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# Excretion of Intracorporeal Cadmium with S-Benzoylthiamin Monophosphate

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Examination was made of the excretion of intracorporeal methylmercury into body hair(Yamamoto, 1990) by the administration of thiamin tetrahydrofurfuryl disulfide(TTFD) which caused significant increase in mercury content in human mustache. The thiamin derivative, S-benzoylthiamin monophosphate(BTMP)(Yamamoto, 1988) failed to have such effect. The mechanism of mercury excretion is thus based on the side chain structure of TTFD, mercaptan.

At the start of the 20th century in Japan, many multiparae were found to have itai-itai disease, the main symptom of which is general pain. The cause of this disease was considered cadmium deposition on the bone from sourced such as soil and river water which contaminated rice and cereals.

The previous system for mercury(Yamamoto, 1990) was applied based on cadmium content variation in human mustache. Although TTFD worked well in the mercury excretion system, the usual dosage of TTFD administered orally did not cause significant increase in cadmium in the mustache. BTMP appeared to exert effect by generating thiol-type thiamin.

Experiments *in vitro* should be conducted to demonstrate the chelating effects of thiol-type thiamin. It is quite important to find evidence for cadmium excretion from human body.

## MATERIALS AND METHODS

As vitamin preparations, thiamin hydrochloride(Merck), BTMP(Sankyo, Japan) were used. For atomic absorption spectrophotometry by a Nihon Jarrell-Ash AA 8200, cadmium and mercury standard solutions(Wako Pure Chemi-

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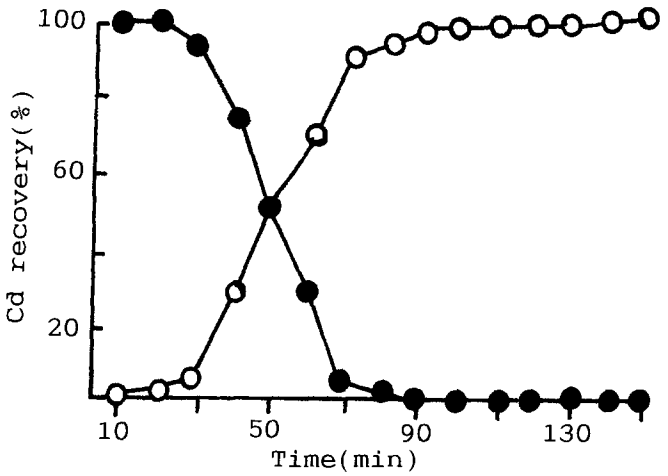


Figure 1. Cd extraction with dithizone from the Cd-$B_1$ mixture at pH 9.5

●—● Cd extracted into the dithizone soln

○—○ Cd in the water layer

cals, 1,000 μg/L each), were used for analysis of cadmium and mercury. "Reagents for harmful metal analysis"(Wako Pure Chemicals) such as hydrochloric acid, sulfuric acid, ammonia water, ammonium citrate dibasic, tin(II) chloride, and "Solvents for pesticide analysis"(Wako Pure Chemicals) such as benzene and chloroform were also used for the purpose.

Chloroform and benzene were washed with sulfuric acid and then with redistilled water to eliminate components such as mercaptan and thiophene. Dithizone chloroform and benzene solution(1,000 mg/L, each) were purified with ammonium citrate(metal-free), followed by dilution to 100 mg/L.

The procedure for determining the chelating effect of thiol-type thiamin is as follows: Two gram of thiamin hydrochloride were dissolved in a small amount of water, to which 200 μg cadmium and 20 mL of 40 % ammonium citrate solution were added. pH of the solution was adjust to 6.0, 7.0 and 9.5 with hydrochloric acid solution and ammonia water, followed by dilution to 200 mL with ammonia water. Ten milliliters of the solution were pipeted out every 10 min for 150 min with mixing, and extracted with 10 mL dithizone benzene solution(30 mg/L) with 5 min shaking. The dithizone extract was separated by centrifugation at 2,000 rpm for 5 min. Cadmium in the benzene extract(4 mL) was placed in 4 mL of nitric acid solution(1 %) by centrifugation after shaking. Cadmium

Table 1. Cd content in mice organs in ppb (Mean + S.D., n = 10)

| | Control group | $B_1$ dose group | BTMP dose |
|---|---|---|---|
| Liver | 4.06 + 0.40 | 4.03 + 0.30 | 3.03 + 0.39 *** |
| Kidney | 15.6 + 1.46 | 16.2 + 1.89 | 12.6 + 1.56 *** |

*** Significant at 0.1 %.

content in the water layer was determined by flame atomic absorption spectrophotometry(Flame AAS).

