Cadmium Binding Components in the Supernatant Fraction of the Small Intestinal Mucosa of Rats Administered Cadmium

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INTRODUCTION

Renal damage due to an accumulation of the metal is an important factor in chronic cadmium poisoning. Absorbed cadmium from the intestine contributes to the accumulation of cadmium in the kidney of normal individuals who are free from other occupational exposures to the metal. Thus, information about intestinal cadmium absorption is important for our understanding of chronic cadmium poisoning.

Few studies have investigated the absorption of cadmium. It is necessary to investigate the rate, mechanism, sites of absorption, and dietary factors that influence the absorption of cadmium. The retention rates of orally administered cadmium have been reported1-4). And, intestinal cadmium absorption is known to be influenced by various factors5,6).

In previous reports we confirmed that the absorption rates of newly administered 109Cd decreased in mice which were orally administered cadmium for set periods. This phenomenon is a convenient and suitable function in the bodies of mice.

Recent investigations have shown that a low molecular weight Cd-binding protein, isolated from the kidney and the liver cytosol7-11) is induced by a cadmium injection. This protein is a metallothionein which characteristically is composed of at least 25% cysteine. A similar protein has been identified in rat intestinal cytosol12) However, the biological role of this protein is not understood.

The data presented here identify the intestinal Cd-binding factor induced by the oral administration of cadmium as a metallothionein. This protein may function in the regulation of intestinal cadmium absorption.

METHOD

Experiment 1: Distribution pattern of cadmium in the small intestinal mucosa. Male rats of the Wister strain (average weight, 488 gm) were used. The animals were given CdCl2 (100 μg Cd/ml) for 3 months, then CdO (200 μg Cd/ml) for 9 months, mixed with commercially available lab feed. They were killed by decapitation. The entire small intestine was immediately removed and placed on an ice-cold plate. It was then slit open, rinsed thoroughly with 100 ml of ice-cold 0.9% NaCl, blotted with the filter paper, and weighed. Mucosal cells were harvested by scraping the intestine with a glass slide. These cells were homogenized in 4 volumes of ice-cold 0.25 M Sucrose with a Potter-Elvejhem homogenizer with Teflon pestle. The homogenate was centrifuged at 105,000 × g for 1 hr at 4°C, and the supernatant fluid (cytosol) was recovered. The principle portions of the samples with the exception of the supernatant fractions, were wet ashed by heating them with 2 ml of sulfuric acid and 10-15 ml of nitric acid. The metal in each sample was analysed with an atomic absorption spectrophotometer.

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Experiment 2: Cadmium binding substance in the cytosol of mucosal cells of the small intestine of rats. Male rats of the Wister strain were used. The animals were given CdCl₂ (100 μg Cd/ml) in their drinking water ad libitum continuously for 1, 3, 6, 9, 32, and 96 days. Two animals per group per period were decapitated. The entire small intestine and the whole liver were removed. Intestinal mucosal tissue was processed as described above. The resulting supernatant fraction was applied to a 2.6×95 cm column of Sephadex G-75 equilibrated with 0.05M Tris-HCl buffer (pH 8.6) at 4°C. The flow rate in the G-75 separation was 15 ml per hour. To avoid bacterial growth, 100 mg of sodium-azide per litre was added to the buffer. Fractions were collected in a refrigerated fraction collector, and monitored for the absorbances at 250 and 280 nm and for their Cd²⁺ contents with an atomic absorption spectrophotometer.

RESULTS

Experiment 1: The weight of and cadmium contents in the small intestine are shown in Table 1, as are the distributions among the fractions. The average weight of mucosal cells and others were 1.265 and 1.814 gm, respectively. The mean values and their standard deviations are given in μg of Cd per gm of each fraction. The average amounts of cadmium in the cytosol and others were 8.16±1.71 μg, and 4.04±1.65 μg, respectively.

