# Use of Inert Gases to Study the Interaction of Blood Flow and Diffusion during Passive Absorption from the Gastrointestinal Tract of the Rat

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ABSTRACT Measurement of the relative absorption rates of inert gases ($H_2$, He, $CH_4$, $SF_6$, and $^{133}Xe$) was used to investigate the interaction between diffusion and blood flow during passive absorption from the stomach, small bowel, and colon of the rat. If uptake is blood flow limited, the gases should be absorbed in proportion to their solubilities in blood, but if diffusion limited, uptake should be proportional to the diffusion rate of the gases in mucosal tissues.

The observed absorption data were fitted to a series of models of interaction between perfusion and diffusion. A simple model accurately predicted the absorption rates of the gases from all segments of bowel. In this model, gas is absorbed into two distinct blood flows: one which flows in proximity to the lumen and completely equilibrates with the lumen, and a second which is sufficiently rapid and distant from the lumen that its gas uptake is entirely diffusion limited. The fraction of the total absorption attributable to the equilibrating flow can be readily calculated and equalled 93%, 77%, and 33% for the small bowel, colon, and stomach, respectively. Thus the rate of passive absorption of gases from the small bowel is limited almost entirely by the blood flow to the mucosa, and absorption from the stomach is largely limited by the diffusion rate of the gases. The flow which equilibrates with the lumen can be quantitated, and this flow may provide a useful measure of "effective" mucosal blood flow.

## INTRODUCTION

A wide variety of substances are absorbed from the gastrointestinal tract by the purely passive mechanism of nonfacilitated diffusion. The rate of such absorption will be determined by the interaction of three factors: first the rate of blood flow to the mucosa,[1] second, the distance ($L$) between the bulk luminal contents and the mucosal vessels, and third, the rate of diffusion ($D$) of the substance through the unstirred layer and the mucosa.

The limits of this interaction range from complete perfusion limitation to complete diffusion limitation. At one extreme, where $D/L$ is very large relative to blood flow, the concentration of a substance in the blood leaving the mucosa equals its luminal concentration. In this perfusion-limited situation, uptake is proportional to the rate of blood flow and independent of $D/L$. At the diffusion-limited extreme, $D/L$ is very small relative to blood flow, and uptake is proportional to $D/L$ and independent of blood flow. In the intermediate case, uptake will be dependent upon both rate of blood flow and $D/L$.

In this paper, the rate of absorption of a series of gases from the stomach, small bowel, and colon of the rat will be presented. The data will be analyzed in terms of various models of the interaction between diffusion and blood flow. It will be shown that one model provides a good fit to the data and makes it possible to predict the absorption rate of a gas from the stomach, small bowel, or colon, simply from knowledge of its diffusion rate in tissue and solubility in blood. This model defines a unique blood flow for each organ which is equilibrated with the luminal gases. This blood flow may provide a useful measure of "effective" mucosal blood flow.

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[1] "Mucosa" will be used to represent the region of the bowel adjacent to the lumen, where appreciable exchange of gas between the blood and lumen occurs, rather than the anatomically defined mucosa.

## METHODS

### Experimental design

Known volumes of mixtures of gases were injected into isolated segments of the gastrointestinal tract of the rat. The rate of uptake of each gas into the blood was determined by measuring the rate of pulmonary excretion of each gas into a closed system. The blood/air solubility ratios of these gases are such that greater than 90% of each gas should be excreted in a single passage through the lung, and the concentration of the gas in the arterial blood entering the mucosa will be negligible relative to its luminal concentration.[2] Thus, if we assume no metabolism of the gases, under relatively steady-state conditions the rate of pulmonary excretion of each gas will approximate its absorption rate. This study is based on relative absorption rates of gases, and minor deviations from the steady state caused by influx of $CO_2$ or $O_2$ into the lumen would not influence the relative absorption rates.

The possibility that the liver might appreciably influence the measured absorption rates seems unlikely. Hepatic metabolism of gases was ruled out by recovery studies. There is no known mechanism whereby the liver could actively concentrate or store the inert gases. If such a hypothetical storage mechanism did exist, it should become saturated with time. There was, however, no significant difference between the absorption ratio of the gases when measured at 10, 20, and 40 min.

Thus, the influence of the liver on the excretion rate of the gases appears to be limited to the quantity of gas that physically dissolves in hepatic tissues. The rate of equilibration of the liver with portal blood will be determined by the partition coefficient ($\lambda$) of the gases between liver and blood and the rate of blood flow per gram of liver. Although $\lambda$ was not measured in the present studies, the bulk of each of the gases is dissolved in the water phase of blood or liver tissue. Thus $\lambda$ for the gases should be slightly less than 1.0. Even a highly lipid-soluble gas, such as $^{133}Xe$, has a $\lambda$ of 0.74 (2). Hepatic blood flow in the rat is about 1.9 ml/g/min (3). It can be calculated, therefore, that within 3 min the partial pressure of the gases in liver tissue will have reached 95% of their final equilibrated concentrations. Because the $\lambda$'s for each of the gases are roughly equal, similar percentages of the gases will leave the blood and enter the liver, and the absorption ratios will be minimally altered during hepatic equilibration.

After the initial 3–4 min equilibration period, the volumes of gas entering and leaving the liver will be equal under steady condition. At any moment, the liver will contain only about one-half the quantity of gas excreted per minute. Thus changes in the quantity of gas dissolved in the liver due to alterations in the steady state (secondary to changes in blood flow or absorption rate of the gases) will have only a minor influence on quantity of gas excreted over a 10- or 20-min study period. In addition, since the $\lambda$'s of the gases are roughly similar, disturbances in the steady state would cause nearly equal percentages of each gas to leave or enter the liver, and changes in the measured absorption ratios of the gases would be negligible.

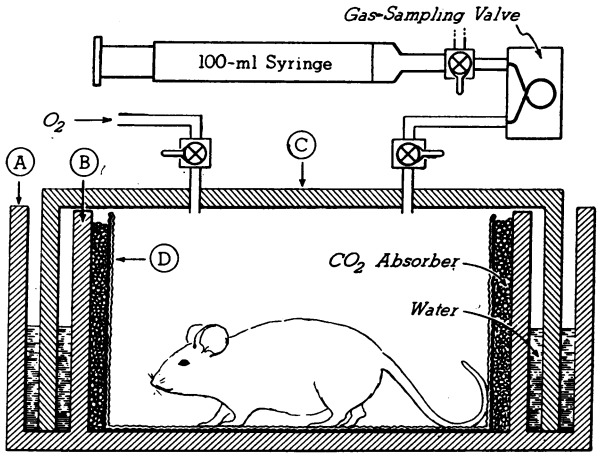


FIGURE 1 Closed system used to collect and sample expired air.

