Experimental Localization of Intestinal Uptake Sites for Metals (Cd, Hg, Zn, Se) In Vivo in Mice

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The intestinal uptake process consists of two separable steps: transport over the lumen membrane into epithelial cytoplasm and transport over the basolateral membrane into serosal fluid. A compound’s residence time in mucosal epithelial cytoplasm depends on rates of the two transport processes and, if the rate of the second step is low, on the rate of mucosal sloughing. Using γ-emitting metal isotopes, in vivo labeling profiles of the intestinal tract were obtained from mice eating their normal diet. The results pertain to processes in the functioning, undisturbed intestinal tract. Single-dose chase experiments indicated that intestinal uptake processes were in fact studied. The labeling profiles varied considerably for different metals. Thus, Cd++ was absorbed mainly in duodenum and early jejunum, while Zn++ was taken up in jejunum and ileum. The uptake profile of Hg++ indicated most rapid uptake in proximal jejunum. Selenomethionine labeled the entire intestinal tract, most rapidly the duodenum, the following intestinal segments were labeled with falling rate. This experimental method is rapid and simple. Further studies aim at developing a quantitative model suited for studying interactions between essential and toxic metals at the level of intestinal metabolism. — Environ Health Perspect 102(Suppl 3):199–206 (1994).

Key words: localization, intestinal uptake, metals, mice, cadmium, mercury, selenium, zinc

Introduction

Intestinal uptake is the absorption route for essential metals and is important for toxic metals as well. Quantitative measurements of fractional intestinal uptake have been performed mainly by using γ-emitting isotopes and whole-body counting. Numerous interactions between dietary components and metal uptake have been demonstrated using this technique.

A deeper understanding of mechanisms of intestinal metal uptake and the nature of these interactions may be gained from studies using isolated intestinal segments in vitro or in situ. These studies rely, however, on the representativeness of the selected intestinal segment for the overall uptake process in the entire intestinal tract. Also, they are performed during conditions in the intestinal lumen (washed sacs perfused with buffer) that are far from those present during the natural intestinal function (digestion and absorption of food components), and most often, the metal concentrations and species used differ widely from those relevant for human exposure. These issues were recently discussed in detail for cadmium (1) and mercury (Hg) (2).

This article describes the first results from a series of investigations aimed at facilitating comparison of whole-body studies and studies using isolated intestinal segments, by employing an intestinal labeling model in intact mice. The model employs mice eating their normal diet ad libitum, and metal uptake is studied in the intestinal tract with its normal content during its normal, unperturbed digestion. Recently published (2,3) as well as previously unpublished results are reviewed below. The presentation describes the experimental model employed to study the uptake of different metal compounds and will discuss the validity of the results obtained. It is the hope of the authors that this very simple and unlabored technique may be useful in combination with classical fractional uptake estimates by whole-body counting and intestinal perfusion studies for studying mechanisms operating in intestinal metal uptake processes.

The overall intestinal uptake process for toxic metals has been described by Foulkes (4) as consisting of a step 1, transport from lumen over the luminal epithelium into cytoplasm of intestinal mucosa; and a step 2, transport over the basolateral membrane into serosal fluid. However, either of these steps may involve more than one molecular event and may operate by different mechanisms. For toxic metals, facilitated mechanisms are unlikely to exist; however, carriers are known to be involved in uptake of essential elements such as zinc.

Available evidence recently summarized by Foulkes (5) indicates that step 1 for toxic metals may be divided into 2 steps, 1A a nonspecific step leading to binding to the outside of the cell membrane; and 1B is a step leading to internalization of the metal ion depending on the activity of mobile carriers. The presence of nonspecific binding sites has been described for cadmium (6); and in relation to inorganic these binding sites would probably also have to compete with other binding possibilities due to the higher affinity of inorganic mercury than of cadmium for thiol-containing ligands and proteins present in the feed.

The mechanism behind the release of the metal ions from luminal binding sites and whether these binding sites are distributed evenly along the intestinal tract is presently unknown, because most studies published so far are in vitro or in situ studies using only parts of the jejunum.

