Effect of Intraperitoneal Administration of *Vibrio parahaemolyticus* on Serum Lipid Level in Mice¹⁾

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Serum lipid level was investigated in mice by the intraperitoneal injection of *Vibrio parahaemolyticus*. At a sublethal dose (0.3 mg wet cells) a considerable increase in triglyceride content was observed in mouse serum at 1 day postinjection. In total cholesterol levels no appreciable difference was shown between the control and injected groups. However, serum triglyceride level in mice injected with non-pathogenic strain also increased in serum at 4 hr postinjection similarly to that injected with pathogenic strain.

Serum triglyceride level in mice changed effectively with increasing doses (0.2–0.8 mg) of the microorganism, and a marked increase of the level at 6 hr postinjection was seen in the injected animals with an increase in doses, but total cholesterol levels did not change in both the control and injected groups.

The heatstable hemolysin from pathogenic strain could not cause an appreciable change in triglyceride level in mouse serum. When *Vibrio parahaemolyticus* was administered into mice and after ten minutes the endotoxin antiserum was injected, the triglyceride content in serum was not different from that of the control mice.

Judging from the hypertriglyceridemia of mouse by the endotoxin in the previous report and the results in the present study, it is presumed that the endotoxin is responsible for the increase in serum triglyceride level in mice injected with *Vibrio parahaemolyticus* pathogenic strain.

Keywords—*Vibrio parahaemolyticus*; serum lipid level; serum triglyceride; total cholesterol; intraperitoneal injection; endotoxin; Kanagawa positive strain; heatstable hemolysin

The pathogenic strain of *Vibrio parahaemolyticus* exhibits “Kanagawa phenomenon” positive on the specific blood agar, and is known to excrete hemolytic toxins in culture medium.³⁻⁵⁾ In the last ten years, it has been

confirmed that there was a relationship between the production of the heatstable hemolysin and the pathogenicity of this strain to man in the case of food poisoning.⁶⁾

Pathogenic strain also shows lethal toxi-

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city to mouse by intraperitoneal injection. Toxic substance elaborated by pathogenes can bring about numerous biochemical disorders in host metabolisms. It may be, therefore, interesting to see metabolic changes in mice injected with *Vibrio parahaemolyticus* which elaborates the endotoxin and hemolytic toxins. Although many investigations of *Vibrio parahaemolyticus* have been made, there is little or no information on the biochemical changes in animals as a consequence of the administration of microorganism. In the previous report,¹⁾ we have observed that mice injected intraperitoneally with *Vibrio parahaemolyticus* endotoxin exhibited an increase in the serum triglyceride level at 14–24 hr postintoxication, and the level of serum free fatty acid (FFA) in the poisoned mice was lower than that in the fasted control mice.

The present study describes the effects of intraperitoneal administration of *Vibrio parahaemolyticus* pathogenic or non-pathogenic strain on serum lipid in mice.

Material and Method

Organism and Cultivation—*Vibrio parahaemolyticus* strain No. 128–72 (Kanagawa phenomenon positive) and strain No. 156–72 (Kanagawa phenomenon negative) were kindly provided by Dr. R. Sakazaki (National Institute of Health, Tokyo) and used throughout this study. The microorganisms were cultivated on solid medium which contained 1% polypepton (Daigo Eiyo Co., Ltd., Tokyo), 1% meat extract (Kyokto Seiyaku Co., Ltd., Tokyo) and 3% NaCl in distilled water.

Heatstable Hemolysin—Heatstable hemolysin was obtained from culture filtrate of *Vibrio parahaemolyticus* Kanagawa phenomenon positive strain No. 128–72, and purified partially by the fractionation

with ammonium sulfate, diethylaminoethyl (DEAE)–Sephadex A-50 and Sephadex G-100 column chromatography according to a modification of Miwatani method.³⁾ The partially purified hemolysin was heated at 100° for 10 min, quickly iced and used for the experiment.

