STUDIES ON THE EFFECT OF CARBON TETRACHLORIDE ON THE LIVER CELLS

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It is experimentally well known that fat particles were accumulated in the liver after treatment with carbon tetrachloride ***in vivo***. Its mechanism, however, was not so clear.

Judah *et al.*[1] attempted to explanate this mechanism on the base of mitochondrial degeneration. On the other hand, Recknagel *et al.*[2~4] emphasized the degeneration of endoplasmic reticulum by means of electron microscope and measurement of enzyme activity in mitochondria. They pointed out that fat particles in the cells appeared at earlier stadium than that of the mitochondrial inactivation, and they concluded that the fatty liver depended on the disturbance of fat secretion. Furthermore, Dixon[5] speculated the importance of the ratio of phospholipid to fat, with regard to the mechanism of accumulation of the fat in the cells.

Based on this Dixon's view, in order to clear the mechanism of appearance of fatty liver, the effect of carbon tetrachloride on the phospholipid of cell particles in the liver was studied in this paper.

EXPERIMENTAL

Male albino rats weighing 120 to 130 g, obtained from Moriyamaso Laboratory (Nagoya), were used throughout the study. The hormone preparation used were commercial preparation of epinephrine and cortisol (Upjohn's Solcortef).

In most experiments, carbon tetrachloride in a 1 : 1 mixture with olive oil was introduced into the stomach at a dose of 0.5 ml of the mixture per 100 g of body weight. Adrenalectomy was done skillfully and promptly. 100 μc of P^{32} was injected subcutaneously and its specific activity was measured at 3 hours after injection. Organic P was measured by Handler's method[11].

The animals were killed by cervical section and exsanguinated. The liver was homogenized with a homogenizer of the Potter-Elvehjem type in 0.25 M sucrose at 0–2°C.

The nuclear fraction was removed by centrifugation at 600×g for 15 minutes. The nuclei-free homogenate was centrifuged for 30 minutes at 12000×g. And then the supernatant was centrifuged for 2 hours at 70000×g.

Two above fractions were extracted with two hundred volumes of chloroform-methanol (2 : 1 v/v), and phosphorus of the extracts was measured.

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RESULTS

As shown in Fig. 1, administration of CCl_4 gave rise to the decrease of content of the phospholipid in mitochondrial fraction (Fract. I) and microsomal fraction (Fract. II) in a short time, but subsequently this content of the

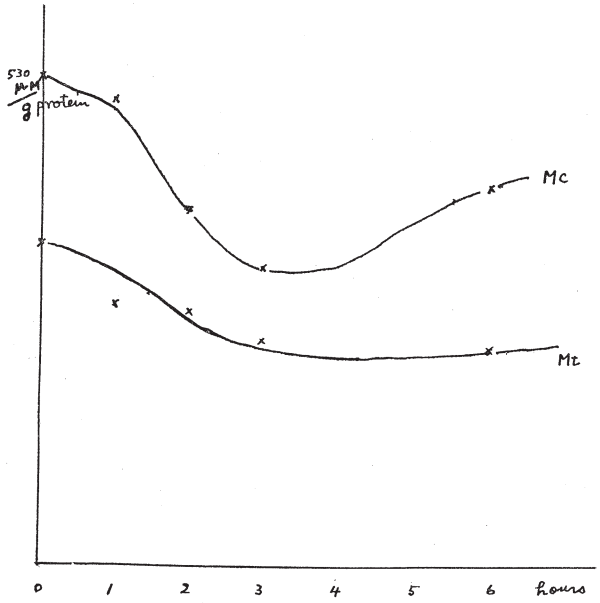

FIG. 1. Effect of carbon tetrachloride on microsomal (Mc) and mitochondrial (Mt) fractions.

Content of the phospholipid decreased in both Mc and Mt fractions after administration of carbon tetrachloride.

Each value is the mean of 8 experiments.

