Quantitation of Countercurrent Exchange during Passive Absorption from the Dog Small Intestine

EVIDENCE FOR MARKED SPECIES DIFFERENCES IN THE EFFICIENCY OF EXCHANGE

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ABSTRACT The present investigation was designed to quantitatively assess the possible influence of countercurrent exchange on passive absorption from the small intestine of the dog. Villus blood flow was measured with a modification of the microsphere method. Simultaneously, the absorption from the gut lumen of five diffusible gases (H_2, He, CH_4, ^{133}Xe, and CO) was determined. Villus blood flow averaged 0.247±0.03 (SEM) ml/min per g. The observed absorption of H_2, He, CH_4, and ^{133}Xe was only 16.2±1.8, 12.8±2.3, 12.0±1.8, and 15.8±1.4%, respectively, of what this villus blood flow could carry away if it reached perfect equilibrium with the luminal gases. This low absorption rate could result from diffusion limitation to absorption or countercurrent exchange. The diffusive permeability of the barrier separating the luminal gases and villus blood flow was assessed by measuring the absorption rate of CO. Because absorbed CO binds tightly to hemoglobin, it cannot exchange, and when present in low concentrations its uptake is entirely diffusion limited. Knowledge of the diffusion rate through tissue of the unbound gases relative to that of CO made it possible to calculate the degree to which each of the unbound gases should equilibrate with villus tip blood. The percentage equilibration between lumen and blood at the villus tip for H_2, He, CH_4, and ^{133}Xe was 99.7, 99.9, 75.6, and 36.0%, respectively. Each of these values greatly exceeded the percentage equilibration of blood leaving the villus (calculated from the observed absorption rate and villus blood flow) and indicated an exchange of 83.8, 87.2, 84.1, and 56.1% of initially absorbed H_2, He, CH_4, and ^{133}Xe. This result is in accord with theoretical calculations which suggest that countercurrent exchange should be exceedingly efficient in the dog.

The striking effect of countercurrent exchange on passive absorption in the dog differs from our previous studies in the rabbit where no exchange was demonstrated. This marked species difference may result from anatomical differences in villus architecture. The dog has long, densely packed villi while the rabbit has broad, widely spaced villi. In the dog, only the villus tips may equilibrate with the lumen, hence a countercurrent gradient may be established in the villus. The entire villus of the rabbit may equilibrate with the lumen and no gradient for countercurrent exchange can therefore be established.

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INTRODUCTION

The blood vessels supplying the villi of the small bowel form a hairpin curve so that the entering arterial vessels run close to the departing venous vessels for a relatively long distance (1). Because of this arrangement, it has been postulated that countercurrent exchange takes place within the villi (2). Such exchange would decrease the rate of absorption of readily diffusible substances from the gut lumen because a fraction of what enters the blood at the villus tip would diffuse back to the arterial vessels. Likewise, such an exchange would slow the rate of delivery of blood borne, diffusible substances to the villus tip since some of the material arriving in arterial blood would be shunted across to venous blood. Swedish investigators have employed a variety of

ingenious techniques to document the existence of such exchange in the cat (3–5).

To quantitate the influence of countercurrent exchange on absorption, one must compare the concentration of an absorbed substance in villus tip blood with the concentration of this substance in blood leaving the villus. In the present study performed in the dog, we simultaneously measured villus blood flow with microspheres, and the absorption rate of a series of five gases. These gases included four inert gases which should exchange freely and carbon monoxide (CO) which will not exchange because of its tight binding to hemoglobin. In addition, this binding causes CO uptake to be diffusion limited and, hence, absorption of CO can be employed to determine the diffusive permeability of the gut analogous to measurement of the diffusion capacity in the lung. Knowledge of this diffusive permeability and villus blood flow made it possible to then predict the concentration attained by the unbound gases in villus tip blood. These values were two to eight times greater than the concentration of the gases in blood leaving the villus and, hence, there is an efficient countercurrent exchange for inert gases in the dog.

METHODS

Adult, mongrel dogs weighing between 12 and 16 kg were fasted for 24 h and then anesthetized with intravenous sodium pentobarbital. A cardiac catheter was passed through one femoral artery, positioned in the left ventricle by pressure monitoring, and attached to a three-way stop cock. A second polyethylene catheter was inserted 4 cm into the opposite femoral artery and was connected via a three-way stop cock to a pressure transducer for the purpose of monitoring arterial blood pressure. Each catheter was flushed periodically with heparinized saline. Throughout each study the animal's temperature, respiration, pulse, electrocardiogram, and arterial blood pressure were monitored.

Gas absorption studies. The rates of absorption of the five gases, CO, He, H_2, CH_4, and ^{133}Xe were determined by measuring their rate of disappearance from isolated segments of jejunum by a previously described method (6). Briefly, through a midline abdominal incision, a 30-mm segment of midjejunum was tied off at both ends with umbilical tape, with care taken to preserve the blood supply. Cannulas attached to three-way stop cocks were tied into the lumen at either ends of the gut segment and the segment was carefully washed clean by perfusing it with warm saline.

In most studies the gas mixture consisted of 5% CO and trace quantities of ^{133}Xe, the remainder consisting of equal parts of H_2, He, and CH_4. To study the relation between P_{CO} and CO absorption, additional studies were performed with mixtures containing 20% CO. All absorption studies were carried out for 30 min.

At the beginning of each absorption period, 15 ml of the mixture of test gases was instilled into the gut segment which was then placed back into the peritoneal cavity. At the end of the absorption period all gas remaining in the loop was stripped into a 20-ml syringe and its volume was recorded. The gas was then quantitatively transferred to a 100-ml syringe and, to insure complete washout of residual gas in the loop, an additional 80 ml of air was perfused through one cannula and collected in the 100-ml syringe.

The fractional absorption rate (percent per minute) for each gas was calculated from the volume of the gas absorbed divided by the logarithmic mean volume of the gas present in the lumen and the time interval of the study. The logarithmic mean gas volume of the loop was calculated from the initial volume (15 ml) and the final measured volume.

