Use of Carbon Monoxide to Measure Luminal Stirring in the Rat Gut

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Abstract. We used carbon monoxide (CO) as a probe to quantitatively measure intestinal unstirred water layers in vivo. CO has several features that make it uniquely well suited to measure the unstirred layer in that its tight binding to hemoglobin makes uptake diffusion limited, and its relatively high lipid solubility renders membrane resistance negligible relative to the water barriers of the unstirred layer and epithelial cell. The unique application of CO was the measurement of the absorption rate of CO both from the gas phase as well as a solute dissolved in saline. Several lines of evidence showed that a gut stripped free of saline and then filled with gas contained a negligible unstirred layer. Thus, absorption of CO from the gas phase measured resistance of just the epithelial cell. Subtraction of this value from the resistance to CO absorption from saline provided a direct measure of unstirred layer resistance. Studies in the rat showed for a 3-min absorption period that the conventionally calculated apparent unstirred layer for the jejunum was 411 μm and for the colon was 240 μm. However, this conventionally calculated unstirred layer resistance did not truly depict the situation in the rat gut, since there was a continuing depletion of CO from outer surfaces of luminal contents throughout the experiment period. This produced a continually increasing diffusion barrier with time. Calculation of expected absorption rate from unstirred cylinders with the dimensions of the rat gut indicated that there was virtually no stirring in the small intestine and minimal stirring in the colon. The technique described in this paper appears to be simpler and to require fewer assumptions for validity than other techniques previously used to measure unstirred layers in vivo.

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Introduction

The movement of solutes between intestinal bulk luminal contents and mucosa requires that the solute diffuse across an unstirred water layer adjacent to the mucosa. The role of this layer in vitro has been extensively studied (1–3), primarily by using the osmotic transient technique (4). This technique is not, however, readily employed in vivo, nor has any other simple technique been shown to accurately measure the unstirred layer effect in the intact gut. Since the mixing induced by villus wriggling and gut motility is not readily simulated in vitro, it is not clear if unstirred layer measurements obtained in vitro are applicable to the in vivo situation. Thus, the precise influence of unstirred layers on the absorptive process in the intact gut remains to be quantitated.

The present report describes studies carried out with carbon monoxide (CO)[1] which suggest that this gas provides a unique probe with which to quantitate unstirred layer effects in vivo.

Methods

Studies were carried out in 300 g Sprague-Dawley rats fasted for 16 h. Using pentobarbital anesthesia, ~10-cm segments of jejunum or colon were isolated between ligatures. A 0.7-mm internal diameter cannula was tied in the proximal end of the segment, a 1.5-mm internal diameter cannula was tied into the distal end, and three-way stopcocks were attached to each cannula. To minimize deadspace in the infusion line, the proximal stopcock was filled with epoxy and then a new 0.7-mm lumen was drilled through the epoxy.

A 50-ml syringe containing isotonic saline (37°C) was attached to one arm of the proximal stopcock and a 1-ml syringe containing the infusate was attached to the other arm. The gut segment was then flushed clean of debris with saline.

For studies with a gaseous infusate the saline in the infusion line was cleared using a small quantity of argon. The proximal stopcock was then closed and the saline in the gut was removed via the distal stopcock by twice gently stripping the gut in a proximal to distal direction between thumb and forefinger. The distal stopcock was then closed and 1 ml of argon containing 0.1% CO was rapidly infused into the segment. The proximal stopcock was then closed, the length of the

1. *Abbreviations used in this paper:* Q_{sal}, absorption rate of CO from the saline; R_{ul}, apparent resistance of the unstirred layer; CO, carbon monoxide; R_{muc}, measure of the resistance of the mucosa; PD, potential difference; Q_{gas}, rate of CO absorption from the gas phase.

segment was measured, but no efforts to produce mixing were employed. At the end of the 20-min absorption period, the lumen was rapidly emptied by stripping the gas into a 50-ml syringe attached to the distal stopcock and then flushing the gut with 50 ml of argon which was also collected in the 50-ml syringe. Studies with CO dissolved in saline were carried out in similar fashion, with the exception that great care was taken initially to remove all gas bubbles from the infusion line and the gut segment by means of the saline flush. The gut was then stripped free of saline in identical fashion to that described for a gaseous infusate and 1 ml of saline containing CO at a P_{CO} of ~76 mmHg (~0.001 ml CO/ml saline) was infused. The length of the gut segment was measured, and once again the gut was not disturbed during a 3-min absorption period. At the end of the absorption period, luminal contents were rapidly stripped into a 50-ml syringe and the gut was flushed with 5 ml of saline followed by 45 ml of argon which was collected in the 50-ml syringe. Four additional studies were carried out using a 1-min absorption period for jejunal gut segments.

Absorption of CO was measured by difference. Samples which were even faintly blood tinged were discarded. After vigorous mixing, the quantity of CO in the perfusate plus the argon wash was determined by analysis of the gas phase in the 50-ml syringe (the partition coefficient of CO between gas and saline is such that virtually all CO will be in the gas phase). The analysis for CO was carried out using a gas chromatograph equipped with a gas sampling valve and a 3′ × ⅛″ stainless steel column packed with molecular sieve at an oven temperature of 100°C, argon as the carrier gas, and a reduction detector (Trace Analytical). To exclude the possibility that an appreciable fraction of the CO was binding to mucosal heme compounds (as opposed to being absorbed into the blood), three additional jejunal absorption studies were carried out using 5% CO in argon.