Analytical Procedures—Male dd-mice, ranging in weight from 20 ± 2 , were used in this study. Before use in experiments, mice were fasted overnight, unless otherwise indicated, and during this period the animals had free access to water.

Vibrio parahaemolyticus suspended in 3% saline were injected intraperitoneally into mice at a tested dose, and the control mice were injected intraperitoneally with 3% saline. Thereafter, mice in each group were sacrificed at various intervals, and the serum obtained was used for chemical analysis.

The blood sugar was determined by the *o*-aminobiphenol method.⁷⁾ Serum FFA was estimated by the modified method of Elphick.⁸⁾ Estimation of serum triglyceride was carried out by the acetylacetone method of Fletcher,⁹⁾ and total cholesterol by the modified method of Zurkowski.¹⁰⁾

Endotoxin Antiserum—The endotoxin from *Vibrio parahaemolyticus* was extracted and purified according to the method of Lüderitz.¹¹⁾ Mice was immunized 3 times with subcutaneous injection of the endotoxin in Freund's complete adjuvant at a concentration of mg/ml, and the antiserum obtained was used for the experiment.

Result

(1) Effect of Intraperitoneal Administration of Viable Cells on Blood Sugar, Triglyceride and FFA Levels in Mice Sera

Vibrio parahaemolyticus Kanagawa phenomenon positive (pathogenic) or negative (non-pathogenic) strain suspended in 3% saline was injected intraperitoneally into fasted mice at a dose of 0.4 mg, respectively. Control

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TABLE I. Comparison of Blood Sugar, Triglyceride and FFA Levels in Mouse injected with Kanagawa-Phenomenon Positive and Negative Strains of *Vibrio parahaemolyticus*

Experimental treatment		Serum		
Injected with		Glucose (mg/dl)	Triglyceride (mg/dl)	FFA (μ Eq/liter)
Control	1 hr	135.2 $\pm$ 6.0	55.2 $\pm$ 6.3	510 $\pm$ 8
	4 hr	125.6 $\pm$ 6.2	46.4 $\pm$ 3.1	540 $\pm$ 10
Kanagawa (+) strain (pathogenic)	1 hr	185.3 $\pm$ 10.4	61.6 $\pm$ 10.2	555 $\pm$ 20
	4 hr	140.5 $\pm$ 4.6	70.3 $\pm$ 4.6	575 $\pm$ 17
Kanagawa (-) strain (non-pathogenic)	1 hr	155.2 $\pm$ 7.2	53.4 $\pm$ 8.2	505 $\pm$ 15
	4 hr	130.0 $\pm$ 7.4	65.6 $\pm$ 6.5	530 $\pm$ 22

Viable cells were injected intraperitoneally to mice at a dose of 0.4 mg, and control mice were injected with 3 % saline.

The result represents mean value $\pm$ S.E from 9 mice.

TABLE II. Effect of Inoculation Dose of Viable Cells (pathogenic strain) on Serum FFA, Triglyceride and Total Cholesterol Levels

Weight of wet cells (mg)	Content in serum					
	FFA (μ eq/liter) Postinjection		Triglyceride (mg/dl) Postinjection		Cholesterol (mg/dl) Postinjection	
	2 hr	6 hr	2 hr	6 hr	2 hr	6 hr
0 (Control)	456 ($\pm$ 25)	450 ($\pm$ 25)	70.5 ($\pm$ 5.0)	63.0 ($\pm$ 5.0)	115.2 ($\pm$ 6.0)	115.2 ($\pm$ 3.0)
0.2	435 ($\pm$ 30)	490 ($\pm$ 20)	65.1 ($\pm$ 6.1)	68.2 ($\pm$ 3.1)	115.0 ($\pm$ 10.2)	102.0 ($\pm$ 3.2)
0.4	435 ($\pm$ 25)	505 ($\pm$ 25)	63.5 ($\pm$ 4.0)	78.0 ($\pm$ 3.0)	102.4 ($\pm$ 4.0)	110.4 ($\pm$ 4.0)
0.8	455 ($\pm$ 40)	455 ($\pm$ 20)	54.0 ($\pm$ 7.1)	85.5 ($\pm$ 5.4)	130.0 ($\pm$ 9.5)	113.0 ($\pm$ 3.2)

Viable cells of *V. parahaemolyticus* were injected intraperitoneally into mice at the dose indicated in Table, and control mice were injected with 3 % saline.

