chloroform. Our study has been done independent of their report, and form A and form B described by them are similar to form I and $CHCl_3$–II type, respectively, in our study. Effect of the nature of the medium on the dissolution rate and *in vivo* bioavailability are currently being investigated in our laboratories.

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A Remarkable Elevation of Serum Calcium Concentration Induced by Ligation of Bile Duct

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The change of calcium concentrations in the serum, liver, and bile of rats was investigated after a single oral administration of calcium chloride. The administration of calcium produced a corresponding increase of calcium concentration in the serum and liver, and this increase was dose dependent. A remarkable elevation of calcium content in the bile was caused by the administration of calcium. By the ligation of bile duct, the calcium concentration in the serum was markedly enhanced after the administration of calcium (10 and 50 mg/100 g) when compared with that of the sham-operated rats. These results suggest that the excretion of calcium into the bile prevents the elevation of calcium concentration in the serum after the absorption of calcium from the intestine.

Keywords——serum calcium; liver calcium; bile calcium excretion; hypercalcemic effect by bile duct ligation; calcium metabolism in hepatic bile system

There are several studies in relation to the regulation of serum calcium after the calcium absorption from the intestine.[2,3)] However, the mechanism to decrease the calcium concentration in the serum increased by the ingestion of calcium containing diet has not been fully resolved. On the other hand, the physiological significance of the bile excretion of calcium absorbed from the intestine is little known reportedly, although a large amount of calcium is contained in the bile. The present study was therefore undertaken to investigate the alteration of calcium concentration in the serum, liver, and bile of rats orally administered calcium chloride. We found that calcium is excreted into the bile and that the serum calcium concentration is markedly enhanced by the ligation of bile duct.

Materials and Methods

Animals——Male Wistar rats, weighing approximately 120 g, were used. The animals were kept at a room temperature of 25±1° and fed commercial laboratory chow containing 7.4% carbohydrate, 1.1% calcium and 1.1% phosphate (Oriental Test Diet Co., Tokyo) and tap water *ad libitum* until the day of testing.

Drug——Calcium chloride was dissolved in demineralized water to the concentration of 10 or 50 mg of Ca/ml. This solution was given by a single oral administration (1 ml/100 g) to rats. Control rats was administered orally demineralized water.

Surgical Procedures——The abdomen was opened by a incision after the intraperitoneal administration of 25% urethane (0.6 ml/100 g). The common bile duct was then cannulated with PE-10 tubing and the bile duct ligated, and the incision was closed with wound clips. The animals were put on the thermostatic

1) Location: *2–1, Oshika 2-chome, Shizuoka, 422 Japan.*
2) G. Coen, G. F. Mazzuoli, I. Antonozzi, and A. Scada, *Metab. Clin. Exp.*, **23**, 709 (1974).
3) R. Swaminathan, J. Ker, and A.D. Care, *J. Endocrinol.*, **61**, 83 (1974).

water bath (38±1°).[4] The animals were orally administered calcium chloride solution by stomach tube immediately after the cannulation, and they were not fed or watered throughout the experiments.

Also, the common bile duct of rats was ligated under light ether anesthesia and the incision closed with wound clips. The animals were orally administered calcium chloride solution by stomach tube 1 hr after the ligation of bile duct and were killed after 30 min.

Analytical Methods——The animals were bled by cardiac puncture under light ether anesthesia. Liver was perfused with cold 0.25 M sucrose solution after bleeding and removed immediately. The concentration of calcium in the liver was determined by atomic absorption spectrophotometry (Perkin-Elmer, Model 303) with a reversed air-acetylene flame after chloric acid digestion.[5]

Blood samples obtained by cardiac puncture were centrifuged immediately after the collection. The serum was separated and analyzed immediately. Determination of calcium was made on 0.1 ml aliquots of serum by atomic absorption spectrophotometry.[6]

Bile volume was measured by means of pipet graduated in 0.01 ml. The amount of bile calcium was determined by atomic absorption spectrophotometry after precipitation with 10% trichloroacetic acid. The bile calcium content was expressed as the excreted calcium (μg) per 100 g body weight of rats.

Statistical Methods——The significance of the difference between the values was estimated by Student's *t* test. p values less than 0.05 were considered to indicate statistically significant differences.

Results

Change of Serum and Liver Calcium Concentrations after Calcium Administration

The effect of calcium chloride at four dose levels on the serum and liver calcium concentration was examined 30 min after a single oral administration of calcium to rats that had fasted for 3 hr (Fig. 1). At a dose level of 10 mg/100 g body weight as Ca, the serum calcium concentration increased significantly when compared with the values obtained from rats given demineralized water, while the liver calcium concentration did not elevate significantly. However, the liver calcium concentration markedly ($p<0.01$) increased at dose level of 50 mg/100 g as Ca.

