Effect of Phenobarbital on the Absorption of Copper by Rat Small Intestine

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ABSTRACT. The effect of phenobarbital (20, 40 and 80 mg/kg/day intraperitoneally for 5 days) on the absorption of copper from the rat small intestine was studied using an in situ recirculation technique. In untreated animals 45.7% of the initial amount of copper was absorbed after a 90-min recirculation. The pretreatment with phenobarbital resulted in a significant increase in the absorption of copper and at the end of 90 min of recirculation 56.7%, 62.7% and 70.0% of the initial amount were absorbed in the animals treated with phenobarbital at the dosages of 20, 40 and 80 mg/kg, respectively. To determine the absorption of copper from different sites of the small intestine, the intestine was divided into three segments of duodenum, jejunum and ileum. The copper absorption varied markedly in different segments and was the largest in duodenum followed in order of jejunum and ileum. Phenobarbital treatment resulted in a marked increase in the copper content in duodenum and jejunum as well as liver and kidney. Together with previous informations, the present data suggest that the phenobarbital-mediated increase in the copper absorption is related to increased synthesis of a carrier protein which may be involved in copper transport.

The copper absorption takes place in stomach or upper intestine in most mammals, although the site of maximal absorption may vary slightly among species. In rat intestine maximal copper absorption is observed in duodenum [15]. Gitlin et al. [4] demonstrated that the intestinal absorption of copper in mice did not result exclusively from simple diffusion. Subsequently, Cramp ton et al. [1] observed two separate mechanisms for the copper absorption in the mucosa of isolated hamster intestine and described that copper was transported from the mucosa to the blood by an energy-dependent mechanism. Furthermore, Evans [3] demonstrated that mucosal proteins facilitated the small intestinal absorption of copper. Regarding copper that is bound to the intestinal mucosa and subsequently absorbed into plasma, several investigations [12, 16] indicate that the intestinal absorption of copper is in part active transport mediated by proteins. Recently, it has been reported that phenobarbital treatment results in a significant increase in the intestinal absorption of iron [14], sugar [5] and bile salt [9]. Since there is evidence suggesting that phenobarbital may induce synthesis of small intestinal microsomal proteins [10], the question was raised whether phenobarbital treatment would influence the intestinal absorption of copper by virtue of its effect on protein synthesis. Accordingly, the present study was conducted using rat intestine to determine the effect of phenobarbital treatment on the small intestinal absorption of copper in situ.

MATERIALS AND METHODS
Animals: Male Sprague-Dawley rats weighing 180 to 220 g were used for this
study and maintained on pellets containing approximately 0.74 mg of copper per 100 g of dry weight. The animals were pairmatched by weight into five groups. Three groups were given sodium phenobarbital dissolved in 0.9% saline by intraperitoneal injection at dosages of 20, 40 and 80 mg per kg of body weight per day for 5 days, respectively. Untreated group, which was used for in situ absorption experiments, was given 1 ml of 0.9% saline per kg of body weight per day by intraperitoneal injection. The control group was used for the determination of copper content in tissues. Absorption experiments were conducted at 18 to 22 hr after the last injection.

Absorption experiments: The animals were anesthetized with 20% urethane given intraperitoneally (1 g/kg), and the abdomen was opened by a mid-line incision. Then the bile duct was ligated at the distal end in order to prevent the bile from flowing into the intestinal tract, because there is a fact that the absorbed copper is rapidly excreted in the bile and a mixture of copper and bile salts reduces the intestinal absorptive ability for copper [6]. The entire length of the small intestine, from the proximal end of duodenum to the distal end of ileum was used for the absorption experiments. The length of the small intestine was approximately 75 cm. Glass cannulae having inside diameters of 2.5 mm and outside diameters of 3.5 mm were inserted through small slits at the proximal end of duodenum and the distal end of ileum, respectively. The stomach and cecum were closed by ligation, care being taken not to occlude major blood vessels. For cleaning the gut lumen, 0.9% saline was passed gently through it until the effluent became clear. The cannulae were then connected to a flask containing the test solution which had been kept at 38°C. The test solution (pH 7.2) was prepared by dissolving 25.0 mg of CuSO₄·5H₂O in 100 ml of 0.9% saline (63.5 μg Cu/ml). The copper concentration in the test solution was set according to our previous study (unpublished data). This copper solution was then continuously recirculated through the intestinal lumen for 90 min at the rate of 5 ml/min, using the circulation apparatus shown in Fig. 1. One milliliter of the sample solution was pipetted at 0, 10, 20, 30, 45, 60, 75 and 90 min after the recirculation was started, and the copper concentrations in the solutions were determined with an atomic absorption spectrophotometry. Immediately after the absorption experiments were finished,

Fig. 1. Apparatus used for recirculation experiments of rat small intestine.
brain, heart, lung, liver, spleen, kidney, testis, and pieces of duodenum, jejunum and ileum were excised and the copper contents in these tissues were determined.