The volume of fluid remaining in the gut segment after stripping was studied by two independent methods. First, saline containing ^{14}C-inulin plus 0.1 g/dl carrier inulin was infused into small bowel or colonic segments and the fluid was allowed to equilibrate for 5 min. The luminal contents were then stripped out of the gut in the usual fashion. The gut segment was then removed from the rat, opened longitudinally, and allowed to equilibrate with 5 ml of saline. The concentration of ^{14}C-inulin in this saline wash and the ^{14}C-inulin concentration in the fluid stripped from the gut were then measured by scintillation counting. The volume of fluid which remained in the lumen after stripping was calculated from the residual ^{14}C-inulin, and the concentration of ^{14}C-inulin in the fluid stripped from the gut. The mean thickness (d) of the water layer remaining after stripping was calculated from the volume and length of the gut, which assumed the surface to be a smooth cylinder.

The volume of residual fluid after stripping also was assessed in a different fashion by instilling 1 ml of saline containing fluorescenated dextran into segments of jejunum or colon. After 5 min, the saline was stripped from the gut in standard fashion. 1 ml of argon was then instilled into the gut segment and after 20 min (the standard absorption period for gas infusate) the segment was frozen with liquid nitrogen in situ. The segment was then removed, frozen cross sections were cut, and the cross sections examined under a fluorescent microscope to assess the thickness of the fluorescent layer.

The possibility that histological or physiological damage resulted from the stripping process was investigated as follows. Six segments of jejunum and six segments of colon were isolated in standard fashion and the debris was flushed from the segment with saline. The saline was then stripped from the lumen of three small bowel and three colonic segments in the same fashion used for absorption studies. These segments, as well as segments which had not been stripped, were then removed, fixed in formalin, sectioned longitudinally, and stained with hematoxylin and eosin. The sections were coded and examined for evidence of histological damage.

To determine if stripping produced physiological damage to the jejunum, glucose absorption was measured from stripped and unstripped gut segments. Jejunal segments were filled with 1 ml of saline containing 100 mg percent glucose. After a 5-min period, the luminal solution was flushed from the gut and glucose disappearance was measured using the glucose oxidase technique. The height of the epithelial cells of fixed sections of small bowel and colon was measured using a calibrated eyepiece.

Calculation of resistance of diffusion barriers. Based on the observation (see results) that virtually no luminal fluid remains in the gut after stripping, the rate of CO absorption from the gas phase (Q_{gas}) should provide a measure of the resistance of the mucosa (R_{muc}) to CO absorption. On the other hand, the absorption rate of CO from the saline infusate (Q_{sal}) is determined by R_{muc} in series with the apparent resistance of the unstirred layer (R_{ul}) to CO diffusion. Thus, the difference between Q_{gas} and Q_{sal} is determined by R_{ul}.

Resistances were calculated in conventional fashion from absorption rates and normalized for partial pressure gradient expressed either as ml/min · mmHg · cm length of gut or ml/min · mmHg · cm^2 surface area of gut, with surface area of the gut calculated for a smooth luminal surface that had the length and volume of the gut segment.

$$R = 1/Q. \quad (1)$$

$$R_{muc} = 1/Q_{gas}. \quad (2)$$

$$R_{ul} = 1/Q_{sal} - 1/Q_{gas}. \quad (3)$$

As will be discussed, this conventionally calculated resistance of the unstirred layer is somewhat misleading in the narrow calibre intestine of the rat. No steady state is achieved during absorption since there is a continually increasing depletion of the fluid layer adjacent to the mucosa. Thus, the conventionally calculated resistance and thickness of the unstirred layer are not constants but increase with increasing length of the absorption period.

Thickness of diffusion barriers for an unstirred layer of constant thickness. The thickness of the unstirred layer and the epithelial cell were calculated from the standard formula for gas absorption:

$$Q = 1/R = [(K)(A)(P_{CO})]/[d], \quad (4)$$

where d equals thickness of diffusion barrier, A equals surface area, K equals Krogh's diffusion coefficient for saline (for calculating unstirred layer thickness from R_{ul}) or Krogh's coefficient for tissue (for calculating mucosal thickness from R_{muc}). Krogh's coefficient represents the milliliter per minute of gas that will diffuse through a barrier which is 1 cm thick and 1 cm^2 in surface area when the partial pressure gradient is 1 mmHg. The value employed for K for CO in saline at 37°C was 3.2×10^{-5} and K for CO in frog muscle tissue was 0.95×10^{-5} (5, 6).

Calculation of luminal mixing for continuously increasing unstirred layer. As will be discussed, there was a continually increasing unstirred layer during the course of our experiments as the water layer adjacent to mucosa was depleted of CO. The degree of luminal mixing in such a situation can be estimated from the following formula, which was originally derived for heat transfer from the lumen of a cylinder to its inner surface.