Experiment 2: The Sephadex G-75 elution profile for the intestinal mucosal cell cytosol is shown in Figures 1-6. The cytosol produced a single cadmium peak. When blue dextran (molecular weight, 1.5 million) was eluted from the same G-75 column, it showed up in the forty-sixth tube (3 ml/tube) as a blue peak. The void volume of this column was calculated as 138 ml. Cadmium almost always appeared in a region which had a ratio of elution volume (Ve) to void volume (Vo) between 2.06 and 2.39, even in samples of the 1-day group, but the Cd-containing peak had low adsorbances at 250 and 280 nm (Fig. 1). Subsequently, we observed that the Cd-containing peak increased slowly as the periods of oral administration

<table>
<thead>
<tr>
<th>Rat</th>
<th>Intestine</th>
<th>weight</th>
<th>Cadmium</th>
<th>Mucosa</th>
<th>Cadmium</th>
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<tr>
<td></td>
<td></td>
<td>(g)</td>
<td>μg</td>
<td>μg/g</td>
<td>(μg)</td>
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<tr>
<td>No. 1</td>
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<td>4.90</td>
<td>3.37</td>
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<td>8.82</td>
<td>11.00</td>
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<td>Mean±S.D.</td>
<td>Mucosa</td>
<td>12.20±3.18</td>
<td>9.87±2.33</td>
<td>Sup.</td>
<td>8.16±1.71</td>
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<tr>
<td></td>
<td>Others</td>
<td>6.13±2.53</td>
<td>3.39±0.96</td>
<td>Sed.</td>
<td>4.04±1.65</td>
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</table>
of cadmium increased, but it had low absorbances at 250 and 280 nm (Figs. 2, 3, 4 and 5). In the sample of the 95-day group, the Cd-containing peak had a high absorbance at 250 nm and a low one at 280 nm (Fig. 6).

After calibrations of the column with horse heart cytochrome C, bovine pancreas ribonuclease, and bovine serum albumin, we estimated that the molecular weight of the Cd-containing material was approximately 8,700. When liver cytosol was applied to the same G-75 column, the Cd-containing material first appeared in a sample obtained from rats treated daily over a period of six days (Fig. 7).

Fig. 1 Gel filtration of the supernatant fraction of the intestinal mucosa homogenate of the 1-day group

Fig. 2 Gel filtration of the supernatant fraction of the intestinal mucosa homogenate of the 3-day group

Fig. 3 Gel filtration of the supernatant fraction of the intestinal mucosa homogenate of the 6-day group

Fig. 4 Gel filtration of the supernatant fraction of the intestinal mucosa of the 9-day group
Results indicate that the cadmium concentration in the intestinal mucosal cells of rats fed a diet containing cadmium for 12 months, was three times as great as in the lower layers. Of the total amount of cadmium in the mucosal tissue about two-thirds was associated with the supernatant fraction obtained by centrifugation at 105,000×g, and the remainder was retained in the sediment. The remainder probably consists of the nucleus, mitochondria, and cell membrane. In studies by Shaikh and Lucis\(^{10}\) (1972), somewhat higher amounts of \(^{109}\text{Cd}\) were found in the soluble fraction of livers obtained from rats 24 hours after a single subcutaneous dose of cadmium chloride \(\text{Cd 109}\). They found that 81.1% of the \(^{109}\text{Cd}\) was present in the soluble fraction. Considering that the present study was done with the small intestine, the difference in results is not great.

When the intestinal mucosal cell cytosol obtained from rats orally administered cadmium for set periods was chromatographed on Sephadex G-75, we found that, the cadmium was almost always bound to proteins whose molecular weights ranged from 5,400 to 9,800, even in samples of the 1-day group. In contrast, the Cd-containing peak in the liver cytosol first appeared in a sample obtained from rats treated daily over a period of six days. We infer that the cadmium in the cytosol of the small intestinal mucosal cell and that in the liver are in the same binding state. But, as there is a lag period between the induction of the Cd-binding protein in both tissues, the protein in the small intestinal mucosal cell may be induced in the intestinal mucosal cell, itself, by contact with cadmium.
However, the chemical forms of cadmium excreted into the lumen of the gastrointestinal tract by various routes of secretion may influence the form of cadmium in the small intestinal mucosal cells. Since cadmium is accumulated to a considerable degree in the liver, the contribution of the bile and pancreatic juice has been considered. Lucis et al.\textsuperscript{13} (1969) reported that the appearance of cadmium in the lumen of the small intestine might be a result of biliary, pancreatic and intestinal secretions. And, Caujolle\textsuperscript{14}\ et al. (1971) observed a relatively constant rate for the biliary excretion of cadmium after intraperitoneal administration. But, Cherian\textsuperscript{15} (1977) has reported that most of the cadmium in bile is associated with a compound having molecular weight below 2,000. When a single dose of 3 mg Cd/kg of body weight was injected intraperitoneally into rats, their liver cytosol bound to the same Cd-binding material identified above. Injections of cadmium stimulate the synthesis of metallothionein in the livers of animals\textsuperscript{9–11,15–18}. Therefore, our results indicate that the Cd-binding material isolated from the intestinal mucosal cells of rats can be classed as a metallothionein. Tanaka et al.\textsuperscript{12} (1973) reported that a cadmium peak appeared at the position of thionein in the gel filtration of intestinal mucosa of rats fed with water containing cadmium for 30 days. Furthermore, Richards and Cousins\textsuperscript{19} (1977) reported that the intestinal protein induced by parenteral zinc, is a metallothionein.

