

## Notes

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### Physiological Significance of Calcium Excretion into the Bile of Rats

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The changes of radiocalcium in the blood, liver, kidneys, femur, and bile were investigated after a single subcutaneous administration of <sup>45</sup>CaCl<sub>2</sub> to fasted rats. The radioactivity (dpm/g wet tissue) of <sup>45</sup>Ca in the blood, liver, and kidneys gradually increased after the administration of <sup>45</sup>CaCl<sub>2</sub>, while the radioactivity of <sup>45</sup>Ca in the femur and bile was markedly elevated. The increase of radioactivity of <sup>45</sup>Ca in the bile was greater than that in the femur. Meanwhile, the radioactivity of <sup>45</sup>Ca in the whole liver per 100 g body weight was markedly increased 15 min after the administration of <sup>45</sup>CaCl<sub>2</sub>. However, the radioactivity of <sup>45</sup>Ca in the bile per 100 g body weight was only slightly increased 15 min after the administration of <sup>45</sup>CaCl<sub>2</sub>, and then began to increase sharply. Furthermore, the excretion of radiocalcium into the urine and feces was examined after a single subcutaneous administration of <sup>45</sup>CaCl<sub>2</sub> to rats. The radioactivity of <sup>45</sup>Ca in the feces was about 30 times that of the urine. It seems likely that the excretion of calcium into the feces through the bile plays a physiological role in the regulation of calcium metabolism.

**Keywords**—calcium metabolism; liver calcium; bile calcium; urine calcium; fecal calcium

### Introduction

It is well known that the bone, kidney, and intestine are the regulatory organs of calcium metabolism in mammals. However, it is not known whether the liver participates in calcium metabolism. Previously, we found that calcitonin, a calcium-regulating hormone, increases the calcium concentration in the liver of rats.<sup>2)</sup> More recently, we reported that calcium orally administered as calcium chloride is excreted into the bile of rats, and that the serum calcium is markedly increased by ligation of the bile duct.<sup>3)</sup> Based on these investigations, it is assumed that the bile pool of the hepatocytes participates in the regulation of calcium metabolism. The present study was therefore undertaken to examine the physiological significance of calcium excretion into the bile. We found that the calcium is markedly excreted into the feces through the bile.

### Materials and Methods

Male Wistar rats, weighing 200–300 g, were kept at a room temperature of 25 ± 1° and fed commercial laboratory chow containing 1.1% calcium and 1.1% phosphate (Oriental Test Diet Co., Tokyo) and tap water *ad libitum* until the day of testing.

The animals were fasted for 24 hr before the experiment. The abdomen was opened after the intraperitoneal administration of 25% urethane (0.6 ml/100 g body weight). The bile duct was cannulated with PE-10 tubing which was secured in place, and then the incision was closed with wound clips. The animals were put on a warm water bath (38 ± 1°),<sup>4)</sup> and the bile was collected. The animals were subcutaneously administered <sup>45</sup>CaCl<sub>2</sub> (0.2 mCi/1.61 mg Ca/1 ml/kg body weight) immediately after the cannulation, and they were not given food or water throughout the experiments.

- 1) Location: a) 2-1, Oshika 2-chome, Shizuoka; b) 18-1, Kamiyoga 1-chome, Setagaya-ku, Tokyo.
- 2) M. Yamaguchi, Y. Takei, and T. Yamamoto, *Endocrinology*, **96**, 1004 (1975).
- 3) M. Yamaguchi and T. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **26**, 2568 (1978).
- 4) R.J. Roberts, C.D. Klaassen, and G.L. Plass, *Proc. Soc. Exp. Biol. Med.*, **125**, 313 (1967).

The bile was collected for 15, 30, and 60 min after the administration of  $^{45}\text{CaCl}_2$ , and blood samples were obtained by cardiac puncture after bile collection. The liver, kidneys, and femur were immediately removed after bleeding. The tissues were dissolved in Soluene (Packard), and the radioactivity of  $^{45}\text{Ca}$  in the tissue was determined with a liquid scintillation counter.

In other experiments, the animals were subcutaneously administered  $^{45}\text{CaCl}_2$ , and kept in metabolic cages. The urine and feces were collected for 24 hr after the administration of  $^{45}\text{CaCl}_2$ . The radioactivities of  $^{45}\text{Ca}$  in the urine and feces were measured with a liquid scintillation counter.

The data were subjected to analysis of variance, and SE values were calculated from the residual error term. The significance of the difference between the values was estimated by Student's *t* test. *P* values lower than 0.05 were considered to indicate statistically significant differences.

## Results and Discussion

The changes of radiocalcium in the blood, liver, kidney, femur, and bile after a single subcutaneous administration of  $^{45}\text{CaCl}_2$  to fasted rats is shown in Table I. The radioactivity (dpm/g wet tissue) of  $^{45}\text{Ca}$  in the blood or kidney gradually increased during 60 min after the administration of  $^{45}\text{CaCl}_2$ . The radioactivity of  $^{45}\text{Ca}$  in the femur was greatly increased 30 and 60 min after the administration of  $^{45}\text{CaCl}_2$ , though physicochemical exchange of calcium between the blood and bone mineral might contribute significantly to the increase of  $^{45}\text{Ca}$  radioactivity. Meanwhile, the radioactivity of  $^{45}\text{Ca}$  in the liver and bile reached a maximum 30 min after the administration of  $^{45}\text{CaCl}_2$ , and was slightly decreased at 60 min. The increase in the radioactivity of  $^{45}\text{Ca}$  in the bile was the largest among all of the tissues, and that of  $^{45}\text{Ca}$  in the liver was the smallest. The bile is formed in the liver and secreted into the intestine, so it seems likely that the radiocalcium in the liver is transferred to the bile.

