Hemodynamics of gastric microcirculation in rats

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METHODS

The methods used to prepare the submucosal and deep mucosal layers of the gastric muscle for micropuncture studies are described briefly below. Details of this technique have been described in a recent report (29).

Animal Preparation

Male Wistar rats, weighing ∼200 g, were fasted overnight with free access to water. Rats were anesthetized with thiobutabarbitral sodium (Iactin-BYK, 120 mg/kg body wt ip) and placed on a micropuncture table, and body temperature was maintained at 37 ± 0.5°C by means of a heating pad controlled by a rectal thermistor probe. A tracheotomy was performed, and the trachea was intubated to facilitate spontaneous breathing. Systemic arterial blood pressure was monitored continuously through a PE-50 catheter placed in the left femoral artery and connected to a Statham electromanometer (model P23). A long, heparinized catheter (PE-50) was inserted into the left femoral vein, and Ringer solution (1.5 ml/h−1 100 g body wt−1) was infused throughout the surgical preparation and experimental periods; this preparation was also used to measure systemic venous pressure.

Tissue Preparation

The abdomen was opened via a 5-cm midline incision, the stomach was gently exteriorized, and surrounding gastric
ligaments were cut. The exposed stomach was continuously bathed in warmed Ringer solution to avoid desiccation and irreversible cessation of blood flow in the superficial blood vessels. The animal was positioned on its right side on the micropuncture table, and the stomach was placed in a gastric chamber. The heating pad and continuous superfusion (1 ml/min) of warm Ringer solution kept the vascularly intact and innervated stomach in a constant environment with high humidity and a temperature of 37 ± 1°C. To stabilize the preparation with the posterior wall facing up, four small pins were inserted into the chamber wall through the esophagus, antrum, forearm, and corpus (see diagram of gastric chamber and instrument setup in Ref. 29). However, to avoid respiration-induced gastric movements and changes in macrovascular parameters (e.g., venous pressure elevation) due to stretch, the esophagus was not tightly fixed. Care was taken to prevent compression or excessive manipulation of the left gastric artery, vein, and nerves to the stomach.

Vessels in different layers of the gastric wall were approached from the serosal side. A very light rubber ring was mounted over the gastric surface to serve as a reservoir for a filter fluid that was necessary for the micropuncture technique (see diagram of gastric chamber and instrument setup in Ref. 29). The narrow gap between the rubber ring and the preparation was sealed with agar gel. The area inside the ring functioned as a working window, where the tip of the superfusion system was located. The working window consisted of two parts, the seromuscular and submucosal areas, each supplied by different small arteries. The seromuscular area served for micropuncture measurements in vessels of the muscle layer. To approach the submucosal and deep mucosal vasculature, a 1-cm-diameter piece of seromuscular tissue on the posterior wall of the corpus was removed by careful dissection from an area free of large vessels. In a small area of this submucosal preparation, we totally removed the submucosal connective tissue and the superficial part of the muscularis mucosa. In this way, the microvessels in the basal mucosa also became accessible through the remaining thin muscularis mucosa. After completion of the experimental setup, the tissue was allowed to equilibrate for 1 h before any experiments were attempted. Vascular reactivity was well preserved, and the minimal exteriorization procedure did not have significant adverse effects on the vessels under study, as have been assessed and tested in our recent report (29).

**Measuring Techniques**

The preparation, illuminated with an fiber-optic illuminator (Intralux 4000-1, Volpi), was visualized using a zoom stereomicroscope (model M8, Wild). Pressures were measured in microvessels with a servo-null transducer (model PVM-145E, Sony). Pressures were recorded simultaneously on a recorder (model OH-50A5M, Radelkis). Microvessel images were viewed through the microscope with a television camera (model SSC-M370CE, Sony), continuously displayed on a television monitor (model PVM-145E, Sony), and recorded on a videocassette recorder (model SLV815VP, Sony). Vessel dimensions were determined using the microscope eyepiece micrometer and measured in video recordings displayed on the television screen with a caliper.