### Analytical techniques

The absorption rates of hydrogen ($H_2$), methane ($CH_4$), helium (He), sulfur hexaflouride ($SF_6$), and xenon-133 ($^{133}Xe$) were studied. Because of the inability to measure $SF_6$ in the presence of $H_2$ and He, two different mixtures of these gases were employed. One mixture consisted of 50% He, 37.5% $H_2$, 12.5% $CH_4$, and tracer quantities of $^{133}Xe$. The second mixture consisted of 12.5% $CH_4$, 87.5% $SF_6$, and tracer quantities of $^{133}Xe$.

$^{133}Xe$ concentration was determined by injecting 2 ml of gas (ambient temperature, 25°–27°C, and pressure, dry) into an evacuated, stoppered glass test tube. The test tube was counted in a Picker Autowell II scintillation counter[3] to at least ±2% accuracy.

The concentration of each of the other four gases was determined using a gas chromatograph equipped with a 2-ml gas-sampling valve, a thermal conductivity detector (for $H_2$, He, and $SF_6$), and a hydrogen-flame detector (for $CH_4$) in series. A 9-foot × ⅜-inch column packed with molecular sieve at a temperature of 105°C was employed. The carrier gas was argon (20 ml/min) for measurement of $H_2$, He, and $CH_4$, and helium (30 ml/min) for $SF_6$ and $CH_4$.

The concentration of each gas was determined by comparison with standard curves constructed from dilutions of the mixture of gases injected into the rat. The standard was always dried and analyzed at the ambient temperature and pressure, as were the samples obtained from the closed system.

*Closed system.* The system was designed to contain a small gas volume yet allow for the removal and subsequent return of the relatively large quantity of gas required to flush the gas sampling valve without appreciably influencing the pressure or volume of gas in the system. The system (Fig. 1) was constructed out of Lucite and consisted of two concentric cubicles

[2] In the steady state, the amount of gas taken up by the blood flowing through the gut segment must equal the amount removed by the lungs: $\lambda F_I(P_I - P_v) = \lambda F_S(P_v - P_a)$ where $F_S$ is the systemic blood flow; $F_I$ is the blood flow to the gut segment; $P_I$, $P_v$, and $P_a$ are, respectively, the partial pressures of the intestinal gas in the intestinal venous blood, pulmonary artery, and pulmonary vein, and $\lambda$ is the partition coefficient. When this equation is combined with the steady-state relation between $P_v$ and $P_a$ (reference 1): $P_v/P_a = 1 + (1/\lambda)(\dot{V}_A/Q)$ (where $\dot{V}_A/Q$ is the ventilation:perfusion ratio) yields the equation $P_I/P_a = 1 + (F_S/\lambda F_I)\cdot(\dot{V}_A/Q)$. Substituting minimum values of 1 for $\dot{V}_A/Q$, 10 for $F_S/F_I$, and a maximum value of 0.1 for $\lambda$, one finds that $P_I/P_a$ has a minimum value of 100. Since $P_I$ must be less than $P_L$ (the partial pressure of gas in the lumen), $P_a$ is negligible relative to $P_L$.

[3] Picker Corp., Cleveland, Ohio.

(A and B) attached to the same base. A box (C), when inverted, would fit between the two cubicles and serve as a lid for the inner cubicle (B). Water served as seal for the inner rat chamber. The rat was placed in a screened box (D) which was surrounded with bara lime as a $CO_2$ absorber. Ultrapure $O_2$[4] (containing negligible quantities of the gases under study) under a pressure of 2 cm of $H_2O$ was added to the system as the pressure in the chamber dropped as a result of $O_2$ utilization and $CO_2$ absorption.

An outlet on the top of the box was connected to the gas-sampling valve of the chromatograph which in turn was connected to a 100-ml syringe. When measurement of the concentrations of the gases in the closed system was desired, the $O_2$ outlet was closed, and with a 100-ml syringe, 80 ml of gas was alternately aspirated and reinjected several times to achieve mixing. This procedure resulted in displacement of water between the outer and inner chamber while the volume and pressure of gas in the closed system remained nearly constant. After the final aspiration, a 4-ml portion was removed from the 100-ml syringe for the $^{133}Xe$ determination, and the remainder of the gas in the syringe was reinjected into the rat chamber. The 2 ml of gas present in the gas-sampling valve was then injected into the chromatograph. Thus, a total of 6 ml of gas was removed from the system for each series of measurements.

The gas space of the closed system, determined from the dilution of a known quantity of He, was 840 ml. The density of the rat was assumed to be 1.00 and, with the rat in the system, the volume of the gas space equalled 840 ml minus the weight of the rat in grams. The weight of the rats ranged from 180 to 230 g.

*Distribution space of gases in the system.* The possibility that appreciable quantities of the excreted gases might be dissolved in the tissues of the rat was directly studied by placing the rat in the closed system, adding a known quantity of gas to the system, and sampling the concentrations of gas in the system at intervals up to 4 h. In the two rats studied, no detectable fall in concentration of He, $SF_6$, or $CH_4$ occurred, although $^{133}Xe$ concentration fell to about 94% of the initial concentration and then remained constant. The volume of distribution of $^{133}Xe$ was therefore considered to be 106% of the gas space of the closed system. Since equilibration with the tissue space was not instantaneous, the correction probably would lead to a slight overestimate of the volume of $^{133}Xe$ excreted.

*Solubilities of gases in blood.* Values for the Bunsen solubility coefficient ($\alpha$) in blood are available in the literature for $^{133}Xe$ (4), $H_2$ (5), and He (6), and were employed. Values of $\alpha$ for $CH_4$ and $SF_6$ in blood were estimated as follows. 20 ml of blood from a fasting human was deoxygenated in a 100-ml syringe by multiple equilibrations with nitrogen. This step, as well as all others, was carried out in a 37°C waterbath. The gaseous $N_2$ was forced out of the syringe and 40 ml of a 4:3:1 mixture of $H_2$, He, and $CH_4$ was introduced. The syringe was agitated for 1 h at 37°C and then the gas phase plus 5 ml of blood was forced out of the syringe. $N_2$ was slowly aspirated into the syringe with agitation at 37°C until a total gas phase of 40 ml was obtained, and the syringe was then agitated at 37°C for 1 h. The concentrations of $H_2$, He, and $CH_4$ in the gas phase were determined by gas chromatography. The solubility of $SF_6$ was similarly determined with the exception that 40 ml of pure $SF_6$ was originally equilibrated with blood.

The Bunsen solubility coefficient ($\alpha$) of each gas was calculated from the formula:

$$\alpha = \frac{40}{15}\frac{P_2/760}{P_1 - P_2}, \text{ corrected for STP,}$$

where the numerator ($40 \cdot P_2/760$) is the volume of gas (in milliliters) removed from 15 ml of blood when the partial pressure of the gas was reduced from $P_1$ to $P_2$.

The Bunsen coefficients calculated for $CH_4$, $SF_6$, $H_2$, and He, respectively, were $0.032 \pm 0.0014$ (1 SD), $0.0056 \pm 0.00051$, $0.016 \pm 0.0016$, and $0.0094 \pm 0.00052$ ml/ml of blood at 37°C. The values obtained for $H_2$ and He by this rather crude technique are similar to those cited in the literature (0.0149 for $H_2$ [5] and 0.0088 for He [6]) and suggest that the $\alpha$'s for $CH_4$ and $SF_6$ are reasonably accurate.

