Jejunal tissue oxygenation and microvascular flow motion during hemorrhage and resuscitation

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Pajk, Werner, Birgit Schwarz, Hans Knotzer, Barbara Friesenecker, Andreas Mayr, Martin Dünter, and Walter Hasibeder. Jejunal tissue oxygenation and microvascular flow motion during hemorrhage and resuscitation. Am J Physiol Heart Circ Physiol 283: H2511–H2517, 2002. —The relationship between flow motion and tissue oxygenation was investigated during hemorrhage/retransfusion with and without dopamine in 14 pigs. During 45% bleed, jejunal microvascular hemoglobin O2 saturation (HbJ02) and mucosal tissue PO2 (P02muc) were recorded in seven control and seven dopamine-treated animals. Mean arterial pressure and systemic O2 delivery decreased during hemorrhage and returned to baseline after retransfusion. Hemorrhage decreased P02muc from 33 ± 2.8 to 13 ± 1.6 mmHg and HbJ02 from 53 ± 4.9% to 32 ± 3.9%, respectively, in control animals. During reperfusion, P02muc and HbJ02 remained low. Dopamine increased P02muc from 28 ± 4.3 to 45 ± 4.6 mmHg and HbJ02 from 54 ± 5.7% to 69 ± 1.5% and attenuated the decrease in P02muc and HbJ02 during hemorrhage. After retransfusion, dopamine restored P02muc and HbJ02 to baseline. Control animals developed rhythmic HbJ02 oscillations with increasing amplitude (frequency, 4.5 to 7.6 cycles/min) and showed an inverse relationship between P02muc and HbJ02 oscillation amplitude. Dopamine prevented regular flow motion. The association between decreased P02muc and increased oscillations in HbJ02 after normalization of systemic hemodynamics and O2 transport in control animals suggests a cause-and-effect relationship between low tissue PO2 and flow motion activity within the jejunal microcirculation.

hemorrhagic shock; vasomotion; dopamine; microcirculation; jejunum

Vasomotion defined as regular rhythmic oscillations of arteriolar microvessel diameters has been described by many authors (3, 20, 28) using different techniques under physiological and pathophysiological conditions. Vasomotion induces alterations in red blood cell velocity called flow motion, which affects microcirculatory transit times and facilitates gas exchange between microvessels and tissue (14). In previous studies, we observed rhythmic oscillations in microvascular hemoglobin O2 saturation (HbJ02) within the jejunal microcirculation in hemodynamically stable pigs. These rhythmic oscillations were unrelated to systemic hemodynamic parameters, respiratory frequency, and intestinal peristalsis and occurred at a frequency of 3.4–5 cycles/min suggesting a flow motion-related phenomenon.

Dopaminergic drugs such as dopamine and fenoldopam significantly attenuated flow motion and at the same time increased jejunal microvascular hemoglobin O2 saturation and mucosal tissue PO2 (P02muc) suggesting a change in microcirculatory blood flow pattern from alternate periodic vasoconstriction/vasodilation to fixed vasodilation (8).

Recent investigations suggest that flow motion becomes more obvious at the lower range of arterial blood pressure at which blood flow autoregulation is seen. Therefore microvascular hemoglobin O2 saturation and P02muc were recorded in a pig model of stepwise hemorrhage and retransfusion with and without treatment with dopamine to investigate the relationship between the magnitude of flow motion and tissue oxygenation. We hypothesized that flow motion activity is dependent on tissue oxygenation and that dopamine is able to attenuate flow motion during hemorrhage in the pig jejunum. Flow motion within the jejunal wall was indirectly determined by analyzing tracings of HbJ02, whereas tissue oxygenation was assessed by measurement of P02muc using Clark-type multiwire O2 electrodes.

