1, Fig. 1), ii) the plasma triglyceride levels signifi-

cantly decreased until 25 hours after the exposure, then increased gradually with recovery of hepatic triglyceride levels and were much higher than the control levels in the period between 70 and 90 hours later (Table 2, Fig. 1), and iii) both liver and plasma phospholipid levels decreased gradually until 25 hours after the exposure, then recovered slowly (Fig. 2).

In 1968, RAO *et al.* (10) found a marked diene conjugation absorption in liver microsomal lipids 5 minutes after carbon tetrachloride administration to the rats, and proposed the lipoperoxidation hypothesis for carbon tetrachloride-fatty liver.

In the present study, the author also observed the absorption spectra of microsomal lipids 90 minutes after 1, 1, 2, 2-tetrachloroethane and carbon tetrachloride inhalations, and found that the former had the maximum absorption at 225 mμ, while the latter at 230 mμ on the difference spectrum (Figs. 3 and 4). These findings suggest that the inhalation of 1, 1, 2, 2-tetrachloroethane or carbon tetrachloride by the mice will lead to a little decomposition of microsomal lipids in the liver.

On the other hand, *in vivo*, WAGLE *et al.* (13) reported the inhibition of protein biosynthesis in vitamin B12-deficient rat liver in 1958. In 1966, VIVIANI *et al.* (14) stated that a fatty liver produced in the lysine- and threonine-deficient rats. These reports seem to suggest that the vitamin B12-deficiency *in vivo* could lead to a fatty liver. However, the author has not yet obtained any evidence to support this hypothesis, in the case of the 1, 1, 2, 2-tetrachloroethane-fatty liver.

CONCLUSION

Female Cb mice weighing 20—23 g were exposed to 800 ppm (in average) of 1, 1, 2, 2-tetrachloroethane for 3 hours. Both triglyceride and phospholipid in the liver and plasma were determined at varying times after the exposure. On the other hand, there were observed the ultraviolet absorption spectra of microsomal lipids in the liver at 90 minutes after the 1, 1, 2, 2-tetrachloroethane or the carbon tetrachloride exposure. The results thus obtained are summarized as follows: 1. The increase of hepatic triglyceride contents attained the maximum level in the period between 20 and 25 hours after the exposure and declined to the initial levels at 90 hours later. 2. The plasma triglyceride levels decreased until 25 hours after the exposure, then tended to increase significantly and were much higher than the control levels in the period between 70 and 90 hours later. 3. Both liver and plasma phospholipid levels decreased gradually up

to 25 hours after the exposure, then slowly recovered with almost the same rate of increase. 4. It was suggested that the inhalation of the above vapors induced a little change in microsomal lipids in the liver.

ACKNOWLEDGEMENT

The author wishes to acknowledge the support and encouragement given by Professor MASANA OGATA.

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