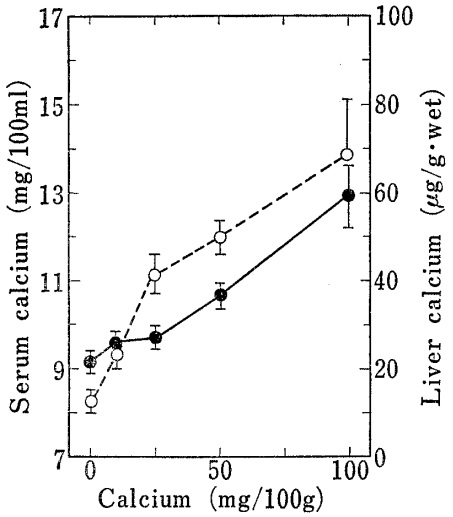

Fig. 1. Effect of a Single Oral Administration of Calcium Chloride on the Serum and Liver Calcium Concentrations of Rats

Rats were killed 30 min after the administration of calcium. Each point represents the mean of 5 animals. Vertical lines represent the SE.
---○---; serum calcium, —●—; liver calcium.

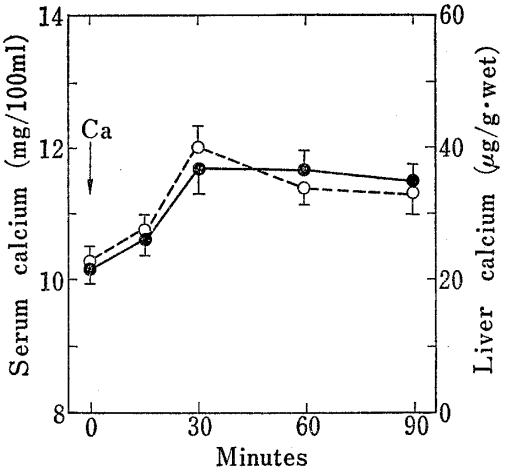

Fig. 2. Time Course of the Increased Calcium Concentration in the Serum and Liver induced by the Administration of Calcium Chloride in Rats

Rats received a single oral administration of calcium (50 mg/100 g). Each point represents the mean of 5 animals. Vertical lines represent the SE.
---○---; serum calcium, —●—; liver calcium.

4) R.J. Robert, C.D. Klaassen, and G.L. Plaa, *Proc. Soc. Expt. Med. Biol.*, **125**, 313 (1967).
5) E.T. Backer, *Clin. Chim. Acta.*, **24**, 233 (1969).
6) J.B. Willis, *Nature* (London), **16**, 249 (1960).

In order to investigate the time course of the serum and liver calcium concentrations, calcium chloride (Ca, 50 mg/100 g) was orally administered to rats that had fasted for 3 hr and their blood was obtained at varying periods after calcium administration (Fig. 2). Figure 2 shows that, as early as 15 min after calcium administration, there was a significant ($p<0.05$) increase in the serum calcium concentration. The serum and liver calcium concentrations continued to increase reaching a maximum at 30 min, and then began to decrease gradually.

Increase of Bile Calcium Content after Calcium Administration

The change of calcium content in the bile of rats orally administered with calcium chloride (Ca, 50 mg/100 g) is shown in Table I. The bile was collected three times at 1-hr intervals after the administration of calcium. The bile calcium content of control rats decreased gradually after the start of bile collection. On the other hand, the bile calcium content of rats administered calcium increased markedly when compared with that of control rats, while the bile volume decreased slightly. The total content of calcium excreted into the bile of rats administered calcium during 3 hr after the bile collection showed approximately 1.5 times ($p<0.01$) in comparison with that of control rats.

Table I. Concentration of Calcium in the Bile of Rats after a Single Oral Administration of Calcium Chloride

Treatment	Number of rats	Bile calcium content (μg/100 g)		
		(0—1)hr	(1—2)hr	(2—3)hr
Control[a)]	5	45.2±4.7[c)]	38.5±3.8	33.8±4.4
Calcium[b)]	6	62.0±3.3[d)]	59.0±4.9[d)]	45.4±5.3

a) Control administered orally demineralized water.
b) Calcium (50 mg/100 g) was administered orally.
c) Each value represents the mean ± S.E.
d) Differs from respective control mean, $p<0.01$.