Determination of the diffusion rates of the unbound gases relative to that of CO. The rates of diffusion of the unbound gases through small intestinal tissue relative to that of CO was determined by a previously described method (7). Briefly, a mixture of CO plus the four other gases was placed in the lumen of a closed segment of rabbit jejunum. The segment was then rapidly dissected and placed in a stoppered flask containing Krebs-Ringer bicarbonate previously gassed with 95% O_2-5% CO_2. The rates of diffusion of each gas relative to that of CO was then determined by comparing the concentration of the gases appearing in the flask with the concentrations in the original gas mixture placed in the gut segment.

Rabbit, rather than dog jejunum, was employed for two reasons. First, the diffusion rate through the thick bowel wall of the dog was so slow that accurate measurements were difficult to obtain. Second, we were primarily interested in the diffusion rate through mucosal rather than muscular tissue. A much greater proportion of the rabbit intestinal wall consists of mucosa than is the case with the dog.

Gas analysis. Xenon concentration was determined by injecting 2 ml of gas (ambient temperature and pressure, dry) into an evacuated, stoppered glass test tube. The tube was counted in a scintillation counter to at least±2% accuracy.

The concentration of each of the other four gases was determined with a gas chromatograph equipped with a 2-ml gas-sampling valve, a molecular sieve column, a thermal conductivity detector (for H_2, He, and CO), and a hydrogen flame detector (for CH_4) in series. Argon was used as the carrier gas at a flow rate of 30 ml/min.

Blood flow studies. Blood flow to the gut segment was measured at the midpoint of the absorption period by a modification of the microsphere technique developed by Sircar et al. (8). Hydrocarbon microspheres (7–10 μm in diameter) labeled with strontium-85 (Minnesota Mining, & Manufacturing Co., St. Paul, Minn.) were suspended in saline by sonication and 1 ml, containing $2–4 \times 10^6$ spheres (approximately 15–20 μCi) was injected rapidly into the left ventricle via the cardiac catheter which was then flushed thoroughly with an additional 15 ml of normal saline. Beginning just before the injection of the spheres, blood was constantly withdrawn from the femoral artery at a rate of 5 ml/min for 120 s with an infusion-withdrawal pump.

After sacrifice of the animal the absorption segment was removed and weighed. All of the villi from a preweighed piece of the segment were carefully removed by scraping with a surgical blade and placed in a counting vial. The adequacy of villus removal was checked by examination with a hand lens as well as by examining permanent sections. The remaining villus-free gut and an additional section of unscraped whole gut were weighed and placed in separate counting vials. The radioactivity of each gut sample and of the blood withdrawn from the femoral artery was then determined by a gamma scintillation spectrometer.

Blood flow to each gut sample was then calculated with the formula:

(1) Blood flow/gram tissue per minute

$$= \frac{\text{dpm/gram tissue}}{\text{dpm in femoral artery sample}} \times 5 \text{ ml/min.}$$

The statistical significance of differences was determined by means of Student's t test.

RESULTS

Villus blood flow. Fig. 1 shows a representative photomicrograph of an isolated sample of pure intestinal villi and the remaining, villus-free gut wall. As demonstrated, scraping the surface of the mucosa with a surgical blade resulted in adequate separation of the villus layer from the remaining mucosa.

Villus blood flow averaged (±1 SEM) 0.247±0.03 ml/min per g of whole intestine in 20 studies. The total gut flow averaged 0.468±0.05 ml/min per g.

Observed absorption of inert gases. The observed fractional absorption rate of each of the inert, intestinal gases is shown in Table I. If the gases in the lumen equilibrate with villus blood flow, the absorption rate will be determined by:

$$(2) \qquad Q_X = F\alpha_x P_x$$

where Q_X is the expected absorption rate of gas x, F is the villus blood flow/gram of intestine (measured with microspheres), and α_x is the solubility of the gas in blood (7), and P_x is the partial pressure of the gas in the lumen.

Expression of this relation in terms of fractional absorption rate (C_x) of a gas rather than absolute absorption rate (Q_x) requires the following manipulation. The volume of gas x in the lumen (A_x) equals (VP_x/P_B-47) where V is the total volume (STP) of gas in the loop and P_B is barometric pressure. The predicted fractional absorption rate for gas x can be calculated from:

$$(3) \qquad C_x = \frac{Q_x}{A_x} = \frac{(P_B - 47)\alpha_x F}{V}$$

Fig. 2 compares the observed absorption rate of the unbound gases (Table I) with the absorption rate which