Step 2 has been found to be considerably slower than the initial binding and internalization for Hg (7). Foulkes (4) found that internalization of Hg bound to the active sites at the cell membranes must be rather fast, in agreement with previous...
work (6, 8). Based on studies using jejunal segments, Foulkes (9) proposed a rapid absorption of cadmium from the intestinal lumen into mucosal cells and a 100-fold slower transfer of cadmium to the systemic circulation.

The hypothesis behind the experimental model employed in this article is that the amount of mucosal labeling by a γ-emitting metal compound is, during some specifically defined experimental conditions, a quantitative indicator of the rate of the intestinal metal uptake process. The magnitude of intestinal mucosal labeling will depend both on the rate of step 1 of the intestinal uptake process and on the ratio between step 1 and step 2. Therefore, this hypothesis is not generally applicable for quantitative comparison of the intestinal uptake rates, for different metals, for different species of the same metal, or for the same metal species during different physiological conditions. As an example, the results obtained using this model would be compromised if the ratios of intestinal rates of step 1 and 2 varied extensively between different parts of the intestinal tract. Such variation could be caused by effects of physiological processes on step 1, i.e., acidity of intestinal content, intestinal motility, biliary and pancreatic secretion, and composition of digesta.

Using washed and perfused rat jejunal sacs, Foulkes (10) demonstrated different uptake rates for Cd at different jejunal sites, the highest uptake rate occurring in central jejunum. Results presented below indicate, however, selective uptake of cadmium in duodenum and a decreasing uptake rate through jejunum in the undisturbed mouse intestine filled with its normal content during its normal function.

In perfused intestinal sacs, the rate of step 2 for Hg** and Cd** has been estimated to be considerably lower than the rate of step 1 (7, 9). The ratio between steps 1 and 2 for cadmium may be altered by induction of metallothionein (11). For lipophilic metal compounds such as CH3Hg**, the rates of steps 1 and 2 are not expected to differ by orders of magnitude. Also for essential metals taken up by facilitated processes, the two rates would be expected not to differ widely.

The results obtained from jejunal perfusion studies pertain to hydrated species of the metal compounds employed for the experiment. However, it is largely unknown which species are of importance during intestinal uptake of mercury, cadmium, and other toxic metals from the digested intestinal contents.

**Experimental Procedures**

Mice eating rodent pellets or a well-defined semisynthetic diet ad libitum were exposed orally to a salt of an essential or toxic metal labeled with a γ-emitting isotope, either by administration of a single dose in a small volume by stomach tube or by addition of the γ-labeled metal salt to the drinking water. Inbred CBA/Bom or outbred Bom_NMRI mice were used as indicated in the experiments. Since NMRI mice grow to become extensively larger than CBA mice, the size of the intestinal tract varies somewhat between different experiments. Whole-body counting was used to estimate the whole-body retention (WBR) of the dose given to each animal. After sacrifice by cervical dislocation, blood, organ, and intestinal metal deposition and retention were quantitated by γ-counting.

The detection limit was calculated for each isotope as the mean background value plus 3 standard deviations based on 40 background countings.

The labeling profiles of the entire intestinal tract at various times after single-dose exposure (chase experiment) or after continuous exposure through drinking water was determined after careful washing. The whole intestinal tract was washed with 0.9% NaCl containing 1 mg/L of the chelating agent CaNa2DTPA at pH 7 to remove intestinal contents including nonabsorbed metal. Washing continued until feces were no longer observed in the eluate. Metal ions loosely bound to the luminal side of the intestinal epithelial cell membrane were expected to be removed by chelation. After washing, the entire intestinal tract was cut into 2-cm fractions and γ-counted. After counting, the mucosa was removed by scraping each intestinal fraction carefully with a scalpel blade and the fractions were recounted. The amount of mucosal label was then calculated by subtracting remaining radioactivity after scraping from the total radioactivity present. The labeling profiles may be displayed either as fractional deposition, i.e., percent of total intestinal radioactivity or, by using...
a standard of known intensity, as pmole metal per fraction.