It is assumed that the intestine can be represented by a cylinder of radius (a) which has an initial concentration of c_o inside and a

concentration of $c = O$ at the outer surface. The general solution for the concentration in the cylinder as a function of the position (R) and time (t) is taken from Carslaw and Jaeger (7). The solution is written in terms of the dimensionless variables: $C = c/c_o$: the concentration relative to the initial concentration, $T = Dt/a^2$, where D is the diffusion coefficient. $r = R/a$: the outer surface of the cylinder is at $r = 1$. $A(T)$: the fraction of the initial contents absorbed between $T = O$ and $T = T$.

The general solution is:

$$C(r, T) = 2 \sum_{n=1}^{\infty} e^{-b_n^2 T} J_o(b_n r)/(b_n J_1[b_n]), \quad (5)$$

where J is the Bessel function of the first kind and b_n is the nth root of the equation; $J_0(b) = 0$. The absorption rate per unit length (J) of intestine is then equal to:

$$J = 2\pi a_o D \frac{dc}{dr}\bigg| r = 1. \quad (6)$$

Using these two equations it can be shown that $A(T)$ is described by:

$$A(T) = 1 - 4 \sum_{n=1}^{\infty} \frac{1}{b_n 2} e^{-b_n^2 T}. \quad (7)$$

For the times used in these experiments (>1 min), only the first term in the summation is important, and A can be approximated by:

$$A(T) = 1 - 0.69e^{-5.8T}. \quad (8)$$

Results

Volume of residual luminal fluid after stripping. Based on residual ^{14}C-inulin after stripping, an average of 11 μl of fluid remained in the jejunum and 9.4 μl of fluid in the colon. In intestinal segments which are roughly 10 cm long and 1 cm^3 in volume, these residual volumes would form unstirred layers of mean thickness of ~9 μm. Studies with fluorescenated dextran confirmed this observation, in that residual fluorescence after stripping formed an irregular mucosal coating which was usually <10 μm in thickness. Thus, the residual fluid after stripping should provide a nearly negligible unstirred water layer barrier relative to that of the mucosal cell, which has a thickness of ~30 μm and a lower diffusion coefficient for CO than does saline.

Study of possible injury to gut from stripping. Study of initial histological sections of small bowel and colon from stripped and unstripped gut showed no detectable evidence of histologic injury. The absorption rate of glucose from jejunal segments which had been previously stripped (0.012±0.001 mg/cm gut per min) was not significantly different from that of unstripped gut (0.011±0.003 mg/cm gut per min).

Absorption rates, resistance, and calculated thickness of unstirred layer and mucosa. The observed absorption rate of CO from the gas phase and from saline, (3-min absorption period), the resistances of the mucosa and the unstirred layer, and the calculated thicknesses of the unstirred water layer and the mucosa are shown in Table I. The absorption rate of CO from the jejunum was about four times faster from the gas phase than from saline. Since resistance equals the inverse of the absorption rate, the resistance to absorption from saline was ~4 times greater than that from the gas phase, the difference equaling the resistance of the unstirred layer. For the colon, the resistance to CO absorption from a saline infusate was ~3 times that of the gas phase. The calculated resistance of the jejunal unstirred layer ($R_{sal} - R_{gas}$) was 9.5 × 10^5 min · mmHg · cm^2/ml, while the resistance for the colon was 5.7 × 10^5 min · mmHg · cm^2/ml.

Table I. Absorption Rate of Gases, Resistance and Diffusion Barriers in Rat Intestine

	CO Absorption rate		Resistance		Diffusion barrier thickness	
					Mucosa	Unstirred water layer
	ml/min · mmHg · cm	*ml/min · mmHg · cm²*	*min · mmHg · cm/ml*	*min · mmHg · cm²/ml*	*μm*	*μm*
Small bowel						
Gas	4.5 × 10^{-6} ±0.24	4.1 × 10^{-6} ±0.28	2.5 × 10^5 ±0.12	2.5 × 10^5 ±0.15	31 ±2.0	—
Saline	1.0 × 10^{-6} ±0.09	0.83 × 10^{-6} ±0.03	10.6 × 10^5 ±0.70	12 × 10^5 ±0.44	—	411 ±19
Colon						
Gas	3.5 × 10^{-6} ±0.22	3.2 × 10^{-6} ±0.19	3.3 × 10^5 ±0.21	3.2 × 10^5 ±0.19	40 ±2.4	—
Saline	1.3 × 10^{-6} ±0.06	1.2 × 10^{-6} ±0.05	7.8 × 10^5 ±0.36	8.9 × 10^5 ±0.37	—	240 ±16

* All values reported mean±SE.

The jejunal absorption rate of CO from argon containing 5% CO (3.0×10^{-6} ml/min · mmHg · cm^2) did not differ significantly from the value observed for the 0.1% CO used in the bulk of the studies. Four additional studies of CO absorption from the jejunum were carried out using a 1- (rather than 3-) min absorption period. The percentage absorption per minute was 46%, the unstirred layer resistance was 3.6×10^4 min · mmHg · cm^2/ml, and the thickness of the unstirred layer was 240 μm in these studies.

Thickness of diffusion barriers. The mean thicknesses of the unstirred layer which would yield the observed resistance were calculated from the diffusion coefficient of CO in saline or tissue (see Table I). For the small bowel, the mean unstirred water layer was 411±19 μm thick and for the colon it was 240±16 μm. The calculated thickness of the jejunal epithelium was 31 μm and the colonic epithelium was 40 μm. The actual measured thickness of the jejunal epithelial cells at the villus tip was 34 μm and of the colon was 31 μm.