Results of gel filtration suggest that the metallothionein in the cytosol of the intestinal mucosa may play an important role in the absorption of cadmium, because no other forms of this metal existed in the cytosol of the intestinal mucosa. Suso and Edwards\textsuperscript{20} (1971) suggested that low molecular weight ligands in the small intestine are involved in zinc transport. Richard and Cousins\textsuperscript{21} (1975) proposed that the intestinal metallothionein regulates zinc absorption by acting as an intracellular sequestering agent. But, Valberg et al.\textsuperscript{22} (1977) found that Cd-thionein produces extensive necrosis in the absorptive cells of the small intestine of mice. The intracellular binding of cadmium to metallothionein is thought to provide protection against the toxic cadmium ion (Kimura et al.\textsuperscript{23} 1974; Webb and Versehoyle,\textsuperscript{24} 1976). However, it has not been established that metallothionein actually serves this function in the intestine.

**SUMMARY**

Cadmium binding protein was isolated from the supernatant fraction of the small intestine of rats continuously administered cadmium for 1, 3, 6, 9, 32, and 96 days. The following were the results:

(1) The cadmium concentration in the mucosal tissue scraped from the small intestine was three times that of the lower layers, and of the total amount of cadmium in the mucosal tissue about two-thirds was associated with the cytosol.

(2) When the cytosol of the intestinal mucosal tissue was chromatographed on a Sephadex G-75 column, cadmium was almost always bound to proteins whose molecular weights ranged from 5,400 to 9,800, even in samples of the 1-day group.

(3) Cadmium in the liver and in the intestinal mucosa were in the same binding state, but as there was a lag period between the induction of the Cd-binding proteins of both tissues, the protein of the small intestinal mucosal cell must have been induced at the intestinal mucosa, itself, by contact with cadmium.

(4) The cadmium binding protein of the small intestinal mucosal cell may play an important role in absorbing cadmium, because no other form of this metal was found in the mucosal cells of the small intestines.
REFERENCES


カドミウム投与ラットの小腸粘膜上皮細胞中の
カドミウム結合蛋白質

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飲水とともに Cd (100 ppm) を1, 3, 6, 9, 32, 96日間継続投与したラットの小腸粘膜を剥ぎとり、その上清
分画をゲルろ過し (G-75 Sephadex)、次の結果を得た。

1) Cd 経口投与により、小腸管壁中にとり込まれた Cd の大部分 (％) は、その粘膜にとり込まれたものであ
り、粘膜内ではその％が細胞質中に分布していた。

2) 剥ぎとった小腸粘膜上清分画を Sephadex G-75 のカラムに添加すると、Cd 投与1日群ですぐに、elusion
factor 2.2 (分子量約8,700) の位置に Cd peak がみられた。

3) 肝臓および小腸粘膜の上清分画中の Cd は同じ結合状態を示したが、両 Cd 結合蛋白質誘導産生に log
period の存在することより、小腸粘膜上皮細胞内においても Cd 結合蛋白質が誘導産生されることが示唆され
た。

4) 小腸粘膜上皮細胞中に存在する Cd の大部分は分子量約8,700の蛋白質と結合した状態で存在しており、
他の形態ではほとんど Cd が存在しないことより、この Cd 結合蛋白質が Cd の吸収に重要な役割をはたしてい
ることが示唆された。

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