In a separate series of experiments, we studied intramural blood flow distribution between gastric muscle and submucosal-mucosal layers using the Dye-Trak microsphere technique (3, 24). All reagents and disposable supplies were obtained from Fisher Scientific. Approximately 10^6 red microspheres, 15 µm diameter (Triton Technology, San Diego, CA) and suspended in 1 ml of saline containing 0.05% Tween 80, were injected into the ascending aorta over a 10-s period through a catheter placed into the right carotid artery. At 5 s before microsphere injection, a 30-s microsphere reference sample was collected by free-flow technique into a heparin-coated glass tube from the distal abdominal aorta through a cannula inserted into the left femoral artery. Two minutes after injection, the rat was killed, and the stomach was removed and divided into muscle and submucosal-mucosal layers by manual dissection. Each tissue and reference blood sample was weighed and then digested in glass tubes with 7 ml of 4 M KOH. The volume of the reference blood sample was calculated by weight according to the specific gravity (30). The digested tissue solution was filtered through a polyester filter with a pore size of 10 µm (Triton Technology) in a glass microanalysis filter holder (model 09-735G, Fisher Scientific). After filtration, microspheres were quantified by their dye content recovered by addition of 300 µl of dimethylforamide. The photometric absorption of each dye solution was determined using a spectrophotometer (model 8452A, Hewlett-Packard), and blood flow to each layer was calculated from the following relationship

\[ \dot{Q}_b = \frac{100 (AUL)/(AU_{muc})}{R} \]

where \( \dot{Q}_b \) is blood flow (ml·min\(^{-1}\)·100 g\(^{-1}\)), \( AUL \) is absorbance unit per layer sample, \( R \) is the percent ratio of total gastric blood flow and vessel dimensions were measured using a colloid osmometer as described by Aukland and J ohn sen (4).

Plasma colloid osmotic pressure was measured from plasma samples using a colloid osmometer (model OH-50A5M, Radelkis). Plasma samples and reference blood sample from 10.220.33.6 on September 21, 2017 http://ajpheart.physiology.org/ Downloaded from
Submucosal and deeper mucosal vasculature. Through the process of anastomoses, small arteries form the main submucosal arterioarterial anastomotic plexus or primary arcade of submucosal arterioles (SMA1) and average 75.5 ± 6.1.8 µm in diameter. In turn, these vessels form smaller and smaller branches, which interconnect with each other and the parent vessels forming a secondary and a tertiary arcade of submucosal arterioles (SMA2 and SMA3, 42.3 ± 1.6 and 24.4 ± 0.8 µm, respectively). These arcades form a very extensive, interconnecting arterial network in the submucosa. The tertiary arcade gives rise to small mucosal terminal arterioles (MTA, 15.5 ± 0.7 µm), which run perpendicularly through the muscularis mucosae and, on entering the mucosa, divide into the hexagonal mucosal capillary plexus. Collecting veins (CV) run perpendicularly through the mucosa and, on cross section, appear as dark round dots that are 36.4 ± 1.1 µm diameter. Within the deeper mucosa they drain into the venous anastomosis, which, on entering the muscularis mucosae, gives rise to the secondary arcade of submucosal venules (SMV2). This observation of the mucosal venous architecture is somewhat different from previous descriptions (12, 17, 32); however, the existence of a deep mucosal venous anastomosis is further supported by a recent study (27). Interestingly, collecting venules were larger in diameter (36.4 ± 1.1 µm) than the deeper mucosal venous anastomosis (31.5 ± 1.3 µm) or the initial part of SMV2. SMV2 are larger than their respective arteries (55.8 ± 1.3 µm) and interconnected in a similar manner. SMV2 enter the primary arcade of submucosal venules (SMV1, 87.5 ± 1.6 µm), which follow the same course as the primary arterioles and return blood to the small veins (SV, 99.1 ± 1.8 µm). These SV run parallel with SA, penetrate the external muscle layers, and leave the superficial submucosa.

