Functional heterogeneity of oxygen supply with blood and hemoglobin-based oxygen carriers in porcine models of hemorrhagic shock
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Abstract
The aim of this study was to investigate the relation between microvascular and venous oxygen pressures during hemorrhagic shock and resuscitation in the pig intestine. To this end microvascular PO$_2$ ($\mu$PO$_2$) was measured by quenching of Pd-porphyrin phosphorescence by oxygen and validated for the intestines. In addition, mesenteric venous blood gasses, blood flow, ilial CO$_2$ production and global hemodynamics were also measured. In one group ($n=11$), moderate shock was induced by withdrawal of 40% of the circulating blood volume. Seven of these animals were resuscitated with a crystalloid solution and four with the withdrawn blood. In a second group of three animals, a more severe shock was induced by withdrawal of 50% of the circulating blood volume; these animals were not resuscitated. Baseline mesenteric venous PO$_2$ and $\mu$PO$_2$ values were similar (60 ± 9 and 60 ± 11 mmHg, respectively). During moderate shock, $\mu$PO$_2$ dropped significantly below mesenteric venous PO$_2$ (26 ± 10 versus 35 ± 8 mmHg). After resuscitation with crystalloid solution, $\mu$PO$_2$ and mesenteric venous PO$_2$ rose to 44 ± 9 and 44 ± 6 mmHg, respectively. In the group that received the withdrawn blood, values were 41 ± 9 and 53 ± 12 mmHg, respectively. Severe shock resulted in a drop in the mesenteric venous PO$_2$ ($n=3$) to a value similar to that seen in the moderate shock group, but the gut $\mu$PO$_2$ dropped to a much lower value than that of the moderate shock group (15 ± 5 versus 26 ± 10 mmHg). The results indicate that the oxygenation of the microcirculation of the gut can become lower than the venous PO$_2$ under conditions of hemorrhagic shock.

Introduction
Intestinal ischaemia resulting from shock is particularly hazardous because it can cause loss of barrier function, which could contribute to the development of sepsis. The oxygenation of the microcirculation can show deterioration in advance of changes in global parameters of oxygenation. However, the behaviour of microcirculatory oxygenation in shock and resuscitation remains largely unknown. There-
Therefore methods are needed to assess the microvascular \( \text{PO}_2 \) (\( \mu \text{PO}_2 \)). The application of the quenching of Pd-porphyrin phosphorescence by oxygen provides a new promising optical method. This method of oxygen measurement has been applied to measure the \( \text{PO}_2 \) of the microcirculation of the eye\(^{21}\), the isolated rat heart\(^{22,23}\), brain\(^{24,25}\), muscle\(^{20,26}\), and tumour\(^{27}\). However, it has not as yet been applied to the intestines. The quenching of phosphorescence technique provides a quantitative measure of the amount of oxygen in the microcirculation. In this technique, the decay time of the phosphorescence is measured after pulsed excitation. The Pd-porphyrin is bound to albumin and when this molecular complex is injected intravascularly the dye is confined to the circulation and enables measurement of the \( \text{PO}_2 \) of this compartment.\(^{21}\) We recently calibrated and characterized the use of Pd-porphyrin phosphorescence for measurement of \( \mu \text{PO}_2 \) with a newly developed phosphorimeter.\(^{28}\) The main purpose of this study was to investigate the relation between the microvascular oxygenation of the pig ileum, measured by the quenched phosphorescence of Pd-porphyrin, and regional and global parameters of oxygenation, during hemorrhagic shock and resuscitation. Pd-porphyrin phosphorescence quenching by oxygen was validated for measurement of \( \mu \text{PO}_2 \) in the intestine by comparison with surface electrode measurements. The results presented in this study show that \( \mu \text{PO}_2 \) becomes lower than the venous \( \text{PO}_2 \) during hemorrhagic shock, indicating that oxygen bypasses the microcirculation. This indicates that venous \( \text{PO}_2 \) is not a good measure of the oxygenation of the intestine under all circumstances.

**Methods**

*Quenching of phosphorescence*

The quenching of the phosphorescence of Pd-porphyrin by oxygen is based upon the principle that a Pd-porphyrin molecule which has been excited by light can either release this absorbed energy as light (phosphorescence) or transfer the absorbed energy to oxygen. As a result of the energy transfer to oxygen, the Pd-porphyrin molecule releases the absorbed energy without phosphorescence, resulting in a phosphorescence intensity and decay time which is dependent on the oxygen concentration. The Stern-Volmer relation gives the relation between the decay time and oxygen concentration: \( \tau_0/\tau=1+\tau_0k_q[\text{O}_2] \) (eqn. 1). In this relation, \( \tau_0 \) (in \( \mu \text{s} \)) is the decay time in the absence of oxygen, \( \tau \) (in \( \mu \text{s} \)) is the decay time measured in the presence of \( [\text{O}_2] \) (\( \mu \text{M} \)), and \( k_q \) (\( \mu \text{M}^{-1}\mu \text{s}^{-1} \)) is the quenching constant. The temperature-dependent calibration constants \( \tau_0 \) and \( k_q \) have been determined *in vitro*.\(^{28}\) The Stern-Volmer relation is defined for oxygen concentration rather than oxygen partial pressures. To compare the oxygen concentration ([\( \text{O}_2 \)]) with oxygen partial pressure (\( \text{PO}_2 \)) given conventionally, the oxygen concentrations from eqn (1) can be converted to \( \text{PO}_2 \) values: \( [\text{O}_2]=\alpha/V_mP_g\text{PO}_2 \) (eqn. 2), where \( \alpha \) is the Bunsen coefficient, \( V_m \) is the molar volume of oxygen at 0°C (22.4 l mol\(^{-1}\)) and \( P_g \) is the standard pressure (760 mmHg).
For the *in vivo* studies, the oxygen solubility in serum (α₂) was used, which is 10% lower than the α of pure water. ²⁹