TABLE I. Tissue Distribution of Radiocalcium after the Administration of  $^{45}\text{CaCl}_2$  to Rats<sup>a)</sup>

Tissue	Radiocalcium (dpm/g wet tissue)		
	15 min	30 min	60 min
Blood	96111 ± 4668	104102 ± 13163	142770 ± 9579 <sup>b)</sup>
Liver	47640 ± 2049	73502 ± 10180 <sup>b)</sup>	63304 ± 15079
Kidney	73955 ± 12019	108027 ± 17950	124495 ± 16543 <sup>b)</sup>
Femur	78256 ± 11224	194805 ± 21579 <sup>b)</sup>	367786 ± 102683 <sup>b)</sup>
Bile	61664 ± 14254	455790 ± 59252 <sup>b)</sup>	436840 ± 47703 <sup>b)</sup>

a) Rats were fasted for 24 hr. The animals each received a single subcutaneous administration of  $^{45}\text{CaCl}_2$  (0.2 mCi/kg). Each value represents the mean of 4 animals.

b) Differs from the value obtained 15 min after  $^{45}\text{CaCl}_2$  administration,  $p < 0.01$ .

The changes of radiocalcium content in the whole liver and the excretion of radiocalcium into the bile after a single subcutaneous administration of  $^{45}\text{CaCl}_2$  in fasted rats were examined, and the results are shown in Fig. 1. The radioactivity (dpm/100 g body weight) of  $^{45}\text{Ca}$  in the whole liver increased dramatically 15 min after the administration of  $^{45}\text{CaCl}_2$  and was much the same at 30 min, but it began to decrease 60 min after the administration. On the other hand, the radioactivity of  $^{45}\text{Ca}$  in the bile was only slightly increased 15 min after the administration of  $^{45}\text{CaCl}_2$ , but a marked elevation was observed 30 min after the administration of  $^{45}\text{CaCl}_2$ . Thus the increase of radiocalcium in the bile after the administration of  $^{45}\text{CaCl}_2$  followed the uptake of radiocalcium in the whole liver. These results clearly indicate that much of the radiocalcium taken into the liver is excreted into the bile.

Furthermore, we examined the excretion of radiocalcium into the urine and feces after a single subcutaneous administration of  $^{45}\text{CaCl}_2$  in fed rats (Fig. 2). The urine and feces were collected for 24 hr. The radioactivity of  $^{45}\text{Ca}$  in the feces was about 30 times that of the urine. This result suggests that the calcium in rats is largely excreted into the feces. Presumably, biliary calcium secreted into the intestine may be excreted into the feces.

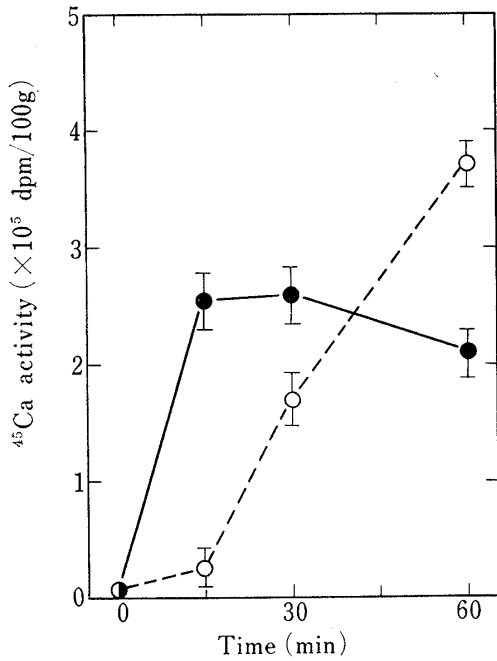


Fig. 1. Changes of Radiocalcium in the Whole Liver and of Radiocalcium Excreted into the Bile after the Administration of <sup>45</sup>CaCl<sub>2</sub> to Rats

Rats were fasted for 24 hr. The animals each received a single subcutaneous administration of <sup>45</sup>CaCl<sub>2</sub> (0.2 mCi/kg). Each point represents the mean of 4 animals. Vertical lines represent the SE. —●—, liver radiocalcium; --○--, bile radiocalcium.

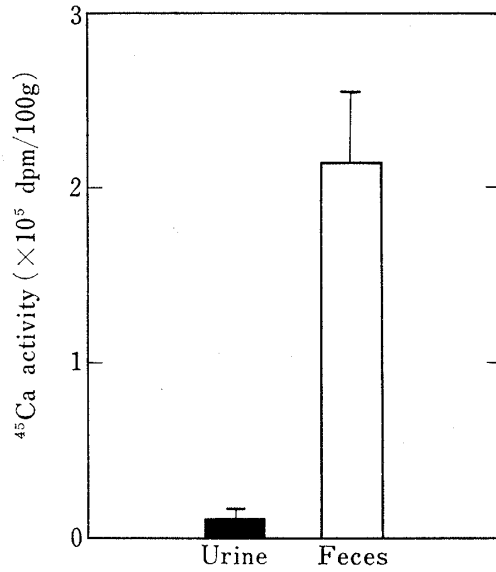


Fig. 2. Radiocalcium Excreted into the Urine and Feces after the Administration of <sup>45</sup>CaCl<sub>2</sub> to Rats

Rats each received a single subcutaneous administration of <sup>45</sup>CaCl<sub>2</sub> (0.2 mCi/kg). The urine and feces were collected for 24 hr. Each bar represents the mean of 5 animals. Vertical lines represent the SE. ■, urine; □, feces.

Based on the results of the previous<sup>3)</sup> and present studies, the excretion of calcium into the bile through the liver from the blood may play a physiological role in the regulation of calcium metabolism.