

## Effect of Subchronic Oral Treatment with Terbium on Gastrointestinal Uptake of Calcium and Phosphorus

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Basic and applied studies to develop pioneering technology have been widely performed. Recently, rare earth elements have become regarded as promising new basic materials for superconductors, ceramics and amorphous substances, and should become important in modern industries. However, increased exposure to rare earth elements, might adversely affect human health.

The metabolism and toxicity of rare earth elements have conventionally been studied with radioisotopes (Schubert et al. 1950; Laszlo et al. 1952; Durbin 1960; Magnusson 1963), but these methods have inherent problems in that the radioisotopes used may form radiocolloids and their radioactivity is toxic (Schweitzer and Jackson 1952; Ellis 1977). Recently, metabolism of dysprosium, europium, ytterbium and yttrium by ICP-AES (Nakamura et al. 1991a; Nakamura et al. 1991b; Nakamura et al. 1991c; Nakamura et al. 1991d) and the biological effects of lanthanum (Ogawa 1992) have been reported. However, the studies on biological effects of rare earth elements have just begun, so very few have been reported.

We therefore investigated effects of rare earth element, terbium (Tb) on the excretion into the urine and feces, as well as the distribution of calcium (Ca) and phosphorus (P) in the liver, pancreas, spleen, kidney, lung, heart, thymus, brain, bone, and blood of male rats.

### MATERIALS AND METHODS

Terbium chloride ( $\text{TbCl}_3 \cdot 6\text{H}_2\text{O}$ ) was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.).  $^{45}\text{Ca}$  and  $^{32}\text{P}$  were purchased from ICN Biomedicals Inc. (Costa Mesa, CA, U.S.A.). Soluene-350 was purchased from Packard Instrument Co. Inc. (Downers Grove, IL, U.S.A.). 2,5-diphenyloxazole (DPO) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). All other reagents used were the highest quality commercially available.

Male Wistar rats, weighing 100–120 g were obtained from Nippon Bio-Supp. Center (Tokyo, Japan). The animals were fed laboratory chow and water ad libitum and housed in stainless cages at a constant temperature (23–25 °C) and humidity (50–60%) under a 12 hr light-dark cycle (light: 7:00 a.m. to 7:00 p.m.). The experiments were performed upon 5 rats per group.  $\text{TbCl}_3$  was administered orally

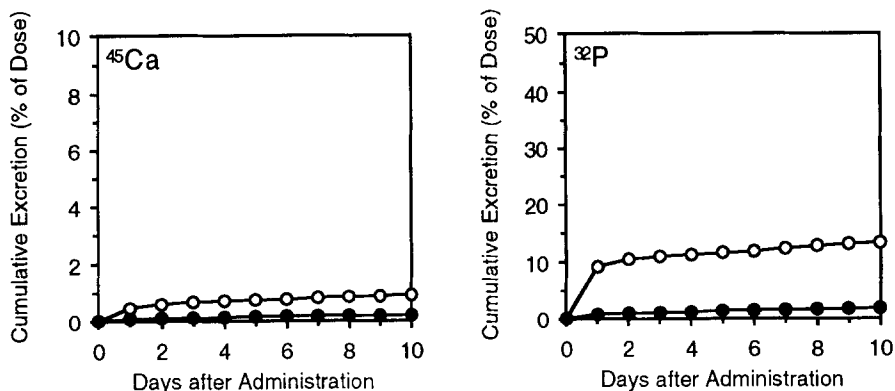


Figure 1. Cumulative urinary excretion of  $^{45}\text{Ca}$  and  $^{32}\text{P}$  in terbium-pretreated rats  
○ Control rats, ● Terbium-pretreated rats.  
Each points represents the mean of 5 rats.