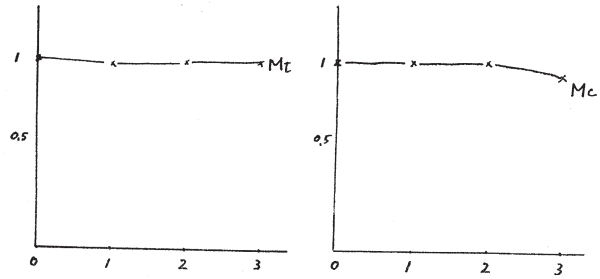

FIG. 2. Effect of carbon tetrachloride on adrenalectomized rats.

Carbon tetrachloride was administered 3 hours after adrenalectomy. Control was measured 2 hours after administration of carbon tetrachloride. These figures indicate the ratio of content of the phospholipid to that of control.

Each value is the mean of 4 experiments.

phosphorus was recovered or preferably elevated.

According to hypothesis of Brody *et al.*[6~8], the participation of the adrenal gland to this phenomenon was investigated, using adrenalectomized rats. Fig. 2 shows these results. No remarkable decrease of content of phosphorus was observed. These results show that the decrease of phospholipid may mainly depend upon the adrenal gland.

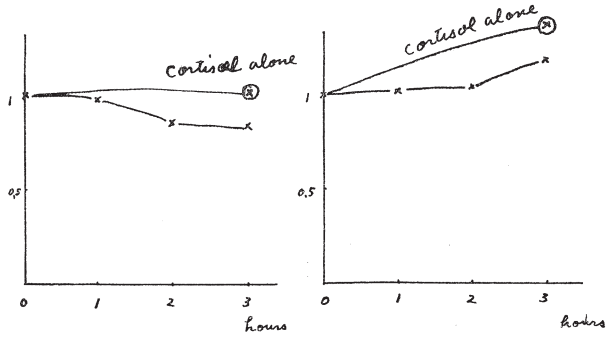

FIG. 3. Effect of cortisol on content of the phospholipid in adrenalectomized rats.

After adrenalectomy carbon tetrachloride and cortisol were administered. 5 mg of cortisol per 100 g of body weight was injected.

Each value is the mean of 4 experiments.

Fig. 3 shows the effect of cortisol on content of the phospholipid in adrenalectomized rats. Phospholipid of microsomal fraction was rather slightly increased by injection of cortisol alone.

The effects of histamine and adrenalin on the uptake of P^{32} to nucleotide (organic phosphorus) and phospholipid were summarized in Table I. The uptake was inhibited in the injection of histamine and adrenalin.

TABLE I. Effects of Adrenalin and Histamine on the Uptake of P^{32}

	Nucleotide P^{32}	Lipid P^{32}
Control	100**	100*
Adrenalin	82*	80*
Histamine	80**	82*

0.1 mg of adrenalin per 100 g of body weight was injected subcutaneously and 6 mg of histamine per 100 g of body weight was injected intraperitoneally.

* is the mean of 4 experiments.

** is the mean of 8 experiments.

But some different effect was observed in the case of cortisol. Fig. 4 shows the effect of cortisol. In microsomal fraction, the uptake of P^{32} to organic phosphorus and phospholipid was elevated. The results were convenient to elucidate the changes of the phospholipid in adrenalectomized rats (Fig. 3).

The effect of adrenalin on content of the phospholipid in microsomal fraction was shown in Fig. 5. The decrease of the phospholipid

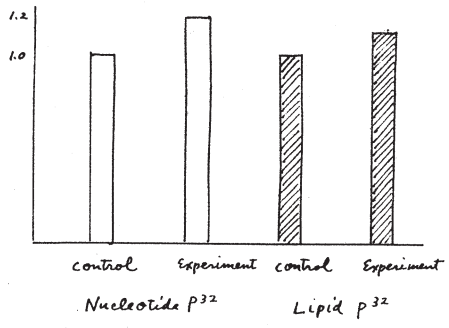

FIG. 4. Effect of cortisol on the uptake of P^{32}.

5 mg of cortisol per 100 g of body weight was injected and P^{32} was measured 3 hours after injection. Nucleotide P^{32} of the supernatant at 12000×g centrifugation was measured. Lipid P^{32} was measured with the preparation from whole cells.

(□) Each is the mean of 8 experiments.

(▩) Each is the mean of 4 experiments.

