Intestinal Cadmium Permeability in Mature and Immature Rats (43285)

E. C. FOULKES,*,†,[1] T. L. MORT,* AND C. R. BUNCHER*

Departments of Environmental Health and Physiology and Biophysics,† College of Medicine, University of Cincinnati, Cincinnati, Ohio 45267-0056*

Abstract. **To compare the intrinsic permeability properties of the small intestine in adult (average body wt 300 g) and 25- to 27-day-old (average body wt 50 g) male rats, the uptake rates of cycloleucine and of cadmium were measured in intestinal segments isolated *in situ* with their blood supply intact. Uptake rates were expressed on the basis of that of ethanol, a solute whose absorption depends primarily on the size, rather than the composition, of the available surface area and on the presence of unstirred layers. These layers may be concluded to affect movement of cycloleucine, cadmium, and ethanol to the same extent. The ratio of uptake rates, therefore, provides in arbitrary units a measure of the intrinsic permeability of the luminal surface area to cadmium and to cycloleucine. On this basis, no developmental change in cycloleucine permeability could be detected. In contrast, the rate of cadmium uptake relative to that for ethanol decreased with age by about 50%. Possible mechanisms are discussed for this significant change in the intrinsic cadmium permeability of the jejunum in post-"closure" animals.**

[P.S.E.B.M. 1991, Vol 197]

The immature intestine has repeatedly been reported to permit more complete absorption of heavy metals than does the mature organ (1). Evidence for this conclusion comes mostly from the measurement of metal retention in young, as compared to adult, animals. However, diet, and therefore the composition of the luminal contents, is likely to differ between the two age groups; diet is well-known to influence intestinal metal absorption (2). In addition, other important variables might also play a role in solute uptake; these include the thickness of unstirred layers and the passage time through the gut. For all these reasons, differences in metal retention cannot be confidently attributed to differences in the intrinsic permeability of the intestinal barrier to metals.

Intrinsic permeability is defined here as the rate of uptake of a solute per unit of absorbing surface area, independent of unstirred layers, luminal contents, etc. Direct measurement of such permeabilities, and their possible changes with development, are difficult to obtain and to evaluate. With age, the tissues change in thickness (mg/mm segmental length) as well as in diameter, and, therefore, in absorbing surface area (cm^2/mm segmental length). As a result, neither absorption per unit weight nor per unit length can serve as a basis for comparison. Procedures have been described for calculation of approximate intestinal surface areas (3), but, as will be developed in the Discussion, they lead to values of the total area, not only the area actually exposed in a given segment. Furthermore, normalizing absorption to a constant surface area ignores the potentially important influence of changes in unstirred layers (4) and other variables with age.

The present paper submits a functional basis for comparison of intrinsic permeabilities of mature and immature rat jejunum to Cd. This basis is provided by the rate of ethanol absorption. It will be shown that such a measure of permeability can provide a basis upon which to compare intrinsic activity of jejunum toward certain solutes in mature and immature animals. The proposed technique was tested here with Cd; the nonmetabolizable amino acid analog cycloleucine was studied as control, and to provide information on the specificity of any changes observed with Cd.

Materials and Methods

Male Sprague-Dawley rats, either adults (average body wt approximately 300 g, Group 1) or 25- to 27-

[1] To whom correspondence and requests for reprints should be addressed at Department of Environmental Health, College of Medicine, University of Cincinnati, Cincinnati, OH 45267-0056.

Received September 4, 1990. [P.S.E.B.M. 1991, Vol 197]
Accepted May 17, 1991.

0037-9727/91/1974-0477$3.00/0

day-old immature animals (average wt 50 g, Group 2) were obtained from Harlan Sprague Dawley, Inc., Indianapolis, IN, and maintained on Purina rat chow and tap water *ad libitum*. Unfasted animals were anesthetized with phenobarbital (Nembutal, 65 mg/kg ip), and the jejunum was flushed out with approximately 5 ml of saline, followed by air. To test the assumption that in both groups of animals uptake of Cd and cycloleucine from the lumen is a first-order process, 4 ml of saline containing one of these solutes together with ^{3}H-polyethylene glycol (PEG; mol wt 4000) as nonabsorbable volume marker were recirculated at 1.5 ml/min through an isolated jejunal segment, as described previously (5); uptake rates were calculated from the decrease in ^{109}Cd to ^{3}H or ^{14}C to ^{3}H ratios in consecutive samples collected from the perfusate over 15–20 min.