The result was represented as mean value $\pm$ S.E from 12 mice.

mice received 0.1 ml of 3 % saline alone. All mice were sacrificed by decapitation one and four hr after treatment, and the serum obtained was used for the determination of blood sugar and lipid levels. The result was shown in Table I. Both blood sugar levels in mice injected with pathogenic and non-pathogenic strains showed an increase at 1 hr postinjection but decreased at 4 hr. However, as shown in the table, the rate of decline of the glucose level in mice injected with the former strain was larger than that of the latter. On the contrary, serum triglyceride level in mice administered with each strain showed an increase at 4 hr postinjection as compared with that of the fasted control mice. An appreciable difference was not observed in sera

FFA levels between the pathogenic and non-pathogenic strains at 4 hr postinjection, though serum FFA in mice injected with the former strain showed a slight increase at 1 hr postinjection.

(2) Effect of Varying Administration Doses of Viable Cells on Serum Lipid Levels in Mice

Table II shows the effect of increasing doses (0.2—0.8 mg) of viable cells on the serum lipid level in mice.

At 2 hr postinjection, a decrease in triglyceride level in serum was seen in inverse proportion to increasing amount of the administered dose of the pathogenic strain. However, at 6 hr postinjection a marked increase of the triglyceride level was observed

TABLE III. Effect of Heatstable Hemolysin Administration on Blood Sugar and Triglyceride Levels in Mice

Treatment	Blood sugar (mg/dl) Postinjection		Serum triglyceride (mg/dl) Postinjection	
	2 hr	14 hr	2 hr	14 hr
Control	115.0 $\pm$ 6.5	106 $\pm$ 14.0	65.5 $\pm$ 12.1	68.3 $\pm$ 11.6
Hemolysin	130.2 $\pm$ 15.6	88.6 $\pm$ 21.4	65.7 $\pm$ 19.8	82.6 $\pm$ 32.3

Mice were injected intravenously with heatstable hemolysin from *Vibrio parahaemolyticus*, and sacrificed 2 and 14 hr postintoxication. Thereafter, analysis was performed. Each result represents mean value $\pm$ S.E from 16 mice.

in the treated animals at the dose response to viable cells. On the other hand, no change of total cholesterol level was observed in both the control and treated groups.

(3) Time Course of Serum Triglyceride and Total Cholesterol Levels in Mice injected with Viable Cells

The sublethal dose (0.3 mg wet cells) of *Vibrio parahaemolyticus* was injected intraperitoneally into mice. Control mice received sterile 3% saline alone. Animals had free access to food during the experimental period. Before decapitation at the desired time after injection, mice in each group were starved for 14 hr, and thereafter sacrificed. The result is shown in Fig. 1. At the tested dose a considerable increase of serum triglyceride was seen in mice at 1 day postinjection. In total cholesterol levels no appreciable difference was observed in both the control and treated groups. Similar result was also seen with non-pathogenic strain.

(4) Effect of Heatstable Hemolysin Administration on Blood Sugar and Lipid Levels in Mice

Table III showed that the intravenous injection of *Vibrio parahaemolyticus* heatstable hemolysin into mice could not cause an appreciable change in serum triglyceride amount 2 hr after treatment, and that there was a tendency to increase slightly in the level at 14 hr postinjection. However, it is not clearly for the reason that the value of serum trigly-

Fig. 1. Time Course of Serum Triglyceride and Total Cholesterol Levels in Mice injected with Viable Cells of *Vibrio parahaemolyticus* (Pathogenic Strain)

The results represent the increase or decrease to the level (100%) of control.