MATERIALS AND METHODS

Animal preparation. Animal experiments were approved by the National Ministry of Science and Research. Thirteen domestic pigs weighing between 35 and 40 kg were fasted for 12 h with free access to water. The animals were anesthetized with 20 mg/kg im ketamine HCl, orally intubated, and mechanically ventilated with a positive end-expiratory pressure of 5 cmH2O. Tidal volume and respiratory frequency were adjusted to maintain normocapnia; inspiratory O2 concentration was chosen to keep PaO2 levels between 100 and

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150 mmHg. Anesthesia was continued with an infusion of 0.8 mg·kg⁻¹·min⁻¹ midazolam and 20 μg·kg⁻¹·min⁻¹ fentanyl. Neuromuscular block was produced by bolus injections of 0.15 mg/kg vecuronium.

After induction of anesthesia, the right carotid artery was cannulated for continuous recording of arterial blood pressure and for intermittent blood sampling. A balloon-tipped thermodilution pulmonary artery catheter (Baxter Healthcare; Irvine, CA) and a separate 14-gauge catheter were inserted via the right internal jugular vein for measurements of cardiac output, central venous pressure, pulmonary arterial pressure, pulmonary capillary occlusion pressure (PAOP) and intermittent sampling of mixed venous blood, and infusion of dopamine. An 8.5-Fr catheter was inserted into the left internal jugular vein for withdrawal of 45% of calculated blood volume in three steps.

After midline laparotomy was performed, a segment of the jejunal mucosa was exposed by a antimesenteric incision. The boundary of the mucosa was sutured to the oval opening of a cork plate. A 16-gauge catheter was placed into a mesenteric vein for intermittent blood sampling. Except for the exposed mucosa, the intestine was reintroduced into the abdominal cavity and the abdomen was closed for the most part. With this preparation, the mucosa is placed directly above the skin surface of the abdomen and is covered with a humidified servo-controlled chamber heated to 37°C.

Measurement techniques. Arterial pulmonary artery and central venous pressure were measured using pressure transducers (model P10EZ, Spectramed-Statham; Bilthoven, The Netherlands). Cardiac output was determined in triplicate by the thermodilution method. Heart rate, blood pressure, and core temperature were continuously recorded. Arterial, central venous, and mesenteric venous blood gases and acid-base status were analyzed using an automatic blood gas analyzer (model 995, AVL Biomedical Instruments; Graz, Austria). Hemoglobin O₂ saturation was measured with a hemoximeter (Cooximeter, AVL Biomedical Instruments). Hemoglobin concentration and microhematocrit were determined by standard hematological methods.

Measurements of jejunal tissue oxygenation. Methodology for the measurement of F0₂muc and HBjO₂ has been described in detail in previous studies (8, 13). Briefly, F0₂muc was measured by two Clark-type multwire surface electrodes (Eschweiler; Kiel, Germany), which were calibrated using pure nitrogen and room air in a 37°C warmed water bath. A single electrode consists of eight platinum wires, each 15 μm in diameter, representing eight individual measuring points and an Ag-AgCl reference electrode. An Erlangen microlight guide spectrophotometer (EMPHO II, BGT; Überlingen, Germany) was used for determination of HBjO₂. The measuring principle is based on the use of one illuminating and six detecting microlight guides (each 250 μm in diameter) and a rapidly rotating bandpass interference filter disk for the generation of monochromatic light in 2-nm steps within the spectral range of 502–628 nm representing 64 different wavelengths. Absolute values of HBjO₂ were calculated with the use of an algorithm described in detail by Frank et al. (6), which has been validated in a previous study (13). All sensors are introduced into the measurement chamber and onto the mucosal surface via small openings at the top of the chamber, which are sealed with small caps after the sensors have been correctly positioned. All sensors were kept in place by adhesion using small polyvinylchloride caps, which hold the sensor and are surrounded by a transparent thin (2 cm in diameter) rubber patch to avoid exposure to room air.

Experimental procedure. After surgical preparation and a resting period of 120 min, baseline measurements of hemodynamics, blood gases, and intestinal tissue O₂ supply were performed (time (t) = 0 min). Animals were randomized to one of two experimental groups: group C (n = 7) served as controls, whereas group D (n = 7) animals received a continuous intravenous infusion of dopamine (16 μg·kg⁻¹·min⁻¹) beginning after baseline measurements and continued throughout the experimental period. Animals were given Ringer lactate and gelatin intravenously to maintain PAOP at 12–14 mmHg. At t = 30 min, infusion of Ringer lactate and gelatin was stopped and 45% of the calculated blood volume was removed in three equal steps in all animals at t = 60, 90, and 120 min, respectively. Measurements of systemic and regional parameters were repeated after every bleeding step. After 30 min (t = 150 min), the shed blood was retransfused and an additional infusion of crystalloids and colloids was given to restore pulmonary artery occlusion pressure to baseline values. Measurements were performed at t = 180 min and t = 210 min. At the end of the experiments anesthetized animals were euthanized by central venous bolus injection of 40 μg/kg KCl.