To investigate the absorption of copper from different sites of the small intestine, the entire small intestine was divided into three segments of duodenum, jejunum and ileum and cannulated separately. Starting at the pylorus, the first 15 cm was regarded as duodenum, the next 30 cm as jejunum, and the final 30 cm as ileum. If the intestine was appreciably longer or shorter than 75 cm, the segments were divided in similar proportions. The absorption experiments were performed on each of three segments by the same means as described above.

**Determination of copper content in tissues:** The excised tissues were rinsed with 0.9% saline and stored at −20°C until copper analysis. The tissues were dry ashed by the method of Shiraishi et al. [11]. The ashed residue was dissolved in 0.5N HNO₃, and the copper content was measured by an atomic absorption spectrophotometry. In this procedure, quartz glassware was immersed for 24 hr in 2N HNO₃ and then washed with distilled water.

**Results**

**Effect of phenobarbital on the intestinal absorption of copper:** The effect of pretreatment with phenobarbital on the small intestinal absorption of copper is shown in Fig. 2. The absorption of copper increased rapidly during the first 30 or 45 min, and 39.0% of the initial amount of copper in the test solution was absorbed after a 45-min recirculation even in the untreated animals. After that, however, an increase in the absorption was very slight, and at the end of a 90-min recirculation only 45.7% of the initial amount was absorbed. On the other hand, the pretreatment with phenobarbital for 5 days resulted in a significant increase in the absorption of copper compared with the untreated animals. In the animals treated with phenobarbital at the dosages of 20 and 40 mg/kg, 56.7% and 62.7% of the initial amount were absorbed at the end of 90 min of the recirculation, respectively. Particularly in the animals treated with a 80-mg/kg dose of phenobarbital, the absorption increased at such a rapid rate that more than 70% of the initial amount was absorbed for the first 60 min.

**Absorption from different sites of the small intestine:** The absorption of copper from different sites of the small intestine was shown in Fig. 3. At the end of 90 min of the recirculation, the absorbed amount of copper varied in different sites and it was the largest in duodenum, followed in order of jejunum and ileum. In duode-
num the absorption was significantly accelerated by the pretreatment with phenobarbital and the absorbed amounts reached to 71.2 (p < 0.01), 87.5 (p < 0.005) and 99.8 μg per cm of intestine (p < 0.005) in the animals treated with phenobarbital at the dosages of 20, 40 and 80 mg/kg, respectively. The accelerated effect was also observed in jejunum but slighter than that in duodenum. On the other hand, there was no difference in the copper absorption from ileum between the treated and untreated animals.

Copper content in different sites of the small intestine: The copper content in different sites of the small intestine is shown in Fig. 4. In the treated rats, the copper contents in duodenum and jejunum were increased by about 1.5 times than that in the untreated rats. The effect of phenobarbital on the copper content was relatively small in ileum as compared with that in duodenum and jejunum.

Cooper content in other tissues: In control animals the copper content was relatively high in brain, heart, liver, spleen and kidney (Table 1). Recirculation of the copper solution caused significant increases in the copper contents in liver (p < 0.05) and kidney (p < 0.01). The hepatic and renal copper contents were significantly elevated by the pretreatment with phenobarbital.

DISCUSSION

It has been shown that the intestinal mucosa is important in regulating the absorption of copper [1, 4]. Several investigations indicate that in the intestinal lumen copper exists as either a copper-amino acid complex or ionic copper [7, 12, 16]. They also showed that the ionic copper combined with amino acids, and then the complexed copper was actively
Fig. 4. Effect of phenobarbital on the copper content in different sites of the rat small intestine. Values represent the mean±SD.

Table 1. Effect of phenobarbital on tissue copper content in rats

<table>
<thead>
<tr>
<th>Dose of phenobarbital</th>
<th>Brain (μg/g)</th>
<th>Heart (μg/g)</th>
<th>Lung (μg/g)</th>
<th>Liver (μg/g)</th>
<th>Spleen (μg/g)</th>
<th>Kidney (μg/g)</th>
<th>Testis (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlb</td>
<td>2.27±0.11</td>
<td>4.31±1.35</td>
<td>1.24±0.29</td>
<td>4.49±0.51</td>
<td>1.71±0.41</td>
<td>4.97±0.84</td>
<td>1.61±0.35</td>
</tr>
<tr>
<td>Untreated</td>
<td>2.18±0.16</td>
<td>3.89±1.34</td>
<td>1.28±0.15</td>
<td>6.70±0.50c</td>
<td>1.79±0.42</td>
<td>6.33±1.04d</td>
<td>1.73±0.41</td>
</tr>
<tr>
<td>20 mg/kg/day</td>
<td>2.25±0.43</td>
<td>4.42±0.50</td>
<td>1.23±0.49</td>
<td>8.31±1.28cgs</td>
<td>1.97±0.42</td>
<td>8.74±1.29cgs</td>
<td>1.70±0.51</td>
</tr>
<tr>
<td>40</td>
<td>2.04±0.33</td>
<td>4.78±0.49</td>
<td>1.33±0.52</td>
<td>9.13±0.58frh</td>
<td>1.88±0.88</td>
<td>8.77±1.08frh</td>
<td>1.77±0.11</td>
</tr>
<tr>
<td>80</td>
<td>2.49±0.17</td>
<td>4.71±0.40</td>
<td>1.28±0.12</td>
<td>9.25±0.57frh</td>
<td>1.88±1.36</td>
<td>9.24±0.92frh</td>
<td>1.68±0.24</td>
</tr>
</tbody>
</table>

