The Transport Mechanism of Cadmium by the Small Intestine of Rats

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INTRODUCTION

Numerous factors influence cadmium absorption from the gastrointestinal tract1-3), but, investigations of cadmium absorption by the small intestine have produced conflicting results. For example, measurements of cadmium absorption showed that zinc enhanced, and mercury decreased cadmium absorption4). When chicks fed a vitamin D deficient diet get a supplement of vitamin D, cadmium absorption is enhanced5), but it also has been shown that in vitamin D deficient animals cadmium absorption is enhanced6). Very little is known about the mechanism of cadmium absorption from the intestine. Sahagian et al.7) (1967) suggested that the movement of cadmium and several other trace elements across the intestinal membrane is controlled merely by the diffusibility characteristics of the individual ions. To investigate the mechanism of cadmium absorption from the intestine, we studied the effect of various cadmium concentrations on the transport of radioactive cadmium and on the transport characteristics of radioactive cadmium in rats treated preliminarily with stable cadmium.

METHOD

Effect of various cadmium concentrations on the transport of radioactive cadmium. Male Sprague-Dawley rats (body weight, 190-230 g) were used. The animals were starved for 24 hours before being killed by decapitation, then the small intestine was immediately resected. The resected intestine was carefully cleaned of mesenterium rests and adipose tissue.

A segment of the everted intestine was processed by Wilson and Wiseman's method8). Sacs (10 cm long) were prepared from different regions of the small intestine, the total length of which was approximately 80 cm. Segments of the everted gut were incubated in 100 ml Elrenmyer flasks containing 40 ml of medium to which stable and radioactive cadmium was added. Incubations were at 37°C for 90 minutes in the presence of oxygen. The composition of the medium was 145 mM NaCl, 5 mM KCl, glucose 200 mg/dl, 2 mM MgCl₂, and 10 mM tris buffer, pH 7.0. The concentration of cadmium (as CdCl₂) was varied between 0.05 and 50 ppm to determine the concentration dependence of transport, all other conditions were constant. Cadmium-109 (New England Nuclear Corp) was added in the chloride form. The activity of the solution was 0.1 μCi/ml. The same solution (1.0 ml) without stable or radioactive cadmium was placed inside the tied segments. The fluid inside the everted sacs was termed serosal fluid, and that on the outside mucosal fluid.

At the end of the incubation period, the everted intestinal strips were washed in ice-cold 0.9% NaCl solution, then blotted with filter paper to remove any adhering solution before radioactivity readings were taken. The radioactive cadmium content in samples of the serosal and mucosal solutions and its retention in the intestinal wall were determined by

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a well-type scintillation counter (the gamma-ray scintillation counter).

Transport characteristics of radioactive cadmium in rats pretreated with stable cadmium. Male Sprague-Dawley rats (body weight, 217–385 g) were used. The animals were given drinking water that contained 0.5, 5 and 50 ppm cadmium in the form of cadmium chloride in distilled water for 3 and 6 months. Two rats were included in each group per period. The control animals (two rats per period) were given drinking water which contained no known cadmium. On the final day of these preliminary treatments the animals were decapitated and everted sacs were prepared by the procedure described above.

Samples were incubated in a 100 ml Erlenmeyer flasks containing 40 ml of medium to which was added $^{109}$CdCl$_2$ at the rate of 0.1 µCi/ml, at 37°C for 90 minutes in the presence of oxygen. When $^{109}$CdCl$_2$ was added, it was added only to the mucosal fluid.

The radioactive cadmium content in samples of the serosal and mucosal solutions and its retention in the intestinal wall were determined by the same scintillation counter.

**RESULTS**

Experiments were carried out with simple everted sacs to determine the ratio of transport of $^{109}$-Cd through the intestinal wall and the retention of $^{109}$-Cd by the wall for different concentrations of cadmium in incubation media.

In the first series of experiments, $^{109}$-Cd was initially present in the mucosal fluid only, thus, the amount transported to the serosal fluid was measured. The transfer of $^{109}$-Cd from the mucosal to the serosal fluid is shown in Table 1 as S/M ratios.

$^{109}$Cd transport was increased at all cadmium concentrations except in the control group. There was no statistical difference between the 0.05 and 0.5 ppm group. The 5 ppm group was significantly higher (p < 0.05) than the 0.05 ppm group, and the 50 ppm group was also significantly higher (p < 0.01) than the 5 ppm group. The higher the initial concentration of cadmium in the mucosal fluid, the greater the transfer of $^{109}$-Cd in relation of it. At an initial cadmium concentration of 50 µg/ml, the $^{109}$-Cd concentration in the serosal fluid exceeded that in the mucosal fluid.