Calculation of degree of luminal mixing. Using Eq. 8, the expected percentage absorption of CO for the small and large bowel for a 3-min absorption period can be calculated for the situation when there is no luminal mixing. The value used for the radius of the cylinder in this formula was 0.01 cm greater than the observed luminal radius to account for the roughly 30 μm thickness of the epithelial cell which has a threefold lower diffusion coefficient for CO than does saline. Values employed were 0.18 cm for the radius of the small bowel and 0.19 cm for the colon, $D = 2.7 \times 10^{-5}$ cm^2/s, and $t = 180$ s; $T = 0.15$ for the small bowel and 0.13 for the colon. Substituting these values of T into Eq. 8 yields $A = 0.71$ for small bowel and $A = 0.68$ for colon. Thus, 71% of the CO should have been absorbed from the small bowel and 68% from the colon if there were no luminal mixing. The actual observed percentage absorption was 69% for the small bowel and 75% for the colon. Thus, it is apparent that there was negligible luminal mixing in the small bowel and perhaps slight mixing in the colon. If there were perfect stirring, then CO flux is described by the following equation:

$$J = 2\pi aDc/L = \frac{d}{dt}(\pi a^2 c), \tag{9}$$

where L is the thickness of the unstirred layer and the last equality comes from the assumption that the luminal concentration is the same everywhere (perfect stirring). This equation can be integrated and the result written in terms of the same dimensionless variables used above, defining a new variable: $d = L/a$, which is the fraction of the intestinal radius that is unstirred:

$$C = e^{-2T/d}. \tag{10}$$

Example for the jejunum: At a time of 180 s and using a D for the mucosal cell one-third that of free solution, then $T = Dt/a^2 = 0.36$. If the unstirred thickness is that of the mucosal cell (30 μm), $d = 0.02$. Then, from Eq. 6, 99% of the CO should be absorbed in 150 s. A more useful calculation is to solve for the time it would take to absorb the observed 69% of the CO if luminal contents were perfectly stirred. Solving Eq. 10, this would take ~33 s (rather than the observed 180 s).

Discussion

Understanding of the role of the unstirred water layer in vivo has been limited by the lack of a simple technique which can accurately measure this layer in the intact gut. The present report describes experiments in which we used CO as a probe to investigate the diffusion barriers of the small and large bowel of the rat.

For a variety of reasons, CO is particularly well suited for study of diffusion barriers in the gut. First, upon reaching the blood stream, this gas binds avidly to hemoglobin, thus reducing the P_{CO} of blood to a very low level. Since the P_{CO} of blood is negligible relative to that of the lumen, the P_{CO} gradient used for measuring resistance to absorption simply equals the luminal P_{CO}. In contrast, the concentration of other passively absorbed solutes may rise to an appreciable level in villus blood. Since it is not possible to determine the concentration of the probe molecule in villus blood, the concentration gradient is unknown and permeability cannot be accurately calculated.

The binding of CO by hemoglobin is particularly important for measuring the permeability of the small bowel mucosa, which has a vascular arrangement that favors very efficient countercurrent exchange between the efferent and the afferent vessel of the villus (8). The negligible P_{CO} level of the blood draining the villus prevents appreciable countercurrent exchange (9), which thus obviates a problem which bedevils permeability measurements with other passively absorbed solutes.

A second useful feature of CO is that this gas has appreciable solubility in both lipid and water (lipid/water partition coefficient equals 3:1, unpublished observation). Since the roughly 10A° thickness of the lipid membrane represents only ~1/30,000 of the 30 μm thickness of the tissue water barrier of the epithelial cell, membrane resistance will be negligible for solutes with roughly equal lipid and water solubilities, such as CO and O_2. For example, Huxley and Kutchai (10) found in the study of O_2 uptake by erythrocytes that the resistance of the membrane was nearly negligible relative to an unstirred water layer of only 2 μm thickness. Since the gut epithelial cell represents a 30-μm unstirred water barrier, it seems likely that membrane resistance to CO absorption is negligible relative to this water barrier. The capillaries of the mucosa are closely applied to the basal surface of the epithelial cell; therefore, the mucosal barrier to CO absorption roughly should equal the resistance of a tissue water barrier of the thickness of the gut epithelial cell.

The possibility that the observed CO uptake results from binding to heme compounds in the epithelial cell rather than

diffusion through the cell to the blood can be excluded by the following argument. The CO binding capacity of the liver (cytochrome P450 plus cytochrome A in mitochondria) is ~40 nm (Holtzman, J., personal communication). This value probably represents a maximal estimate of the ability of the gut epithelial cell to bind CO. The total CO binding of the 30 μm thick cylinder of epithelial cells in the gut segments employed can thus be calculated to be ~30 nl. About 500 nl of CO was absorbed during the experiments carried out using the standard CO concentration of 0.1%, which indicated that only a small fraction of the absorbed CO could be accounted for by binding. This conclusion is supported by the finding that the percentage uptake of CO was similar at concentrations of 0.1 and 5%, the latter concentration yielding a CO absorption rate several hundred times that of the calculated CO binding capacity of the epithelial cells. If an appreciable fraction of the absorbed CO had been bound in the epithelial cell at the lower CO concentration, the percentage uptake should have been appreciably greater than at the higher CO concentration, where CO would have had to diffuse through the entire epithelial cell thickness.