Intravascular Pressure Distribution

Microvascular pressure was measured for up to 5 h with no signs of deterioration of the tissue as judged by the absence of leukocytes sticking and rolling along the walls of venules or progressive dilatation of arterioles and large-amplitude vasomotion. In most animals, spontaneous gastric muscle contractions and respiratory movements were small enough to allow micropuncture measurements. The results of microvascular pressure and dimension measurements are summarized in Figure 3. A total of 155 vessels from 25 animals were sampled. Systemic arterial and venous blood pressure averaged 108 ± 2 and 7 ± 0.5 mmHg, respectively.

Submucosal and deeper mucosal vasculature. Pressures in submucosal SA averaged 77.8 ± 2.6 mmHg. Only slightly lower pressures were measured in SMA1 (74.6 ± 2.5 mmHg), which is not surprising, since SMA1 are shunt vessels between adjacent small arteries. About one-half of the systemic pressure was found in SMA2 (74.1 ± 1.8 mmHg). Pressures in SMA3 averaged 34.4 ± 1.6 mmHg, significantly less than in SMA2 but similar to the pressures in MTA (32.4 ± 1.2 mmHg). Pressures in CV, SMV2, SMV1, and SV were 26.6 ± 1.1, 21.8 ± 1.6, 17.1 ± 0.8, and 14.4 ± 0.6 mmHg, respectively. Mucosal capillary pressure was estimated from input (MTA) and output (CV) pressures.
of the mucosal capillary network by calculating the mean and resulted in 28 mmHg. The validity of this estimation was tested using intravascular pressure data obtained from the muscle microvasculature. Because the difference between calculated (24.3 mmHg) and measured (23.6 ± 1.4 mmHg) MC pressure was not significant, the value of 28 mmHg for mucosal capillary pressure was accepted.

Muscle vasculature. Pressures were not measured in ascending arterioles or descending venules because of technical difficulties. However, the greatest pressure drop was apparent across these arterioles, since the MA pressure was 30.5 ± 1.4 mmHg, less than one-half of SMA1 pressure. Microvascular pressure further decreased to 23.6 ± 1.4 mmHg in the MC and to 18.2 ± 0.9 mmHg in MV. Figure 4 compares precapillary, capillary, and postcapillary hydrostatic pressures from muscle and mucosal layers.

Intramural Blood Flow Distribution

Absolute and relative blood flows to gastric muscle and mucosal layers were measured in 11 additional animals. Because the submucosal and mucosal microvasculatures are coupled and cannot be separated by manual dissection, we report mucosal and submucosal flows as a single value. However, the scarcity of submucosal capillaries would suggest that submucosal flow is only a small fraction of total gastric blood flow. Absolute flows to the muscle and submucosal-mucosal regions averaged 70.4 ± 22.3 and 120.8 ± 18.8 ml·min⁻¹·100 g⁻¹, respectively. From calculation of the relative blood flow distribution, the gastric muscle layer received 16 ± 3% and the submucosa-mucosa 84 ± 3% of total gastric blood flow. These results are comparable to those from other studies (3, 5, 11).

DISCUSSION

Microvascular pressures have been measured in different organs, including the small intestine (13), but studies from gastric microvessels are lacking. Previously, gastric mucosal hydrostatic capillary pressure has been estimated from the balance of Starling forces, resulting in a value of 10.6 mmHg (15, 16). In contrast, we found that mean capillary pressure in different regions of the gastric vasculature ranges from 20 to 30 mmHg, values that are significantly higher than previously calculated. This discrepancy may be explained

![Fig. 2. Micrograph of gastric microvasculature injected with india ink. See Fig. 1 legend for vessel identification.](image)

![Fig. 3. Intravascular pressure distribution in gastric vasculature. Data were recorded from muscle (○) and mucosal blood vessels (●) at systemic arterial pressures of 100–110 mmHg. See Fig. 1 legend for vessel identification. Values are means ± SE; n, number of vessels. A total of 155 vessels from 25 animals were sampled.](image)
partly by inherent limitations in the methods used in previous calculations. For example, the rate of lymph flow was used as an estimate of net capillary filtration rate (6). However, lymph flow may not accurately reflect capillary filtration rate if significant transepithelial fluid secretion or absorption occurred during the period in which lymph flow was measured. In secreting organs, such as the stomach, capillary filtration rate could be significantly underestimated by lymph flow, since a proportion of the capillary filtrate would be removed from the interstitial spaces via the transporting epithelia rather than the lymphatics. Furthermore, previous calculations of Starling forces used a value of interstitial fluid pressure measured with microcapsules implanted in the gastric submucosa (1). Because this layer of the gastric wall has only a few nutritive capillaries compared with the dense mucosal capillary system, tissue factors governing transcapillary fluid exchange in the submucosa are most probably different from those of the mucosa.

