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Studies on the Influence of Calcium on Cadmium Absorbed by Rat Liver and the Resistivity Against the Toxicity of Cadmium in Rat

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Recent years, scientists from various countries have respectively issued many papers about the toxicological test of Cadmium(Cd) on animals. Some authors reported that Cd can inhibit the uptake of Calcium (Ca) in the tissues. To determine the effects of Cd on the intestinal absorption of Ca, everted gut sacs from rats were incubated in bathing media containing 4×10^{-5} M Ca and Cd in concentration of 10^{-4} to 10^{-2} M, the Ca absorbed in the tissue and in the serous fluid decreases with the increase of Cd concentration (Chertok et al. 1981). In an attempt to approach the effect of Cd on the intestinal brush border, some authors suggested that Cd inhibits the uptake of Ca by inhibiting Ca-stimulated ATPase in the intestinal brush border (Sugawara et al.1975). On the other hand, there were some reports on the effect of Ca on the uptake of Cd, a low dietary uptake of Ca can result an increasing accumulation of Cd in the kidney (Lorentzon et al. 1977) and can increase the susceptibility to the deleterious effects of environmental Cd (Nevenka 1977). However, up to now, we haven't seen any report on what role Ca plays in the uptake of Cd after supplementary Ca is added to the diet. One of the purposes of this work was to compare the amount of Cd in the livers of rats which were fed by rations cotaining various amounts of supplementary Ca (Ca-Cd test in brief). In addition, it is interesting to note what was observed by Yuhas et al. (1978), they applied an in situ single-pass intestinal perfusion technique to an anaesthetic rat to determine the intestinal absorption in Ca. This observation shows that at 1 or 10 ppm of Cd, there were no effect on net Ca absorption. However, at 100 ppm of Cd solution, there was an increase in Ca absorption. We continue their work, using drinking water containing 100 ppm Cd to feed the rats for seventeen days (Cd treatment in brief) and then in vitro determined the absorption capability of Cd in the duodenum, at the same time, checked the result against the control (Cd-Cd test in brief).

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MATERIALS AND METHODS

The animals used were Wistar rats from an inbred colony obtained from the Liaoning Province Epidemic Prevention Station.

All chemicals were of analytical grade and were obtained commercially (made in Peking Chemical Plant).

The rats of Ca-Cd test groups were fed with general maize flour, and the rats of Cd-Cd test groups were fed the basal diet which was composed of (in g/100 g of the basal diet) maize flour, 50; Chinese sorghum, 10; barley, 10; bean cake, 20; fish meal, 5; bone dust, 3.5; table salt, 0.5 and cod-liver oil, 1. These foods were prepared by mixing powdered food and distilled water equal to the powder weight.

On Ca-Cd test, the experiments were performed on six groups of 340 to 350 g male rats. The groups were divided according to the level of Ca supplementation in the diet (Table 1), the control group was not supplied with Ca. After being on the diet for 3 days, each rat, in the experimental and control groups, was administered 6 ml of water containing 500 ppm Cd ($CdCl_2$) by stomach tube. The rats were killed on the 8th day, livers were obtained and individually analyzed for Cd by atomic absorption spectrometry after chemical pretreatment (George, 1972).

On Cd-Cd test, thirteen female Wistar rats, 330-340 g body weight, were used in this experiment and divided into two groups randomly. In experimental group, the animals were administered drinking water containing 100 ppm Cd and basal diet as being mentioned before ad libitum for 17 days. In the control, the animals did not received drinking water containing Cd, but received tap water and the same food as the experiment group.

An everted intestinal sac method adapted the procedure of Wilsom and Wisemen (1954) was used in the experiment. The rats were fasted for 24 hr on 18th day after the beginning of the drinking water containing Cd administration. The animals were anesthetized with ether and about 12-cm segment of the intestine immedieatly distal to the pyloric sphincter was freed from mesenteric atachments, removed and everted on a stainless steel rod (300 mm long, 1.5 mm diameter). The everted intestine was then slipped off the steel rod, a length of everted intestine (5 cm long) was tied off at one end by a thread ligature and a second ligature was placed loosely around the other end (ready for tying). A blunt needle, attached to a 1 ml syringe (tuberculin type), was introduced into the intestinal lumen and the loose ligature being pulled tight over the needle. About 0.5 - 0.8 ml of isotonic saline (physiological saline) was then injected into the sac and as the needle was withdrawn, the ligature was tied tight. then we took two sacs on each rat as duplicate determinations of a specimen. These distended sacs were placed in Warburg flask with 5 ml of isotonic saline containing 100 ppm Cd and incubated in a water bath at

Table 1. The influence of calcium dose on cadmium absorption in the liver.

Animal group	Ca(mg)/day/rat	No. of Animals	Cd content of the liver X_g ppm* Mean ± S.E.M.	Inhibition ratio
Control	0	5	3.73 ± 0.44	0
1	2.5	6	3.25 ± 0.36	12.9
2	5.0	6	3.60 ± 0.42	3.5
3	10.0	6	2.02 ± 0.38	45.8
4	20.0	6	2.34 ± 0.34	37.2
5	40.0	6	2.29 ± 0.74	38.6

*The cadmium content of the liver is represented by the geometry mean of the specimen data in each group and calculated according to the following formula:

$$X_g = \lg^{-1}\left[\frac{1}{n}\sum_{i=1}^{n}\lg X_i\right]$$

37° C. The center well of the Warburg flask contained 0.2 ml 6 N NaOH and a filter paper for CO_2 absorption. Before and periodically during the incubation, the saline containing Cd was gassed with 95% O_2 and 5% CO_2. The flasks were shaken at 90 oscillations/min(total excursion 4 cm). Thirty-min after the beginning of incubation, the sacs were removed from the bathing solution, blotted on absorbent tissue, severed and each content drained into a 25 ml volumetric flask and was diluted with a 1:99 nitric acid solution, brought to a final volume and filtered. Then the atomic absorption of Cd was read in a WYX model 401 spectrophotometer.