Thirty ICR mice of 4 week old were divided into three groups: BTMP dose group, $B_1$ dose group and the control. They were provided basic feed(Crea Japan) for 30 days. The feed included Cd 0.014 ppm, Cu 2.7 ppm, Mn 1.9 ppm and Zn 13.5 ppm. BTMP water(50 mg/L) was given to the BTMP group, $B_1$ water to the $B_1$ group and hydrochloric acid water(pH 2.0) to the control group. At the end of the feeding period, some internal organs were removed and decomposed by sulfuric and nitric acid. Cadmium content in an organ was determined after dithizone extraction followed by extraction with diluted nitric acid.

BTMP treatment was conducted for 30 days. Cd source was rice(Cd 0.21 μg/g). To six adult men with body weight ranging from 53 - 86 Kg, 41 to 61 years, BTMP at 100 mg was administered everyday for the second 10 days of the course. TTFD 100 mg everyday 40 days administered following termination of BTMP treatment. At every one or two days throughout the study period, mustache was sampled, and cadmium content in the sample(10 mg) was ashed with 1 mL of nitric acid(20 %) with heating at 250 °C on a sand bath. Cadmium content in the ash was determined by flameless AAS.

**RESULTS AND DISCUSSION**

Cadmium in the thiamin solution(pH 9.5) was extracted with dithizone, in the amount shown in Figure 1. Within 30 min preincubation, cadmium was completely extracted by dithizone, and from 40 min incubation, cadmium extraction was much less since thiamin had been converted to thiol- type $B_1$ in alkaline solution. After 90 min incubation, 99.5 % of cadmium remained in the water layer. Cadmium extraction with dithizone benzen from the thiamin-cadmium mixture(pH 5.0 and