Another factor that could significantly modify microcirculatory hydrostatic pressures is muscle activity. Many studies have shown that the motor activity of the intestine can affect blood flow (8–11, 18, 23, 33). In these experiments a large increase in intramural pressure produced by distension or prolonged tonic contraction was accompanied by an increased local vascular resistance. Changes in blood flow have been explained as a result of passive changes in vessel caliber due to changes in vascular transmural pressure subsequent to alterations in motility. In our preparation, muscle contractions can also increase passive resistance of blood vessels. SA, SV, and ascending arterioles and descending venules run perpendicularly to the outer muscle layers. Furthermore, MTA and SMV2 penetrate the muscularis mucosae in a similar manner. Ample evidence has been presented (1) that the basal tone of gastric muscles and peristaltic waves also increase the interstitial fluid pressure. In comparison to earlier methods (7, 17, 32), in which gastric or intestinal wall and muscle were transected and the mucosa was exteriorized, in our preparation the whole stomach was fixed in the gastric chamber without surgery. Thus the effect of tonic muscle contraction on vascular and interstitial hydrostatic pressure was included in our experimental results. However, because of this lack of exposure, we could not measure mucosal interstitial fluid pressure by direct micropuncture. On the other hand, we could not document the possible effect of peristalsis on intravascular pressure (i.e., oscillation in pressure), because, as a result of contractions, small vessels could be penetrated for a relatively short period (15–20 s). Also, oxygen is a potent vasoconstrictor, and high oxygen superfuses (such as those equilibrated with room air) have long been known to cause arteriolar constriction and decreased blood flow in exteriorized tissues (22, 25, 34). Most intravital microscopy studies use a superfuse equilibrated with a 0 or 5% oxygen gas mixture to prevent abnormally high oxygen levels in the tissue. This ensures that tissue oxygenation is achieved solely by blood oxygen delivery (as it is in situ) and that the superfuse is not a source of additional oxygen. Therefore, our high-oxygen superfuse could have had some effect on arteriolar tone and the microvascular pressure profile, although we did not observe any indication of such vasoconstriction.

Digestive juices are secreted each day by the salivary glands, stomach, pancreas, liver, and small intestine. Although emphasis has been placed on the importance of the epithelium in secretory transport of fluid in digestive organs, the role of the microcirculation in transporting fluid to and from the epithelium has received relatively little attention. We found that mean capillary pressure in the gastric mucosa was 28 mmHg, whereas plasma oncotic pressure was 18.9 ± 1.7 mmHg. With the use of these values together with additional data from other studies, it is possible to estimate filtration forces in the gastric mucosal microcirculation. If we consider the values of gastric lymph protein concentration and osmotic reflection coefficient (28) and assume a small positive value for the interstitial fluid pressure (1), there is a 10- to 15-mmHg net driving force for transcapillary filtration. A total safety factor against edema formation (increased lymph flow, interstitial fluid pressure, transcapillary oncotic pressure gradient) has been described for intestinal mucosa and ranges from 12 to 15 mmHg (26). Thus, from this information, gastric mucosal interstitium may be well hydrated and close to fluid imbalance under resting conditions. These conditions may thereby supply the fluid necessary for active epithelial secretion. Further increments in mucosal capillary pressure in excess of 15 mmHg, which may happen during peristaltic muscle contractions, particularly those of the muscularis mucosae, may lead to enhanced passive gastric secretion. Evidence has been shown that increases in interstitial fluid pressure are associated with alkaline fluid secretion across the gastric mucosa (2). Flow of interstitial fluid across the gastric mucosa (so-called gastric filtration) has been observed under a variety of experimental conditions, e.g., elevation of arterial and venous pressure and intra-arterial infusion of ACh, which leads to increased capillary filtration and strong muscular activity (2). Thus one can further speculate that, during peristaltic muscle contractions, high mucosal capillary