RESULTS AND DISCUSSION

The effects of Ca on the Cd absorption in vivo is shown in Table 1 and Figure 1. Cd absorption decreased with increasing levels of the Ca supplement among some experiment groups, although there is not exact linear relationship, it is possible that Ca could inhibit the Cd absorption.

The determined data (Table 1) can be expressed by the Lagrange formula (Hildebrand 1974) and a linear equation as follows:

Original formula:

$$y = \frac{(x-x_1)(x-x_2)\cdots(x-x_k)}{(x_0-x_1)(x_0-x_2)\cdots(x_0-x_k)} f(x_0) + \frac{(x-x_0)(x-x_2)\cdots(x-x_k)}{(x_1-x_0)(x_1-x_2)\cdots(x_1-x_k)} f(x_1)$$

$$+\cdots\frac{(x-x_0)(x-x_1)\cdots(x-x_{k-1})}{(x_k-x_0)(x_k-x_1)\cdots(x_k-x_{k-1})} f(x_k)$$

Where x is the arbitrary amount of Ca supplement, which is expressed at horizontal ordinate in Figure 1, x_0, x_1, $x_2 \ldots x_k$ are the amount of Ca supplement at the various experiment-points, y is the value at longitudinal coordinate, which corresponds to an independent variable x at horizontal ordinate. As a result, we have the relation: (the mathematics formula of the Ca-Cd test)

$$y = \begin{cases} \{(x-0)(x-5)(x-10) \times 6.93 - (x-2.5)(x-5)(x-10) \times 2.98 \\ \quad - (x-0)(x-2.5)(x-10) \times 5.76 + (x-0)(x-2.5)(x-5) \\ \quad \times 5.39 \times 10^{-1}\} \times 10^{-2} \\ \qquad (\text{When } 0 \leqslant x \leqslant 10) \\ 1.90 + 0.012x \\ \qquad (\text{When } 10 \leqslant x \leqslant 40) \end{cases}$$

Where the given values in the parentheses are the amounts of Ca supplement at each experiment-point (mg/day/rat). The multipliers in formula are those which are obtained first on the substitution

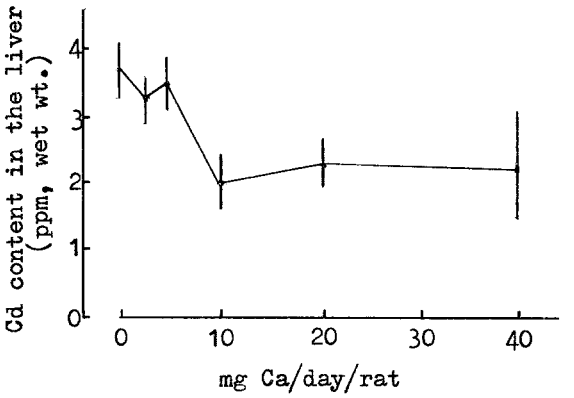

Figure 1. The dosage-content curve of the Ca supplement and Cd absorption on each rat.

of the known numbers into Lagrange fomula and then simplified.

In Cd-Cd test, the average amounts of Cd in the everted gut sacs of the control and experimental groups were 12.3 ± 0.78 and 3.04 ± 0.15 ug (mean ± S.E.M.) respectively. (the numbers of animals in the control and experimental groups were seven and six respectively).

The level of statistical significance of the difference between control mean and experimental group mean was determined by means of a t test ($p < 0.001$). These results show the average amount of Cd in everted gut sacs which were removed from the experimental group rats was less than that from the control group rats.

As for Ca-Cd test, the mechanism for the inhibition of Ca on Cd may be the same with that of Cd on Ca, Which has recently been recognized. This phenomenon is attributed to a competition one another between Cd and Ca, because of the function of the selective permeability of the biological membrane for various substances. One of us and others (Tang et al. 1984) also reported that on the biological membrane absorption, there were displacement or competition between certain cation metals, because this two metals (Ca; Cd) lie in the same group of the periodic table, their chemical properties; numbers of the outer electrons and other electric properties are similar.

As for Cd-Cd test, why is the average amount of Cd absorption in gut sacs which were removed from the experimental group rats (the treatment in vivo of rats with Cd) far less than those which were removed from the control group rats? The mechanism, as some authors have revealed, may be due to the capacity of absorpting Ca, that is to say, at the treatment in vivo of rats with 100 ppm of Cd, animals have higher ability absorpting Ca compared with the control group (Yuhas 1978). Thus Cd was inhibited by Ca displacement.

Recently, a study (Schenkel, person communication) had been reported that there were a negative correlation between the quantities of both Ca intake and in organs on pigs. This finding is in agreement with the results of our experiment on the rats. Claes Post et al. (1984) and Sharma (1983) had shown that Cd, having entered the organism, is binded rapidly by low-molecular-weight proteins i.e., Cd-binding proteins (CdBP) which play a role in the detoxification of Cd within the cell, it is the defence mechanism of the organism.

In present work (Cd-Cd test), we performed only one concentration group, but according to this physiological phenomenon as being mentioned above, we wander whether there is a local construction of the feedback in the intestine, or an organism with resistivity against the toxicity of Cd. If this function is the same as immunity, "irritability" and resistance to drugs etc., whether can we call all of these "Underminer-Resistance property" action? This problem needs to be verified further by the toxicologists, physiologists and the hygienists.

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