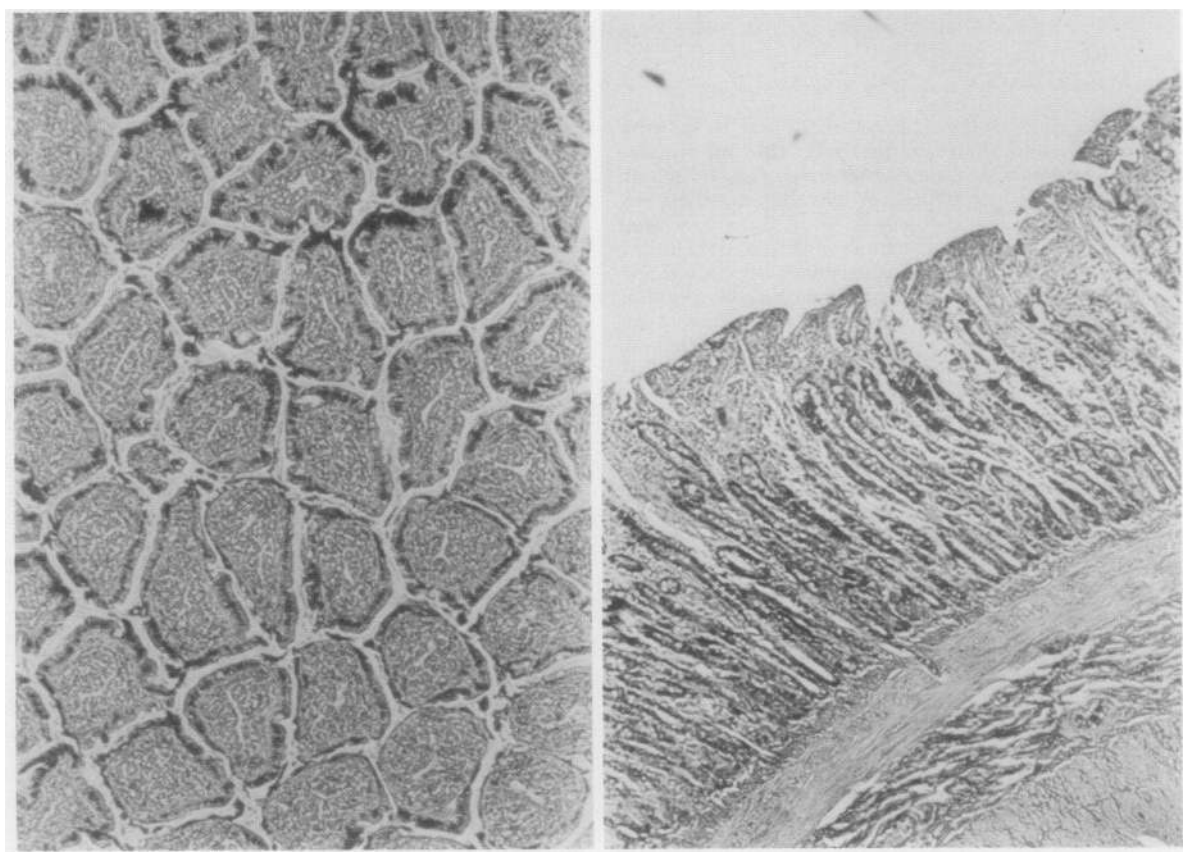

FIGURE 1 Histologic sections of dog small intestine demonstrating separation of villus layer. Left: scrapings from mucosal surface consisting of isolated villi. Right: bowel after removal of villus layer.

TABLE I
Absorption Rate and Relative Diffusion Rate of Gases

Gas	Fractional absorption rate	Diffusion rate in tissue relative to CO (K_x/K_{CO})*
	*ml/min g**	
H_2	0.0039±0.0004 (20)	2.99±0.10 (10)
He	0.0018±0.0005 (20)	2.19±0.08 (10)
CH_4	0.0061±0.0009 (20)	1.58±0.08 (10)
133_{Xe}	0.043±0.0027 (20)	2.00±0.21 (10)
CO	0.046±0.0023 (20)	—

( ), Number of experiments.
* Mean±1 SEM.

would occur if luminal gases perfectly equilibrated with villus flow. The observed absorption of H_2, He, CH_4, and ^{133}Xe was only 16.2±1.8, 12.8±2.3, 12.0±1.8, and 15.8±1.4%, respectively, of what the villus flow could carry away with complete luminal equilibration.

Carbon monoxide absorption. The relation of CO absorption to luminal P_{CO} was studied at an initial P_{CO} of 38 mm Hg and at 152 mm Hg. The fractional absorption rates were not significantly different at these two P_{CO} values averaging 0.046±0.005 and 0.041 ±0.004 (SEM) percent/minute per gram, respectively, in 20 studies.

The fact that raising the luminal CO concentration resulted in a proportional rise in the absolute quantity of CO absorbed indicates that the P_{CO} of the blood must be much less than the saturating value of about 2 mm Hg (9). Thus the blood P_{CO} is negligible with respect to the luminal P_{CO} and absorption of CO is effectively diffusion limited in these studies.

Calculated concentration of gases in villus tip blood. The theoretical rate at which the unbound gases would be absorbed if their uptake were limited only by diffusion (as is the case with CO) was calculated from the observed absorption rate of CO in each study and the diffusion rate of each gas relative to CO (K_x/K_{CO}) (Table I):

(4) Diffusion limited absorption of gas_x

$$= \text{CO absorption (percent/minute per gram)} \times \frac{K_x}{K_{CO}}$$

These values for H_2, He, CH_4, and ^{133}Xe were, respectively, 0.137±0.007, 0.101±0.005, 0.073±0.004, and 0.092±0.007 %/min per g. It should be stressed that these diffusion limited absorption rates are only a theoretical concept since the partial pressure of these gases in villus blood rises appreciably and thus the gradient for diffusion decreases and absorption rate falls. We next compared this theoretical diffusion limited uptake value with the mean rate that each gas could be carried away if there were perfect equilibration between villus blood and the lumen. The diffusion limited rate for H_2, He, CH_4, and ^{133}Xe was, respectively, 5.7±0.3, 7.2±0.4, 1.4±0.1, and 0.46 ±0.03 times greater than the rate at which these gases could be carried away by an equilibrated villus blood flow. It is apparent from the rapid rate of diffusion of the unbound gases relative to their maximal possible removal rate by villus blood flow that the partial pressure of these gases in villus blood will rise and there will be appreciable perfusion limitation to absorption. The percentage equilibration between lumen and villus tip blood for each unbound gas was calculated from formula 5A in the Appendix assuming P_a (the partial pressure of the inert gas in the arterial blood) is zero:

(5) Fractional equilibration

$$= 1 - e^{-\left[\frac{Q_{CO}}{P_{CO}} \times \frac{1}{\alpha_x} \times \frac{K_x}{K_{CO}} \times \frac{1}{F}\right]}$$

Where F is villus flow, Q_{CO} is observed absorption of CO, α_x is solubility of gas (x) in blood, $\frac{K_x}{K_{CO}}$ is the diffusion rate of gas (x) relative to CO. The calculated percentage equilibration between lumen and blood at the villus tip for H_2, He, CH_4, and ^{133}Xe was, respectively, 99.7, 99.9, 75.6, and 36.0%. These values are minimal estimates since countercurrent exchange would result in partial saturation of blood before arrival at the villus tip (see Appendix). Fig. 3 diagrammatically shows the calculated percentage equilibration of blood at the villus tip and the percentage equilibration of blood leaving the villus, (calculated from the observed absorption rate and villus blood flow) and the percentage of the initially absorbed gas which is shunted by a countercurrent exchange mechanism. For H_2, He, CH_4, and ^{133}Xe the percent shunting from the efferent to the afferent blood supply was 83.8, 87.2, 84.1, and 56.1%, respectively.