The $^{109}$-Cd retention in the intestinal wall is given in Table 2. Results are presented as percentages of the initial mucosal fluid activity. Retention was significantly higher for the groups with added cadmium (except for the 50 ppm group) than for the control group. No difference in retention was observed in the 50 ppm group when compared with the control group.

In the second series of experiments, $^{109}$-Cd was initially present in the serosal fluid of the non-everted segments, transport into the mucosal fluid was measured.

<table>
<thead>
<tr>
<th>Cd concentration</th>
<th>No. of samples</th>
<th>S/M* ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm (control)</td>
<td>5</td>
<td>0.124 ± 0.043</td>
</tr>
<tr>
<td>0.05 ppm</td>
<td>4</td>
<td>0.210 ± 0.086</td>
</tr>
<tr>
<td>0.5 ppm</td>
<td>4</td>
<td>0.231 ± 0.085</td>
</tr>
<tr>
<td>5 ppm</td>
<td>4</td>
<td>0.565 ± 0.138</td>
</tr>
<tr>
<td>50 ppm</td>
<td>4</td>
<td>2.325 ± 0.228</td>
</tr>
</tbody>
</table>

* The serosal $^{109}$Cd content divided by the mucosal content

<table>
<thead>
<tr>
<th>Cd concentration</th>
<th>No. of samples</th>
<th>%#</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>5</td>
<td>2.24 ± 0.43</td>
</tr>
<tr>
<td>0.05 ppm</td>
<td>4</td>
<td>4.91 ± 0.98</td>
</tr>
<tr>
<td>0.5 ppm</td>
<td>4</td>
<td>3.65 ± 0.70</td>
</tr>
<tr>
<td>5 ppm</td>
<td>4</td>
<td>6.50 ± 1.57</td>
</tr>
<tr>
<td>50 ppm</td>
<td>4</td>
<td>2.60 ± 0.75</td>
</tr>
</tbody>
</table>

Retention of the $^{109}$Cd content in the intestinal wall × 100
Figure 1 compares the transport ratios of $^{109}$Cd from the mucosal to the serosal side, and from the serosal to the mucosal side in each experimental group. Transport from the mucosal to the serosal fluid was greater than transport in the opposite direction for all groups.

The effects of preliminary treatment with CdCl$_2$ on the movement of $^{109}$Cd from the intestinal wall into the serosal fluid, and on retention of $^{109}$Cd in the intestinal wall, are shown in Tables 3 and 4. In animals preliminarily treated for 3 months, the effects on transport of $^{109}$Cd into the serosal fluid were small, though there was a tendency for this to increase. The 50 ppm group was the only group showing a significant decrease in the retention of $^{109}$Cd in the intestinal wall.

In animals preliminarily treated for 6 months, the ratios of $^{109}$Cd passing into the serosal fluid were reduced only in the 50 ppm group. There were no statistical differences among the other groups and the control group. Retention was significantly lower for animals preliminarily treated with cadmium.

**DISCUSSION**

The results show (Table 1) that cadmium transport from the mucosal to the serosal side of the small intestine of the rat is substantially enhanced in the 0.05 ppm group when it is compared to the control. This is especially marked for the S/M ratio in the 50 ppm group, their cadmium transport being eighteen times that of the controls.

$^{109}$Cd transport was not proportional to the cadmium concentration on the mucosal side, nor to the concentration of cadmium taken up by the intestinal wall. At an initial cadmium concentration of 50 $\mu$g/ml, there was no significant effect on the retention of $^{109}$Cd in the intestinal wall, in spite of an extremely large increase in the amount of $^{109}$Cd transport into the serosal fluid. Characteristically cadmium could be transported across the intestinal wall against a gradient when the initial cadmium concentration on the mucosal side was raised to 50 $\mu$g/ml. This showed that passage of $^{109}$Cd across the intestinal wall could not be the result of uptake by the wall from the mucosal fluid, followed by simple diffusion into the

**Table 3** Influence of a preliminary 3-month Cd treatment on $^{109}$Cd transport through and retention within the intestinal wall

<table>
<thead>
<tr>
<th>Cd concentration</th>
<th>No. of samples</th>
<th>S/M* ± S.D.</th>
<th>%# ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>4</td>
<td>0.127±0.039</td>
<td>1.59±0.42</td>
</tr>
<tr>
<td>0.5 ppm</td>
<td>4</td>
<td>0.169±0.023</td>
<td>1.46±0.50</td>
</tr>
<tr>
<td>5 ppm</td>
<td>4</td>
<td>0.118±0.068</td>
<td>1.42±0.37</td>
</tr>
<tr>
<td>50 ppm</td>
<td>4</td>
<td>0.156±0.037</td>
<td>1.08±0.23</td>
</tr>
</tbody>
</table>