Once the exponential rates of solute uptake had been confirmed, half-times ($t_{1/2}$) of removal could be computed from the fractional recovery of each solute following stationary incubation *in situ*. For this purpose, an average of three jejunal segments per animal were tied off *in situ* with minimal handling. The infusate (0.2 ml of 0.15 *M* saline) contained 0.2 μCi of ^{3}H-PEG, plus either (i) 0.1 μCi of ^{109}Cd and $CdCl_2$, 2 nmol/ml, or (ii) 0.1 μCi of [^{14}C]ethanol and carrier, 20 μmol/ml or as stated, or (iii) 0.1 μCi [^{14}C]cycloleucine, final concentration 0.5 μmol/ml; it was introduced into the segment through a thin needle. The incubation was terminated after periods of incubation chosen to approach the $t_{1/2}$ value obtained in preliminary studies for each solute in either age group. Fractional recoveries (FR) were calculated from the ratios of ^{109}Cd or ^{14}C to ^{3}H-PEG. Segment length at the end of incubation was measured with the excised tissue resting on filter paper. The first order rate constant (k) was computed as usual from the formula

$$ln(\text{FR}) = tk$$

and $t_{1/2}$ was equated to 0.693/k.

In both the recirculation and the stationary incubation procedures just described, it was the disappearance of solutes from the lumen which was determined. Loss of cycloleucine and also of ethanol (see Discussion) essentially measures their net absorption. In contrast, practically all Cd removed from low concentrations in the lumen could be recovered from the tissue, at least in adult rats (6). In addition, control experiments using EDTA to separate intracellular Cd from that externally bound to the apical membranes (7) showed that after 10 min of perfusion, about 90% of total tissue Cd had been accumulated in the cells (unpublished results). It follows that uptake of Cd cannot be equated to its absorption, and the term "Cd permeability" refers only to the luminal surface area as defined by ethanol absorption.

Radioactivity was counted in disintegrations per minute on a Packard model 2000 Liquid Scintillation Spectrometer. Radiochemicals were purchased from New England Nuclear, Boston, MA.

Statistics

The relationship between $t_{1/2}$ and segment length was studied in the two groups of animals for each of the three different solutes. A combined slope was fitted to all six groups, and was found not to differ significantly from zero. In addition, covariance analysis of the six separate lines did not reject the conclusion that they are parallel. Accordingly, calculating a mean $t_{1/2}$ for each condition, without correction for segment length, was an effective analysis. Although not shown, the corrected analysis was also calculated, but the results were essentially the same. The relative intrinsic permeability of the tissues could then be expressed by the ratio of the means of $t_{1/2}$ for Cd or cycloleucine to $t_{1/2}$ (ethanol). These ratios were then compared between adult and immature animals, and the significance of any difference was evaluated by the bootstrap statistical comparison (8).

Results

Does Uptake of Cycloleucine, Cd, and Ethanol Follow First-Order Kinetics?. The following studies were carried out to answer this question. Labeled solute (20 μM $^{109}CdCl_2$ or 0.5 m*M* [^{14}C]cycloleucine, together with ^{3}H-PEG in saline) was recirculated *in situ* through isolated segments of jejunum; duplicate samples were removed for counting at the times indicated, and fractional uptake of Cd and cycloleucine was calculated from the fall of their concentration ratio to PEG. Results are shown in Figure 1 for two immature animals. In a total of four rats with cycloleucine and five with Cd, similar straight lines were obtained. However, as noted previously (9), interanimal variability in such experiments is so high that the average of values for individual animals possesses little meaning.

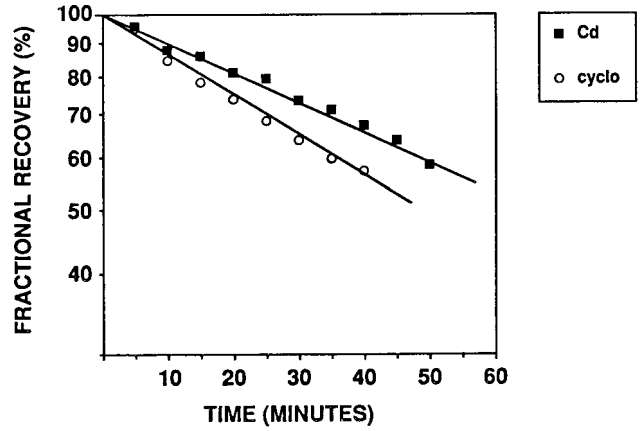

Figure 1. Uptake of Cd and of cycloleucine from the perfused jejunum in two individual immature rats.