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**Fig. 4.** Precapillary, capillary, and postcapillary hydrostatic pressures in muscle (○) and mucosal regions (●) of gastric vasculature. See Fig. 1 legend for vessel identification. Values are means ± SE; n, number of vessels. *P < 0.05 compared with corresponding segment in muscular circulation (by ANOVA).
pressure results in elevated interstitial hydrostatic pressure and an enhanced alkaline fluid secretion, and this may play an important role in protection against luminal acidity.

We found that capillary pressure in the gastric muscle layer was significantly less (23.6 ± 1.4 mmHg) than the calculated mean capillary pressure in the mucosa (28 mmHg) at normal systemic arterial pressures (100–110 mmHg; Fig. 4). This suggests that the Starling forces across the muscle microcirculation may be closer to equilibrium. This idea is further supported by the anatomic difference between the two capillary beds; i.e., mucosal capillaries are of the fenestrated type, whereas those in the muscularis are of the less permeable continuous type. Our findings are consistent with direct pressure measurements by Gore and Bohlen (13, 14) from the rat small intestine, which showed regional differences in capillary hydrostatic pressure between muscle and mucosal layers. They suggested that mesenteric capillaries are primarily a filtering network; intestinal muscle capillaries are normally in fluid balance, whereas, at rest, intestinal mucosal capillaries are primarily absorptive. These findings imply that there are regional differences in transcapillary fluid exchange in the gastrointestinal tract, whereas the whole organ is essentially in fluid balance.

The gastric vasculature is composed of two structurally different microvascular beds, which are connected in parallel at the level of the submucosa. Microsphere measurements indicated that, under resting conditions, the mucosa-submucosa receives 84 ± 3% of the total gastric blood flow and the muscularis 16 ± 3%. These findings are consistent with previous work using similar techniques (3, 5, 11). Differences in intravascular pressure distribution between the muscle and the mucosal region may be explained in terms of differences in vascular morphology, architecture, and relative resistances in the two regions. In Fig. 3 the arterial pressure drop down to the precapillary arterioles was almost identical; pressure was 32.4 ± 1.2 mmHg in MTA and 30.5 ± 1.4 mmHg in MA (P = NS). However, there was a significant difference in capillary pressures: 23.6 ± 1.4 mmHg average MC pressure and 28 mmHg calculated mucosal capillary pressure. We suspect that the difference in mucosal and MC pressure profiles was due to a significant postcapillary venous resistance in the mucosa, because a large pressure drop was observed from CV (26.6 ± 1.1 mmHg) through the SMV2 (21.8 ± 1.6 mmHg) and into the SMV1 (17.1 ± 0.8 mmHg). We found that CV, on the mucosal side of muscularis mucosae, were larger in diameter (36.4 ± 1.1 µm) than the following deeper mucosal venous anastomosis (31.5 ± 1.3 µm) or initial part of SMV2, on the submucosal side of muscularis mucosae. Semiquantitative evidence for our argument can be obtained by calculating simple resistance ratios from the pressure and relative flow data.

Assume that gastric muscle and mucosal vasculatures can be represented by two simple parallel circuits with a common input at the level of the SMA1 and a common output at the level of the SMV1. As we described, the drop in arterial pressure down to the MA and MTA was identical. Therefore, intravascular pressures in these arterioles were considered the input values. It is a simple matter to calculate resistance ratios in this model using relative flow data from the microsphere experiments and intravascular pressure data from Fig. 3. Four different resistance ratios were calculated as follows

\[
R_{a\text{mus}}/R_{a\text{muc}} = \frac{(Q_{\text{muc}}/Q)_{(P_{\text{MA}} - P_{\text{c\text{muc}}})} / (Q_{\text{mus}}/Q)_{(P_{\text{MTA}} - P_{\text{c\text{muc}}})}}{1} (1)
\]