Results are presented as medians and statistically compared using the nonparametric Mann-Whitney U-test.

Results

Mercury

Figure 1 shows the result of a chase experiment, in which a single oral dose of 5 μmole/kg body weight of 203Hg-labeled mercuric chloride was given to groups of female Bom:NMRI mice, killed at the times indicated. The time course for uptake into the intestinal epithelium and passage through the gastrointestinal tract is illustrated in relative terms as percent of total gastrointestinal mercury present in each intestinal segment (Figure 1A) and in absolute terms as amount of mercury in each segment (Figure 1B).

The small intestine is divided into duodenum (segments 1 to 5) with excretion from the gall bladder and pancreas (segment 3 or 4), jejunum (segments 6 to 15), and ileum (segments 16 to 22). Mercury deposition was not observed in the first two segments below pylorus (4 cm). Distally from segment 3, the mercury deposition and retention increased at all postdosage time intervals examined. Maximum labeling occurred between the fifth and the ninth segment (between 10 and 18 cm below pylorus; Figure 1). The maximum mercury deposition occurred in the proximal jejunal segments at 3 hr after dosing. However, 24 and 48 hr postdosage, measurable relative maxima in retention were observed here as well, even though mercury was absent in the remaining small intestine (Figure 1A).

A second epithelial deposition maximum was observed at 36 cm and 38 cm below pylorus at 0.75 and 1.5 hr postdosage and in cecum (the distinct peak at segment 23) from 1.5 hr postdosage. After 6 hr, mercury started to appear in the large intestine. At this time after dosage, the WBR of the dose as determined by whole-body counting started to decrease, indicating initiation of fecal elimination of nonabsorbed mercury. After this time the WBR decreased rapidly (Table 1). Maximum blood concentrations of mercury occurred at 3 and 6 hr, and mercury in liver and kidneys reached maximum levels at 6 and 12 hr postdosage, respectively (Table 1). The relative deposition of mercury in kidneys increased throughout the experimental period. At 48 hr, the liver and gastrointestinal tissue still contained relatively large amounts of mercury.

In another experiment, the effect of 300 mg tetraethyl thiuram disulfide (disulfiram, antabuse [TTD]) per kg feed on mercury absorption was studied in mice given 3 μmole 203Hg-labeled mercuric chloride per liter of drinking water for 3 days before killing. A control group was treated identically, except that TTD in feed was omitted. The Hg labeling profiles (Figure 2) from both groups displayed maxima in the proximal jejunal 14 to 16 cm below pylorus as well as in the cecum. Accordingly, TTD did not change the deposition pattern, but increased the intestinal mucosal deposition of mercury. As both the WBR and hepatic Hg deposition was increased by TTD, an enhanced intestinal uptake of mercury is indicated. TTD affected neither the blood level nor the kidney deposition. Recounting of intestinal segments after scraping demonstrated that mercury was not deposited at levels beyond the detection level in muscles and connective tissue of the intestinal tract during the 3 days exposure period, neither in controls nor in TTD-treated mice (Figure 2). Accordingly, in this and shorter lasting experiments, counting of intestinal segments gives a valid estimate of the deposition of mercury in the intestinal mucosa. Scraping and subtraction to determine epithelial mercury appear to be unnecessary.

Cadmium

In an initial experiment, 10 male CBA mice received 0.04 μmole CdCl2 labeled with 109Cd per liter of drinking water for 3 days. After sacrifice, the median intestinal labeling profile of the group was constructed. Removal of the intestinal mucosa by scraping the luminal side and recounting demonstrated that less than 5% of the total label remained. Accordingly, scraping was considered unnecessary and was omitted in later experiments.

