Some Changes of Serum Lipid in Mice injected with 
*Vibrio parahaemolyticus* Endotoxin

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Some changes of serum lipid in mice were observed by the intraperitoneal injection of endotoxin extracted from *Vibrio parahaemolyticus*. A remarkable increase in the serum triglyceride content was shown in the poisoned mice after 12–24 hr. FFA level in the control mice gradually increased in response to the fasting periods for 12–24 hr whereas the level of FFA in the endotoxin–poisoned mice was lower than that in the control mice. Total cholesterol, however, did not show an appreciable difference in the level of the control animals. The hepatic triglyceride content in the poisoned mice increased at 14 hr postintoxication, and the lysosomal triglyceride lipase activity slightly increased 3 hr after intoxication, and declined after 14 hr.

There was not very much change of the triglyceride level in the serum in cortisone plus endotoxin-injected mice.

An increase of serum triglyceride in CCl₄-poisoned mice was not observed at 14 hr postinjection of endotoxin in spite of its high level in the serum of endotoxin-injected mice.

The mechanism responsible for the development of the hypertriglyceridemia in mice after injection of endotoxin is unknown at present.

Endotoxins (LPS) of gram-negative bacteria has been shown to possess numerous biological activities which have similar common effects in mammals. For example, as non-specific effects of these endotoxins depending on the dose and animal tested, a leucopenia is shown, followed by a polymorphonuclear leucocytosis and endothelial damage resulting in haemorrhage. In the previous report, like other bacterial endotoxins, mice injected with *Vibrio parahaemolyticus* endotoxin initially exhibited hyperglycemia, maximally in a few hours, then declined into a hypoglycemia with a concomitant decrease in hepatic glycogen 15 hr postintoxication.

Woods, *et al.*, had observed in the *in vitro* experiment that endotoxin stimulated an aerobic glycolysis by insulin–like action on the metabolism of animals. Lipid metabolism in mammalian has a close contact with carbohydrate metabolism, and is greatly affected by hormones. For example, insulin inhibits the release of free fatty acid (FFA) from adipose tissue, and enhances lipogenes.

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2) Location: 4–4–1 Komatsujima, Sendai.
Therefore, it is interesting to investigate a change of lipid metabolism induced by the administration of endotoxin into experimental animals.

The present study was undertaken to see whether endotoxin from *Vibrio parahaemolyticus* had an influence on mice sera lipids such as FFA, triglyceride and total cholesterol, or not.

**Material and Method**

**Organism and Cultivation**—*Vibrio parahaemolyticus* strain No. 6 was cultivated on solid medium which contained 1% polypepton (Daigo Eiyo Co., LTD, Tokyo), 1% meat extract (Kyokuto Seiyaku Co., LTD, Tokyo) and 3% NaCl in distilled water. After growing for 48 hr at 37°C, the organism was harvested, washed 3 times with 3% NaCl followed with acetone, and dried in vacuo.

**Endotoxin (LPS)**—The endotoxin was extracted with 45% phenol salt solution (90% C₆H₅OH : 3% NaCl = 1 : 1) at 65°C for 20 min, and purified according to the method of Lüderitz.⁹

**Analytical Procedures**—The male dd-mice, ranging in weight from 18 to 20 g, were fasted overnight, unless otherwise indicated, and during this period the animals had free access to water. Mice were injected intraperitoneally with a test dose (0.3 mg) of endotoxin, and the control mice received the intraperitoneal injection of saline. At various intervals thereafter, mice in each group were sacrificed, and the serum was used for chemical analysis.

Blood glucose was estimated by the o-aminobenzoic acid method.¹⁰

Serum FFA was estimated by the modified method of Elphick.¹¹ Triglyceride in serum was determined by the acetylacetone method,¹² and total cholesterol by the modified method of Zurkowski.¹³

Liver tissue was homogenized in twenty volumes of chloroform–methanol (2 : 1) as described by Folch, et al.¹⁴ and the filtrate was washed with 0.017% MgCl₂ solution. The lower phase of the mixture was used for the determinations of FFA and triglyceride. After removal of solvent, FFA was extracted with chloroform, passed through on silica column,¹⁵,¹⁶ and estimated by the same method that was used for serum FFA. Triglyceride was extracted with isopropanol according to the method of Flecher,¹⁷ passed through on Zeolite column containing copper sulfate and estimated by acetylatedon method.

Liver lysosome was prepared with the procedure described by Weissmann.¹⁸ The pellet obtained at 20000 × g for 20 min was resuspended in a constant volume of water and the aliquots were taken for the measurement of lyosomal lipase activity, and the activity was measured by the determination of glycerol²² released from triolein.

Glutamic pyruvic transaminase (GPT) in serum was estimated by Reitman–Frankel method.¹⁹

**Results**

(1) Effect of Administration of *Vibrio Parahaemolyticus* Endotoxin on Blood FFA, Triglyceride and Total Cholesterol Levels

Fig. 1 shows the effects of intraperitoneal injection of endotoxin on lipid levels in mouse serum. FFA value in the control mice sera gradually increased in response to the starvation periods for 12–24 hr, whereas the FFA level in the endotoxin–poisoned mice remained lower than that in the control mice.

No appreciable difference in the total

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cholesterol level was observed in the sera of endotoxin-poisoned mice in comparison with that in the control. However, it was noted that a remarkable increase in serum triglyceride was observed in the poisoned mice 12—24 hr postinjection.