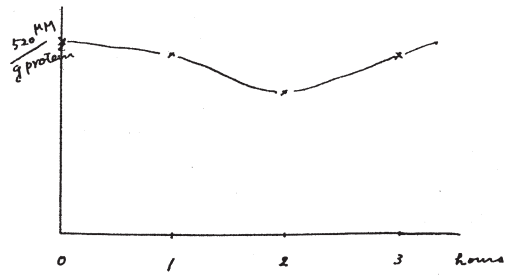

FIG. 5. Effect of adrenalin on content of the phospholipid.

0.05 mg of adrenalin per 100 g of body weight was injected. Lipid P of microsomal fraction was measured.

Each value is the mean of 4 experiments.

was observed in the same type with administration of carbon tetrachloride.

These results suggest the participation of the adrenal medulla to the decrease of phospholipid, when carbon tetrachloride was administered in rats.

DISCUSSION

Though there were many reports about the mechanism of origin of carbon tetrachloride fatty liver, it is difficult to analyze the phenomenon because of the complicated integrity of a living body.

For instance, Judah *et al.*[1] pointed out the degeneration of mitochondria, but the author must answer to the question, what is meaning of the degeneration of mitochondria, and how is the mechanism of the escape of NAD (nicotinamide-adenine dinucleotide).

Here must be considered a initiative mechanism. Furthermore, if the disturbance of fat secretion according to Recknagel's hypothesis depends upon the degeneration of the ER (endoplasmic reticulum)[4], then it gives rise to the

problem, how does carbon tetrachloride induce its disturbance.

Present experiments show that administration of carbon tetrachloride lowers content of the phospholipid of microsomal fraction and mitochondrial fraction of the liver cells. However since no remarkable change in content of the phospholipid was observed by adrenalectomy (Fig. 2), these results suggest that the effect of carbon tetrachloride may be influenced through the adrenal gland. Furthermore, concerning the role of the adrenal gland by administration of carbon tetrachloride, there occurs the problem whether it depends upon the medulla or the cortex.

Fig. 4, 5 and Table I show the medulla is chiefly effective to these decrease; histamine and adrenalin inhibit the uptake of P^{32} to ATP and phospholipid, and this may suggest the inhibition of formation of ATP and phospholipid. And Fig. 5 shows that the injection of adrenalin decreases the phospholipid in microsomal fraction and it is the same type with administration of carbon tetrachloride.

On the other hand, cortex hormone (cortisol) shows another effect (Fig. 4); in microsomal fraction content of the phospholipid rather increases.

The lowering of formation of ATP and phospholipid may depend upon anoxia through the adrenal medulla. It was reported that anoxia by binding the vessel resulted in the fatty liver of the same type as carbon tetrachloride[9]. The lowering of formation of ATP and phospholipid (Table I) may concern with the transport of cell membrane, and these changes relatively correspond with the electron microscopic features after administration of carbon tetrachloride[10], therefore the disturbance of fat secretion by Recknagle *et al.*[4] may concern to these phenomena. This fact agrees with Dixon's hypothesis[5] that he speculates the decrease of phospholipid in the cell may occur deposition of fat and disturbance of its transport.

Brody *et al.*[6~8] suggested participation of adrenal gland to the effect of carbon tetrachloride from the fact that the grade of fatty liver was mitigated by adrenalectomy and administration of antihistamine. And present experiments do not conflict them. But these experiments need more detailed investigation in some points.

SUMMARY

1. By administration of carbon tetrachloride to rats content of the phospholipid in both microsomal fraction and mitochondrial fraction decreased within short time, and these tendencies relatively agree with the changes of electron microscopic features of cell particles after administration of carbon tetrachloride.

2. In the adrenalectomized rats these tendencies by carbon tetrachloride almost disappeared. These results suggest that the decrease of the phospholipid may be caused mainly through the adrenal gland.

3. Injection of histamine and adrenalin inhibited the uptake of P^{32} to nucleotide and phospholipid. On the other hand cortisol operates in other way.

4. By injection of adrenalin content of the phospholipid in microsomal

fraction decreased. And these tendencies are the same type with carbon tetrachloride.

From these results the decrease of the phospholipid by administration of carbon tetrachloride may mainly depend upon the adrenal medulla. The decrease of phospholipid may be an important factor to the origin of carbon tetrachloride fatty liver.

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