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Effect of Phospholipid on the Release of Beta-lipoprotein in Orotic Acid-induced Fatty Liver

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The content of liver lipids and protein, and serum beta-lipoprotein were determined in control and orotic acid-fed rats. Further, the effect of phosphatidylcholine on the release of beta-lipoprotein from liver slices into the incubation medium was examined in both groups. Results are summarized as follows:

1. The amount of liver glycerides increased significantly by ingestion of orotic acid, but liver phospholipid and protein decreased.
2. A 50 per cent depression of the content of serum beta-lipoprotein was observed in rats fed orotic acid.
3. The release of beta-lipoprotein from liver slices decreased when orotic acid was added to the diet. This decrease was partly restored by the addition of phosphatidylcholine.
4. When liver slices were pre-treated with phospholipase C, the amount of released beta-lipoprotein increased in both control and orotic acid-fed rats. The treated liver slices from orotic acid-fed rats released the same amount of lipoprotein as the control by the addition of phosphatidylcholine.

From these observations, phosphatidylcholine appears to take part in the release of beta-lipoprotein. Accordingly, it was concluded that fatty liver induced by orotic acid resulted from the impaired phosphatidylcholine synthesis in the liver.

Supplementation of semisynthetic diet with 1 per cent orotic acid results in the accumulation of fat in rat liver and a reduction in the concentration of circulating lipids, particulary beta-lipoprotein.[2-9] It has been confirmed that fatty liver induced by orotic acid results from a block in the release of hepatic betalipoprotein,[10] but its mechanism is little understood. Roheim, *et al.* have demonstrated that liver from the rats fed orotic acid failed to utilize apoprotein of betalipoprotein in perfused liver study.[11,12] On the other hand, phospholipid is now known to be essential component of serum lipoprotein. Phospholipid molecule, which has both polar and non-polar groups, is believed to form a bridge between the polar protein and non-polar constituents such as neutral lipids.[13] In fact it has been found that the combination of phospholipid with apoprotein is the first step in the synthesis of high density lipoprotein[14] and later, neutral lipids are assumed to be bound to phospholipid-protein matrix.[15] Beta-lipoprotein may be synthesized through similar steps.

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It is known that methylation of phosphatidylethanolamine is a major pathway in the biosynthesis of liver phosphatidylcholine in female rats.[16] Our previous results demonstrated that orotic acid induced a marked fatty liver and a marked depression in *in vivo* incorporation of methyl group of methionine into liver phosphatidylcholine and serum beta-lipoprotein in female rats, but not in males.[17]

These findings suggest that a decreased release of beta-lipoprotein by orotic acid may be due to an inhibition of synthesis of phospholipid moiety of beta-lipoprotein in the liver. Accordingly, the present study was undertaken to evaluate the effect of phospholipid on the release of beta-lipoprotein in liver slices from orotic acid-fed rats.

Experimental

Animals and Diet——Female rats of Wistar strain weighing from 180 to 210 g, were fed a semisynthetic diet,[3] consisting of (in per cent), casein 18.0, sucrose 72.8, corn oil 2.0, vitamin mixture 2.2, and salts 5.0, for 2 days. Groups of animals then received a dietary supplement of 1 per cent orotic acid for 7 days. Control rats continued on a semisynthetic diet.

Release of Beta-lipoprotein by Rat Liver Slices——Rats were decapitated and bled, and their liver was quickly removed and perfused with cold saline. Liver slices were prepared, washed in cold saline, transferred to incubation flask, and incubated in Krebs-Ringer bicarbonate buffer at 37° in O_2–CO_2 (95: 5, by vol.). Each incubation flask contained two liver slices totalling approximately 200 mg tissue in 2.5 ml of the medium. In one experiment, liver slices were pre-incubated with phospholipase C (E.C. 3.1.4.3.), 250 μg/ml medium for 30 min at 37°. After phospholipase C treatment, the slices were washed 3 times with cold saline and then incubated in the Krebs-Ringer medium added with liver phosphatidylcholine (P 50 μg/ml medium) for 120 min at 37°. Phosphatidylcholine was prepared from rat liver by the method of Hanahan, *et al.*[18]