Table 1. Time course for whole-body and organ deposition of mercury in mice given a single oral dose of 5 μmole HgCl2/kg body weight by stomach tube.

| Hours postdosage | 0.75 | 1.5 | 3 | 6 | 12 | 24 | 48 |
|------------------|------|-----|---|---|----|----|----|
| WBR              | 167  | 156 | 156 | 111 | 45.7 | 22.1 | 12.2 |
| Washed Gl        | 24.3 | 21.6 | 27.6 | 19.4 | 11.3 | 3.35 | 2.44 |
| Liver            | 0.33 | 1.15 | 2.58 | 4.09 | 3.36 | 1.51 | 2.37 |
| Kidney           | 0.26 | 1.12 | 2.66 | 4.52 | 4.94 | 2.49 | 3.86 |
| Kidneys/liver    | 0.79 | 0.97 | 1.03 | 1.11 | 1.47 | 1.65 | 1.54 |
| Blood            | 0.112 | 0.215 | 0.301 | 0.360 | 0.273 | 0.131 | 0.129 |

*Results are given as medians (n = 5) and expressed as nmole Hg in each organ or nmole Hg/g blood at the time of sacrifice. Results are given as medians (n = 5) and are expressed as nmole Hg in each organ or nmole Hg/g blood at the time of sacrifice.
In this initial experiment and in an experimental repeat, similar labeling profiles were observed (Figure 3); and in both cases, the four proximal duodenal segments of the intestine contained as an average about 90% of the total activity deposited in the intestinal mucosa.

In a third experiment, groups of mice receiving a single oral dose of 2 μmole 109Cd-labeled CdCl₂ per kg body weight by stomach tube were killed at various intervals postdosage. The data are expressed as nmole Cd/segment (Figure 4).

The intestinal labeling profiles in these figures indicate preferential labeling of the duodenum (segments 1 to 4) at all postdosage intervals studied, with maximum labeling of segments proximal to the excetration from the gall bladder and pancreas (segments 2 to 4). The “shoulder” seen in Figure 4 indicates some deposition of Cd in the jejunum (segments 5 to 12), whereas stomach, ileum (segments 13 to 17), and colon were not labeled. Apparently, more Cd is deposited in the cecum than in ileum, but this can be explained by the difference in size, as cecum has a much larger surface area than ileum. The time-dependent changes in labeling profiles strongly suggest that intestinal uptake kinetics are displayed in the figures.

The time-course for cadmium in blood could not be studied, as all values were below the detection limit. The WBR indicates that fecal elimination of nonabsorbed cadmium started between 4 and 9 hr postdosage, and after 72 hr only 1% of the dose was retained in the body. The washed gastrointestinal tract retained considerable amounts of cadmium during the entire experimental period. The deposition of cadmium in the liver and kidney increased throughout the experimental period. The ratio of the amount of Cd in kidneys to that in liver gradually increased from 0.06 at 1 hr to 0.33 at 72 hr postdosage (Table 2).

Addition of 300 mg TTD per kg of feed for days along with 0.04 μmole CdCl₂ per liter of drinking water changed the intestinal-labeling profile; thus the preferential duodenal labeling was much less pronounced, and the rest of the intestinal tract was much more intensively labeled (Figure 5). The intestinal labeling profile in the control group, treated identically except for omission of TTD in the feed, was almost identical to the profile observed in the preceding 3 days’ labeling experiments (Figure 3). TTD increased the deposition of Cd in the intestine by a factor of 4.72 compared to the control group. The WBR was increased by a factor of 2.1.

Selenium

In the experiments studying intestinal uptake of 75Se-labeled seleno-L-methionine (SEM) described below, scraping and recounting of intestinal segments were performed. In later experiments, to be reported elsewhere, scraping was omitted, as the amount of label remaining after scraping in experiments with 3 days exposure to SEM in the drinking water was only 2 to 3% of total intestinal label. This label could represent incorporation of SEM into intestinal basal membrane and muscle proteins.

Figure 6 shows the intestinal-labeling pattern at different time intervals after administration of a single oral dose of 4 μg SEM to NMRI mice. The chase kinetics indicate that the preferential labeling of duodenum and jejunum is due to an uptake process, not merely incorporation of SEM into intestinal tissue protein.