$SF_6$ has a high solubility in lipid (0.21 ml/ml at 25°C [7]) relative to water. The mean fasting plasma lipid of the rat (230 mg/100 ml [8]) is about 400 mg/100 ml less than that of man (622 mg/100 ml [9]), whereas the concentration of lipids in the erythrocytes are roughly equal (10,11). Thus, the observed solubility coefficient of 0.0056 ml/ml for $SF_6$ in human blood was reduced to 0.0051 ml/ml to take into account the decreased lipid content of rat blood.

$^{133}Xe$ solubility has been thoroughly studied and is influenced by the hemoglobin concentration. The hematocrit of the rat was determined at the end of each experiment, and $\alpha$ for $^{133}Xe$ was calculated from the formula of Rochester, Brown, Wichern, and Fritts (4):

$\lambda = 0.0996 + (0.000853 \times \text{hematocrit in percent})$ where $\lambda$ = ml of $^{133}Xe$, at 37°C and 760 mm Hg, dissolved in 1 ml of whole blood.

*Determination of relative diffusion rates of the gases.* The relative rates of diffusion of each gas through small intestinal tissue was estimated as follows. Under ether anesthesia, the distal end of the small bowel of a rat was ligated and a polyethylene tube was tied into the proximal end. The intestine was then rapidly dissected from the rat, 2 ml of a gas mixture was injected into the segment, and the polyethelene tube was sealed. The gut was then placed in a sealed 100-ml flask containing 20 ml of Krebs-Ringer bicarbonate that had been previously gased with 95% O and 5% $CO_2$. The flask was maintained at 37°C, and the fluid in the flask was vigorously mixed with a magnetic stirrer. After 20 min, 50 ml of gas was withdrawn from the flask through the gas-sampling valve into a syringe. This 50 ml of gas was displaced by Krebs-Ringer bicarbonate (equilibrated with 95% $O_2$ and 5% $CO_2$). 4 ml of gas was removed from the syringe for $^{133}Xe$ determination, and the gas in the syringe was then injected back into the flask, displacing Krebs-Ringers bicarbonate. The gas remaining in the gas-sampling valve was injected into the chromatograph. A second analysis of gas concentration was made at 40 min. The relative diffusion rates of the gases were similar at 20 and 40 min, indicating that the intestinal tissue remained intact.

## Recovery studies

The use of pulmonary excretion of gases to measure uptake requires that the gases are not metabolized by the rat. Recovery studies were carried out by injecting known quantities of gas into the small bowel, peritoneal cavity, or colon, and then measuring the volume of gas excreted at intervals over the subsequent 8 h. For the small bowel and peritoneal cavity, recovery was nearly complete (>90%) for each of the gases.

Similar findings were obtained in the colon for He, $SF_6$, $^{133}Xe$, and $CH_4$. However, in each of four rats there was clear-

[4] Matheson Co., Inc., Joliet, Ill.

cut utilization of $H_2$ in the colon, as evidenced by very low recoveries of $H_2$ (less than 40%). For this reason, data for $H_2$ uptake from the colon are invalid for purposes of the present study. This metabolism of $H_2$ in the large bowel, but not the small bowel or peritoneal cavity, strongly suggests bacterial utilization of $H_2$.

## Absorption studies

Rats were deprived of food for 24 h before study to reduce endogenous $H_2$ production and clear the bowel of solid material. On the day of study, the endogenous $H_2$ and $CH_4$ of the rats were determined in the closed system, and only those rats with negligible production of these gases were studied.

Under ether anesthesia, one end of the gut segment to be studied was ligated and a polyethylene tube was tied into the opposite end of the segment. The portions of the gut used were the entire stomach, the upper half of the small intestine beginning just beyond the ligament of Treitz, and the large intestine from just distal to the cecum to the rectosigmoid junction. The polyethylene tube was brought through the incision, which was then closed with sutures, and the rat was allowed to regain consciousness. A gas mixture of known composition and volume was then injected into the segment, the polyethylene tube sealed, and the rat placed in the closed system. These manipulations took less than 1 min. Gas in the system was sampled at 10, 20, and 40 min.

The fractional excretion rate (percent per minute) of a given gas for each interval was calculated by measuring the volume of this gas excreted during that period and dividing by the logarithmic mean volume of this gas present in the gut during that interval. The quantity of the gas excreted during each interval was determined from the change in concentration of the gas excreted into the closed system and the amount initially instilled into the gut segment. Except where noted, the data have been normalized by plotting uptake rate of each gas relative to the uptake of $CH_4$.

## Models of interaction of blood flow and diffusion

The data were fitted to a series of models of the possible interaction between diffusion and blood flow.

*Model I: blood flow-limited case.* In this model, diffusion is so rapid relative to blood flow that blood leaving the mucosa is completely equilibrated with luminal contents. Thus the rate of gas absorption equals $\alpha F P_B$, where $F$ equals rate of blood flow, $\alpha$ is the solubility coefficient of the gas in blood, and $P_B$ is the partial pressure of gas in the blood, which equals its partial pressure in the lumen ($P$). The rate of absorption ($Q$) can then be written as:

$$Q = \alpha F P. \tag{1}$$

If the rate of absorption of gas "$x$" is compared to that of $CH_4$, when the luminal partial pressures are equal (or absorption is expressed as fraction of intraluminal gas absorbed), one finds:

$$\frac{Q_x}{Q_{CH_4}} = \frac{\alpha_x}{\alpha_{CH_4}}. \tag{2}$$

Thus for the blood flow-limited case, if one plots $Q_x/Q_{CH_4}$ versus $\alpha_x/\alpha_{CH_4}$ where $x$ refers to $H_2$, He, $SF_6$, or $^{133}Xe$, all points should lie on a straight line with a slope of 1.

*Model II: diffusion-limited case.* In this model, blood flow is so rapid relative to diffusion that all gas absorption can be considered to be caused by diffusion to a sink where the partial pressure of the gas is negligible relative to its luminal concentration. The absorption rate of a gas will equal its diffusion rate, which is given by $DA(C_1 - C_2)/L$, where $D$ is the diffusion coefficient in tissue, $A$ is the mucosal area, $C_1$ is the concentration of the gas in tissue at the luminal mucosal interface, $C_2$ is the concentration in the sink, and $L$ is the thickness of the diffusion path. $C_1 = \alpha' P$ where $\alpha'$ is the solubility of the gas in the tissue, and, for the diffusion-limited case, $C_2 = 0$. Thus the rate of uptake is equal to

$$Q = \frac{\alpha' A D P}{L}. \tag{3}$$

The ratio of the absorption rate of gas $x$ to $CH_4$ equals

$$\frac{Q_x}{Q_{CH_4}} = \frac{\alpha'_x D_x A/L}{\alpha'_{CH_4} D_{CH_4} A/L} \cdot \frac{P_x}{P_{CH_4}}. \tag{4}$$

The ratio of $(\alpha' x D_x A/L)/(\alpha'_{CH_4} D_{CH_4} A/L)$ equals the ratio of the relative diffusion rates of the gases in tissues $(k_x/k_{CH_4})$. $(P_x/P_{CH_4})$ cancels out when absorption is expressed as fractional absorption rate. Thus, in the diffusion-limited case, a plot of $Q_x/Q_{CH_4}$ versus $k_x/k_{CH_4}$ should give a straight line with a slope of 1.