Analysis of Medium, Serum, and Liver——After incubation, liver slices were separated from the medium by centrifugation, and beta-lipoprotein fraction was isolated by precipitation with mepesulfate (sodium salts of sulfated polygalacturonic acid methyl ester methyl glycoside) as described by Florsheim, *et al.*[19] Serum was obtained by centrifugation of blood by heart puncture under ether anesthesia. Serum beta-lipoprotein was separated as described for the medium. Lipids in beta-lipoprotein fraction were extracted by the procedure of Folch, *et al.*[20] Liver lipids were extracted once with 80 per cent ethanol, once with 100 per cent ethanol, twice with chloroform–ethanol (1: 1, by vol.), and once with ether. Lipid phosphorus was determined by the method of Chen, *et al.*[21] after digestion with perchloric acid. Glycerides were hydrolyzed in 0.5 N alcoholic potassium hydroxide for 30 min at 70° and liberated glycerol was enzymatically estimated as described by Eggstein.[22] Protein was determined according to Lowry, *et al.*[23]

Results

As shown in Table I, the content of liver glycerides of rats given orotic acid was significantly higher than that of animals given semisynthetic diet alone. In contrast, liver phospholipid and protein decreased by ingestion of orotic acid. Moreover, the level of serum beta-lipoprotein was decreased in rats fed a diet supplemented with orotic acid. These results are in accord with the finding of others.[5,6,8,10–12,24–26]

The release of protein and phospholipid moieties of beta-lipoprotein into the incubation medium is shown in Fig. 1. Liver slices from orotic acid-fed rats released less amount of beta-lipoprotein than those from control animals. By the end of 2 hr of incubation, liver

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slices from orotic acid-fed rats released 0.60 as much protein moiety and 0.46 as much phospholipid moiety of betalipoprotein as liver slices from control rats. In rats fed orotic acid, a decreased release of lipoprotein from liver slices seemed to reflect the depression in the content of serum beta-lipoprotein (Table I).

Table I. Effect of Orotic Acid on Liver Lipids and Protein, and Serum Beta-lipoprotein

	Control	Orotic acid	Per cent of control
Liver			
Glyceride (mg glycerol/g liver)	2.02±0.136	20.9 ±0.85[a)]	1030
Phospholipid (mg phosphorus/g liver)	1.03±0.096	0.701±0.0141[b)]	68.0
Protein (mg protein/g liver)	99.1±2.58	76.2 ±2.42[b)]	76.8
Serum beta-lipoprotein			
Phospholipid (μg phosphorus/ml serum)	17.9±0.88	8.95 ±0.822[a)]	50.0
Protein (mg protein/ml serum)	1.77±0.052	1.04 ±0.091[a)]	58.9

Results are given as mean±standard error.
a) mean significant difference from control ($p<0.01$)
b) mean significant difference from control ($p<0.001$)

In the following experiment, examination was made on whether or not the addition of phosphatidylcholine, which is a major component of phospholipid moiety of lipoproteins,[27,28] increased the release of beta-lipoprotein into the incubation medium. These results are shown in Fig. 2. In the control group, release of betalipoprotein was not changed by the addition of phosphatidylcholine, whereas the release of beta-lipoprotein increased 2-fold by the addition of phosphatidylcholine in liver slices from orotic acid-fed rats (Fig. 2A).

Liver slices treated with phospholipase C released a larger amount of beta-lipoprotein in both control and orotic acid-fed groups as compared with non-treated liver slices (Fig. 2A, B). Such an increased release of lipoprotein may be due to the destruction of biological membrane barrier by phospholipase. In spite of increased release, liver slices from orotic acid-fed rats released less than those from the control group. However, the release of lipoprotein from treated liver slices of orotic acid-fed rats was increased the addition of phosphatidylcholine until the amount of released lipoprotein was almost equal to that in control rats (Fig. 2B).

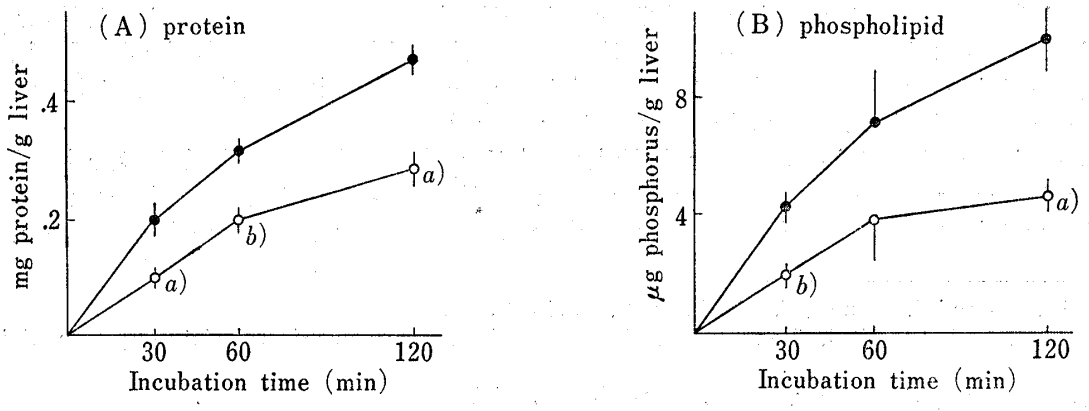

Fig. 1. Effect of Orotic Acid on the Release of Beta-lipoprotein by Rat Liver Slices