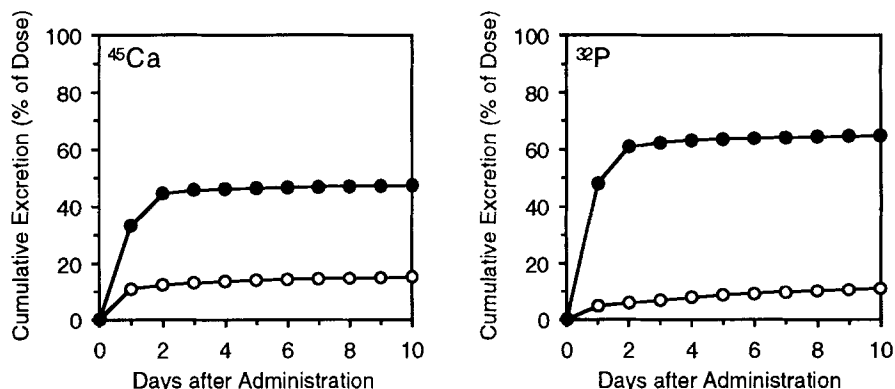


Figure 2. Cumulative fecal excretion of  $^{45}\text{Ca}$  and  $^{32}\text{P}$  in terbium-pretreated rats  
○ Control rats, ● Terbium-pretreated rats.  
Each points represents the mean of 5 rats.

at a dose of 100 mg/rat/day as Tb metal for 4 weeks. The  $^{45}\text{Ca}$  and  $^{32}\text{P}$  were given to the rats once orally at a dose of 10  $\mu\text{Ci}/\text{rat}$ . The actual dosages for Ca and P are 1.3 and 2.3  $\mu\text{g}/\text{rat}$ , respectively. Thereafter, urine and feces were collected daily for 10 days. The rats were sacrificed at 10 days after treatment with  $^{45}\text{Ca}$  and  $^{32}\text{P}$ . The rats were anesthetized, then the blood was collected, and brain, lung, heart, liver, kidney, spleen, pancreas, thymus and bone were removed. The urine, feces and organs were put into vials, and reduced to wet ash with nitric acid and hydrogen peroxide.

The ashed solutions were added to 1 ml Soluene-350 and 10 ml toluene scintillator (0.65%, 0.04% POPOP), then the  $^{45}\text{Ca}$  radioactivity was counted using a liquid-scintillation counter.  $^{32}\text{P}$  was counted without toluene scintillator after the addition of 5 ml distilled water.

## RESULTS AND DISCUSSION

The effects of Tb on  $^{45}\text{Ca}$  and  $^{32}\text{P}$  tissue distribution and excretion into urine and

feces were investigated.

The cumulative excretion into the urine and feces of  $^{45}\text{Ca}$  and  $^{32}\text{P}$  is shown in Figure 1. In control rats, the ratios of  $^{45}\text{Ca}$  and  $^{32}\text{P}$  in the urine for 10 days were 0.9 and 13.3%, respectively. On the other hand, those in Tb-pretreated rats were 0.2 and 1.8%, respectively. The cumulative excretion into the feces of  $^{45}\text{Ca}$  and  $^{32}\text{P}$  are shown in Figure 2. The  $^{45}\text{Ca}$  and  $^{32}\text{P}$  fecal ratios in rats treated with Tb for 4 weeks increased remarkably compared with control rats (15.1→47.4% and 10.9→64.8%, respectively).

The residues of  $^{45}\text{Ca}$  in control and Tb-pretreated rats 10 days after the administration of  $^{45}\text{Ca}$ , were 84.0 and 52.4%, respectively, those of  $^{32}\text{P}$  were 75.8 and 33.4%, respectively (Figure 1, 2). The regression curves of  $^{45}\text{Ca}$  and  $^{32}\text{P}$  remaining in the rats are summarized in Table 1 and 2. The slope at 1-4 days in Tb-pretreated rats has a high value compared with that of control rats. However, the slopes at 4-10 days in control and Tb-pretreated rats were similar. These results indicate that the biological half-lives of  $^{45}\text{Ca}$  and  $^{32}\text{P}$  after 4th day in control and Tb-pretreated rats are similar.