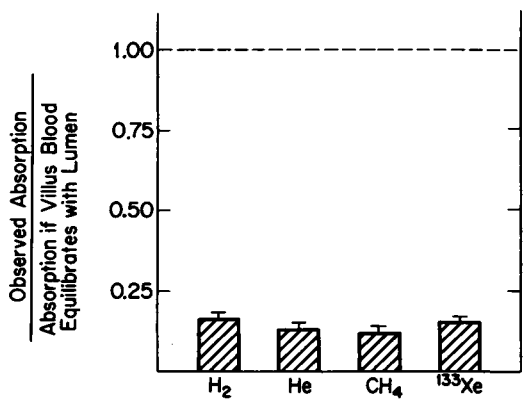

FIGURE 2 Comparison of the observed absorption rates of H_2, He, CH_4, and ^{133}Xe with the absorption that would occur if the gases in the gut lumen perfectly equilibrated with villus blood flow.

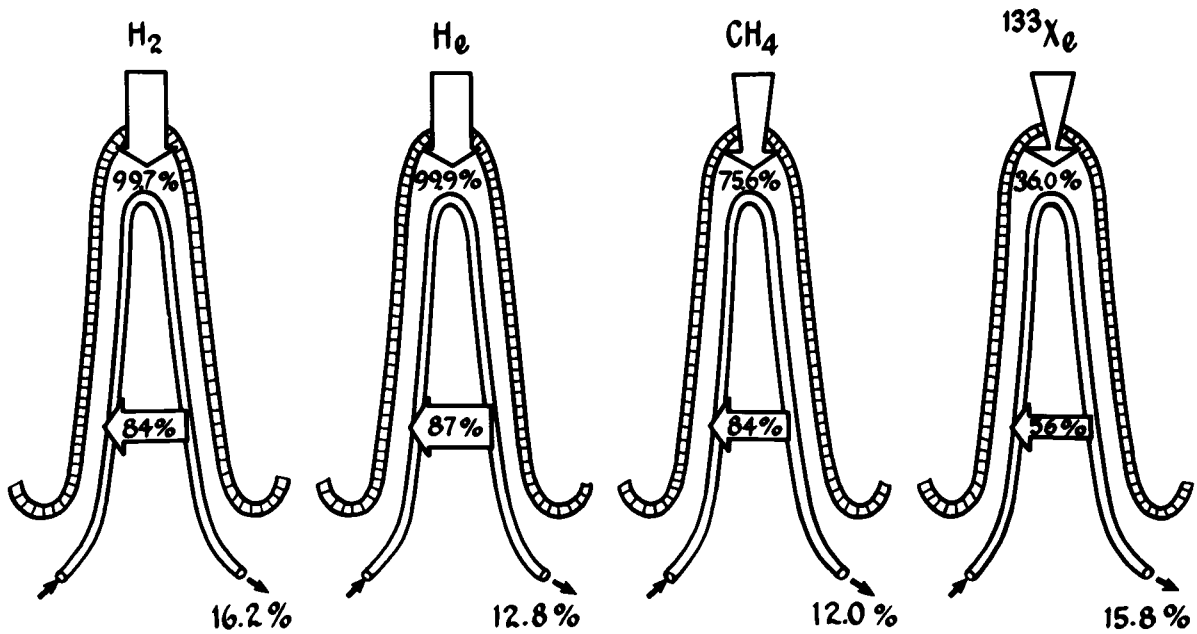

FIGURE 3 Diagram of absorption into the dog villi of H_2, He, CH_4, and ^{133}Xe comparing the calculated percentage equilibration of blood at the villus tip with that of blood leaving the villus, and showing the percentage of the initially absorbed gas which is shunted by a countercurrent exchange mechanism.

Theoretical calculations. Employing certain assumptions concerning the anatomy of the villus architecture it is possible to theoretically calculate the degree to which countercurrent exchange should limit absorption of the unbound gases. In addition, it is possible to calculate the absorption which might be expected from longitudinal diffusion of gas along the long axis of the villus. The model of the villus that will be assumed in this analysis is shown in Fig. 4. The villus has been divided into two regions: a region at the tip of the villus ("absorptive") where exchange of gas between the blood and lumen occurs, and a region ("countercurrent") in which countercurrent exchange between the single central arteriole and the peripheral capillaries and veins occurs. This peripheral capillary and venous network will be approximated by a uniform cylindrical shell of flowing blood. This model is a reasonable approximation to the actual anatomical arrangement (1). It will be assumed that the resistance to exchange is determined by diffusion through the tissue water and that the resistance of the cell membranes (epithelial and endothelial) is negligible. This is a good approximation for these gases since they have appreciable solubility in both lipid and water and the thickness of the lipid layer is only a small fraction of the water layer.

As shown by the calculations in the Appendix, villus countercurrent exchange should theoretically be extremely efficient. The fraction of initially absorbed gas which would be predicted to exchange ($1\text{-}P_v/P_v$, Table II) would be 96.6, 95.6, 87, and 76% for He, H_2, CH_4, and ^{133}Xe, respectively. These values for exchange are somewhat greater than the values determined experimentally (see Fig. 3).

Similarly, the theoretically predicted absorption rate in villus flow (j_F, Table II) is less than the experimentally determined value for each gas (see j_{exp}, Table II and Fig. 3). This discrepancy is particularly large for the rapidly diffusing gases, H_2 and He.

The above discrepancy could be explained by the absorption of gas which is longitudinally diffusing in the villus to deeper blood flows in the crypt region. As demonstrated in the Appendix, such diffusion (j_D, Table II) could account for the major portion of gas uptake of rapidly diffusing gases such as He and H_2. It should be emphasized that the rapid rate that gas reaches the subvillus region by diffusion is an indirect result of countercurrent exchange which sets up the gradient in the villus. The theoretically predicted total uptake rate of gas (j_T) by the villus exchange mechanism and the longitudinal diffusion can be compared to the observed absorption rate (j_{exp}) in Table II.

