Intestinal villus blood flow measured with carbon monoxide and microspheres

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Microspheres (3M Company, Saint Paul, Minn.) 7-10 microns in diameter were injected into the superior mesenteric artery, carotid, and aorta, and then the femoral and carotid arteries were cannulated with polyethylene catheters filled with heparinized saline. The femoral and carotid arteries were cannulated with polyethylene catheters filled with heparinized saline. The femoral artery catheter was inserted about 4 cm into the artery that placed its orifice just below the aortic arch in the ascending aorta. The femoral catheter was connected to a syringe in an infusion-withdrawal pump and the carotid catheter was connected to a stopcock.

The absorption of CO was determined by instilling 5 ml of pure CO into a 15-cm segment of mid small intestine isolated between ligatures. A 20-minute absorption period was used and the loop was then rapidly flushed with 100 ml of air. Techniques employed for the measurement of CO absorption were identical to those previously described (2). Flco was calculated from the observed absorption of CO (Qco), the binding of CO by hemoglobin (1.36 ml CO/g Hb (3)), and the hemoglobin concentration of femoral artery blood

\[
Flco = \frac{Qco}{1.36 \times Hb}
\]

Villus blood flow was determined with hydrocarbon microspheres (3M Company, Saint Paul, Minn.) 7-10
μm in diameter that were injected during the final minute of the CO absorption period. The spheres were suspended in saline by sonication, and 1 ml, containing about 2.5 × 10⁷ spheres, was injected into the aorta via the carotid catheter, which was then flushed with an additional 1 ml of saline. Just prior to the injection of the spheres, blood was begun to be constantly withdrawn from the femoral artery at a rate of 5 ml/min for 30 s. Then 30 ml of a latex solution (16) was injected into either the carotid or the superior mesenteric artery and 20 min allowed for the latex to set. The gut segment between the ligatures was then dissected free, opened, blotted dry, and weighed.

Three pieces of gut, weighing approximately .05 g each, were cut from different locations in the segment, weighed, and examined under a dissecting microscope. The vascular architecture appeared white because of the latex, and the microspheres stood out as black dots in the vasculature (Fig. 1). The arterial supply of the villus consists primarily of a central arteriole that ascends to the tip before giving off capillaries (15); therefore, nearly all spheres in the villi were present near the tips. The total number of spheres (~1,200) in the villi of each specimen were counted utilizing a grid, and the average number of villus spheres per gram of intestinal tissue was calculated.

The blood sample from the femoral artery was hemolyzed by a fourfold dilution with distilled water and then centrifuged. The supernatant was removed and water was added to the precipitate containing the spheres to yield a volume of 1.1 ml. This mixture was then sonicated and a 10-μl sample was placed on a glass slide and sealed with a cover slip that was greased at the edges to produce a slight elevation from the slide. The spheres were counted utilizing a dissecting microscope and grid. A total of at least 700 spheres were counted. Villus flow was calculated from the formula

\[
\text{villus flow/g intestine} = \frac{\text{spheres/g intestine}}{\text{total spheres in femoral artery sample}} \times 5 \text{ ml/min}
\]

The shunting of the spheres across the intestine was determined by injecting a known quantity of spheres into the superior mesenteric artery of four rabbits and collecting the drainage of the superior mesenteric vein. The spheres in mesenteric vein blood were counted as previously described.

RESULTS

In Fig. 2, the villus flow calculated from microspheres is plotted against the flow calculated to equilibrate with CO (F\text{VCO}). Villus flow measured by spheres averaged 0.087 ± 0.011 ml/(min × g) (SE), which was not significantly different from F\text{VCO} that averaged 0.082 ± 0.010 ml/(min × g). The correlation coefficient was 0.83. The percentage of spheres that shunted averaged 16 ± 3%.

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**Fig. 1.** Photomicrograph of rabbit small bowel mucosa showing microspheres lodged in vascular architecture of villi that are filled with latex.
Studies of CO uptake at increasing luminal CO suggested that the uptake of this gas might provide a measure of villus blood flow. As luminal PCO is increased, absorption increases linearly until a PCO of about 400 mmHg is reached, at which point absorption remains constant despite further elevations of PCO to 700 mmHg (4). This finding indicates that at a PCO of 400 mmHg or greater, the entire absorption of CO occurs into a distinct blood flow that is saturated with this gas. There is negligible uptake by other, partially saturated flows, since such blood should absorb increasing quantities of CO as the partial pressure of the gas is increased from 400 to 700 mmHg (2, 4). Villus blood flow, because of its intimate and prolonged exposure to the lumen, seemed the logical candidate for this discrete flow that becomes saturated with CO.

In order to determine the relation of $F_{I_{CO}}$ to villus blood flow, one must have an independent means of accurately assessing villus flow. The only previous technique purported to measure villus flow is a recently described procedure that employs an intra-arterial injection of $\beta$-emitting isotopes and a scintillation counter (1). The accuracy of this technique rests upon several assumptions, some of which have not yet been entirely validated.