Each point represents the mean of three rats and the vertical line represents standard error of the mean.
significant difference from control: *a*) $p<0.05$, *b*) $p<0.01$
●—●: control, ○—○: orotic acid

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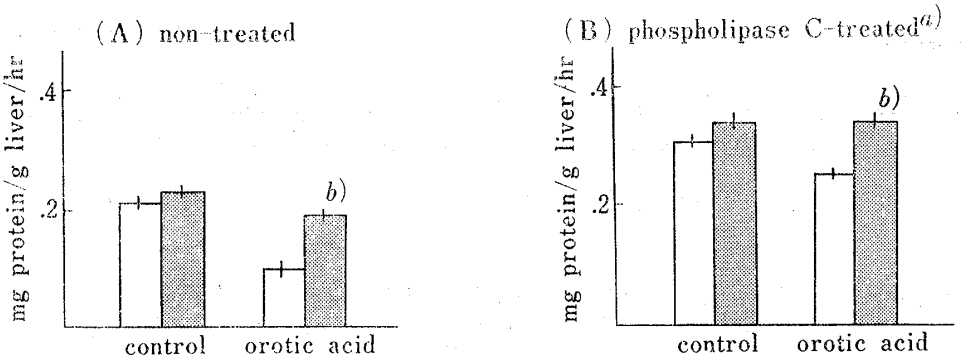

Fig. 2. Effect of Phosphatidylcholine on the Release of Beta-lipoprotein from Liver Slices in Control and Orotic Acid-fed Rats

a) Liver slices were pre-incubated with phospholipase C for 30 min. Phosphatidylcholine was added to 50 μg phosphorus per ml of incubation medium. Height of the bar represents the mean ± standard error.
b) significant difference at $p < 0.01$
□: Krebs-Ringer bicarbonate buffer (KRB)
▨: KRB + phosphatidylcholine

Discussion

Accumulation of fat in the liver from rats which ingested a semisynthetic diet supplemented with orotic acid was largely glycerides. On the contrary, phospholipid content of rats given orotic acid was less than that of animals given a semisynthetic diet alone (Table I). These observation have been confirmed in several laboratories.[5–7,29] However, it has been shown that supplementation of orotic acid did not change the rate of fatty oxidation[7] and an increased incorporation of acetate [1-^{14}C] into total lipids in the liver by orotic acid was only partially reflected in a marked fat accumulation in the liver.[17] Circulating serum beta-lipoprotein was depressed by ingestion of orotic acid (Table I). Moreover, a decreased release of beta-lipoprotein from liver slices in orotic acid-fed rats was also observed (Fig. 1). Similar results were obtained in perfused liver.[7,10] These evidences support the hypothesis that accumulation of glycerides in the liver results from an inhibition of the release of beta-lipoprotein by ingestion of orotic acid. However, it is little known how the release of lipids out of the liver can be inhibited. Hankin found no accumulation of lipoprotein in the liver of orotic acid-fed rats which were accumulating a large amount of lipids.[30] Moreover, Roheim, *et al.* showed that the synthesis of the protein moiety of lipoprotein was unaffected by ingestion of orotic acid.[11,12] They suggested that glyceride accumulation in the liver from orotic acid-fed rats may be due to a failure to utilize apoprotein to form lipoprotein. The present work showed that a decreased release of beta-lipoprotein was partly restored by phosphatidylcholine (Fig. 2A). These observations suggest that phosphatidylcholine, especially endogenous, plays an important role in the release of lipoprotein. Phosphatidylcholine seems to serve as a bridge between neutral lipids and apoprotein of lipoprotein, because its molecule has both hydrophilic and hydrophobic groups. On the other hand, Aizawa, *et al.* have shown that phospholipid activated RNA polymerase and facilitated uptake of amino acid.[31,32] Accordingly, observed curative effect of phosphatidylcholine may also be due to the restored synthesis of specific protein which takes part in lipoprotein formation even though total protein synthesis in the liver is little changed by ingestion of orotic acid.

From the results of this and the previous studies,[17] it suggests that the failure in the release of betalipoprotein in orotic acid-fed rats is due to depressed synthesis of liver phosphatidylcholine and as a result, liver glycerides are accumulated.

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