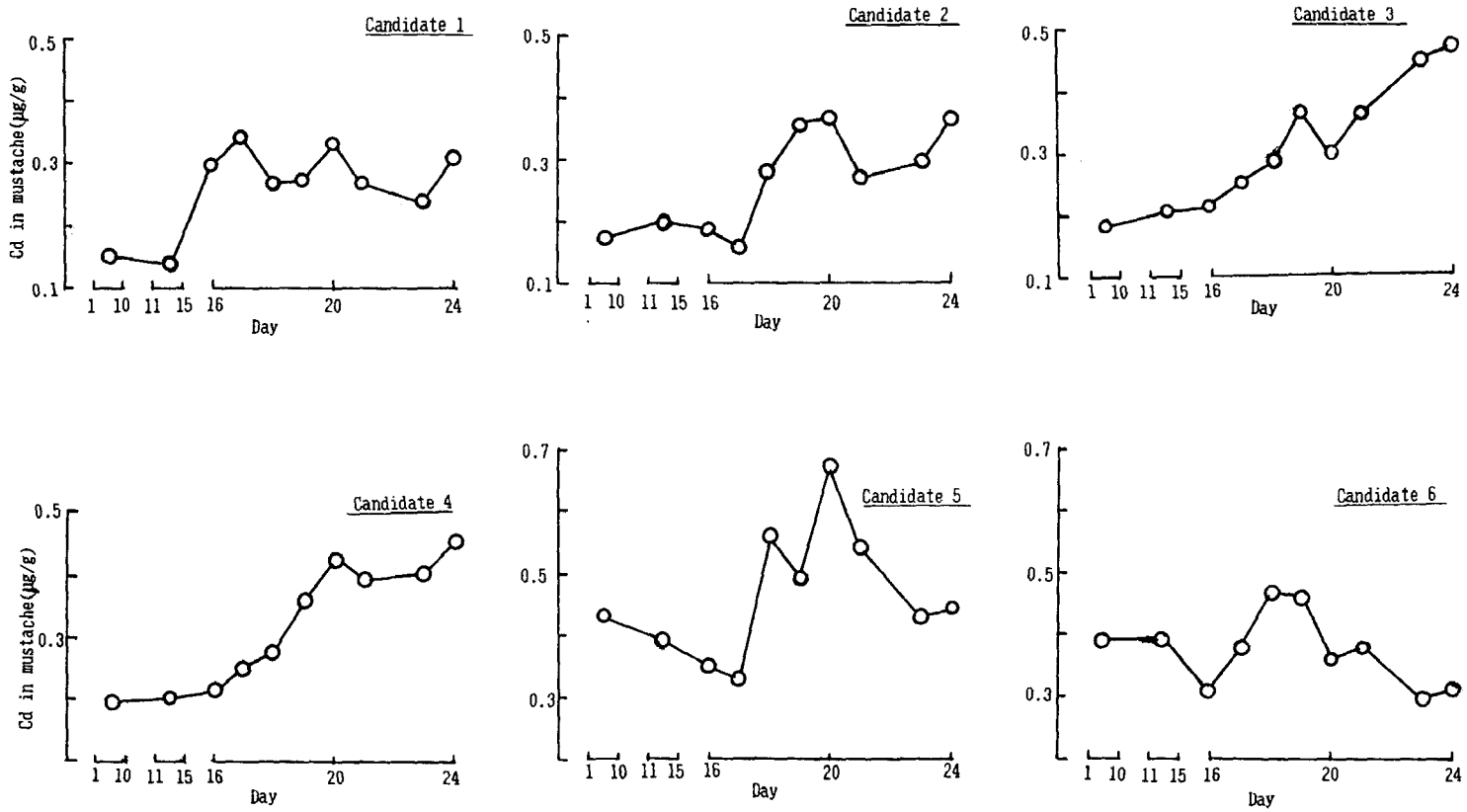


Figure 2. Cd excretion in mustache after BTMP administration. BTMP was given to 6 adults each day from the 10th to 19th day throughout the 40 days of the study period.

Table 2. Cd excretion in mustache

| | Days | | | | | |
|---|---|---|---|---|---|---|
| | 17th | 18th | 19th | 20th | 21th | 24th |
| Increase in Cd(μg/g) in mustache | | *** | ** | * | * | * |
| | 0.03 | 0.101 | 0.131 | 0.150 | 0.115 | 0.100 |

Note 1. Experimental conditions were the same as in Figure 2.
2. Increase in Cd(μg/g) in mustache means average Cd increment in the 6 adults.
Increment(μg/g) of each = (Cd in mustache) - (Average Cd in mustache during first 10 days)
3. Symbols "*", "**" and "***" indicate significance at risk of rate of 5, 1 and 0.1 %, respectively.

7.0) was completed within a short incubation period. The present results clearly indicate that cadmium and thiol-type $B_1$ form a chelate. That is, the binding of thiol to cadmium atom is much stronger than that to atoms of dithizone.

As shown in Table 1, cadmium content in the liver and kidney of the control and $B_1$ dose group was not significant. Cadmium content in the BTMP dose and control groups in internal organs differed greatly from one another. Even in blood from portal vain and liver, S-Benzoylthiamin (SBT), the metabolite of BTMP, has been found(Nakajima, 1968). Thiol-type $B_1$, just formed from SBT in the organs, by esterase appeared to bind to cadmium atoms.

Variation in cadmium content in mustache of the 6 adults within 30 days BTMP treatment is shown in Fig.2 with time. After 5 to 7 days, cadmium in the mustache increased for 10 days, but no increase occurred TTFD administration. Average increase in Cd in mustache(μg/g) during the 30 day period is shown in Table 2. Mean Cd content in mustache during the first 10 days used as the control value. From the 18th to 21th day, Cd in mustache significantly increased, the risk rate being at 5 to 0.1 %. Thus possibly, some Cd may be excreted into mustache due to BTMP administration.

Mineral poisoning is usually treated with chelating reagents such as BAL and d-penicillamin with fairly

good results. Delay in treatment is without effect. Even though these reagents have high chelating activity toward some heavy metals, the reagents should combine with the metals before the SH group of tissue protein does so with the metals. BTMP is a derivative of vitamin $B_1$ and not a powerful chelating reagents. BTMP administration immediately following a meal may possibly diminish cadmium incorporation from rice and cereals.

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