Since the resistance of the cell membrane to CO is negligible relative to that of the thickness of the epithelial cell, the cross-sectional area of epithelial cells exposed to the lumen, rather than membrane surface area, determines epithelial cell resistance. Increased surface area produced by microvilli should have no influence on the absorption rate of CO. In the presence of an unstirred water layer, the importance of the increased surface area provided by villi also becomes relatively unimportant to the CO absorption process. For example, Fig. 1 compares a schematic diagram of villi (with microvilli) beneath an unstirred layer of appreciable thickness with a diagram of a flat mucosal surface under a similar unstirred layer. The absorption rate of CO will be determined largely by the cross-sectional area of the unstirred layer and by the cross-sectional area of the epithelial cell at the villus tip as well as distance CO must diffuse from bulk luminal contents to reach mucosal blood. It is apparent that these values are roughly the same for both schematic models. The marked increase in surface area due to the villi and microvilli should be relatively unimportant. Thus, in the absence of gross undulations of the gut surface, it seems likely that the surface area can be considered to be flat for calculations of unstirred layer thickness.

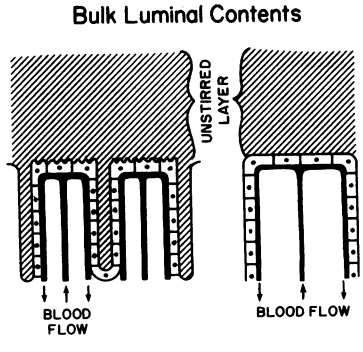

Figure 1. Schematic representation of a mucosa with villi and microvilli and a flat mucosa. If the membrane offers negligible resistance to absorption of a solute (such as is the case with CO), absorption rate will be determined by the thickness of the diffusion barrier separating blood and bulk luminal contents. This thickness is roughly similar for each of the schematic models.

Lastly, we utilized the ability of CO to exist either as a gas or as a solute dissolved in saline. The underlying rationale of the technique employed is that there is a nearly negligible unstirred layer during absorption of CO from a gaseous infusate. Therefore, CO absorption from the gaseous infusate measures epithelial cell resistance. On the other hand, CO absorption from a liquid infusate measures unstirred layer plus epithelial resistance. Thus, the difference in the rates of CO absorption from gaseous and liquid infusates provides a unique, quantitative measure of the permeability of the unstirred layer to CO absorption. In contrast, it is difficult to distinguish epithelial cell from unstirred layer resistance for other solutes whose absorption can only be measured from the liquid phase.

Several independent lines of evidence support the concept that stripping of the gut removes virtually all luminal fluid (and does not damage the bowel in the process), so that negligible fluid remains to produce an unstirred layer when a gaseous infusate is instilled into the gut. First, saline containing ^{14}C-inulin was allowed to equilibrate with luminal contents and was then stripped from the gut. Based on the ^{14}C-inulin remaining in the small bowel or colon after stripping, sufficient fluid remained to produce a layer of mean thickness of only ~10 μm over a smooth luminal surface. This remaining fluid layer was negligible in comparison to the 250–400 μm unstirred layers which were measured during absorption from liquid infusates. This layer is also negligible when compared with the 30 μm thick tissue water layer of the epithelial cell which has a diffusion coefficient for CO of ~⅓ that of saline. A second line of evidence that minimal unstirred layers exist during absorption of CO from a gaseous infusate was provided by experiments in which a saline solution containing fluorescenated dextran was allowed to equilibrate with luminal contents. This solution was then stripped from the gut. Examination of frozen cross sections for fluorescence showed only a thin (usually <10 μm) layer covering the mucosa. The third piece of evidence derives from calculation of the thickness of tissue which would have been required to yield the observed absorption of CO from the gas phase, assuming the surface of the gut to be smooth. This calculated thickness of tissue should roughly equal the measured thickness of the epithelial cell if no unstirred layer exists during absorption from the gas phase. The tissue thickness that would have produced the observed absorption rate in the small bowel was ~30 μm and in the colon ~40 μm. For the jejunum, this value was roughly equal to the actual measured height of the epithelial cells (34 μm). For the colon, the calculated tissue thickness (40 μm) was slightly greater than the measured height of the epithelial cell (31 μm), a result which may be explained by the innumerable crypts which stud the colonic mucosa.

Evidence that the gut was not injured by the stripping procedure was provided by both anatomical and physiological studies. No anatomical differences were apparent when longitudinal sections of control and stripped gut segments were compared, and glucose absorption was similar in stripped and control segments of jejunum.

Based on the above evidence, we conclude that stripping the gut removes virtually all luminal fluid without injury to the gut mucosa. If there is negligible luminal fluid during absorption from the gas phase, it follows that the resistance to CO absorption must be provided solely by the thickness of the epithelial cells of the mucosa. CO absorption from saline is determined by the resistance of the unstirred layer and mucosa in series. The difference between these two resistances yields a direct measure of the resistance of the unstirred layer.

The resistance of the unstirred layer is expressed conventionally in terms of the mean thickness of water which would produce the observed resistance to absorption. Such a thickness is readily calculated from the resistance of the unstirred layer, provided its surface area is known. Presumably, the cross-sectional surface area of the unstirred layer can be considered to be approximately equal to the surface area of a smooth cylinder which has the dimensions of the luminal surface of gut.