Statistical analysis. Results are presented as means ± SD. Comparison between baseline values was made using unpaired t-test. Overall effects within and between groups were evaluated by repeated measurements of variance (ANOVA). In case of significant differences, further comparisons were made with paired t-test (within group to baseline) and unpaired t-tests (between groups at individual time points). Values of P ≤ 0.05 were considered significant. The Bonferroni-Holm procedure was used for correction of multiple comparisons.

Fast Fourier transform analysis was performed for every single time series of HBjO₂ to obtain a quantitative description of main oscillatory frequency components and amplitudes. Frequency resolution was determined by the signal-sampling interval of 189 s and by limiting the number of Fourier frequencies from 4 to 33, resulting in an oscillatory frequency range of 1.27 to 10.5 cycles/min. To simplify further statistical analysis, frequencies were divided into three groups: group I (1.27–4.4 cycles/min), group II (4.5–7.6 cycles/min), and group III (7.7–10.5 cycles/min). Mean values of oscillatory amplitudes were calculated for each frequency group and every measurement period. Because group II oscillations demonstrated by far the most important changes in oscillatory amplitude further statistical analysis of vasomotion amplitudes was limited to group II frequencies. Mean values of amplitudes were used for statistical comparisons.

RESULTS

No differences in systemic hemodynamics, O₂ transport variables, and acid-base status were observed between group C and D animals at baseline (Table 1). Stepwise hemorrhage significantly decreased mean arterial pressure, cardiac index, pulmonary capillary occlusion pressure, and systemic O₂ delivery in both groups, whereas retransfusion restored these parameters to baseline values. Systemic O₂ consumption and arterial blood gas variables remained constant throughout the experiment.

There were no differences in baseline Po₂muc, HBjO₂, mesenteric venous pH, mesenteric venous Po₂ (Po₂muv), and jejunal O₂ extraction ratio between control and dopamine-treated animals (Table 2). Hemorrhage significantly decreased Po₂muc from 33 ± 2.8 mmHg (t = 0
stepwise bleeding, and after resuscitation. Mucosal PO2, HBjO2, P O2mv, and jejunal O2 extraction ratio at baseline, during and after arterial oxygen tension; PaCO2 arterial carbon dioxide tension; pHa, arterial pH; DO2sys, systemic oxygen delivery; VO2sys, systemic oxygen consumption; Hct, hematocrit. *P < 0.05 vs. baseline.