Figure 7 shows the intestinal-labeling pattern after exposure of mice to different concentrations of SEM in drinking water for 3 days. This experiment demonstrates that within the dose range used in the present experiment, the labeling profile indicating SEM uptake is independent of dose size.

The labeling profiles are very similar in the two experiments, indicating preferential labeling of duodenum and early jejunum. The amount of mucosal labeling is reduced with distance from pylorus.

As the preferential labeling of the duodenum and jejunum is independent of dose size, the pattern at low dose is not determined by availability of SEM, but rather indicates the site at which the uptake rate is largest.

Zinc

In initial experiments with 3 days’ exposure to 65Zn-labeled ZnCl₂ in drinking water, removal of the intestinal mucosa by scraping the luminal side and recounting indicated very limited deposition of Zn label into intestinal connective and muscle tissue. Accordingly, scraping was considered unnecessary and omitted in later experiments.

Figure 8 shows the chase kinetics after a single oral dose of 30 μmole/kg b.w. ZnCl₂ given to NMRI mice by stomach tube. The labeling profiles are rather complex and indicate uptake into intestinal mucosal epithelial cytoplasm in the entire intestinal tract, with maxima at several positions. The labeling kinetics are very similar to those observed after single dose administration of
HgCl₂. The extensive peak in ileum segments 15 to 16 at 1 hr postdosage probably signifies the position of the peristaltic wave at this time postdosage. These experimental data on intestinal Zn uptake are preliminary. The labeling of cecum already at 1 hr postdosage indicates a need for independent experimental repeat. The labeling profiles seem to indicate, that the proximal (duodenaljejunal) part of the small intestine is important to Zn absorption, because the intensive labeling at early intervals after dosage is followed by rapid disappearance of the label.

Discussion
The present study describes a new experimental in vivo model for investigation of the localization and molecular mechanisms of gastrointestinal absorption of metals. The model employs the intact gastrointestinal tract with its natural content during the undisturbed digestion process. The results obtained with two toxic and two essential elements indicate that very different intestinal labeling patterns are obtained with different metals; for each metal the pattern is in agreement with previously published studies of the uptake site. Together with the chase kinetics, this supports, that the results obtained indicate intestinal metal uptake processes, not merely unspecific binding of metal to mucosal tissue. The model is still not fully validated, since the results obtained need confirmation by independent methods.

Mercury
Previous studies aimed at identifying the intestinal uptake site for inorganic mercury employed perfused rat intestinal segments in vitro or in situ. By this technique Endo et al. (12) found that duodenum was the major absorption site for mercuric oxide (HgO) and mercutry chloride (HgCl₂).

The jejunal distribution pattern for mercury observed at 0.75 hr postdosage could be due to preferential binding of mercury to the luminal side of the epithelium in complexes resistant to chelation by DTPA in the flushing fluid. At this time after dosage, relatively low levels of mercury are found in liver and kidneys, and the blood level of mercury has not yet reached a maximum. The mercury peak in the central part of the ileum represents the position of the front of the peristaltic wave at this time after dosage, as no mercury was found in cecum or large intestine. Since the decrease in mercury label in ileum from 0.75 hr to 1.5 hr postdosage was not followed by increases in organ and blood levels of mercury, ileal mercury may not be absorbed. The second intestinal-labeling peak had moved further down the small intestine and into the cecum at 1.5 hr postdosage and indicates the position of the peristaltic wave at this time after dosage. Cecum was also the site of the maxima after 3 hr and 6 hr, suggesting a very fast initial movement of feces from stomach to cecum followed by a reduced rate through cecum and large intestine. Accordingly, the WBR of mercury did not decline until 6 hr postdosage. At this time, the blood level of mercury had already peaked.

The mercury deposition in the proximal part of jejunum with a peak at segment 7 and maximum labeling at 3 hr postdosage probably represents internalized mercury. The preferential labeling of the proximal part of jejunum, posterior to the outlets of the bile and pancreatic ducts agrees with results of previous investigations, which demonstrated enhanced absorption of mercury at increasing pH (7,13,14). Thus, the present study using the undisturbed intestinal system filled with digesta supports the studies using ligated perfused intestinal segments in vitro or in situ, indicating proximal jejunum as the major site for uptake of inorganic mercury; however, the absorption seems to occur in
a larger part of jejunum than previously reported.