(2) Effect of Endotoxin on the Concentration of FFA and Triglyceride in Mice Liver and on Hepatic Lysosomal Triglyceride Lipase Activity

Effect of endotoxin injection on FFA and triglyceride levels in mouse liver was examined. As shown in Table I, the hepatic triglyceride content in the endotoxin-treated mice showed an increase to some extent at 14 hr postintoxication as compared with that in the control mice, but the level of FFA in liver did not differ from that in the control mice.

Activity of hepatic lysosomal triglyceride lipase was estimated to see if the endotoxin may have an influence on the activity in the liver of the poisoned mice. When the endotoxin was injected intraperitoneally into mice, the activity of hepatic lysosomal triglyceride lipase showed a slight increase after 3 hr postinjection and decreased after 14 hr (Table II).

(3) Effect of Cortisone on Glucose, FFA and Triglyceride Levels in Serum of Endotoxin-injected Mice
Cortisone promotes a glyconeogenesis and a negative nitrogen balance. The ability of cortisone to protect experimental animals against the toxicity of bacterial endotoxin has been known from a number of investigations.\textsuperscript{19–21)}

Cortisone acetate (Wako Pure Chemical Ind. LTD., Osaka) was administered subcutaneously into mice with the desired dose (5mg), and the endotoxin was injected intraperitoneally. After 14 hr fast, mice were sacrificed and the analyses were performed. Cortisone-injected mice exhibited a hyperglycemia after 14 hr as compared with the control mice (Table III). When cortisone was given together with endotoxin, the blood sugar did not show an appreciable change in contrast to that in the control mice fasted for 14 hr.

There was not very much change of the triglyceride level in the serum in cortisone plus endotoxin-injected mice in spite of a hypertriglyceridermia of endotoxin-poisoned mice.

(4) Effect of Endotoxin Injection on Triglyceride and Total Cholesterol Contents in Serum of CCl\textsubscript{4}-poisoned or untreated Mice.

Acute liver injury was produced in mice as described in Table IV. Liver damage in CCl\textsubscript{4}-administered mice was ascertained by the elevation of serum glutamic pyruvic tran-

<table>
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<tr>
<th>Table III. Effect of Cortisone on Glucose, FFA and Triglyceride Levels in Serum of Endotoxin-injected Mice</th>
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<td><strong>Experimental treatment</strong></td>
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<tr>
<td>Control</td>
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<tr>
<td>Cortisone acetate (5mg)</td>
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<tr>
<td>Cortisone acetate (5mg) + LPS (0.3mg)</td>
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<td>LPS (0.3mg)</td>
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The result was represented as mean ± S.E. from 12 mice. The endotoxin was injected intraperitoneally (Cortisone was given subcutaneously) with a time interval no greater than 10 minutes. After 14 hr fast, mice were sacrificed and analyses were carried out.

<table>
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<tr>
<th>Table IV. Effect of Endotoxin Injection on Triglyceride and Total Cholesterol Contents in Serum of CCl\textsubscript{4}-poisoned or untreated Mouse</th>
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<tr>
<td><strong>Time after injection (hr)</strong></td>
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The result was given as mean value ± S. E. from 12 mice. Mice were divided into four groups and fasted on food. The first group was served as untreated controls. The second group was injected intraperitoneally with 0.3mg of LPS. The third group was injected similarly with 0.3 mg of LPS, and also administrated 0.2 ml of CCl\textsubscript{4}. The fourth group was administrated above dose of CCl\textsubscript{4}. After 14hr, the fasted mice were sacrificed and analyses were carried out.

aminase (GPT) activity.

CCl₄-poisoned mice could not give a hypertriglyceridemia at 14 hr postinjection of endotoxin.

A detectable alteration of total cholesterol level could not be observed in the serum of CCl₄-poisoned mice as a result of endotoxin administration.

**Discussion**

The changes in sera lipids of mice after endotoxin treatment might be a primary effect or a secondary consequence of the biological activities of endotoxin.

It was demonstrated in the present study that mice injected intraperitoneally with *Vibrio parahaemolyticus* endotoxin increased remarkably in serum triglyceride level at 14—24 hr postinoculation. In our previous report¹ a hyperglycemia was found in mice administered with *Vibrio parahaemolyticus* endotoxin, but mice injected with a large quantity (40 mg/kg) of endotoxin showed an evident hypoglycemia after 16 hr.

Woods, *et al.*⁵,⁶ had observed that salmonella endotoxins exerted a stimulatory effect on tumor glycolysis like the action of insulin. On the other hand, Shands, *et al.*²⁵ had reported that no clear-cut evidence of an insulin-like action by endotoxin was found in the *in vivo* setting at a hypoglycemia of BCG-infected mice.

It is known that hormones may play an important role in the regulation of various phase of lipid metabolism. Insulin increases the synthesis of triglyceride in both adipose tissue and liver,¹¹,¹² and decreases the rate of FFA release from adipose tissue. It is known that during fasting some of the plasma FFA arises from depot fat, whereas in hyperglycemia the movement of depot fatty acid to blood FFA decreases.

In our experiment, blood FFA in the endotoxin-poisoned mice showed lower level than that in the fasted control mice for 24 hr, and also it was found that the endotoxin administration had little or no effect on the serum-cholesterol level in mice.

Fourteen hours after the intraperitoneal injection of the endotoxin into mice, there occurred a slight decrease in lysosomal triglyceride lipase activity in liver. Liver injury by CCl₄ administration did not bring about a hypertriglyceridemia in the animals injected with endotoxin.

The mechanism by which the endotoxin increases triglyceride concentration in serum is, however, unknown from the present experimental data. Further experiments must be done.

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