The relative diffusion rate of the gases $(k_x/k_{CH_4})$ in tissues was determined by measuring the relative rate that gases diffused through the entire thickness of the small bowel in vitro. It is assumed in model II, as well as in models III, IV, and V, that this value approximates the relative diffusion rate of the gases through the tissue and unstirred layer separating the bulk luminal contents, and the absorptive blood flow in each of the three organs. Since the main barrier to diffusion of the gases is simply tissue water, this assumption seems reasonable.

*Model III.* In this model, extensively used by Van Liew (12), it is assumed that blood is uniformly distributed to all the tissues so that there is a continuous interaction between diffusion and blood flow.

If it is assumed that the capillary blood is in equilibrium with the surrounding tissue, then the rate of uptake is given by

$$Q = A\sqrt{\alpha \alpha' F D}\, P. \tag{5}$$

The ratio of the absorption of gas $x$ relative to $CH_4$ is

$$\frac{Q_x}{Q_{CH_4}} = \sqrt{\frac{\alpha_x}{\alpha_{CH_4}} \frac{k_x}{k_{CH_4}}}. \tag{6}$$

If this model is correct, then a plot of $Q_x/Q_{CH_4}$ versus $(\alpha_x k_x/\alpha_{CH_4} k_{CH_4})^{\frac{1}{2}}$ should be a straight line with a slope of 1. Van Liew (12) has modified this model by the introduction of the parameter "$K$" which is equal to the fractional equilibration of the capillary blood with the surrounding tissue. With this modification, the ratio of the rates of uptake is given by

$$\frac{Q_x}{Q_{CH_4}} = \sqrt{\frac{\alpha_x k_x K_x}{\alpha_{CH_4} k_{CH_4} K_{CH_4}}}. \tag{7}$$

It is difficult to test this version of the model because the $K$'s are completely empirical and cannot be predicted theoretically. However, qualitatively, one would expect that $K$ should be about 1 for the gases with the higher diffusion coefficients, i.e., lower molecular weights, and should decrease for the heavier molecules. That is, in the plot above, the gases smaller than $CH_4$ should lie above the line and gases larger than $CH_4$ should tend to lie below it.

*Model IV.* In this model (see Fig. 2A), the mucosal blood comes into complete equilibrium with the lumen, so that the

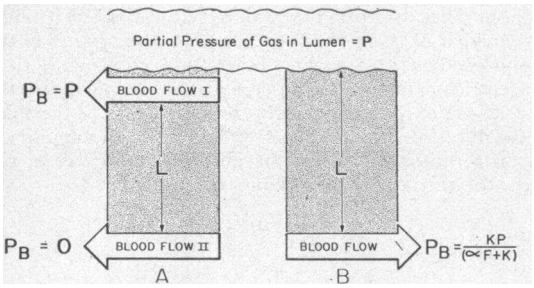


FIGURE 2 Models of interaction of blood flow and diffusion. In *A* (model IV), gas is absorbed into two distinct blood flows. Blood flow I equilibrates with the gases in the lumen, and the partial pressure of each gas in this blood ($P_B$) equals the partial pressure of the gas in the lumen ($P$). Blood flow II is sufficiently rapid and distant from the lumen that the partial pressures of the gases in the blood remains negligible (0) relative to the lumen.

In B (model V) the gases diffuse a distance ($L$) and then all gas is absorbed by a single blood flow. For this flow, $P_B = KP/(\alpha F + K)$ (see text).

concentration of a gas in blood leaving the mucosa equals $\alpha P$, and the rate of removal of the gas by this blood flow is $\alpha PF$. It is further assumed in this model that additional diffusion of gas occurs (without interacting with blood flow) through tissue of width $L$ to a blood flow of sufficient rapidity relative to $D/L$ that the partial pressure of the gas remains negligible. The rate of diffusion is equal to $(C_1 - C_2)AD/L$, where $C_1$ and $C_2$ are equal to the concentration of the gas at the luminal edge of the diffusion barrier and in the sink, respectively.

For this model $C_1 = \alpha' P$ and $C_2 = 0$. Thus the rate that a gas is absorbed by the diffusion-limited portion of this model is equal to $\alpha' ADP/L = kP$ (where $k = \alpha' AD/L$) and the total rate of absorption of gas $x$ is given by

$$Q_x = (\alpha_x F + k_x)P_x. \tag{8}$$

If we divide Eq. 8 by a similar equation for $CH_4$ and note that $P_x = P_{CH_4}$ if $Q$ is normalized for the differing partial pressures in the lumen, we get

$$\frac{Q_x}{Q_{CH_4}} = \frac{\alpha_x F + k_x}{\alpha_{CH_4} F + k_{CH_4}}. \tag{9}$$

To convert this equation to a form that is more suitable for testing as a model, the following simple algebraic manipulations were carried out: first, the denominator on the right is brought into the numerator on the left, and the ratio $\alpha_{CH_4}/\alpha_x$ is factored out

$$\left(F + \frac{k_{CH_4}}{\alpha_{CH_4}}\right)\frac{Q_x}{Q_{CH_4}}\frac{\alpha_{CH_4}}{\alpha_x} = F + \frac{k_x}{\alpha_x}. \tag{10}$$

Then the factor $k_{CH_4}/\alpha_{CH_4}$ is added to and subtracted from the right side and the equation is rearranged:

$$\left(F + \frac{k_{CH_4}}{\alpha_{CH_4}}\right)\left(\frac{Q_x\alpha_{CH_4}}{Q_{CH_4}\alpha_x} - 1\right) = \frac{k_x}{\alpha_x} - \frac{k_{CH_4}}{\alpha_{CH_4}}. \tag{11}$$

Finally, both sides are divided by $k_{CH_4}/\alpha_{CH_4}$ to obtain the final expression

$$\left(\frac{F\alpha_{CH_4}}{k_{CH_4}} + 1\right)\left(\frac{Q_x\alpha_{CH_4}}{Q_{CH_4}\alpha_x} - 1\right) = \frac{k_x\alpha_{CH_4}}{k_{CH_4}\alpha_x} - 1. \tag{12}$$

In Eq. 12, $Q_x/Q_{CH_4}$ is the simultaneously measured ratio of the absorption of the two gases, and $k_x/k_{CH_4}$ is the ratio of the diffusion rates of the two gases through the tissue measured in the nonperfused gut segments studied in vitro. The only unknown is the single parameter, $\alpha_{CH_4}F/k_{CH_4}$, which is the ratio of rate of removal of methane by the blood flow-limited mechanism to the rate of removal by the diffusion-limited mechanism. If this model is correct, a plot of $(Q_x\alpha_{CH_4}/Q_{CH_4}\alpha_x) - 1$ vs. $(k_x\alpha_{CH_4}/k_{CH_4}\alpha_x) - 1$ should give a straight line with a

$$\text{slope } (S) = \frac{k_{CH_4}}{k_{CH_4} + \alpha_{CH_4}F}. \tag{13}$$

$S$ equals the fraction of the total gas uptake which is diffusion-limited. An $S$ of zero indicates complete blood flow limitation, and as the slope increases, there is increasing diffusion limitation until $S = 1$, indicating complete diffusion limitation.