DISCUSSION

If countercurrent exchange influences the absorption rate of a substance, the concentration of the substance

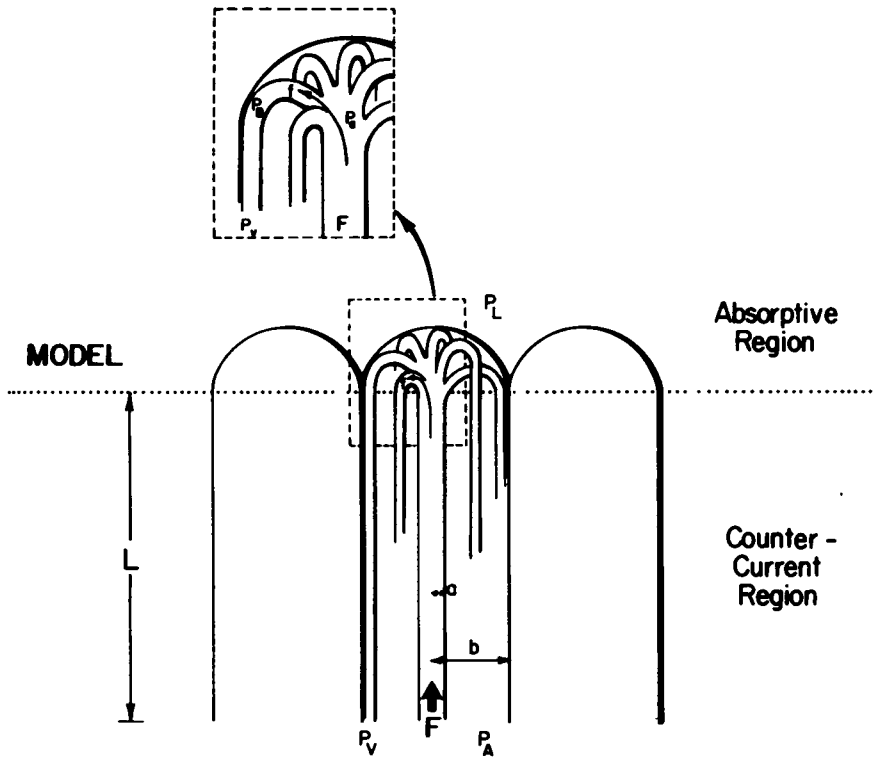

FIGURE 4 Model of the intestinal villus which has been divided into (*1*) an "absorptive region" at the tip where exchange of gas between blood and lumen occurs, and (2) a "counter-current region" where countercurrent exchange between the single central arteriole and the peripheral capillaries and veins occurs. (See Appendix for explanation of notations).

in blood leaving the villi must be less than its concentration in the blood near the villus tip. The difference between these two concentrations provides a quantitative measure of the retarding effect of exchange on the absorptive process.

In the present study we calculated the approximate concentrations of inert gases in blood near the villus tip from measurements of CO absorption and villus blood flow. These concentrations were then compared with the concentrations of the inert gases in blood leaving the villi calculated from the observed absorption rates of the gases and villus blood flow.

Villus blood flow was determined by means of a modification of a microsphere technique developed by Sircar et al. (8). These workers injected unlabeled microspheres (7–10 μm diameter) into the arterial circulation of the rabbit and determined villus blood flow by visually counting the spheres lodged in the villi. The blood supply of the villus consists primarily of a central arteriole which ascends to the villus tip before ramifying into capillaries. Since the diameter of the microspheres is less than that of the arteriole, the spheres pass up the arteriole and can be visually observed to lodge primarily at the villus tip.

The technique of Sircar et al (8), was modified in the present study in that radioactively labeled microspheres were employed. All the villi were sheared off a segment of a gut of known weight and the number of spheres in the villi was then determined by scintillation counting. Examination of the gut segment with a dissecting microscope as well as by fixed microscopic sections showed that nearly all the villi were sheared off at the junction with the crypts (see Fig. 1). While some partially amputated villi might remain, this should have little effect on the flow

TABLE II*

Gas	D	P_V/P_v	P_v/P_L	$j_T = P_V/P_L$	j_D	j_T	j_{exp}
	10^{-5} cm^2/s						
H_2	3.4	0.044	1.0	0.044	0.25	0.29	0.16
He	4.4	0.034	1.0	0.034	0.33	0.36	0.12
CH_4	1.03	0.13	0.96	0.12	0.07	0.19	0.12
133_{Xe}	0.50	0.24	0.70	0.17	0.03	0.20	0.15

* See Fig. 4 and Appendix for explanation of symbols.

measurements since virtually all the villus spheres lodged at the tip.

The observed absorption rates for H_2, He, CH_4, and ^{133}Xe were only 16.2, 12.8, 12.0, and 15.8%, respectively, of the absorption rates that would have resulted had the luminal gases equilibrated with the villus flow. There appear to be only two possible explanations for this surprisingly low absorption rate. First, the unstirred water layer and mucosa separating the lumen and the villus blood flow might cause sufficient diffusion limitation that villus blood only poorly equilibrates with the lumen. The second possibility is that near the villus tip the blood actually comes into close equilibration with the lumen but then loses gas by a countercurrent exchange mechanism as it passes down the villus.

The crucial information required to distinguish between these two possibilities is the diffusive permeability of the barrier that separates the luminal gases and the villus blood flow since this information would permit calculation of the degree to which equilibration is limited by diffusion.