The intestinal-labeling profile after 3 days exposure to HgCl₂ in the drinking water followed by 3 hr without mercuric chloride before sacrifice was very similar to the labeling profiles observed at 3 hr after oral administration of mercuric chloride, despite the very large difference in total amounts of mercury deposited in mice from the two experiments. Also, the relative deposition was very similar.

In the present study, mercury was not observed in the muscular layer around the intestinal wall. However, Endo et al. (7), using perfused intestinal segments in situ observed mercury in the serosa or muscle cells. This discrepancy could be due to the different experimental methods used.

The enhanced intestinal labeling with 203Hg observed in mice given TTD in the feed, indicates enhanced intestinal absorption of mercury. The labeling profile and site of preferential intestinal mercury deposition was, however, not changed, except that the peak of label in proximal jejunum was slightly broadened. Accordingly, the WBR and the hepatic level of mercury were significantly increased in mice given TTD in the feed. The amounts of mercury label in blood and kidneys were similar in the two groups, indicating that diethylthiocarbamate (DDC) formed by cleavage of TTD would form a lipophilic complex with mercury, which is rapidly cleared from the blood into compartments not accessible for immediate renal excretion.

Formation of lipophilic complexes may profoundly affect the intestinal uptake kinetics for metals. Thus, TTD, which is metabolized to two molecules of DDC, increased the fractional intestinal absorption of Cd due to the chelating effect, and TTD enhanced the acute toxicity of oral CdCl₂ (15). Incubation of rat hepatocytes in a medium containing either HgAc, Hg(DDC)₂ or CH₃HgCl demonstrated increased uptake into hepatocytes of Hg(DDC)₂ compared to the other mercury compounds (16).

Kostial et al. (17) found that the site of gastrointestinal deposition of mercury was unaffected by treatment with the chelator 2,3-dimercapto-propane sulfonate, although the amount of mercury retained was significantly reduced.

**Cadmium**

The fractional intestinal Cd uptake is low, about 1% (18) in mice given single oral doses of the same size as used in this investigation, where 1% uptake was found. Orally administered cadmium is initially deposited in the proximal part of the intestinal tract (19–24).

 Autoradiography of the rat intestine shows uniform labeling throughout the mucosa and submucosa (24). Also in the present study, cadmium was selectively and almost exclusively deposited in duodenum, with a maximum in the first segment. The labeling profile in the 3-day drinking water exposure experiment was very similar, strongly supporting the preferential duodenal uptake of cadmium. The mechanism is likely to be related to the existence of different species of cadmium at the low pH in the gastric outlet and posterior to the site of secretion of alkaline pancreatic juice into the intestinal space.

 Autoradiographic studies of Cd movement in the small intestine of rats demonstrate that within 20 min after oral administration of ¹⁰⁹Cd, silver grains reach a maximum level in the brush border and epithelium. However, the amount of silver grains over the lamina propria mucosa reached a maximum at 1 hr. Internalized Cd is distributed over the entire area of the absorptive cells. This could indicate a nonspecific transport mechanism (25). In a previous study (26), the maximum blood levels of cadmium were reached already 30 min after oral administration. About 75% of ¹⁰⁹Cd label was found in plasma rather than in blood cells.

The duodenal preference for intestinal uptake of ionic cadmium demonstrated in this investigation may be explained by the low pH of the gastric content emptying into the duodenum. Distal to the pancreatic duct, pH increases, and cadmium will rapidly be chelated by various dietary components and thus be less available for intestinal uptake. This could also explain the changed organ distribution in mice receiving TTD in the feed. The formation of a lipophilic cadmium (bis)dithiocarbamate complex facilitates the intestinal uptake, as previously demonstrated (27, 28). Due to chelation by DDC, Cd is less accessible for chelation by food components and thereby able to be absorbed to a higher degree. In the present study, the WBR was increased by a factor of 2.1 and the intestinal deposition by a factor of 4.7 by TTD exposure. This is in accordance with results from a long-term study in which the fractional whole-body retention of Cd was increased by TTD (15).