Table 1. Regression line of dosed  $^{45}\text{Ca}$  residues in terbium-pretreated rats

Group	1-4 d	4-10 d
Control	$\ln y = -0.0110x + 4.493$ ( $r = 0.9748$ )	$\ln y = -0.0033x + 4.464$ ( $r = 0.9960$ )
Terbium	$\ln y = -0.0672x + 4.215$ ( $r = 0.8551$ )	$\ln y = -0.0039x + 3.996$ ( $r = 0.9926$ )

The correlation coefficient ( $r$ ) was calculated using linear regression analysis. X-axis: days after administration of  $^{45}\text{Ca}$ , Y-axis: ratio of  $^{45}\text{Ca}$  in the body (% of dose).

Table 2. Regression line of dosed  $^{32}\text{P}$  residues in terbium-pretreated rats

Group	1-4 d	4-10 d
Control	$\ln y = -0.0203x + 4.472$ ( $r = 0.9887$ )	$\ln y = -0.0112x + 4.437$ ( $r = 0.9955$ )
Terbium	$\ln y = -0.1125x + 3.969$ ( $r = 0.8817$ )	$\ln y = -0.0098x + 3.607$ ( $r = 0.9974$ )

The correlation coefficient ( $r$ ) was calculated using linear regression analysis. X-axis: days after administration of  $^{32}\text{P}$ , Y-axis: ratio of  $^{32}\text{P}$  in the body (% of dose).

Nakamura et al. (1991b) reported that high doses of dysprosium, europium, ytterbium and yttrium cause temporary suppression of body weight. This phenomenon may be a result of damage to the digestive organs. Also, it has been reported that the lung and stomach are damaged by lanthanum at a dose of 200 or 1000 mg/kg/day for 28 days (Ogawa 1992). Our previous investigation has shown that excretion patterns into the urine and feces and the distribution of  $^{45}\text{Ca}$  and  $^{32}\text{P}$  in the body of rats given lanthanum, are similar to those of control rats when the administration is stopped (Hanioka et al. 1993). The dose in this study was 100 mg/

rat/day, which may have damaged the stomach or intestine. Therefore it seems likely that absorption of  $^{45}\text{Ca}$  and  $^{32}\text{P}$  is temporarily inhibited.

The distribution in liver, pancreas, spleen, kidney, lung, heart, thymus, brain, bone and blood of  $^{45}\text{Ca}$  and  $^{32}\text{P}$  was examined. In Tb-pretreated rats, the levels of  $^{45}\text{Ca}$  and  $^{32}\text{P}$  in organs except for  $^{45}\text{Ca}$  in the pancreas decreased by 1/2 to 1/7 compared with control rats (Table 3, 4). It seems that the difference in tissue burden of  $^{45}\text{Ca}$  and  $^{32}\text{P}$  between the tissues of the control and Tb-treated rat is due to the difference in the absorption of Ca and P.

Table 3. Distribution of  $^{45}\text{Ca}$  in organs and tissues of terbium-pretreated rats

	(dpm in tissue/total dpm) x 100	
	Control	Terbium
Liver	0.0288 $\pm$ 0.0104	0.0037 $\pm$ 0.0013 <sup>b)</sup>
Pancreas	0.0020 $\pm$ 0.0006	0.0031 $\pm$ 0.0018
Spleen	0.0013 $\pm$ 0.0004	0.0005 $\pm$ 0.0003 <sup>∧)</sup>
Kidney	0.0067 $\pm$ 0.0021	0.0027 $\pm$ 0.0016 <sup>∧)</sup>
Lung	0.0028 $\pm$ 0.0009	0.0012 $\pm$ 0.0003 <sup>a)</sup>
Heart	0.0021 $\pm$ 0.0006	0.0004 $\pm$ 0.0001 <sup>∧)</sup>
Thymus	0.0014 $\pm$ 0.0006	0.0009 $\pm$ 0.0007
Brain	0.0072 $\pm$ 0.0029	0.0036 $\pm$ 0.0011
Bone	83.96 $\pm$ 7.98	46.31 $\pm$ 6.34 <sup>b)</sup>
Blood	0.0051 $\pm$ 0.0020	0.0043 $\pm$ 0.0011

Each value is the mean  $\pm$  S. E. of 5 animals.

a): Significantly different from control (P<0.05).

b): Significantly different from control (P<0.01).