The diffusive permeability of the gut was determined in the present study from the absorption rate of CO analagous to the use of CO to measure the diffusing capacity of the lung. The binding of CO to hemoglobin makes this gas uniquely well-suited for the measurement of the diffusive permeability of layers separating blood and lumen. Carbon monoxide, like the inert gases, passively diffuses across the mucosa and vascular wall. However, upon gaining access to the blood-stream, CO becomes tightly bound to hemoglobin and this binding results in an extremely low P_{CO} in blood relative to its CO content. Since countercurrent exchange is a diffusive process, its rate will be proportional to the partial pressure difference which exists between the limbs of the exchanger. As discussed in a previous paper (6), the low P_{CO} in the efferent blood draining the villus would reduce exchange of CO to negligible levels relative to the rate of exchange of other, unbound substances such as the inert gases. The binding of CO by hemoglobin also insures that at low luminal P_{CO} the absorption of this gas will be entirely diffusion limited since the P_{CO} of the blood will remain negligible relative to that of the lumen. The P_{CO} of villus blood in our experiments can be calculated from knowledge of the villus blood flow and the CO absorption rate. At a hemoglobin concentration of 16 g/100 ml, the observed villus blood flow of 0.25 ml/min per g could bind and remove 0.05 ml of CO/min per g (10). The observed absorption rate of CO was about 0.00035 ml/min per g, therefore the villus blood was about 0.7% saturated with CO. Assuming 50% saturation of hemoglobin and a P_{O_2} of 30 mmHg this percentage of carboxyhemoglobin would be associated with a P_{CO} of only about 0.002 mm Hg (9), which is negligible relative to the P_{CO} of the lumen which averaged about 30 mm Hg. Direct support for the concept that CO was absorbed with entirely diffusion limited kinetics was the observation that a fourfold increase in luminal P_{CO} resulted in an approximate fourfold increase in CO uptake indicating that the absorbing blood was not appreciably saturated with CO.

Since CO uptake is entirely diffusion limited, measurement of CO absorption rate provides a measure of the diffusive permeability of the barrier separating luminal gases from villus blood flow. The inert gases and CO both have appreciable solubility in lipid and water (11). Since the thickness of the water layer is much greater than that of the lipid layer in the absorption barrier, CO and the inert gases should have similar diffusion barriers consisting of the thickness of the water layer which separates blood and luminal contents. The relative diffusion rates of the gases through this barrier were experimentally determined by measuring their rate of diffusion out of closed loops of rabbit jejunum in vitro.

The rate that each of the inert gases would diffuse through the absorption barrier and reach the villus blood flow (providing there was no perfusion limitation) was calculated from the CO absorption rate and the diffusion rate of each of the inert gases relative to CO. However, the gases do not actually reach the villus flow at this calculated rate because the partial pressure of these gases in the blood at the villus tip rises causing appreciable perfusion limitation. The concentration actually attained by each inert gases in villus tip blood was estimated from equation 5. The calculated percent equilibration of villus tip blood with the lumen was 99.7, 99.9, 75.6, and 36.0% for H_2, He, CH_4, and ^{133}Xe, respectively. In contrast, it could be calculated from the observed absorption rate of the gases and villus flow measurements that blood was leaving the villi only 16.2, 12.8, 12, and 15.8% saturated with H_2, He, CH_4, and ^{133}Xe, respectively. Thus approximately 85% of the H_2, He, and CH_4 and 56% of the ^{133}Xe initially taken up by blood at the villus tip was shunted during passage down the villus.

It has been postulated that plasma skimming causes the villi to receive a blood flow relatively poor in erythrocytes. No data are available regarding the possibility that the blood perfusing the villi is similarly relatively poor in microspheres. If this is the case, the microsphere technique might slightly underestimate villus blood flow and the discrepancy between the calculated percentage equilibration of blood at the villus tip and that of blood leaving the villus would be even greater than shown in this paper.

These results in the dog contrast sharply with our previous studies in the rabbit (6). In the latter species

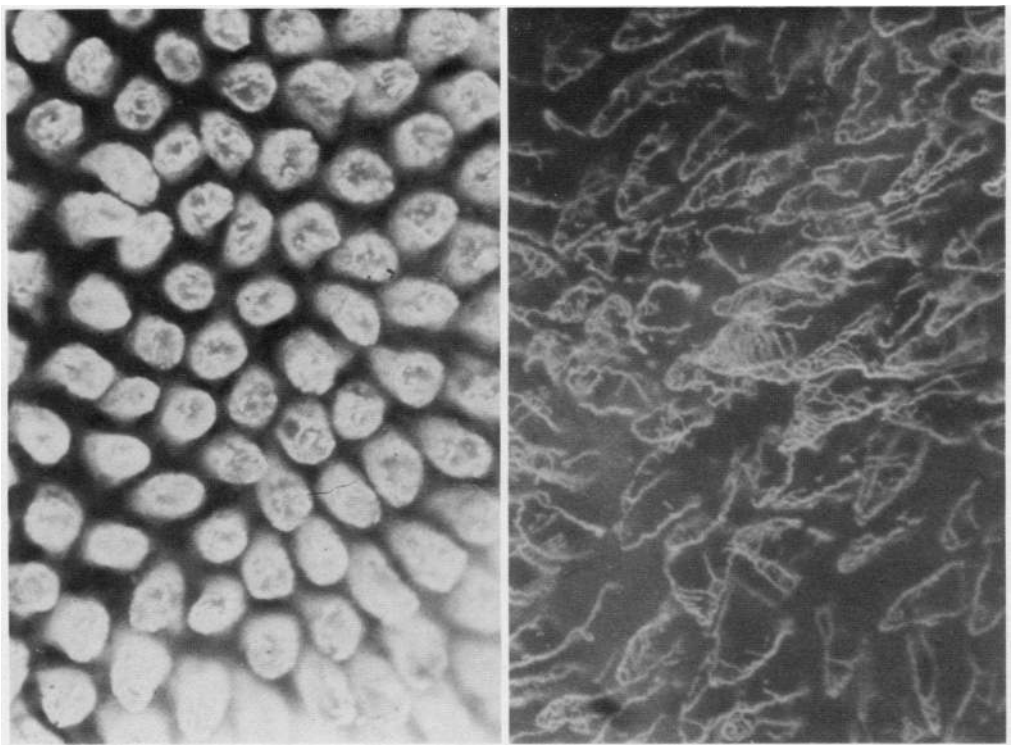

FIGURE 5 Photomicrograph comparing the latex injected small intestinal villi of the dog (left) and the rabbit (right).

villus blood flow per gram of gut measured by microspheres averaged only 0.087 ml/min per g, about ⅓ the value in the dog. However, inert gas absorption (milliliter/minute per millimeter Hg/gram of gut) was 5–10 times faster in the rabbit than in the dog. Whereas in the dog, blood was leaving the villus only about 15% equilibrated with the luminal gas, the flow required to account for the observed gas absorption in the rabbit was several times the measured villus flow. Thus, the luminal gases were presumably saturating not only the villus flow of the rabbits but were also diffusing to deeper blood flows. Since the blood leaving the villus of the rabbit is apparently equilibrated with the lumen, countercurrent exchange does not appear to take place in the villi of this animal.