Table 2. Mucosal PO2, HBjO2, PO2mv, and jejunal O2 extraction ratio at baseline, during and after stepwise bleeding, and after resuscitation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline, min</th>
<th>Hemorrhage, min</th>
<th>After Bleeding (150 min)</th>
<th>Retransfusion, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>30</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>PO2mv, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>33 ± 6.8</td>
<td>30 ± 7.5</td>
<td>23 ± 5.1*</td>
<td>18 ± 3.7*</td>
</tr>
<tr>
<td>Group D</td>
<td>28 ± 10.5</td>
<td>45 ± 11.1†</td>
<td>42 ± 11.1*</td>
<td>33 ± 9.9†</td>
</tr>
<tr>
<td>HBjO2, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>53 ± 11</td>
<td>50 ± 13.9</td>
<td>42 ± 7.2</td>
<td>34 ± 5.6</td>
</tr>
<tr>
<td>Group D</td>
<td>54 ± 13.9</td>
<td>69 ± 3.6†</td>
<td>68 ± 8.2†</td>
<td>59 ± 15.1†</td>
</tr>
<tr>
<td>pH mv</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Group C</td>
<td>7.4 ± 0.02</td>
<td>7.41 ± 0.03</td>
<td>7.41 ± 0.02</td>
<td>7.39 ± 0.02</td>
</tr>
<tr>
<td>Group D</td>
<td>7.45 ± 0.03</td>
<td>7.45 ± 0.03</td>
<td>7.43 ± 0.03</td>
<td>7.39 ± 0.05</td>
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<tr>
<td>PO2mv, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>40 ± 3.6</td>
<td>41 ± 2.7</td>
<td>41 ± 3.5</td>
<td>44 ± 1.5</td>
</tr>
<tr>
<td>Group D</td>
<td>40 ± 3.4</td>
<td>38 ± 4</td>
<td>39 ± 3.7</td>
<td>43 ± 3.9</td>
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<tr>
<td>POmv, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>55 ± 4.4</td>
<td>53 ± 5</td>
<td>48 ± 3*</td>
<td>44 ± 2.4*</td>
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<tr>
<td>Group D</td>
<td>54 ± 9.9</td>
<td>68 ± 9.6†</td>
<td>64 ± 7.3†</td>
<td>53 ± 5.9†</td>
</tr>
<tr>
<td>O2ER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>0.26 ± 0.05</td>
<td>0.29 ± 0.08</td>
<td>0.36 ± 0.05*</td>
<td>0.44 ± 0.02*</td>
</tr>
<tr>
<td>Group D</td>
<td>0.25 ± 0.11</td>
<td>0.12 ± 0.08†</td>
<td>0.18 ± 0.08†</td>
<td>0.32 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SD. PO3mv, mucosal PO2; HBjO2, jejunal microvascular hemoglobin O2 saturation; pH mv, mesenteric venous pH; PO2mv, mesenteric venous CO2 tension; PO2mv, mesenteric venous O2 tension; O2ER, jejunal O2 extraction ratio. †P < 0.05 vs. baseline; ‡P < 0.05 vs. group C.

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min) to 13 ± 1.6 mmHg (t = 150 min), HBjO2 from 53 ± 4.9% (t = 0 min) to 32 ± 3.9% (t = 150 min), and Po2muc from 55 ± 1.8 mmHg (t = 0 min) to 41 ± 1.5 mmHg (t = 150 min) in group C animals. Jejunal O2 extraction ratio (O2ER) significantly increased from 0.26 ± 0.02 to 0.51 ± 0.03. During reperfusion, Po2muc and HBjO2 remained low in group C animals. Dopamine treatment significantly increased Po2muc from 28 ± 4.3 mmHg (t = 0 min) to 45 ± 4.6 mmHg (t = 30 min), HBjO2 from 54 ± 5.7% (t = 0 min) to 69 ± 1.5% (t = 30 min), and Po2muc from 54 ± 4 mmHg (t = 0 min) to 68 ± 3.9 mmHg (t = 30 min). O2ER significantly decreased from 0.25 ± 0.05 (t = 0 min) to 0.12 ± 0.03 (t = 30 min). Po2muc, HBjO2, and Po2muc remained significantly higher and O2ER significantly lower during hemorrhage and retransfusion in group D animals compared with controls. There were no significant differences in PCO2muc and pH between groups.

Figure 1 demonstrates representative original tracings of HBjO2 from one group C and group D animal. During hemorrhage and retransfusion, group C animals developed rhythmic oscillations of HBjO2 with increasing oscillatory amplitude. In contrast, dopamine infusion increased HBjO2 and prevented development of major HBjO2 oscillations.

Changes in HBjO2 oscillation amplitude within groups I–III are shown in Fig. 2. Group II oscillations (4.5–7.6 cycles/min) progressively increased with the severity of hemorrhage and remained high even after retransfusion in group C animals, whereas infusion of dopamine prevented the increase in HBjO2 oscillations.

The relationship between mean Po2muc values and amplitudes of HBjO2 oscillations in the frequency range of 4.5–7.6 cycles/min are shown in Fig. 3. In contrast to group D animals, we observed a significant inverse relationship between Po2muc and HBjO2 oscillation amplitude in group C animals.