The exponential nature of Cd uptake in adult rat jejunum has been frequently illustrated in earlier work (5). As for amino acids, their transport out of the intestinal lumen is well-known to be mediated by typical carrier systems. Accordingly, the rate of cycloleucine uptake at low concentrations should also be characterized by first-order kinetics, a generally accepted fact that we have confirmed in a series of control studies in mature and immature rats (results shown only for immature rats; see Fig. 1).

It can be similarly concluded from *a priori* considerations that ethanol absorption, being a passive process (see Discussion), also follows first-order kinetics. Additional experimental support for this conclusion is provided by the observation that the rate of fractional ethanol absorption, expressed in terms of the half-time of its removal from the lumen, is independent of concentration (see below).

Measurement of $t_{1/2}$. On the basis of the finding that intestinal uptake is a first-order process for each of the solutes studied (cycloleucine, Cd, and ethanol), the half-time of uptake could be determined as detailed in Materials and Methods.

In order to minimize handling of tissues before measuring absorption, tissue lengths in these experiments were only determined after incubation; as a result, a wide variety of lengths of tissue was studied during stationary incubation in each age group. Under our conditions, $t_{1/2}$ varied little with segment length. This is illustrated in Figures 2 and 3 for Cd and ethanol; additional details are collected in Table I. Covariance analysis of the six individual slopes did not reject the hypothesis that the lines are parallel, and the combined slope for all measurements did not differ significantly from zero. As indicated in Statistics, the small influence of length on $t_{1/2}$ made unnecessary a normalization to

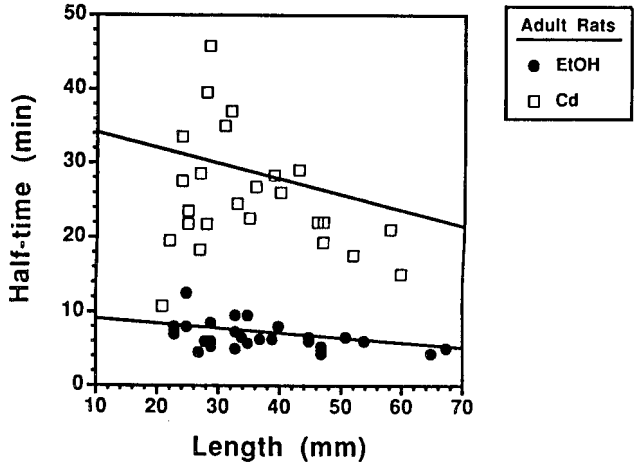

Figure 2. $t_{1/2}$-values for Cd and ethanol in segments of adult jejunum of different lengths (L). The calculated lines of best fit, as indicated, are $(t_{1/2})$Cd = 32.7–0.20 L, $(t_{1/2})$EtOH = 9.0 – 0.06 L.

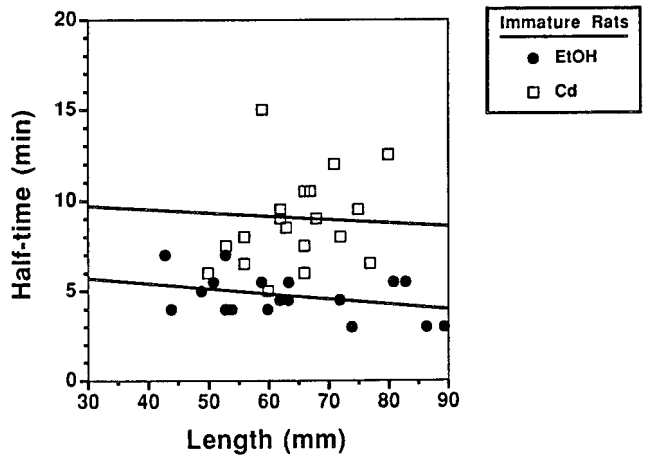

Figure 3. $t_{1/2}$-values for Cd and ethanol in segments of immature jejunum. The calculated lines of best fit, as indicated, are $(t_{1/2})$Cd = 9.6 – 0.01 L, $(t_{1/2})$EtOH = 6.1 – 0.02 L.