This striking species difference in countercurrent exchange may result from anatomical differences in villus architecture. The villi of the rabbit (see Fig. 5) are short, broad, and widely spaced. In contrast, the villi of the dog are long, thin, and very closely spaced. When viewed from the luminal surface, only the tightly packed tips of dog villi are visible (Fig. 5). Thus, in the rabbit it seems likely that luminal contents have ready access to a well-mixed intervillus space and hence the gases can diffuse into the villi from the sides as well as the tips. In this situation the entire villus becomes equilibrated with gases in the lumen. Blood entering the villus would become equilibrated as it entered the villi and remain equilibrated as it passed down from the tip to the base of the villus. In contrast, the densely packed villi of the dog could prevent appreciable stirring of the intervillus space and only the villus tips would equilibrate with the lumen. With this arrangement, blood would not become equilibrated with the lumen until it reached the villus tip. A concentration gradient would therefore exist between the efferent and afferent vessels resulting in countercurrent exchange. The villi of man appear to more closely resemble that of the dog than of the rabbit and appreciable exchange is likely to occur in the mucosa of the human gut.

An arrangement in which only the villus tips are exposed to the lumen creates a very efficient countercurrent exchange. For substances which can penetrate the wall of the central arteriole of the villus as demonstrated by the theoretical calculations in the Appendix, only about 3, 4, 13, and 24% of the initially absorbed of He, H_2, CH_4, and ^{133}Xe would be expected to escape exchange and be absorbed by villus blood flow. The absorption rates of CH_4 and ^{133}Xe in villus blood flow (j_F, Table II) predicted from these calculations of exchange were reasonably close to the observed values (j_{exp}, Table II). However, the observed

absorption of H_2 and He, low molecular weight gases with high diffusivities, was much greater than would be predicted from this theoretical analysis of the countercurrent exchanger (see Table II). This discrepancy appears to result from longitudinal diffusion of gases in the villus which permits the absorption of gas by blood flow in the crypt region in addition to that carried away by the blood flow of the villus. As shown in the Appendix, the rate that gases might reach a subvillus blood flow by diffusion is greatly enhanced by the presence of countercurrent exchange in the villus. The exchanger creates a concentration gradient in the villus which markedly reduces the diffusion path to the crypt blood flow.

As shown in Table II, such diffusion (j_D) would be expected to account for a much greater fraction of the absorption of H_2 and He than would the countercurrent exchanger (j_F). On the other hand, for ^{133}Xe, a high molecular weight gas with a low diffusion coefficient, the diffusive component would be expected to account for only about 15% of total absorption (j_T, Table II).

It is apparent from the above analysis that the passive absorption rate of diffusible substances from the small bowel of the dog (and presumably man) cannot be predicted from the simple flow and diffusion limited kinetics that we previously used with success in the rabbit (6). Rather, to predict absorption one must employ a complicated model which takes into account both countercurrent exchange and longitudinal diffusion in the villus.

APPENDIX

The following notations are used for the theoretical calculations of the appendix and Table II:
f, F, F_T: single capillary flow, flow per villus, villus flow per gram intestine; P_A, C_A, P_V, C_V: partial pressure and concentration of gas in arterial blood entering villus and venous blood leaving villus; P_a, C_a, P_v, C_v: partial pressure and concentration of gas in blood entering and leaving absorptive region of villus tip; P_L, $P_B(x)$: partial pressure in intestinal lumen and capillary blood; α, α_B: solubility of gas in tissue and blood; D: diffusion coefficient in tissue; K: αD; q, Q: absorption by one capillary and per gram of intestine; a, b, L: radius of central arteriole and villus and length of countercurrent region of the villus; k: countercurrent exchange resistance; J_F: expected absorption rate if gas were flow limited and no countercurrent exchange; j_F, j_D, j_T: theoretical absorption via blood leaving villus, via diffusion through villus, and total absorption; all relative to absorption rate if flow limited (J_F).

A. *Uptake of gas in exchange region.* The uptake of gas into a single capillary in the exchange region (Fig. 4, inset) is described by the following differential equation:

$$f\frac{dP_B}{dx} = \alpha D\, A(x)/(\alpha_B \Delta[x])(P_L - P_B[x]) \qquad (1A)$$

where x is the distance along the capillary in the exchange regions, P_L and P_B are the partial pressure of the gas in the lumen and blood, α and α_B are the solubilities of the gas in the tissue space and blood, D is the tissue diffusion coefficient, f is the capillary blood flow, and A and Δ are the "effective" exchange area (per unit capillary length) and diffusion distance, respectively, for a given section of the capillary. These last two parameters include the effect of unstirred layers of the lumen. The solution to this equation is given by:

$$P_v/P_L = 1 - (1 - P_a/P_L)\exp(-\alpha DR/[\alpha_B f]);$$

$$R = \int_0^L A(x)/\Delta(x)dx \qquad (2A)$$

where R is the integral area/(diffusion distance) ratio of the capillary (L = capillary length) and is a constant for all the gases, and P_a and P_v are the partial pressures of the gas entering and leaving the exchange area. For CO, the value of P_B in Equation *1A* is negligible relative to P_L^{CO} and the uptake (q_{CO}) of CO by the capillary is diffusion limited and described by:

$$q_{CO} = D_{CO}\alpha_{CO}\, P_L^{CO}\, R \qquad (3A)$$

Thus, solving Equation *3A* for R and substituting into Equation *2A*:

$$P_v/P_L = 1\text{-}(1\text{-}P_a/P_L)\exp(\text{-}[K/K_{CO}]\,[q_{CO}/f]/\alpha_B P_L^{CO}) \qquad (4A)$$