* Serosal $^{109}$Cd content divided by the mucosal content
# Retention of $^{109}$Cd content in the intestinal wall $\times$100 Initial mucosal solution activity

**Table 4** Influence of a preliminary 6-month Cd treatment on $^{109}$Cd transport and retention within the intestinal wall

<table>
<thead>
<tr>
<th>Cd concentration</th>
<th>No. of samples</th>
<th>S/M* ± S.D.</th>
<th>%# ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>4</td>
<td>0.186±0.092</td>
<td>0.54±0.22</td>
</tr>
<tr>
<td>0.5 ppm</td>
<td>4</td>
<td>0.155±0.022</td>
<td>0.27±0.05</td>
</tr>
<tr>
<td>5 ppm</td>
<td>4</td>
<td>0.187±0.084</td>
<td>0.26±0.07</td>
</tr>
<tr>
<td>50 ppm</td>
<td>4</td>
<td>0.093±0.016</td>
<td>0.25±0.07</td>
</tr>
</tbody>
</table>

* Serosal $^{109}$Cd content divided by the mucosal content
# Retention of the $^{109}$Cd content in the intestinal wall $\times$100 Initial mucosal solution activity
serosal fluid.

Moreover, the experiments with noneverted segments confirmed that the transport of cadmium out of the intestinal wall was mainly unidirectional, and was unlikely to be the result of simple diffusion.

Schacter et al.⁹) (1960) and Dowdle et al.¹⁰) (1960) reported that calcium and iron are absorbed from the small intestine by active mechanisms that require metabolic energy and that are capable of transport against the gradient. Though active transport in the sense of movement against a gradient was not demonstrable under our conditions, the results suggest that a special mechanism is concerned in the absorption of cadmium, because there was no linear relation between the initial concentration of cadmium and the rate of transport across the intestinal wall.

The intestinal wall of the rat appears to undergo changes in permeability caused by the cadmium concentration on the mucosal side. Mason et al.¹¹) (1977) showed that quail exposed to 1 ppm dietary cadmium for 2 days had enteropathy. Valberg et al.¹²) (1977) found that exposure of the proximal intestinal mucosa to 100 μM CdCl₂ for 1 hour produced broadening and shortening of the villi with pseudostratification of the epithelium. But, Loeser and Larke¹³) (1977) reported that rats exposed to 30 ppm dietary cadmium for 3 months showed no sign of alterations in the intestines based on their histopathological investigation.

Moore et al.¹⁴) (1973) found that an increase in the concentration of cadmium resulted in more cadmium being absorbed from the gastrointestinal tract, although the amount absorbed was not proportional to the increase in concentration.

The experiments on transport of ¹⁰⁹Cd across the intestinal wall of rats preliminarily treated with cadmium showed that the cadmium transport through and retention within the intestinal wall was reduced in comparison to that in the control rats. This suggests that the preliminary cadmium treatment induced a change in the permeability of the intestinal wall that facilitated the transmural passage of ¹⁰⁹Cd. In a previous study of cadmium absorption in mice preliminarily treated with cadmium, we found that the absorption rates of newly administered ¹⁰⁹Cd decreased in comparison to those of the control (unpublished data).

Several features of the present work suggest that the uptake of ¹⁰⁹Cd by the intestinal wall and the transport of ¹⁰⁹Cd from the wall into the serosal fluid involve separate processes, and that uptake by the intestinal wall is by a mechanism of limited capacity that can be saturated at relatively low concentrations.

### SUMMARY

The mechanism of cadmium absorption was studied in vitro using sacs of the small intestine of the rat. The relation between the concentration of cadmium in the mucosal fluid and the rate of transport of ¹⁰⁹Cd to the serosal fluid showed that the higher the concentration of cadmium, the greater was the transfer of ¹⁰⁹Cd. Wall uptake of ¹⁰⁹Cd was limited and could be saturated at relatively low concentrations.

It was suggested that a special mechanism is concerned in the absorption of cadmium, because there was no linear relation between the initial concentration of cadmium and the rate of transport across the intestinal wall.

### REFERENCES


シロネズミ腸管によるカドミウム輸送機構

田口徹也*・鈴木庄亮
東京大学医学部保健学科

1. シロネズミの小腸を用い、in vitro でカドミウムの吸収機構について検討した。
2. 粘膜側 Cd 濃度と膜膜側への放射性 Cd の輸送率との関連は、Cd 濃度が高ければ高い程、輸送率も高かった。腸管壁にとり込まれる放射性 Cd 量には限度があり、比較的低濃度の Cd で飽和に達した。
3. Cd 濃度と放射性 Cd 輸送率との間に直線的な相関の認められないことより、Cd の輸送は simple diffusion ではなく、何らかの機構でそこで吸収していることが示唆された。

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