The blood flow which equilibrates with the lumen can be calculated from the expression

$$F = \frac{Q_{CH_4}(1 - S)}{\alpha_{CH_4}P_{CH_4}}. \tag{14}$$

*Model V.* In this model (see Fig. 2B), the gases diffuse a distance $L$ and then are carried away by the blood flow ($F$). The rate of diffusion to this blood is equal to $\alpha' DA\,(P - P_1)/L$, where $P_1$ is the partial pressure of gas in the blood and the other terms are as defined previously. The rate of removal by the blood is equal to $\alpha FP_1$, and in the steady state these two terms must be equal:

$$\alpha FP_1 = \frac{\alpha' DA}{L} \cdot (P - P_1).$$

Solved for $P_1$:

$$P_1 = \frac{\alpha' DAP}{L\left(\alpha F + \frac{\alpha' DA}{L}\right)}. \tag{15}$$

the rate of absorption is given by rewriting the above equation in terms of $k = \alpha' DA/L$:

$$Q = \alpha FP_1 = \frac{\alpha FkP}{\alpha F + k}. \tag{16}$$

With the same sort of manipulation used for model IV:

$$\left(\frac{\alpha_{CH_4}F}{k_{CH_4}} + 1\right)\left(\frac{Q_{CH_4}k_x}{Q_xk_{CH_4}} - 1\right) = \frac{k_x\alpha_{CH_4}}{k_{CH_4}\alpha_x} - 1. \tag{17}$$

If this model is correct, a plot of $[(Q_{CH_4}k_x/Q_xk_{CH_4}) - 1]$ vs. $[(k_x\alpha_{CH_4}/k_{CH_4}\alpha_x) - 1]$ should give a straight line with slope of $1/[1 + \alpha_{CH_4}F/k_{CH_4}]$. A slope of 0 indicates complete diffusion limitation, and a slope of 1 indicates complete perfusion limitation.

## RESULTS

Table I summarizes the solubility and diffusion data, and the results of the absorption studies. For each segment of the gut, relative absorption rates of the gases were measured in seven animals. The slope (±1 SD) and intercept (±1 SD) of the line which best fits (least

TABLE I

*Solubility, Diffusion Rate, and Absorption Rate of Gases**

| Gas | Blood solubility at 37°C | | Diffusion rate in tissue relative to $CH_4$ ($k_x/k_{CH_4}$) | Absorption rate relative to $CH_4$ ($Q_x/Q_{CH_4}$) | | |
|---|---|---|---|---|---|---|
| | $\alpha$ | $\alpha_x/\alpha_{CH_4}$ | | Stomach | Small intestine | Colon |
| | *ml/ml, STP* | | | | | |
| He | 0.0088 | 0.274 | 1.38±0.035 | 1.03±0.032 | 0.351±0.0083 | 0.551±0.0020 |
| $H_2$ | 0.0149 | 0.465 | 1.89±0.024 | 1.51±0.042 | 0.580±0.041 | — |
| $SF_6$ | 0.0051 | 0.159 | 0.062±0.00083 | 0.071±0.0008 | 0.147±0.0071 | 0.138±0.0041 |
| $^{133}Xe$ | 0.126 | 3.94 | 1.64±0.082 | 2.70±0.092 | 3.61±0.12 | 2.94±0.011 |
| $CH_4$ | 0.0320 | | | | | |

* Data expressed as ±1 SE.

squares) the plot for each of the models are shown in Table II. The smaller the coefficient of variation of the slope (1 SD of slope/slope), the better the points fit the line.

The fit of the data to model I is shown in Fig. 3, in which the absorption rate of each gas relative to $CH_4$ is plotted aginst the blood solubility of the gas relative to $CH_4$. As shown in Fig. 3B, the relative rate of absorption of gases from the small bowel appeared to be largely determined by the solubility of the gases in blood, i.e., was largely blood flow-limited. Expressed mathematically, the line that best fits this data has a slope approaching 1 with a low coefficient of variation and an intercept near 0 (Table II). The deviations of the observed data from the line of identity, which was significant ($P < 0.05$) for each of the four gases, were what would be expected if there were also a small degree of diffusion limitation. Thus He and $H_2$, low molecular weight gases with large diffusion coefficients, were absorbed slightly faster than predicted. $SF_6$ and $^{133}Xe$, high molecular weight gases with small diffusion coefficients, were taken up slower than predicted.

Similarly, Figs. 3C and A demonstrate that the colon had a greater degree of diffusion limitation than did the small bowel, and the stomach had a still greater degree of diffusion limitation.

The increasingly greater discrepancy between the perfusion-limited model and the observed absorption data for small bowel, stomach, and colon is demonstrated by the increasing coefficient of variation of the slope and the increasing deviation of the intercept from 0 for these three portions of the gastrointestinal tract.

Inspection of the data for the diffusion-limited model II (Tables I and II) demonstrates that there was a poor fit when $Q_x/Q_{CH_4}$ was plotted against $k_x/k_{CH_4}$ (the relative diffusion rate of the gases in tissue). The gases ($H_2$, He, and $SF_6$) with $\alpha$'s less than that of $CH_4$ were taken up slower than predicted and $^{133}Xe$, which has an $\alpha$ greater than $CH_4$, was taken up faster than predicted. This would result from some perfusion limitation not taken into account in this model.

The absorption data for the stomach, colon, and small bowel, respectively, showed a progressively worse fit to model II, the reverse of what was observed with model I. Thus these three organs had an increasing degree of perfusion limitation.