Therefore, we compared $F_{I_{CO}}$ with villus flow as measured by a recent modification of a microsphere technique (15). The critical feature of the modification is that the microspheres 7-10 $\mu$m in diameter are localized and counted by direct microscopic visualization of the villi. The validity of this technique depends solely upon the assumption that the spheres are distributed to, and lodge in, tissue in proportion to the blood flow which passes through the capillaries of that tissue. Greenway and Murphy (5) have demonstrated that the arterioles leading to the intestinal mucosa of the cat are in "series" with the arterioles of the submucosa. Thus, microspheres greater than 12 $\mu$m in diameter may lodge in arterioles leading to the villi, resulting in an underestimation of villus flow. However, the diameter (7-10 $\mu$m) of the spheres used in the present study is smaller than that of the arterioles and thus these spheres pass through the arteriole system and lodge at the point where the arteriole ramifies into capillaries, as evidenced by the localization of nearly all villus spheres at the villus tips. A second possible problem is that large microspheres migrate towards the center of a flowing stream and thus their distribution to tissue may not accurately reflect blood flow. However, Phibbs and Dong (13) found that this problem was primarily limited to larger microspheres, while those with a diameter of 7-10 $\mu$m had a cross-sectional distribution in the arteriole similar to that of erythrocytes. Thus, it seems likely that the spheres used in the present study will be delivered to tissue in a fashion similar to that of erythrocytes. Jodal and Lundgren (7) have suggested that plasma skimming occurs in the intestinal mucosa, resulting in perfusion of the villi with a blood relatively poor in erythrocytes. Both $F_{I_{CO}}$ and villus flow measured by microspheres are probably measuring villus erythrocyte flow rather than blood flow and a correction for plasma skimming may be required to obtain the true rate of blood flow to the villi.

An average of 16% of spheres injected into the superior mesenteric artery were recovered in the superior mesenteric vein. Whether these heads traversed arteriovenous shunts or for some reason passed through capillaries is not known. In addition we have no data as to the site of the shunt in the intestine. Thus, no conclusions can be drawn concerning the influence of the shunting of spheres on the accuracy of villus blood flow measurements.

In the present study we compared flows determined simultaneously by two entirely independent techniques, both of which intuitively seemed likely to provide a measure of villus blood flow. The relatively good correlation between the results of these two measurements (see Fig. 2) over a threefold range of flows strongly suggests that both techniques were measuring the same flow, namely villus blood flow.

The villus flow observed in the rabbit in the present study is only about one-third to one-sixth that reported for mucosal flow in the dog measured by microspheres (6) or $^{133}$Xe washout (14), or in the cat by means of $^{85}$Kr washout (8). This discrepancy is not totally unexpected since the flow to the entire mucosa rather than just villus flow was determined in these previous reports. In addition, the rabbit has somewhat atrophic, leaf-shaped villi that are much less densely packed than are the fingerlike villi of the dog and the cat.

If counter-current exchange occurs in the villi, readily diffusible substances in the lumen such as the inert gases would equilibrate with blood at the villus tips, but then shunt from the venous drainage of the villus to arterial flow entering the villus. The partial pressure of the gases in the venous outflow of the villi would therefore be reduced below the luminal level and absorption rate would be diminished.

In previous studies in rabbits (2) and rats (11), we found that intestinal absorption of inert gases ($H_2$, $He$, $CH_4$, $^{133}$Xe) was accurately predicted by a two-flow model: one flow equilibrated with the lumen and absorbed with flow-limited kinetics, and a second flow...
absorbed with diffusion-limited kinetics. It was further demonstrated that the absorption rate of inert gases into the equilibrating flow was similar to that predicted from equilibration of the luminal gases with Flco, when this flow was corrected for plasma skimming (2). In view of the present findings that Flco represents villus blood flow, absorption of inert gases (and, presumably, other diffusible substances) from the lumen can be accounted for by the following two-component model: a flow-limited component in which the gases equilibrate with villus blood flow and are then carried away without subsequent counter-current exchange, and a diffusion-limited component in which gases are removed by a nonvillus blood flow presumably in the crypt region or submucosa.

While the observed data could be explained by models involving counter-current exchange, these are far more complicated than the above model. For example, both villus flow and a certain fraction of the crypt flow could initially equilibrate with gas in the lumen. A fraction of the gas absorbed by the villus flow could then be retained by counter-current exchange, reducing the observed equilibrating flow to a value fortuitously similar to that of the villus flow. While it is not possible to rule out such models, our studies indicate that absorption of diffusible substances from the rabbit small bowel can be entirely explained by a simple model that does not invoke counter current exchange.

Counter-current exchange would be favored by the existence of arterioles and veins which run in close proximity for a long distance. In addition, exchange of absorbed materials in the villus requires that only the villus tips are exposed to the luminal material, since if the base of the villus equilibrates with the lumen, the venous blood will leave the villus in equilibrium with the lumen. The rabbit has short, leaf-shaped villi that are much less densely packed than the long, fingerlike villi of man, dogs, and cats. This latter villus architecture would therefore be better suited for counter-current exchange, and it is possible that the influence of exchange on absorption may be species related.

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REFERENCES