Table I indicates that the resistance to CO absorption from the gas phase in the jejunum was only ~25% less than that of the colon, a result to be expected if the resistance of both organs is provided by a functionally flat surface of epithelial cells of roughly similar thickness. If the villi appreciably increased the functional absorptive surface area of the small bowel, resistance should have been much less than colonic resistance.

As shown in Table I, the resistance of the unstirred layer in small bowel and colon under the conditions of our study was roughly 3–4 times greater than that of the mucosal epithelial cell. The resistance of this unstirred layer to any other compound can be calculated from the diffusion constant of the compound in saline, $(R_{\mu l})_{CO}/(R_{\mu l})_x = (D)_x/(D)_{CO}$.

The mean thickness of the unstirred water layer which would have yielded the observed absorption rate of CO from saline (assuming the surface area to be smooth cylinder) for the 3-min study period was ~400 μm for the small bowel and 240 μm thick for the colon. It should be stressed, however, that these values vary with the length of the absorption period. Thus, the calculated mean thickness of the unstirred layer was only 240 μm for the jejunum when the absorption period was 1 min in duration. For a gut with the dimension of the rat small bowel or colon, there is a continuing depletion of CO from the outer layers of luminal contents, with increasing length of absorption period producing a steadily increasing diffusion barrier to absorption. For example, for the first few seconds of the absorption period, CO must diffuse only a very short distance to reach the mucosa, and for this period the apparent unstirred layer would be very thin. With increasing length of absorption period, CO must diffuse greater and greater distances to reach the mucosa, as the most external layers of saline are depleted of CO. Because of the cylindrical shape of the gut, CO molecules near the center of the lumen have to diffuse out into greater and greater volumes of unstirred layer. As a result, these centrally located molecules diffuse through an unstirred layer whose apparent "thickness" may be many times greater than the linear distance between the center of the lumen and the surface of the gut.

A more rational way to assess the mixing of luminal contents in the gut in our study is to compare the observed absorption rate with that which would be expected from a cylinder with dimensions of the rat gut if there were no mixing of luminal contents and if there were perfect mixing (no unstirred layer). Using Eq. 8, it is possible to calculate the percentage absorption of CO which would be expected if there were no stirring of luminal contents. Fig. 2 shows the relationship between length of absorption period and predicted absorption rate for a cylinder which has the dimensions of the rat small bowel. For 1 and 3 min absorption periods, 48 and 71% CO would be expected to be absorbed if there were no luminal stirring. The actual observed absorption percentages, 46 and 69% at 1 and 3 min, were virtually identical to these predicted values. For comparison, Fig. 2 also shows the predicted absorption rate for the rat jejunum if there were perfect mixing (i.e., no unstirred layer). With perfect mixing of luminal contents, the 69% absorption of CO observed over a 3-min absorption period would have occurred in just 33 s. Thus, the luminal contents of the small bowel of the rat (under the conditions of our study) were virtually unstirred. The diameter of the colon was nearly the same as that of the small bowel and the expected absorption rates for CO for mixed and

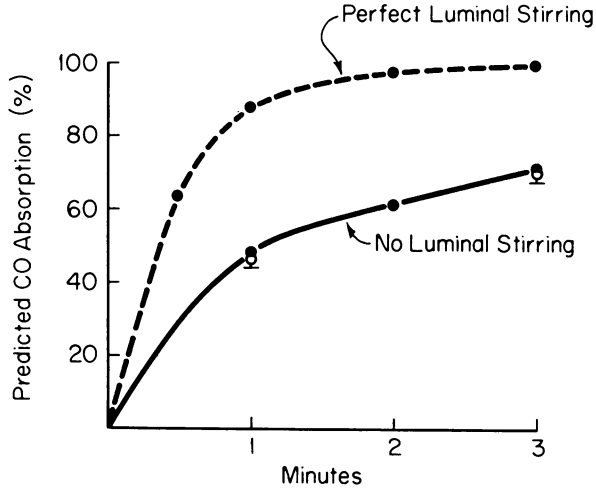

Figure 2. Predicted percentage absorption of CO from the rat gut for 1-, 2-, and 3-min absorption periods. The solid line shows predicted absorption if there were no luminal stirring, and the dotted line indicates CO absorption with perfect stirring. The mean±1 SE of the observed CO absorption rates at 1 and 3 min are indicated by the open circles.

unmixed luminal contents should be roughly similar to that shown in the plots for the small bowel (Fig. 3). The observed CO absorption at 3 min (75%) was somewhat greater than would be predicted with no luminal stirring (68%), but only ~⅓ as fast as would have occurred with perfect stirring. This better luminal mixing in the colon probably resulted from the segmenting muscular contractions which were regularly observed in the colon, while the small bowel showed no such movement. While the bowel was filled with a volume which appeared to produce moderate, uniform distension, it is possible that a lesser degree of filling would have been associated with better luminal mixing.

Although virtually no stirring occurred in the small bowel of the rat, an intestine which has the diameter of that of the rat and a several hour transit time requires no stirring for complete absorption of a compound which has the water and lipid permeabilities of that of CO. For example, with no luminal mixing, >95% absorption of CO would occur in 10 min (see Fig. 3).