It seemed clear that a model which takes into account some interaction between perfusion and diffusion would be required to explain the data. The model used by Van Liew (12) (model III) to take account of this interaction is not very satisfactory. When $Q_x/Q_{CH_4}$ was plotted against $(\alpha_x k_x/\alpha_{CH_4} k_{CH_4})^{\frac{1}{2}}$, a straight line with a

TABLE II

*Fit of Absorption Data* to Various Models of Blood Flow-Diffusion Interaction*

| | Model I | | Model II | | Model III | | Model IV | | Model V | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Slope | Intercept | Slope | Intercept | Slope | Intercept | Slope | Intercept | Slope | Intercept |
| Stomach | 0.531 | −0.683 | 1.053 | 0.0086 | 0.955 | 0.286 | 0.687 | 0.0166 | 0.136 | −0.181 |
| | ±0.208 | ±0.409 | ±0.591 | ±0.845 | ±0.175 | ±0.252 | ±0.0322 | ±0.0852 | ±0.0319 | ±0.0844 |
| Small intestine | 0.901 | 0.0908 | 0.841 | 0.123 | 1.054 | −0.187 | 0.0814 | −0.0137 | 0.742 | 0.121 |
| | ±0.0252 | ±0.0494 | ±1.283 | ±1.83 | ±0.130 | ±0.88 | ±0.0021 | ±0.0071 | ±0.028 | ±0.039 |
| Colon | 0.696 | −0.189 | 1.289 | −0.131 | 1.104 | 0.0419 | 0.276 | 0.0605 | 0.414 | −0.233 |
| | ±0.0813 | ±0.184 | ±1.205 | ±1.491 | ±0.0526 | ±0.0825 | ±0.0141 | ±0.0349 | ±0.0909 | ±0.195 |

* Data are expressed as ±1 SD of slope and intercept.

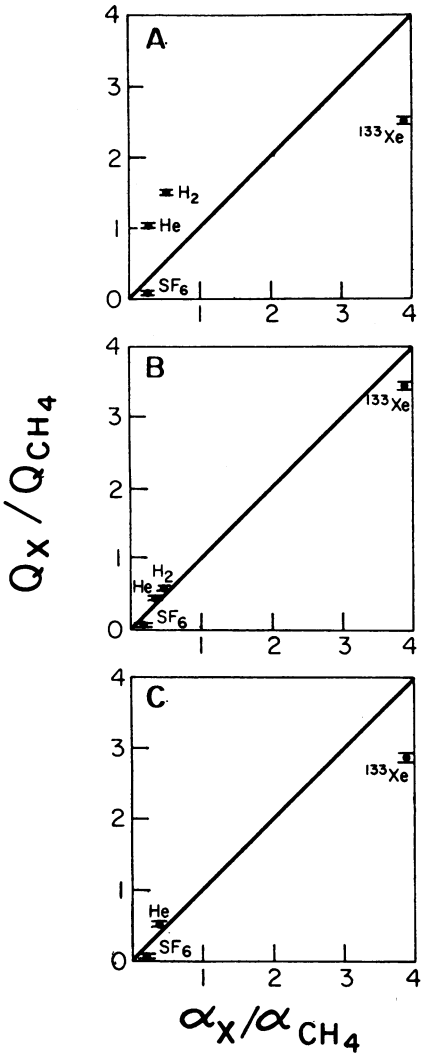


FIGURE 3 Relation of the absorption rate of gases to their solubilities in blood (model I). The observed absorption rate of a gas relative to $CH_4$ ($Q_x/Q_{CH_4}$) is plotted against the ratio of the solubility of the gas in blood to that of $CH_4$ ($\alpha_x/\alpha_{CH_4}$). A, B, and C show data for the stomach, small intestine, and colon respectively. If the absorption rate is determined solely by the solubility of the gas in blood, all points should fall on the line of identity.

slope of 1 and 0 intercept should be obtained (Eq. 6). It can be seen from Fig. 4B that the points for the small bowel did not lie on the line. Van Liew's modified model (Eq. 7) does not improve the fit. One would predict from this modification that the gases smaller than methane should tend to lie above the line and those larger than methane should lie below. Actually just the reverse is true (Fig. 4B). Van Liew's model is also unsatisfactory for the stomach, but provides a fairly good fit to the data for the colon.

Model IV provided an excellent fit to the data for the stomach, small bowel, and colon. For this model (Eq. (12), the points determined by plotting $([Q_x\alpha_{CH_4}/Q_{CH_4}\alpha_x] - 1)$ vs. $[(k_x\alpha_{CH_4}/k_{CH_4}\alpha_x) - 1]$ for each gas should lie on a straight line passing through the origin. The slope of this line is equal to $k_{CH_4}/(k_{CH_4} + \alpha_{CH_4}F)$, which equals the fraction of the total gas uptake which is diffusion limited. This ratio is the only variable parameter of the model. As can be seen from Fig. 5, these predictions were almost perfectly fulfilled in all three organs.

The fit of the data to Model V was not as good as for the model IV. This was evidenced by the greater scatter

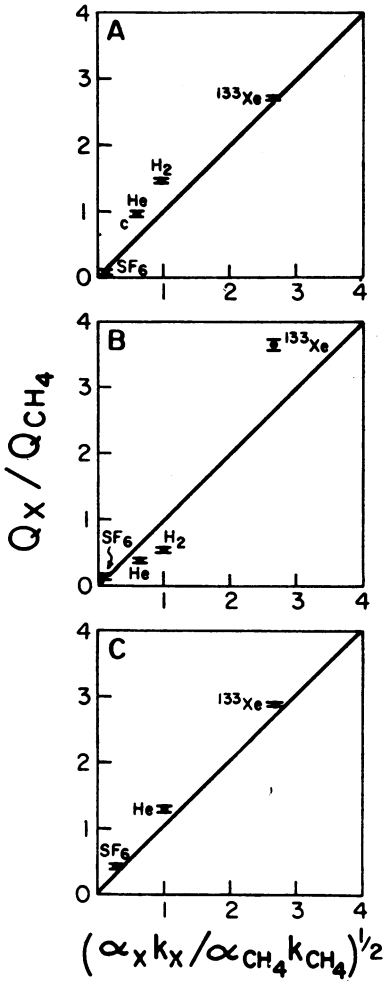


FIGURE 4 Relation of the relative absorption rate of gases to that predicted by model III. The observed absorption rate of a gas relative to $CH_4$ ($Q_x/Q_{CH_4}$) is plotted against $(\alpha_x k_x/\alpha_{CH_4}k_{CH_4})^{\frac{1}{2}}$. A, B, and C represent data for the stomach, small bowel, and colon respectively. If this model accurately predicts the absorption rate, all points should fall on the line of identity.

of the points about the line (greater coefficient of variation of slope) and the fact that the line of best fit had an intercept appreciably further from 0 than was observed with model IV.

## DISCUSSION

To predict the rate at which substances are absorbed from the gastrointestinal tract by passive, nonfacilitated diffusion, one must know not only the diffusion coefficient of the substance in the mucosa but also the interaction between diffusion and perfusion. Winne (13, 14) has approached this problem by investigating the rate of absorption of a series of substances with different intestinal permeabilities. By collecting all the blood draining a perfused segment of rat small intestine, Winne was able to correlate the rate of absorption with the rate of blood flow, which he could alter by changing the blood volume of the rat. He found that the higher the permeability of the solute, the more its absorption was influenced by the blood flow. The absorption of tritiated water (highest permeability) was almost directly proportional to blood flow, while the absorption of mannitol was not at all influenced by blood flow. He concluded from these results that the absorption of tritiated water and other highly permeable solutes (aniline, antipyrine) was nearly blood flow limited in the small intestine.