**Selenium**

The most important natural dietary Se species is SEM, which is absorbed more efficiently in the intestinal tract than selenite (29,30). In experimental studies, the intestinal uptake of SEM has been found to be more than 90%; thus, Griffiths et al. (30) estimated about 96% uptake in rats. According to present theory, SEM may be taken up by the active transport system for neutral amino acids, due to its structural similarity to methionin (MET). The highest intestinal uptake rate for MET is believed to occur in jejunum. Neutral amino acids are believed to be absorbed by several mechanisms, of which Na-dependent carrier transport is quantitatively most important. There are three Na-dependent mechanisms for neutral amino acids, two of which are relevant for MET and thereby presumably also for SEM (31). However, the uptake of SEM may also employ mechanisms unrelated to amino acid uptake. The uptake of MET from lumen involves binding to a membrane-bound receptor followed by energy-dependent transport into cytoplasm followed by liberation (32).

⁷⁷Se-labeled SEM uptake has been studied in hamster intestinal sacs perfused in vitro. The highest rate was found in the region of the jejunoileal junction (33). In their study, McConnell and Cho demonstrated that SEM was absorbed against a concentration gradient. Addition of ouabain to the serosal side inhibited SEM uptake, indicating a sodium dependent energy-requiring transport (34).

The results obtained in the present investigation with ⁷⁷Se-labeled SEM demonstrate, that SEM is taken up in all parts of the small intestinal tract. The uptake rate is highest in duodenum and the first part of jejunum; the rate in ileum falls with increasing distance from duodenum and falls to an even lower rate in the ileum. The rate of uptake is considerably lower in the large intestine (data not shown). The different labeling patterns observed between CBA and NMRI mice is due to a considerable size difference between these two strains.

The highest uptake rate for amino acids is considered to be in jejunum. The present results, though preliminary, therefore offer new information about the intestinal uptake mechanism for SEM and amino acids. Theoretically, the results can be explained, either by assuming a high uptake rate for methionine in duodenum, or by assuming the existence of two different uptake mechanisms for SEM.
Zinc

The site of intestinal zinc uptake is not well described; however, it is presumed to be proximal jejunum (35). Zinc absorption is enhanced by a low molecular weight binding ligand, presumably of pancreatic origin (36). Several compounds, including prostaglandins (37), citric acid (38,39), and picolinic acid (40), have been suggested to be natural zinc-binding ligands.

It is well documented that intestinal zinc uptake is homeostatically regulated (41). Metallothionein is apparently involved in this regulation, as intestinal zinc absorption is inversely related to the mucosal MT level (42). The results of the present study are preliminary and indicate that Zn uptake probably occurs in proximal jejunum.

In this investigation, the intestinal labeling profiles were studied with four metals, two toxic and two essential. The results offer information about uptake from intestinal lumen into mucosal epithelial cytoplasm, and about the chase kinetics showing either basolateral transport into the blood stream or retrograde transport back to intestinal lumen due to sloughing of mature villal cells.

The intestinal labeling profiles varied extensively for the four metals, indicating that different uptake processes were indeed operating, depending on the specific metal studied. The chase kinetics were, for all four metals, in agreement with previous suggestions about the intestinal localization of intense intestinal uptake.

We therefore conclude that this experimental model when properly used describes the intensity of the two steps in intestinal absorption of metals, namely transepithelial transport from lumen into epithelial cell cytoplasm, and basolateral transport into serosal fluid and further into blood. This model therefore offers a tool for tying together the two types of studies presently available for investigating mechanisms of intestinal metal uptake. These are quantitative studies of fractional intestinal uptake of metals performed during very natural conditions in intact animals or humans, and studies of molecular mechanisms of metal uptake performed in isolated perfused intestinal segments during conditions very far from those in the undisturbed intestine filled with digesta during its normal function.

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