a Animals injected intraperitoneally saline or phenobarbital (20, 40 and 80 mg/kg/day) for 5 days.
b Animals without the absorption experiments.
c Values expressed as mean±SD.
d Significantly different from controls (p<0.05).
e Significantly different from controls (p<0.01).
f Significantly different from controls (p<0.005).
g Significantly different from untreated rats (p<0.01).
h Significantly different from untreated rats (p<0.005).
transported across the intestinal mucosa, and the portion of copper that traversed the mucosal membrane as an uncomplexed ion combined with metallothionein or metallothionein-like proteins. These observations suggest the role of a mucosal carrier protein that involves the copper absorption. The present study showed that the copper absorption varied in different sites of the small intestine and that it was the most pronounced in duodenum followed in order of jejunum and ileum. Also, this result indicates that the site of maximal absorptive ability for copper is duodenum and that more carrier protein may be contained in duodenal mucosa than in jejunal or ileal mucosa.

The copper absorption in the untreated rats was greatly reduced after 60-min recirculation and did not increase any more even though the recirculation was continued. This result suggests that the intestinal absorptive capacity for copper has its limit, and the copper absorption by simple diffusion may occupy only the minor part in the intestinal absorption of copper in these rats. Similar finding was shown in the animals treated with a 80-mg/kg dose of phenobarbital, and the absorption rate of copper was about 1.7 times that in the untreated rats. In these animals the copper absorption reached the maximum after a 60-min recirculation. On the other hand, the copper absorption in the animals treated with phenobarbital at the dosages of 20 and 40 mg/kg increased even after a 90-min recirculation. The increased rate of the intestinal absorption of copper may be the consequence of the increase in the intestinal absorptive capacity for copper induced by phenobarbital. However, the stimulatory effect of phenobarbital on the increase in the absorptive capacity for copper has its limit.

The question arises what is the mechanism by which phenobarbital causes increased intestinal absorptive capacity for copper. The data obtained in the present study cannot provide a definite answer to this question. Several investigations have demonstrated that phenobarbital treatment may stimulate protein synthesis and induce an increase in the activity of several enzymes in the small intestine. Thomas et al. [13] reported that phenobarbital treatment for 5 days resulted in a proliferation of intestinal smooth endoplasmic reticulum and an increase in the activity of intestinal microsomal n-demethylase activity. Phenobarbital treatment has also been shown to increase human glycolytic and pentose pathway enzymes in jejunum [10], and to increase cholesterol synthesis in rat intestine [8]. Furthermore, Thomas et al. [14] demonstrated that phenobarbital treatment of rats increased duodenal absorption of both heme and non-heme iron and microsomal heme-splitting activity. The findings that the duodenal and jejunal absorptive capacity for copper can be increased by phenobarbital suggests that copper transport is mediated by a copper binding protein. Thus, the stimulatory effect of phenobarbital may be due to increased synthesis of a carrier protein for mucosal copper transport in the small intestine.

Isolation and identification of a copper-binding protein in rat duodenum demonstrate that copper is associated with two distinct proteins in the cytosol [2]. One fraction of copper is contained within a protein that has the physical properties of the enzyme superoxide dismutase, and the second fraction is bound to a sulfhydryl-rich protein which is very similar to metallothionein. In the present study, phenobarbital treatment resulted in a marked increase in the copper
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concentration in duodenum and jejunum as well as in liver and kidney, suggesting that phenobarbital may have some effect on the synthesis of these copper-binding proteins in the cytosol. Whether the phenobarbital-mediated enhancement of copper absorption is related to induction of a specific microsomal protein will have to await a definite identification of copper mucosal protein complexes.

References

7. Mason, K. E. (1979). A conspectus of research on copper metabolism and require-


要 約

銅の腸管吸収におよぼすフェノバルビタールの影響: 浅野隆司・保間成男（日本大学農芸学部農芸栄養学教室）——In situ のラット小腸循環系を用いて、銅吸収におよぼすフェノバルビタール（20, 40, 80 mg/kg/day を実験前5日間連続腹腔内投与）の影響を検討した。フェノバルビタール無処置群の銅吸収率は摂流流開始90分後に45.7%に達した。処置群の銅吸収率は無処置群に比較し有意に大きく、90分後には20 mg/kg群、40 mg/kg群、80 mg/kg群でそれぞれ56.7％、62.7％、70.0%の吸収率を示した。十二指腸、空腸、回腸、それぞれの部位における銅吸収量には著明な差異が認められ、十二指腸からの吸収量が最も多く、ついで空腸・回腸の順であった。また、フェノバルビタール前処置によって肝臓・腎臓・十二指腸・空腸の銅濃度に著明な増加が認められた。以上の結果から、フェノバルビタールによる銅吸収の増加は、輸送担体としての蛋白の合成促進によるものと推察された。