Fig. 1. Original tracings of microvascular hemoglobin O2 saturation (HBjO2) in a control animal (A) and a dopamine-treated animal (B).

Fig. 2. A and B: changes in HBjO2 oscillation amplitude within the frequency groups I (1.27–4.4), II (4.5–7.6), and III (7.7–10.5). Values of oscillatory amplitudes are means ± SD. *P ≤ 0.05 vs. baseline; †P ≤ 0.05 vs. control animals. cpm, Cycles per minute.

Fig. 3. Relationship between mucosal tissue Po2 (Po2muc) and amplitude of HBjO2 oscillations in the frequency range of 4.5–7.6 cycles/min in control (A) and dopamine-treated animals (B).
DISCUSSION

This is the first study to demonstrate an inverse relationship between tissue O₂ supply as reflected by jejunal Po2muc and flow motion activity as within the jejunal wall in a model of stepwise hemorrhage and subsequent fluid resuscitation. Minor oscillations at baseline progressively increased in amplitude without significant changes in frequency, suggesting increased arteriolar vasomotion within the jejunal microcirculation. Despite fluid resuscitation and normalization of systemic hemodynamics and O₂ transport parameters, mean Po2muc remained low and increased flow motion persisted in control animals. Treatment with continuous infusion of 16 μg·kg⁻¹·min⁻¹ dopamine significantly increased jejunal tissue oxygenation at baseline and attenuated flow motion during hemorrhage and resuscitation. Dopamine significantly preserved Po2muc at 15% and 30% blood loss when compared with controls. However, after 45% blood removal, there were no differences in mean Po2muc between groups.

The biological mechanisms responsible for flow motion are still under investigation. It has been shown that voltage-operated L-type Ca²⁺ channels, cGMP, the Na⁺/K⁺ pump, and the sarcoplasmic reticulum are part of a biological oscillator system forming the basis for arteriolar vasomotion (1, 10, 11). Electromechanical coupling with oscillations of smooth muscle cell membrane potential, intracellular Ca²⁺, and arteriolar diameter has been demonstrated during hyperoxia in the hamster cheek pouch preparation in vivo (29). Blockade of L-type Ca²⁺ channels with nifedipine immediately attenuated oscillations in membrane potential, intracellular Ca²⁺ and abolished vasomotion, thus pointing at a key role of cell membrane Ca²⁺ channels in the regulation of vasomotion. Recently, Peng et al. (22) hypothesized that arteriolar vasomotion is initiated when intermittent unsynchronized release of Ca²⁺ from the sarcoplasmatic reticulum of vascular smooth muscle cells becomes synchronized in the presence of intact endothelium and under certain conditions.

Unfortunately, mechanisms contributing to the evolution of regular, rhythmic flow motion during periods of limited O₂ supply to tissue are much less clear. Under in vitro conditions, moderate hypoxia has been shown to inhibit L-type Ca²⁺ channels in arteriolar smooth muscle cells leading to a decrease in intracellular Ca²⁺, promoting muscle relaxation without vasomotion (5). In contrast, in the hamster skeletal microcirculation, moderate to severe hypoxia has been shown to increase the frequency of arteriolar rhythmic diameter changes (2). Increased flow motion was suppressed by phentolamine, a selective α-adrenoceptor blocker (4).

Because of contrasting experimental results, we can only speculate on the mechanisms of increased flow motion during ischemia and reperfusion. Despite the lack of final evidence, the association between decreased Po2muc and increased oscillations in HbjO₂ in face of normalized systemic hemodynamics and systemic O₂ transport after resuscitation suggests that the magnitude of flow motion is not simply dependent on presence of systemic hypotension or low systemic blood flow. One might speculate about the existence of a cause effect relationship between low tissue Po₂ and flow motion activity involving an O₂-sensing system within the jejunal microcirculation.