The analysis of the relative contributions of diffusion and blood flow to the absorption of these solutes is complicated by the markedly different diffusion coefficients of the solutes in the cell membrane, the cell interior, and the capillary wall. Although these three diffusion coefficients can be summed to yield a single permeability coefficient, accurate measurement of this value is difficult. In addition, quantitative analysis of the absorptive process must involve at least three different diffusion coefficients and several compartments (13, 14).

In contrast to the solutes used by Winne, the inert gases are unusual in that they are a series of substances with a broad range of molecular weights and with solubilities in lipid and water of roughly the same order of magnitude.[5] Because of this lipid solubility, one can neglect the diffusion barrier of the lipid cell membrane and capillary wall, and consider only the diffusion through an aqueous path between the lumen and the blood. The diffusion coefficient of a gas through this aqueous path will be determined solely by its molecular radius, and is easily and accurately measured by determining the rate that gases penetrate the nonperfused bowel wall in vitro. Thus, the analysis of the absorptive process is greatly simplified, and the understanding of the relation between diffusion and blood flow should be sharpened.

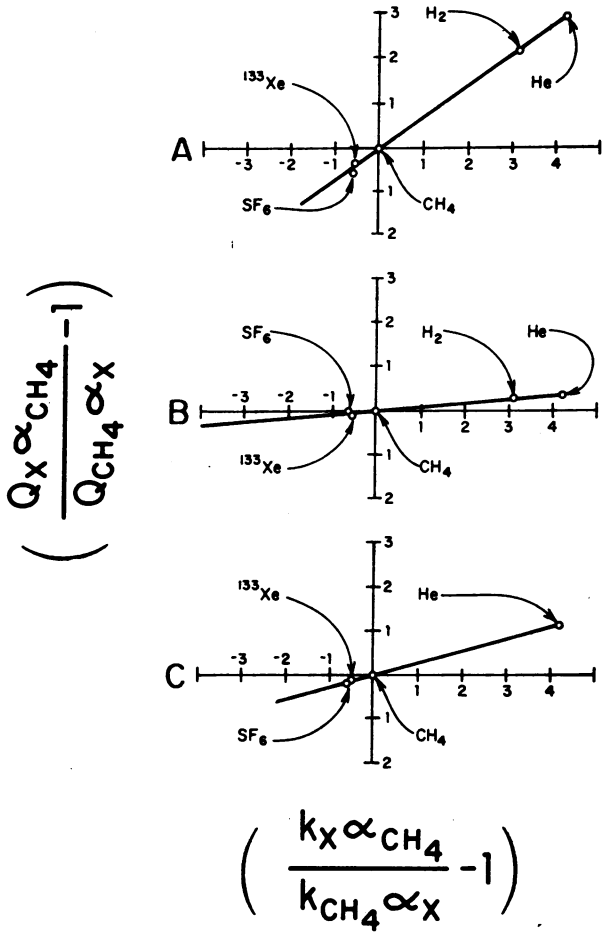


FIGURE 5 Fit of the observed data to model IV. A, B, and C represent the data for stomach, small bowel, and colon respectively. If this model accurately predicts the absorption rates of the gases, all points should fall on a straight line with an intercept of zero. A slope of zero indicates complete blood flow limitation, and a slope of 1 indicates complete diffusion limitation.

In addition, the quantitative pulmonary excretion of absorbed gas makes it technically very simple to study gas absorption in unanesthetized animals. Because appearance rather than disappearance is measured, it is possible to measure accurately the absorption of a small fraction of the luminal contents, and a series of measurements of the simultaneous absorption rate of multiple gases can be performed, with only minor corrections for disappearance of material from the lumen.

The data presented in this paper indicate that different degrees of interaction between diffusion and perfusion exist when gas is absorbed from the stomach,

[5] The approximate lipid-to-water solubility ratios for $^{133}$Xe, SF$_6$, CH$_4$, H$_2$, and He are, respectively: 20, 40, 11, 3.4, and 2.2 (15,6). (The last three ratios are unpublished observations of the authors.)

small bowel, and colon. The relative rate of uptake of gases from the small bowel was almost entirely dependent upon the solubility of the gases in blood, and largely independent of the diffusivity of the gases. Thus, the partial pressure of gas in the lumen and the blood leaving the mucosa seemingly approach equilibrium, and uptake is almost entirely blood flow limited. The diffusivity of the gases played a progressively greater role in determining absorptive rates from the colon and stomach, indicating increasing diffusion limitation.

Therefore, any single model which attempts to explain the absorption of gases from the stomach, small bowel, and colon must take into account varying degrees of diffusion and perfusion limitation. Neither model I (complete blood flow limitation) nor model II (complete diffusion limitation) could adequately explain the observed data.

The model proposed by Von Liew (12) (model III) to explain the rate of absorption of gases from subcutaneous pockets provides for both a flow and a diffusive component by postulating that blood flow is uniformly distributed to the tissue surrounding the pocket, and that there is a continuous interaction between blood flow and diffusion. Since a fixed relation ship exists between diffusion and perfusion in this model, it could not explain the varying degrees of diffusion limitation observed in the present study. Thus, this model provided a fairly good fit for the colonic data, but not for the stomach and small bowel.

There is no a priori reason to assume the existence of a single model that will represent the interaction between diffusion and perfusion for all segments of the gastrointestinal tract. Thus it was somewhat surprising that the simple model shown in Fig. 2A (model IV) very accurately predicted the absorption rate of gases from the stomach, small bowel, and colon.

This model assumes that, functionally, two distinct blood supplies are involved in the absorption of gases. One flow is located in such close proximity to the lumen that complete equilibrium is achieved between the luminal and blood gases. The second flow is sufficiently rapid and distant from the lumen that the partial pressure of the luminal gases remains negligible in this blood. Thus, absorption of gas into this blood flow is entirely diffusion limited. According to Eq. 13, the relative quantities of gas absorbed by each of these two flows is readily calculated from the slope of the plots shown in Fig. 5. The fraction of the total absorption of $CH_4$ taken up by the blood flow-limited mechanism is 93%, 77%, and 33% for small bowel, colon, and stomach, respectively. For gases with lower diffusion coefficients than $CH_4$, these percentages will be lower, and for gases with larger diffusion coefficients these percentages will be higher.

The rate of the blood flow which comes into equilibrium with the lumen can also be readily calculated from Eq. 14, if the partial pressure of a gas in the lumen is known. In the present study, the partial pressure of each of the gases in the mixtures initially instilled into the bowel was known; however, the alteration of these values due to the influx of $CO_2$, $O_2$, and $N_2$ is not known, and thus an absolute value for this flow cannot be accurately calculated.