Table I. Regression of Half-Time on Length[a]

	Adults		Immature	
	r	slope	*r*	slope
Ethanol	−0.35	−0.06	−0.37	−0.02
Cadmium	−0.27	−0.20	−0.29	−0.01
Cycloleucine	+0.05	+0.02	−0.32	−0.05

[a] The combined slope for all animals did not differ from zero and covariance analysis did not reject the conclusion that the six individual lines are parallel. The number of animals for each group is given in parentheses in Table II.

Table II. Rates of Solute Uptake in Jejunum of Adult and Immature Rats

	Ethanol[a]	Cadmium	Cycloleucine
Group 1			
$t_{1/2}$[b]	6.4 ± 1.4 (35)[c]	21.6 ± 7.2 (42)	9.6 ± 2.3 (21)
$t_{1/2}/t_{1/2\,EtOH}$	—	3.4 ± 1.2[d]	1.5 ± 0.5[e]
Group 2			
$t_{1/2}$	4.6 ± 1.2 (16)	9.0 ± 2.8 (18)	7.6 ± 1.9 (17)
$t_{1/2}/t_{1/2\,EtOH}$	—	1.9 ± 0.8[d]	1.6 ± 0.6[e]

[a] Raising the ethanol concentration from 0.02 to 0.50 *M* did not significantly change $t_{1/2}$ (see text).
[b] $t_{1/2}$, half-time in minutes (mean ± SD), with number of observations in parentheses.
[c] The number of animals for each group is in parentheses.
[d] Observed mean bootstrap difference and SD = 1.34 ± 0.30, 98% confidence interval 0.73–2.13, $P < 0.001$.
[e] Difference not significant by same bootstrap procedure.

standard length; instead, mean values for $t_{1/2}$ for each solute were calculated and are shown in Table II.

As an additional test of the conclusion that uptake of ethanol is a passive process, the influence of ethanol concentration on the $t_{1/2}$ of its absorption was determined in 16 jejunal segments in adult rats. In eight of these segments, containing initial ethanol concentrations of 0.02–0.05 *M*, $t_{1/2}$ equaled 4.9 ± 0.7 min. This value did not differ significantly from 5.1 ± 0.9 min,

observed at up to approximately 40-fold higher levels (0.50–0.85 M) in a second group of eight segments in the same animals. This fact fully confirms under our conditions the direct relationship between rate of ethanol absorption and its luminal concentration, as expected for passive diffusion and as demonstrated previously (10).

Discussion

Choice of Ethanol as Probe. Ethanol is highly diffusible and highly soluble in both fat and water, so its rapid permeation is relatively independent of the chemical nature of cell membranes. The rapidity of alcohol (methanol) absorption in the rat jejunum has been described previously (11), and the low reflection coefficient of biological membranes to ethanol is well-documented (12). There is clearly no need to postulate the contribution of any carrier system to alcohol absorption, and the exponential rate of its uptake from the lumen should be determined primarily by the size of the available absorbing area and by the presence of unstirred layers. To the extent that unstirred layers equally affect rate of removal of other solutes from the lumen, the ratio of the rate of Cd uptake to that of ethanol therefore expresses a measure (in arbitrary units) of the intrinsic permeability of the luminal absorptive surface to Cd.

Choice of labeled ethanol as probe presupposes the absence of significant alcohol metabolism in the gut. The likelihood that oxidation of ethanol influenced the rate of ^{14}C absorption under present conditions seems small. Not only was wash-out of gut segments before study designed to reduce bacterial contamination and thus bacterial oxidation of ethanol, but the subsequent incubation was of only a few minutes duration. Furthermore, increasing ethanol concentration approximately 40-fold exerted little influence on $t_{1/2}$. Finally, oxidation of ethanol would first lead to acetaldehyde, a compound as water and lipid-soluble as ethanol, and, therefore, also highly absorbable. Only if, during the short incubation, a large fraction of alcohol in the lumen had been metabolized to acetate and beyond might a noticeable effect on $t_{1/2}$ have been produced; this is an unlikely possibility.