Dopamine was able to block flow motion even in the presence of low tissue Po₂. Experimental studies (7, 9, 18) have shown that catecholamines interfere with arteriolar vasomotion. Norepinephrine, for example, induces vasomotion by converting unsynchronized intracellular Ca²⁺ oscillations into global synchronized changes in vascular smooth muscle cells in the presence of endothelium via a cGMP-dependent mechanism (22). In previous experiments, we have demonstrated that the dopaminergic drugs dopamine and fenoldopam increase Po2muc in a dose-related manner and offset flow motion within the jejunal microcirculation. These effects were blocked by the application of SCH-23390, a selective dopamine-1 receptor antagonist, suggesting that attenuation of flow motion was mediated by a cAMP-dependent mechanism (author’s unpublished observation). Therefore it is conceivable that drugs that increase intracellular cAMP within vascular smooth muscle cells interfere with the activity of flow motion in the jejunal microcirculation even in the presence of limited O₂ supply.

Arteriolar vasomotion of microvessels and increased flow motion has been increasingly observed under conditions of low flow, hypotension, and tissue hypoxia. Increased flow motion is believed to provide adequate temporal and spatial tissue perfusion to maintain nutritional blood flow in tissue under limited O₂ supply. Mathematical model analysis demonstrated that hydraulic resistance of blood vessels exhibiting vasomotion is less compared with vessels showing static diameters with identical average (26). Furthermore, it has been suggested that vasomotion induced flow motion facilitates fluid reabsorption within capillaries and venules during periods of arteriolar vasoconstriction (15). Augmented fluid absorption might be of major importance for attenuating development of tissue edema in particular after ischemia-reperfusion injury. Formation of tissue edema may continuously deteriorate tissue O₂ supply. Rücker et al. (23) demonstrated in a muscle peristium skin preparation in rats that flow motion in critically perfused tissue not only preserves functional capillary density but also protects the adjacent tissue from capillary perfusion failure. Flow motion in different gut segments has been repeatedly demonstrated under physiological and pathophysiological conditions. By means of laser-Doppler velocimetry and reflectance spectrophotometry, Yamaguchi et al. (30) demonstrated regular changes in microcirculatory blood flow, indexes of microvascular hemoglobin oxygenation and concentration at a frequency ranging between 4 and 6 cycles/min in the rat gastric mucosa. These oscillations increased in amplitude and frequency during hemorrhage, whereas gastric motility remained constant. During normothermic cardiopul-
monary bypass (CPB), we reported the onset of HBjO$_2$ oscillations with frequencies of 5–7 cycles/min simultaneously with a significant drop in PO$_{2muc}$ after institution of CPB (12). There were no significant differences in mean arterial pressure and systemic O$_2$ delivery between sham and CPB animals. Therefore, increased flow motion most likely resulted from local alterations in jejunal blood flow induced by the onset of CPB.

Methods for assessing tissue oxygenation and flow motion activity. Measurements of organ surface O$_2$ using Clark-type electrodes have been established in several studies as a suitable instrument for assessing tissue oxygenation in a variety of organs (7, 17, 19, 21). PO$_{2muc}$ measurements require diffusion of O$_2$ through mucosal epithelial cells to the electrode where O$_2$ is consumed in a complex redox reaction (16). The PO$_2$ measured reflects the PO$_2$ in underlying cells, as long as diffusion error of the electrode is small and the pollution of electrode surface by atmospheric O$_2$ can be excluded. O$_2$ consumption of multiwire surface electrodes is small. Measurement errors due to diffusion of atmospheric O$_2$ to the electrode surface can be avoided with the measurement setup used (13). Therefore, multiwire surface electrodes reflect tissue PO$_2$ present in mucosal epithelial cells underneath the electrode. On the basis of scanning electronic microscopy investigation results of microvasculature of jejunal villi in pigs and taking into account the surface area of 19 mm$^2$ of a multiwire surface O$_2$ electrode, the electrode roughly covers a mucosal area containing 380 villi (13).

Reflectance spectrophotometry was introduced by Sato et al. (24) for calculating amount and O$_2$ saturation of hemoglobin in gastric mucosal vessels. By injecting black ink into different layers of the intestinal wall, they demonstrated that the catchment volume of microvascularization of jejunal villi in pigs and taking into account the surface area of 19 mm$^2$ of a multiwire surface O$_2$ electrode, the electrode roughly covers a mucosal area containing 380 villi (13).

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REFERENCES