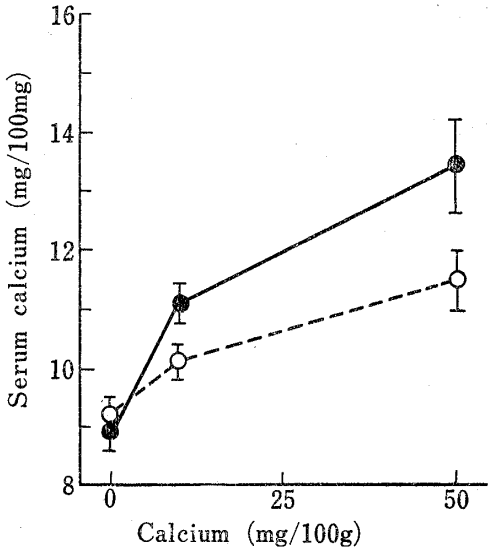

Fig. 3. Effect of a Single Oral Administration of Calcium Chloride on the Serum Calcium Concentration of Rats Ligated the Bile Duct

Rats received the administration of calcium 60 min after the ligation of bile duct and they were killed 30 min after the administration of calcium. Each point represents the mean of 5 animals. Vertical lines represent the SE.
---○---; Sham-operated rats,
—●—; bile duct-ligated rats.

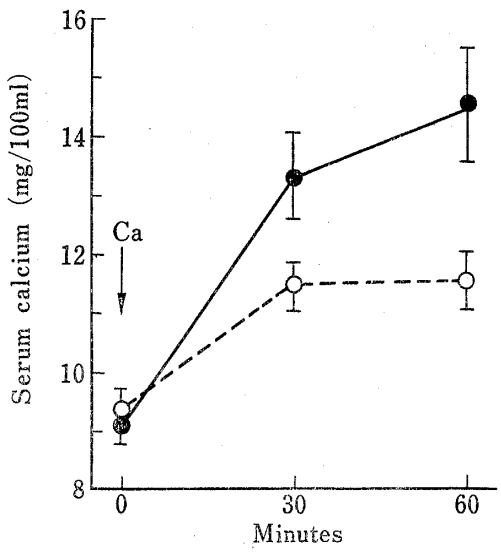

Fig. 4. Time Course of the Increased Calcium Concentration in the Serum induced by the Administration of Calcium Chloride in Rats

Rats received a single oral administration of calcium (50 mg/100 g) 60 min after the ligation of bile duct. Each point represents the mean of 5 animals. Vertical lines represent the SE.
---○---; Sham-operated rats,
—●—; bile duct-ligated rats.

Change of Serum Calcium Concentration after Bile Duct Ligation

The effect of increasing doses of calcium chloride on the serum calcium concentration in rats ligated the bile duct is shown in Fig. 3. The animals were killed 30 min after a single oral administration of calcium. The ligation of bile duct did not shown a significant alteration of the serum calcium concentration. However, the serum calcium concentration of rats ligated the bile duct was significantly elevated by the administration of calcium (10 mg/100 g) when compared with the values obtained from the sham-operated rats.

The time course of the increase of the serum calcium concentration in rats ligated the bile duct after a single oral administration of calcium (50 mg/100 g) is shown in Fig. 4. The animals were killed at varying times after the administration of calcium. The serum calcium concentration in the sham-operated rats reached a maximum level 30 min after the administration of calcium. Meanwhile, the increase of serum calcium concentration produced by the calcium administration was enhanced approximately 2 times by the ligation of bile duct 60 min after the calcium administration.

Discussion

A single oral administration of calcium chloride produces a corresponding increase in the concentrations of calcium in the serum, liver, and bile of intact rats. This result clearly indicates that the calcium absorbed from the intestine was partly secreted into the bile through the liver. Also, the serum calcium concentration in rats ligated the bile duct was markedly enhanced by a single oral administration of calcium chloride when compared with that of the sham-operated rats. Presumably, it appears that a remarkable elevation of serum calcium produced by the ligation of bile duct is caused by the inhibition of transport of calcium from the serum into the bile.

On the other hand, it is known that an increase in the serum calcium concentration produced by the administration of calcium stimulates the secretion of calcitonin from thyroid glands to maintain calcium homeostatis in the serum of rats.[7] Presently, we found that an increase in the serum calcium concentration after the administration of calcium enhanced the excretion of calcium into the bile, and that the ligation of bile duct markedly potentiated the serum calcium concentration increased by the calcium administration to rats. These results suggest that the excretion of calcium into the bile plays a physiological role on the regulation of calcium level in the serum increased after the ingestion of high calcium containing diets.

7) P.D. Broulik and V. Pocovsky, *Endocrinol. Exp.*, **7**, 183 (1973).