Table 4. Distribution of  $^{32}\text{P}$  in organs and tissues of terbium-pretreated rats

	(dpm in tissue/total dpm) x 100	
	Control	Terbium
Liver	0.964 $\pm$ 0.241	0.388 $\pm$ 0.066 <sup>b)</sup>
Pancreas	0.108 $\pm$ 0.030	0.048 $\pm$ 0.009 <sup>a)</sup>
Spleen	0.055 $\pm$ 0.009	0.024 $\pm$ 0.005 <sup>b)</sup>
Kidney	0.137 $\pm$ 0.022	0.061 $\pm$ 0.017 <sup>b)</sup>
Lung	0.063 $\pm$ 0.011	0.029 $\pm$ 0.006 <sup>b)</sup>
Heart	0.053 $\pm$ 0.009	0.024 $\pm$ 0.005 <sup>b)</sup>
Thymus	0.046 $\pm$ 0.017	0.017 $\pm$ 0.004 <sup>a)</sup>
Brain	0.101 $\pm$ 0.019	0.040 $\pm$ 0.011 <sup>b)</sup>
Bone	74.23 $\pm$ 5.69	32.76 $\pm$ 5.75 <sup>b)</sup>
Blood	0.066 $\pm$ 0.029	0.024 $\pm$ 0.004 <sup>a)</sup>

Each value is the mean  $\pm$  S. E. of 5 animals.

a): Significantly different from control (P<0.05).

b): Significantly different from control (P<0.01).

It has been reported that the concentration of rare earth elements and endogenous Ca in liver, spleen and lung of the rat increased about 5-fold by intravenous administration with rare earth elements (Laszlo 1952; Nakamura et al. 1993a; Nakamura et al. 1993b). Suzuki and coworkers reported that the levels of Ca and P in lung of the rat were remarkably increased when lanthanum chloride or yttrium chloride was instilled intratracheally (Hirano et al. 1990a; Suzuki et al. 1992). It has been suggested that the biological half-lives of lanthanum and yttrium in lung are extremely long compared with those of heavy metal compounds such as cadmium chloride (Amanuma and Suzuki 1987), cadmium oxide (Hirano et al. 1989a), cupric sulfate (Hirano et al. 1990b) and zinc oxide (Hirano et al. 1989b). These observations suggest that insoluble complexes of rare earth elements may be formed in the lung. The results of the present study indicate that phenomena caused by non-biological actions, such as inhibition of Ca absorption due to massive amounts of Tb or to complex formation with P and the Tb, may have occurred in the body.

Thus, oral long-term administration of Tb to rats is suggested to inhibit absorption of  $^{45}\text{Ca}$  and  $^{32}\text{P}$ . However, since the inhibited absorption was transient, it was not considered to have been derived from irreversible impairment of the mechanism of Ca and P absorption.