where K is equal to αD. Finally, if it is assumed that the ratio q/f is the same for all capillaries then $q/f = Q/F_T$ where Q is the intestinal CO uptake per gram and F_T is the villus (tip) blood flow per gram. With this assumption, the final equation describing the fractional equilibration of the gas in the exchange region is obtained:

$$P_v/P_L = 1\text{-}(1\text{-}P_a/P_L)\exp(\text{-}B)$$

$$B = K\, Q_{CO}/(K_{CO} F_T\, \alpha_B P_L^{CO}) \qquad (5A)$$

Equation 5A is the maximum gas uptake and will overestimate the actual uptake if the ratio of q/f varies from capillary to capillary. This effect has been discussed in detail in relation to pulmonary diffusion capacity measurements (12). To determine the ratio of P_v/P_L in Equation *5A*, one must know the value of the partial pressure of the gas entering the exchange region (P_a). This value is determined by the degree of countercurrent exchange that occurs and is evaluated in the next section.

B. *Countercurrent exchange.* The exchange between the central arteriole and the peripheral venous shell (Fig. 4) is described by the set of equations:

$$F\frac{dC_V}{dx} = k(C_v - C_a)$$

$$F\frac{dC_a}{dx} = k(C_v - C_a) \qquad (6A)$$

where F is the blood flow per villus, C_v and C_a are the concentrations of the gas in the peripheral shell (referred to as "venous") and the central arteriole, and k is the resistance for exchange and is given by:

$$k = 2\pi\, D/\ln(b/a) \qquad (7A)$$

where a is the radius of the central arteriole and b is the

radius of the peripheral "venous" shell and will be assumed to be equal to the radius of the villus (13). These equations can then be solved for the ratio of the partial pressures of the gas in the venous blood leaving the villus (P_V) to the concentration in blood just entering the countercurrent region (P_v):

$$P_V/P_v = (1 + kL/F)^{-1}, \text{ and } P_a/P_v = 1\text{-}P_V/P_v \qquad (8A)$$

where L is the length of the countercurrent region and it has been assumed that the partial pressure of gas in the arterial blood entering the villus (P_A) is zero. A more general detailed analysis of this countercurrent exchange has been presented by Winne (14). It has been assumed in Equation 6A that the rate limiting process in the exchange is the diffusion through the tissue between the two vascular regions and that the gas that reaches the arteriole instantly equilibrates with the arteriolar blood. An estimate of the error introduced by this approximation is provided by the exact numerical solution for a related problem (15). An examination of this solution indicates that for the conditions in the villus, the additional resistance due to the uptake into the arteriole blood is roughly equivalent to reducing k in Equation 7A by about 20%. Although it is easy to incorporate this effect, a 20% change in k does not change any of the conclusions of this analysis and, considering the other approximations that have been made, is negligible.

The following values have been used to estimate from Equation 8A the degree of countercurrent exchange that occurs. A blood flow per villus of 2.5×10^{-5} ml/villus per min is obtained from the villus blood flow determined in this paper from microspheres (0.25 ml/min per g) and the number of villi (10,000/g).[1] The radius of the villus (b) is about 0.1 mm and the radius of the arteriole (a) is about 10 μm. It will be assumed that the length of the countercurrent region is approximately equal to the length of the villus (1 mm). With these numbers, the value of P_V/P_v determined from Equation 8A have been tabulated in Table II. The values of the tissue diffusion coefficients (D) that were used in this calculation are shown in Table II. The values of D for He and H_2 were obtained by reducing the experimental values for diffusion in water (16) by 30% to correct for solid fraction of the tissue. The values of D for Xe and CH_4 were determined from the value of D for H_2 and the previously determined ratios of K and α (7).

As mentioned above, to determine the degree of equilibration (P_v/P_L) that occurs in the villus tip (Equation 5A) it is necessary to know the value of P_a. Substituting the expression for P_a/P_v (Equation 8A) into Equation 5A, and solving for P_v/P_L:

$$P_v/P_L = (1\text{-}\exp[-B])/(1\text{-}\exp[-B]/[1 + F/kL]) \qquad (9A)$$

where B is defined in Equation 5A. The values of P_v/P_L for the different gases are listed in Table II. Finally, the product of P_v/P_L and P_V/P_v is P_V/P_L which is the fractional equilibration of the blood leaving the villus and is equal to the amount of gas absorbed by the blood relative to what would be expected for the completely blood flow limited case. This ratio is denoted by j_F and is listed in Table II. It can be seen that j_F for the highly diffusible gases He and H_2 is much less than the experimentally observed value (Table II). One explanation for this discrepancy is that there may be significant amounts of longitudinal diffusion in the villus. This effect is considered in the next section.

C. *Longitudinal diffusion in the villus.* The above calculation indicates that there should be an almost perfect countercurrent exchange for the highly diffusible gases H_2 and He. It can be shown from Equation 6A that in this case there should be a linear partial pressure gradient in the villus varying from P_L at the villus tip to approximately zero at the base. There will then be diffusion of the gas down this linear gradient and, therefore, out of the intestinal lumen. The magnitude of this diffusive component of absorption will be estimated in this section. The diffusive absorption per villus (J_D) will be given by:

$$J_D = \pi b^2 \alpha D\, P_L/L \qquad (10A)$$

where it has been assumed that concentration at the villus base is zero (as should be the case for H_2 and He). The absorption per villus that would be expected for a blood flow limited case (J_F) is given by:

$$J_F = \alpha_B F\, P_L \qquad (11A)$$

Thus the magnitude of this diffusive component relative to the flow limited case (j_D) is given by (assuming $\alpha_B = \alpha$):

$$j_D = \pi b^2 D/FL \qquad (12A)$$

The magnitude of this component is shown in Table II. It can be seen that the diffusive transport is the dominant mechanism of transport for the highly diffusible gases.

Finally, the total theoretical absorptive rate (j_T) which is the sum of j_F and j_D is listed in Table II. It can be seen that the agreement between the theoretical values of j_T and the experimental values in this study (j_{exp}) is fairly good and suggest that the model of the villus shown in Fig. 4 has some validity.

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