The Role of Mucus. The possibility was considered that the presence of mucus might influence solute uptake differently in young and adult animals. The significance of mucus layers to solute passage through the gut wall has been considered by several investigators, including Nimmerfall and Rosenthaler (13), for instance. However, a specific effect of mucus seems implausible for diffusion of small and uncharged molecules like ethanol and cycloleucine.

A role of mucus in determining relative rates of Cd uptake under present conditions is also unlikely. If the relatively slow Cd uptake by the adult intestine resulted from the presence of Cd-binding mucus, the uptake should be slowed to the greatest extent at low Cd concentrations. Actually, earlier results showed that the fractional rate of uptake of two heavy metals (Cd^{2+} and Ni^{2+}) in the mature jejunum remains constant as their luminal concentrations are raised from 1 to 20 μM, followed by a gradual decrease, not an increase, at higher levels up to 200 μM (14). If the presence of mucus had determined the rate of Cd uptake, increased saturation of its Cd-binding capacity should have led to an increased rate of fractional Cd uptake at the higher concentrations. This argument assumes, of course, that the Cd-binding capacity of the mucus had been saturated. Additionally, however, Cd in the perfusate should equilibrate reasonably fast with mucus; at that stage, the rate of further Cd removal from the perfusate should no longer be influenced by mucus. Note that in Figure 1, the exponential rate of Cd uptake remained constant for at least 50 min. As the relatively slow rate of Cd uptake in the adult can, therefore, apparently not be attributed to presence of mucus, it follows logically that presence or absence of such substances plays no part in explaining the relatively faster rate of Cd uptake in the immature rats.

Influence of Length on $t^{1/2}$. The length of segments in the stationary perfusion experiments exerted little influence on $t_{1/2}$. Because the size of the absorbing surface area is clearly a major determinant of uptake rates, it follows that the injection of a constant volume of 0.2 ml must have led to exposure of an approximately constant surface area under our conditions, relatively independent of segment length. A reasonable explanation for this fact is provided by the observation that following flushing of the intestinal segments with saline and air, their lumen is completely collapsed. The length of intestine participating in uptake of the test solutes would, therefore, be expected to vary closely with the volume of solution introduced. Clearly, in that case, calculation of the total surface area of the segment (3), as referred to in the Introduction, would not provide a relevant measure of the surface area participating in absorption. If, on the other hand, attempts had been made to fill the whole segment, the risk of stretching the mucosa could not have been avoided.

Relative Permeabilities of Luminal Surface. The use of ethanol as probe in these studies was justified above. It rests on the conclusions that the alcohol is not rapidly oxidized under our conditions, and that its movement across the intestinal barrier is entirely passive. Furthermore, mucus, the main constituent of unstirred intestinal layers of possible relevance to diffusion of the three solutes studied here, does not appear to influence their uptake (see above). In the present context, the only critical factor relating to unstirred layers is, therefore, their thickness, and this should affect movement of the three solutes to a similar extent.

Thus, the rate of fractional absorption of ethanol is seen to be determined primarily by the thickness of unstirred layers, and by the size, rather than the chemical properties, of the surface available for uptake. Thus, relative intrinsic permeabilities of this surface to Cd and cycloleucine can be expressed in arbitrary units as the ratio of their half-time of uptake to that for ethanol; the larger that ratio, the less permeable the surface is to this solute. This permits direct comparison of relative intrinsic permeabilities of the intestine at different stages of development. The conclusion, as documented in Table II, states that the adult intestine absorbs cycloleucine at essentially the same exponential rate per unit absorbing area as does the immature organ; this presumably reflects a similar density of cycloleucine carriers in the membrane. In contrast, the ratio of the rate of Cd uptake to that of ethanol falls with maturation over the age span studied. This is interpreted as a specific decrease in the intrinsic Cd permeability of the luminal absorbing area. Several hypotheses may be suggested to explain such a decrease.

One possibility invokes the presence of paracellular shunt pathways; such pathways do not appear to contribute significantly to Cd uptake in the mature jejunum (11). Another explanation might be based on pinocytotic activity, which has been correlated with metal retention in suckling animals (15), but appears to play only a little role in Cd uptake from the adult intestine (7). A fall in pinocytosis during postweaning development could, therefore, explain the observed change in the rate of Cd uptake. A third suggestion is that rates of Cd uptake are determined by the fluidity of the cell membranes. This hypothesis had been proposed previously as a likely determinant of Cd uptake (7), and the immature organ has been characterized by relatively high membrane fluidity (16). Future work will have to attempt to test these hypotheses.