▨ : triglyceride □ : cholesterol

ceride amount after 14 hr showed the large standard error. On the other hand, blood sugar slightly increased at 2 hr postinjection and after 14 hr the level inversely showed a lower value than that of the control mice.

(5) Effect of Intraperitoneal Administration of Viable Cells on Serum Lipid Levels in Mice immunized with Endotoxin from *Vibrio parahaemolyticus*

Mice were immunized 3 times with subcutaneous injection of *Vibrio parahaemolyticus* endotoxin, and the antiserum obtained was used for the following experiment. The viable

Fig. 2. Effect of Intraperitoneal Administration of Viable Cells on Serum Triglyceride Level in Mice immunized with Endotoxin

A: control mice

B: endotoxin-immunized and viable cells injected mice

C: non-immunized and viable cells injected mice

Each result represents $M \pm SE$ from 8 animals. The organism used was pathogenic strain of *Vibrio parahaemolyticus*.

cells of *Vibrio parahaemolyticus* (pathogenic strain) was administered intraperitoneally at a dose of 0.3 mg into 14 hr-fasted mice, and ten minutes after treatment, 0.1 ml of the antiserum was injected subcutaneously in the animals. Mice were decapitated four hr after the antiserum injection. As shown in Fig. 2, the immunized mice scarcely had any appreciable difference in serum triglyceride level in comparison with that of control mice while an evident difference was observed in the values between the immunized and non-immunized groups. It may be considered that the increase of triglyceride in mice sera mainly results from the action of endotoxin in the microorganism.

Discussion

Although a number of investigations on carbohydrate metabolism have been made in mice injected with pathogenes such as *Salmo-*

nella typhimurium,¹²⁾ *Listeria monocytogenes*,¹³⁾ *Coxiella burnetii*¹⁴⁾ and *B. tuberculosis*,¹⁵⁾ lipid metabolism in injected animals is now scarcely reported. Paretsky, *et al.*¹⁶⁾ have reported that there were some increase in cholesterol and a large increase in glyceride in liver lipid fractions of guinea pigs injected with *Coxiella burnetii* in Rickettsiae.

In our experiment, however, the intraperitoneal administration of *Vibrio parahaemolyticus* into mice resulted in an increase in serum triglyceride, but not in serum total cholesterol at 6 hr postinjection. Heatstable hemolysin has a tendency of a slight increase in the triglyceride level of mouse serum, but this tendency is not clearly elucidated since the large standard error in the value is observed in mouse serum at 14 hr postintoxication. When *Vibrio parahaemolyticus*, however, was administered into mice and after ten minutes the endotoxin antiserum was injected, the amount of serum triglyceride was not different from that of the control mice.

In the previous report,¹⁾ we have observed that the serum triglyceride level in mice markedly increased 14 hr after intraperitoneal administration of endotoxin from *Vibrio parahaemolyticus*. However, no appreciable change in total cholesterol levels was observed in the serum of endotoxin-poisoned mice. Serum triglyceride content in mice injected with non-pathogenic strain also showed an increase of the level similarly to that injected with pathogenic one.

Lipid metabolism in mammalian is closely related to carbohydrate metabolism. We have

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already observed that *Vibrio parahaemolyticus* endotoxin-poisoned mice showed a hyperglycemia in the early stage of postinjection, and thereafter developed to a distinct hypoglycemia.¹⁷⁾ In this report, mice injected with heatstable hemolysin showed a slight decrease in blood sugar level at 14 hr postinjection, but not a hypertriglyceridemia. The decline of blood sugar, therefore, resulting from hemolysin administration is probably dissimilar from that of endotoxin.

Thus, judging from the experimental results by use of the endotoxin antiserum or

heatstable hemolysin, it is considered that the endotoxin is responsible for the elevation of serum triglyceride level in mice injected with viable cells of *Vibrio parahaemolyticus* pathogenic strain.

The mechanism of the change of triglyceride levels in mouse serum by the administration of microorganism remains unexplained by the present experiment.

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