\[
R_{v\text{mus}}/R_{v\text{muc}} = \frac{(Q_{\text{muc}}/Q)_{(P_{\text{c\text{mus}}}/P_{\text{SMV1}})} / (Q_{\text{mus}}/Q)_{(P_{\text{c\text{muc}}}/P_{\text{SMV1}})}}{1} (2)
\]

\[
R_{a\text{mus}}/R_{v\text{muc}} = \frac{(P_{\text{MA}} - P_{\text{c\text{muc}}}) / (P_{\text{MTA}} - P_{\text{c\text{mus}}})}{1} (3)
\]

\[
R_{a\text{muc}}/R_{v\text{muc}} = \frac{(P_{\text{c\text{muc}}}/P_{\text{SMV1}})}{1} (4)
\]

where \(R_a\) and \(R_v\) are the precapillary and postcapillary resistances in the muscle or mucosal vasculature as indicated, \(Q_{\text{mus}}/Q_{\text{muc}}\) and \(Q_{\text{mus}}/Q_{\text{muc}}\) represent the fractional blood flow through the muscle and mucosal layer, respectively, and \(P_{\text{MA}}, P_{\text{MTA}}, P_{\text{SMV1}},\) and \(P_{\text{c}}\) denote the average pressures in MA, MTA, and SMV1 and corresponding capillaries, respectively. Substituting the appropriate values into Eqs. 1–4, we find that

\[
R_{a\text{mus}}/R_{a\text{muc}} = 0.84 (30.5 - 23.6)/0.16 (32.4 - 28) = 8.23 (1a)
\]

\[
R_{v\text{mus}}/R_{v\text{muc}} = 0.84 (23.6 - 17.1)/0.16 (28 - 17.1) = 3.13 (2a)
\]

\[
R_{a\text{mus}}/R_{v\text{muc}} = (30.5 - 23.6)/(23.6 - 17.1) = 1.06 (3a)
\]

\[
R_{a\text{muc}}/R_{v\text{muc}} = (32.4 - 28)/(28 - 17.1) = 0.40 (4a)
\]

The large values of precapillary \(R_{a\text{mus}}/R_{a\text{muc}} = 8.23\) and postcapillary \(R_{v\text{mus}}/R_{v\text{muc}} = 3.13\) resistance ratios of muscle to mucosal circulations indicate low mucosal vascular resistances. This would be expected, because there are approximately five times as many capillaries in the mucosal circulation as in the muscle vasculature. If we assume that the microspheres used to measure the relative blood flows were distributed in direct proportion to the number of parallel channels in each tissue region, \((Q_{\text{mus}}/Q_{\text{muc}})/(Q_{\text{mus}}/Q_{\text{muc}}) = 0.84/0.16 = 5.1\) then, the precapillary-to-postcapillary resistance ratio in the muscle vasculature \(R_{a\text{mus}}/R_{a\text{muc}} = 1.06\) indicates a fairly large equal precapillary and postcapillary resistance in the muscle layer. However, the low mucosal precapillary-to-postcapillary resistance ratio \(R_{a\text{muc}}/R_{v\text{muc}} = 0.40\) suggests that mucosal venules offer high resistance to flow. Therefore, when considered relative to the low mucosal vascular resistances, mucosal venules may be an important determinant of the high mucosal capillary pressure, besides the low precapillary resistance. The relatively high gastric mucosal postcapillary venous resistance is somewhat unique, since very low venular resistances were described in other parts of the intestine (13, 15, 16).

In summary, the present investigation indicates that gastric capillary hydrostatic pressure in the mucosal microcirculation is significantly higher than capillary pressure in the muscle vasculature. Morphological findings and the calculated vascular resistances indicate that the regional difference in capillary pressures may be due to low precapillary, but relatively high postcapillary, resistance in the mucosal microcirculation. Analysis of filtration forces suggests that fluid balance is not maintained in the gastric mucosal microcircula-
tion, which appears to be primarily a filtering network. This conclusion is consistent with the secretory function of this organ.

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REFERENCES