## REFERENCES

- Amanuma K and Suzuki KT (1987) Effect of intratracheal instillation of cadmium chloride on phospholipids in alveolar wash fluid. *Toxicology* 44: 321-328
- Durbin PW (1960) Metabolic characteristics within a chemical family. *Health Physics* 2: 225-238
- Ellis KJ (1977) The lanthanide elements in biochemistry, biology and medicine. *Inorg Perspect Biol Med* 1: 101-135
- Hanioka N, Jinno H, Sekita H, Toyo'oka T, Ando M, Kojima S, Takeda M (1994) Metabolism of calcium and phosphorus in rats after continuous oral administration of lanthanum. *Jpn J Toxicol Environ Health* 40: 26-33
- Hirano S, Tsukamoto N, Kobayashi E, Suzuki KT (1989a) Toxicity of cadmium oxide instilled into the rat lung. I. Metabolism of cadmium oxide in the lung and its effects on the essential elements. *Toxicology* 55: 15-24
- Hirano S, Higo S, Tsukamoto N, Kobayashi E, Suzuki KT (1989b) Pulmonary clearance and toxicity of zinc oxide instilled into the rat lung. *Arch Toxicol* 63: 336-342
- Hirano S, Kodama N, Shibata K, Suzuki KT (1990a) Distribution, localization, and pulmonary effects of yttrium chloride following intratracheal instillation into the rat. *Toxicol Appl Pharmacol* 104: 301-311
- Hirano S, Sakai S, Ebihara H, Kodama N, Suzuki KT (1990b) Metabolism and pulmonary toxicity of intratracheally instilled cupric sulfate in rats. *Toxicology* 64: 223-233
- Laszlo D, Ekstein DM, Lewin R, Stern KG (1952) Biological studies on stable and radioactive rare earth compounds. I. On the distribution of lanthanum in the mammalian organism. *J Natl Cancer Inst* 13: 559-573
- Magnusson G (1963) The behavior of certain lanthanons in rats. *Acta Pharmacol Toxicol* 20 (Suppl. 3): 1-95
- Nakamura Y, Hasegawa Y, Tonogai Y, Kanamoto M, Tsuboi N, Murakami K, Ito Y (1991a) Studies on the biological effects of rare earth elements. I. Method for analysis of dysprosium (Dy), europium (Eu), ytterbium (Yb) and yttrium (Y) from the biological materials. *Eisei Kagaku* 37: 28-38

- Nakamura Y, Tsumura-Hasegawa Y, Tonogai Y, Kanamoto M, Tsuboi N, Murakami K, Ito Y (1991b) Excretion of dysprosium, europium, ytterbium and yttrium in the rat after oral administration. *Eisei Kagaku* 37: 418-425
- Nakamura Y, Tsumura-Hasegawa Y, Tonogai Y, Kanamoto M, Tsuboi N, Murakami K, Ito Y (1991c) Studies on the biological effects of rare earth elements. II. Distribution and histological effects of Dy, Eu, Yb and Y in the rat after intravenous administration. *Eisei Kagaku* 37: 489-496
- Nakamura Y, Tsumura-Hasegawa Y, Tonogai Y, Kanamoto M, Tsuboi N, Murakami K, Ikebe K, Ito Y (1991d) Studies on the biological effects of rare earth elements. III. Fate of chlorides of Dy, Eu, Yb and Y in the rat after intravenous administration. *Eisei Kagaku* 37: 497-506
- Nakamura Y, Tsumura Y, Tonogai Y, Ito Y (1993a) Studies on the biological effects of rare earth elements. IV. Effects of chlorides of Dy, Eu, Yb and Y on rare earth elements, Ca, Mg and P contents in various organs in the rat after intravenous administration. *Jpn J Toxicol Environ Health* 39: 44-55
- Nakamura Y, Tsumura Y, Tonogai Y, Ito Y (1993b) Studies on the biological effects of rare earth elements. V. Relationship between the concentration of rare earth elements and 9 minerals in various organs in the rat after intravenous administration of Dy, Eu, Yb by low or high dose. *Jpn J Toxicol Environ Health* 39: 121-131
- Ogawa Y (1992) Studies on the biological effects of lanthanum. Effects of repeated oral administration tests in rat. *Jpn J Toxicol Environ Health* 38: 545-533
- Schubert J, Finkel MP, White MR, Hirsch GM (1950) Plutonium and yttrium content of the blood, liver, and skeleton of the rat at different times after intravenous administration. *J Biol Chem* 182: 635-642
- Schweitzer GK, Jackson M (1952) Radiocolloids. *J Chem Educ* 29: 513-522
- Suzuki KT, Kobayashi E, Ito Y, Ozawa H, Suzuki E (1992) Localization and health effects of lanthanum chloride instilled intratracheally into rats. *Toxicology* 76: 141-152
- Weiss GB (1974) Cellular pharmacology of lanthanum. *Ann Rev Pharmacol* 14: 343-354