However, luminal mixing becomes increasingly important to the absorption process as the diameter of the gut increases or the diffusion coefficient of the compound in the unstirred layer decreases. Fig. 3 plots predicted CO absorption against length of absorption period for bowel segments with radii of 0.17 cm, 0.32 cm, and 0.56 cm, which, respectively, roughly represent the dimensions of the jejunum of the rat, rabbit, and dog which we have used in previous absorption studies. With no luminal mixing, 95% absorption of CO in the rabbit would take ~35 min, and in the dog ~87 min. Fig. 4 shows similar plots for a molecule whose uptake is limited solely by diffusion in water (i.e., like CO), but which has a diffusion coefficient in water of 7×10^{-6} cm^2/s (roughly the diffusion coefficient of sucrose or decanol) rather than the 2.7×10^{-5} cm^2/s diffusion coefficient of CO. While 95% absorption would occur in 40 min in the unstirred rat gut, such absorption would require ~3 h in the rabbit and 7 h in the dog. Thus, some luminal stirring is required for complete absorption during transit through the small bowel when the luminal diameter becomes great and the diffusion coefficient of the substance in water is low.

It should be noted that the influence of the thickness of the unstirred layer on the absorption process is maximal for

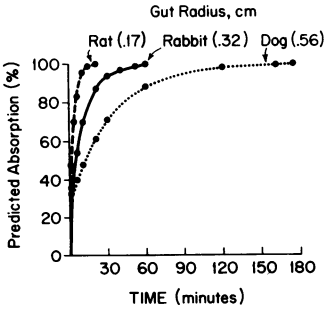

Figure 3. Predicted absorption rate of CO from the intestinal segments with the radii of the rat (0.17 cm), rabbit (0.32 cm), and dog (0.56 cm) if there were no luminal stirring.

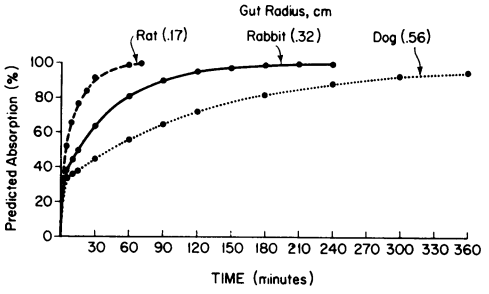

Figure 4. Predicted absorption rate of a solute with high membrane permeability and a diffusion coefficient of 7×10^{-6} cm^2/s when there is no luminal stirring. Values are shown for the jejunum of the rat, rabbit, and dog.

compounds (like CO), where the membrane offers negligible resistance to uptake. As brush border transport becomes the rate-limiting step in absorption, the importance of the unstirred layer is decreased. Thus, for most biologically important substances, the data in Figs. 2–4 are maximal estimates of the importance of luminal stirring to uptake.

It is difficult to compare our measurements of luminal stirring using CO as a probe with other published measures of this diffusion barrier. The literature on unstirred layers in the gut suggests that the osmotic transient technique is the "gold standard" against which all other techniques should be compared. While this technique has been used extensively in in vitro studies, there are problems with the application of the osmotic transient method to the in vivo situation. In this technique, measurement of the potential difference (PD) across the gut is obtained before and after a rapid change in the osmolality of the fluid bathing the luminal surface of the gut. The ½ time required to reach a new steady state PD is used to calculate the unstirred layer thickness. At unstirred layers of several hundred micrometer thickness, this ½ time is quite short. Thus, it is necessary to change the osmolality of luminal solutions very rapidly so as to have a precise measure of the point in time at which the gut is exposed to a new osmolality. Westergaard and Dietschy (1) solved this problem in the in vitro situation by attaching a sheet of gut mucosa to an instrument which could be moved rapidly from a solution of one osmolality to another.

While the osmotic transient technique has been applied to the in vivo situation (11), this application seems questionable because of the inability to rapidly switch bathing solutions. For example, Read et al. (11) perfused a human gut segment in vivo with a solution of one osmolality and then switched to a solution of another osmolality. It would take an appreciable length of time for the second perfusate to wash out the first perfusate, and a gradual shift in the osmolality of bulk luminal contents would occur as the second perfusate moved down the gut, a complex situation from which to analyze ½ time measurements of potential shifts. One could get around this problem by stripping all luminal fluid before infusing the

second solution. This would, however, remove all of the unstirred layer, and thus measurement of ½ time would be meaningless. Thus, the results of the osmotic transient technique in vivo may reflect the method used to change solutions as well as the unstirred layer that exists in the steady state situation. Despite the above problems, Read et al. (11) measured the apparent unstirred layer in the human gut to be ~600 μm, which is a value close to that reported by other workers (12, 13) using different techniques in other species.