It is important to emphasize that our immature animals had passed the stage of "closure" in the structural development of the intestinal mucosa, at which time the intestine ceases to take up macromolecular substances (17). There still remains, however, a significant difference between the intrinsic ability of postclosure immature and adult animals to take up Cd. Forbes and Reina (18) similarly reported that lead is removed more than twice as fast from the gut contents in 27-day-old rats than in adults. It is not known to what extent this represents changes in the intrinsic permeability of the absorbing surface, as has been demonstrated here for Cd. It would also be interesting, as a further extension of the present work, to determine the relative intrinsic rate of Cd uptake in neonatal animals. In any case, our results support the conclusion that, in addition to other possible factors, it is the intrinsic ability of the luminal barrier in the intestine to take up Cd that puts immature organisms at special risk resulting from exposure to this metal.

This research was supported by NIH Grants ES-04840 and ES-00159. We thank Dr. D. Johnson for his interest in this work.

1. Kostial K, Kello D, Jugo S, Rabar A, Maljokovic T. Influence of age on metal metabolisms and toxicity. Environ Health Perspect **25**:81–86, 1978.
2. Rabar I, Kostial K. Bioavailability of cadmium in rats fed various diet. Arch Toxicol **47**:63–66, 1981.
3. Fisher RB, Parsons DS. The gradient of mucosal surface area in the small intestine of the rat. J Anat **84**:272–282, 1950.
4. Thomson ABR, Dietschy JM. The role of the unstirred water layer in intestinal permeation. In: Csaky TZ, Ed., Pharmacology of Intestinal Permeation. Handbook of Experimental Pharmacology, 70/II. Berlin: Springer-Verlag, pp165–269, 1984.
5. Foulkes EC, Voner C. Effects of Zn status, bile and other endogenous factors on jejunal Cd absorption. Toxicology **22**:115–122, 1981.
6. Foulkes EC, McMullen DM. Endogenous metallothionein as determinant of intestinal cadmium absorption: A reevaluation. Toxicology **38**:285–291, 1986.
7. Foulkes EC. On the mechanism of transfer of heavy metals across cell membranes. Toxicology **52**:263–272, 1988.
8. Efron B. The jackknife, the bootstrap and other resampling plans. Soc Ind Appl Math Monograph 38, Philadelphia, 1982.
9. Foulkes EC. Some determinants of intestinal cadmium transport in the rat. J Env Pathol Toxicol **3**:471–481, 1980.
10. Csaky TZ. Intestinal absorption of xenobiotics. In: TZ Csaky, Ed. Pharmacology of Intestinal Permeation. Handbook of Experimental Pharmacology, 70/II. Berlin: Springer-Verlag, pp1–30, 1984.
11. Foulkes EC, McMullen DM. Kinetics of transepithelial movement of heavy metals in rat jejunum. Am J Physiol **253**:G134–G138, 1987.
12. Stein WD. The Movement of Molecules across Cell Membranes. New York: Academic Press, 1967.
13. Nimmerfall F, Rosenthaler J. Significance of the goblet-cell mucin layer, the outermost luminal barrier to passage through the gut wall. Biochem Biophys Res Commun **94**:960–966, 1980.
14. Foulkes EC, McMullen DM. On the mechanism of nickel absorption in the rat jejunum. Toxicology **38**:35–42, 1986.
15. Keller CA, Doherty RA. Correlation between lead retention and intestinal pinocytosis in the suckling mouse. Am J Physiol **239**:G114–G122, 1980.
16. Israel EJ, Pang KY, Harmutz PR, Walker WA. Structural and functional maturation of rat gastrointestinal barrier with thyroxine. Am J Physiol **252**:G762–G767, 1987.
17. Clark RM, Hardy RN. An analysis of the mechanism of cessation of uptake of macromolecular substances by the intestine of the young rat ("closure"). J Physiol **204**:127–234, 1969.
18. Forbes GB, Reina JC. Effect of age on gastrointestinal absorption (Fe, Sr, Pb) in the rat. J Nutr **102**:647–652, 1972.