If it is assumed that there was no endogenous gas in the lumen before instillation of the gases, and that subsequently $P_{O_2}$, $P_{CO_2}$, and $P_{H_2O}$ rapidly reached luminal values of about 40 mm of Hg, 40 mm of Hg, and 47 mm of Hg, respectively, the equilibrating flow per gram of organ can be roughly estimated to be 0.27 ml/min/g for the small bowel, 0.18 ml/min/g for the colon, and 0.020 ml/min/g for the stomach. This flow for the small bowel is similar to the "mucosal" flow reported by Winne in the rat with $D_2O$ absorption (13), and is greater than that obtained from measurements of CO uptake in the rabbit (0.08 ml/min/g) (16), or $^{133}Xe$ uptake in the dog (0.1–0.7 ml/min/10 cm).[6] However, measurements obtained by rubidium clearance in the rat indicated that about 60% of small bowel flow perfuses the mucosa (17). Since the total blood flow per gram of rat small intestine is approximately 1ml/g/min (18), mucosal flow estimated by rubidium clearance appears to be 2–3 times greater than the equilibrating flow measured by gas uptake.

The equilibrating flow for the stomach is only about 1/50 of the gastric mucosal flow of rats reported by Schanker, Shore, Brodie, and Hogben (19), estimating from the clearance of aniline from the blood into the lumen. However, these authors found values similar to those reported in the present paper when flow was calculated from the rate of $D_2O$ or salicylic acid clearance from the gastric lumen into the blood. One possible explanation for this directional difference in clearance is that gastric secretion markedly shortens the diffusion path for aniline. Aniline may merely have to diffuse into the gastric glands and it will then be convected to the gastric lumen. On the other hand, gastric secretion would tend to retard the passive absorption of substances such as gases and salicylic acid from the lumen. Thus, the absorption of gases and salicylic acid appears to be largely diffusion limited whereas aniline clearance is largely blood flow limited.

The models employed in the present study have assumed no exchange of gases between the vessels entering and leaving the mucosa. However, Kampp, Lundgren, and Sjostrand, and Kampp, Lundgren, and Nilsson (20, 21) have postulated that a counter-current

[6] 1 cm of dog small bowel weights approximately 1 g. (Unpublished observation of the authors.)

exchange exists in the small bowel mucosa. Our experiments provide a measure of the possible magnitude of such a process. The exchange process involves the diffusion of the solute from the vein leaving the villus back into the artery (or capillary) entering the villus. The effect of this exchange is to trap the solute in the villus and slow its rate of removal. If this exchange were quantitatively important, one would expect it to show up in our experiments, because of the very high rates of diffusion of gases in the vessel wall. One would expect that the higher the diffusion coefficient of the gas, the more effective the counter-current would be, and the slower the rate of absorption of the gas. That is, $H_2$ and He should be trapped to a greater extent than $SF_6$ and $^{133}Xe$, and thus be absorbed more slowly. This was exactly the reverse of what was observed and thus these results do not support the existence of an appreciable counter-current exchange.

It is possible to imagine arrangements which could yield results consistent with ours and yet still involve a significant amount of exchange. For example, if there were two completely separate blood supplies, one with no counter-current exchange and the other with a perfect exchange, then, because nothing could be removed by the perfect exchanger, our results would apply only to the nonexchanging component. This seems unlikely.

Dietschy, Sallee, and Wilson (22) have recently pointed out that the absorption rate of substances with high permeability coefficients for the lipid membrane, such as the inert gases, could be markedly influenced by the presence of an unstirred water layer between the bulk luminal contents and the mucosal cell.

Such an unstirred layer is represented by our model V, which consists of a diffusion barrier of water placed between the lumen and the blood. The fit of the data to this model was not as good as to model IV, although the data for the small bowel fit both models quite well, and the presence of an unstirred layer in the small intestine cannot be ruled out. However, because uptake from the small bowel was almost entirely blood flow limited, such an unstirred layer could not have played an important role in the present study. It is possible that the influence of such an unstirred layer would have been more evident if absorption of gases from an aqueous rather than a gaseous phase had been studied.

Coburn, Swerdlow, Luomanmaki, Forster, and Powell (23) have used the rate of absorption of carbon monoxide (CO) from the urinary bladder as a measure of the "mucosal" blood flow. They found that the rate of CO absorption was directly proportional to the luminal $P_{CO}$ when the $P_{CO}$ was below about 400 mm of Hg, but at values of $P_{CO}$ above 400 mm of Hg the absorption rate became constant and did not increase with increasing $P_{CO}$. Similar results were obtained in the rabbit ileum (14). The model Coburn et al. used to interpret their results is equivalent to the model (model IV) we find most successful in the gastrointestinal tract. That is, there are two separate blood flows, one which comes into complete equilibrium with the lumen, while the other flow has only a negligible CO concentration at all values of $P_{CO}$. Thus as the $P_{CO}$ is raised, the concentration of CO in the equilibrated flow increases almost linearly until the hemoglobin becomes completely saturated, and then the rate of removal becomes a constant. The sharp transition from the linear to the constant rate of removal implies that a single flow only is removing an appreciable amount of CO. Because of the extremely high "solubility" of CO in blood relative to tissue, the absorption of CO by the flow component obscures any diffusive component, even though the diffusive component might be several times larger than the flow component for a gas that had equal tissue and blood solubilities.

Although model IV provided an excellent fit to the data, it is quite possible that other, more complex, arrangements could also explain the observed data. It should also be emphasized that the two distinct absorptive flows of model IV represent a functional concept, and it remains to be determined whether they have any anatomical significance. However, the remarkable fit of the model to the results in all three organs suggests that there is a blood flow which comes into equilibrium (for the inert gases) with the luminal space. The obvious candidate for this blood flow is the most superficial capillary network, found in the mucosa of all three organs.

Despite the fact that gas absorption from the small bowel was almost entirely blood flow limited, the equilibrating flow appeared to be less than the total mucosal blood flow measured with rubidium (17). This could occur if only the blood perfusing the most luminal aspect of the mucosa equilibrated with the lumen, or if a portion of the mucosal flow was involved in a very efficient counter-current exchange. These two possibilities have been thoroughly considered by Hamilton, Dawson, and Webb (24) to explain the very low mucosal flow rates estimated by $^{133}Xe$ absorption from the canine small intestine. In addition, a similar discrepancy between absorptive flow, measured by gas uptake, and mucosal flow, measured by $^{32}P$-labeled erythrocytes, has been observed in studies in cats by Svanvik.[7] It seems likely that an "effective" mucosal perfusion rate relative to gas absorption, rather than an anatomical mucosal flow, was measured in the present studies.

Although the exact relation between "effective" and anatomical flow remains to be determined, the present model nevertheless provides an accurate means of pre-

[7] Svanvik, J. 1973. To be published in Acta Physiol. Scand.

dicting the absorption rate of gases from various segments of the gastrointestinal tract. It seems likely that this type of model may also be constructed for a variety of other materials absorbed by passive diffusion.

## ACKNOWLEDGMENT

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