There also might be a problem with solution switching in the in vitro situation. In the process of moving the mucosa from one solution to the next, a certain quantity of the initial solution must adhere to the mucosa to provide an unstirred layer. (If no fluid adhered, the new steady state PD should be reached instantaneously, and no unstirred layer would be measured.) Thus, the upper limit of the thickness of unstirred layer which can be measured by this technique may be determined in part by the thickness of the initial fluid layer which adheres to the mucosa. In a totally unstirred beaker, Westergaard and Dietschy (1) reported that the unstirred layer was only ~300 μm, and this unstirred layer decreased by only ~50% with vigorous stirring. While there may have been a small amount of mixing due to convection in the unstirred beaker, it seems unlikely that the unstirred layer could have been only 300 μm thick, particularly in view of the 400–600 μm thickness of unstirred layers measured in the jejunum in the present study as well as by others (12, 13). Thus, it seems possible that the finding of 300 μm unstirred layer in the unstirred beaker might be an artifact of the osmotic transient technique, in that this value was limited by the thickness of the initial solution which adhered to the mucosa during transfer to the second solution. Such an artifact possibly could explain an observation made by Westergaard and Dietschy (1) concerning the uptake of decanol, a compound whose uptake was said to be limited by diffusion through the unstirred layer. Vigorous stirring of the luminal solution yielded a roughly eightfold increase in the uptake of decanol but only about a twofold decrease in the unstirred layer thickness measured with the osmotic transient technique. If decanol uptake were limited by the unstirred layer, the increase in uptake should have been only twofold (commensurate with the halving of the unstirred layer). The authors explain this discrepancy by proposing that stirring increased the surface area of the unstirred layer by fourfold. Another possible explanation could be that the unstirred layer measurement was artifactually low in the unstirred state. Thus, the change in unstirred layer thickness with stirring was actually much greater than measured.

Winne (12) employed a technique in the rat in which air bubbles were infused into the small bowel to induce mixing. The observed increase in absorption rate of 28% for urea and 64% for antipyrine yielded a minimal estimate of unstirred layer thickness of ~500 μm thickness. The lesser increase in absorption rate observed with these compounds with stirring relative to that observed with CO (CO absorption rate increased 400% when the unstirred layer was removed) is explained by the lesser permeability of the brush border membrane for these compounds as compared to CO. This represents an advantage for measurements of the unstirred layer using CO, since the thickness of the unstirred layer is determined by differences in absorption rates between the unstirred and stirred states. In addition, the possible effects of bubbles on the gut surface area in contact with fluid is difficult to calculate. Presumably this bubble technique could not be applied to studies of animals with large calibre intestines, such as dogs or rabbits.

Smithson et al. (13) calculated the unstirred layer in the small bowel of rats by comparing the apparent K_ms for the hydrolysis of sucrose, lactose, and a peptide (gly-leu-gly-gly) in vivo with the K_ms of brush border preparations. From the higher apparent K_m for the in vivo gut, an unstirred layer of ~600 μm was calculated. The authors concluded that an unstirred water layer of this thickness is impossible, since such a layer would indicate that nearly "½ the volume of rat intestinal lumen is occupied by the diffusion barrier", and thus, they postulated a mucous barrier to diffusion of the disaccharides and the peptides. However, based on our calculations of diffusion from a cylinder to its edges, such an unstirred layer thickness seems quite possible, and no mucous barrier need be postulated. In addition, a recent letter by DeSimone (14) commenting on the report of Smithson et al. (13) also points out that an unstirred layer of 600 μm could readily exist in the rat intestine. Application of this technique is somewhat more arduous than the CO method, since calculation of the apparent K_m requires absorption measurements from series of solutions that contain different concentrations of the probe molecule. Unstirred layer measurement with CO requires merely measuring CO absorption rate from the aqueous and gaseous phases.

Lastly, there may be compounds other than CO whose uptake is limited solely by the unstirred layer, and thus can be used to measure the resistance of the unstirred layer. For example, if the active transport of a molecule at the brush border were very rapid relative to diffusion through unstirred layers, passage through the unstirred layer would be the rate-limiting step in absorption. However, it seems unlikely that any compound is actively transported at a rate sufficient to make diffusion through the unstirred layer the limiting step in absorption. For example, in the present study, glucose was absorbed from a 100 mg/dl solution at a rate of <⅓ that of CO from the gas phase. Only a fraction of this discrepancy is accounted for by the difference in diffusion constants of CO and glucose in the unstirred layer, but rather it is due to the fact that the active transport of glucose is slower than the passive diffusion of CO across the gut epithelium.

Compounds other than CO which are passively absorbed might also serve as probes to measure unstirred layer thickness. Presumably, binding or metabolism of these compounds would have to occur in the epithelium or the blood in order to render uptake diffusion limited and avoid problems with countercurrent exchange. Westergaard and Dietschy (1) carried out in vitro studies which suggest that the uptake of the higher

alcohols (such as decanol) is limited solely by diffusion through the unstirred layer, while uptake of lower molecular weight alcohols (with lower lipid/water partition coefficients) seems to be limited by tissue uptake as well as diffusion through the unstirred layer. This observation is surprising since, as discussed previously, the cell membrane is thought to offer negligible resistance to the uptake of alcohols with lipid: water solubilities in the range of butanol. It seems possible that the increased tissue uptake rate of higher alcohols might be attributable to binding of these alcohols in the tissue, or, perhaps, merely dissolution in tissue lipid. This possibility is supported by our calculations of the tissue water concentrations of decanol that were achieved in the study of Westergaard and Dietschy (1), which apparently reached levels of three times that of the incubation solution. If such tissue binding of decanol is not readily saturable, uptake of this alcohol could be a useful probe for measurement of unstirred layers in vivo.

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