Roughly three layers of tissue can be distinguished in the ileum: the muscularis externa, the submucosa and the mucosa. An estimation of the penetration depth of the used excitation light and the attenuation of the phosphorescence intensity was made to determine which microcirculatory layers of the intestinal wall contribute predominantly to the measured PO₂. To this end, a Monte-Carlo simulation of the phosphorescence measurement was performed in which the absorption coefficient (μₐ) and scattering coefficient (μₛ) from human colon tissue were used. ³⁰ The Monte-Carlo simulation simulates the optical pathway of a large number of photons and gives the light distribution within the tissue. ³¹ The calculated light distribution was compared with the geometric dimensions of histological sections of the ileal wall. To this end, sections, stained with Haematoxylin to stain the cell nucleus and Aspholoxin to stain the cytoplasm, were analysed microscopically.

**The animal model**

The Animal Ethical Committee of the Academic Medical Centre of the University of Amsterdam approved the experiments described in this study. After an overnight fast, female cross-bred Land race × Yorkshire pigs (*n* = 14; mean weight, 15 ± 2 kg; Vendrig, Amsterdam, The Netherlands) were sedated with ketamine-HCl (Nimatek; UAV, Cuyk, The Netherlands; 10 mg kg⁻¹i.m.) and intubated. Anesthesia throughout the experiment was, after an initial bolus, maintained by continuous infusion of fentanyl citrate (Fentanyl; Janssen Pharmaceutica, Tilburg, The Netherlands; bolus, 15 μg kg⁻¹; 15 μg kg⁻¹ h⁻¹i.v.) and midozolam-HCL (Dormicum; Hoffmann-LaRoche, Mijdrecht, The Netherlands; bolus, 0.75 mg kg⁻¹; 1.5 mg kg⁻¹ h⁻¹i.v.). No other drugs were administered which could influence the measurements. Muscle relaxation was maintained with vecuronium-bromide (Organon; Teknika B.V., Boxtel, The Netherlands; bolus, 1 mg kg⁻¹; 0.75 mg kg⁻¹ h⁻¹i.v.). During the preparation the heart rate and blood pressure were monitored for signs of stress; if this occurred the level of anesthesia was adapted accordingly. After the depth of anesthesia was established, the infusion rate of the anesthetics was kept constant. Muscle relaxation was assessed by monitoring the eyelid movement and shivering of the animal.

After intubation, ventilation (Dräger AV-1; Drägerwerk A.G., Lubeck, Germany) was performed by intermittent positive pressure ventilation with a mixture of 33% O₂ and 67% N₂. During preparation, artificial ventilation was instituted to maintain an end-tidal PCO₂ of 35–40 mmHg. A positive end-expiratory pressure of 5 mmHg was used to prevent atelectases. All animals received an infusion of 25 ml kg⁻¹ h⁻¹ 0.9% NaCl solution throughout the experiment to compensate for fluid loss. A catheter was
placed in the brachial artery to measure blood pressure and heart rate. A Swan Ganz catheter (Edwards 5 Fr.; Baxter Healthcare Co., Deerfield, KA, USA) was introduced in the right jugular vein for measurement of the cardiac output and central temperature. A catheter in the left jugular vein was used for blood withdrawal and administration of the resuscitation fluids. Blood samples were taken from the brachial artery and the right pulmonary artery for determination of the arterial and mixed venous blood gasses, respectively. In addition, a vein in the mesentery was cannulated to obtain mesenteric venous blood samples. Blood gasses were analysed using an ABL 505 blood gas analyser (Radiometer, Copenhagen, Denmark). Hemoglobin (Hb) concentration and saturation was measured using an OSM 3 (Radiometer, Copenhagen, Denmark). A flow probe (Transonic Systems Inc., NY, USA) was used to measure blood flow in the superior mesenteric artery. The intraluminal PCO₂ of the ileum was measured as an indicator of regional ischaemia. This was done by inserting a CO₂-permeable Silastic balloon (Baxter HealthCare Co.) filled with saline into the lumen of the ileum. After 30 min equilibration, a sample was taken from this balloon and analysed for PCO₂ using the blood gas analyser. Because equilibration of CO₂ in saline is only 77 % complete after 30 min, the PCO₂ values were multiplied by 1.24 to compensate for this effect.

Oxygen measurements
A length of ileum was extracted from the peritoneal cavity via a mid-line laparotomy. The fibre of the phosphorimeter and a multiwire surface oxygen electrode (GMS, Kiel-Mielkendorf, Germany), with built-in thermocouple, were placed on the serosa of the last 10 cm of the ileum. The oxygen electrode was calibrated before and after the experiment with air- and nitrogen-saturated water. A rubber ring was placed around the electrode in contact with the tissue to isolate the electrode from atmospheric oxygen. The fibre phosphorimeter used to measure μPO₂ by quenched phosphorescence of Pd-porphyrin has been described elsewhere. Since the calibration constants kₚ and τ₀ are temperature dependent, the temperature of the intestine surface was measured throughout the experiment and used for continuous correction of kₚ and τ₀. Pd meso-tetra (4-carboxyphenyl) porphine (Porphyrin Products, Logan, UT, USA) was dissolved in 3 ml DMSO (67 mg ml⁻¹) and added to 50 ml of a human albumin solution in saline (40 g l⁻¹). This mixture was brought to a pH of 8 using Tris base. Eight hours later, HCl was used to buffer the solution to a pH of 7.4. This method of preparation avoids pH dependency of the calibration constants. From this solution, 12 mg (kg body weight)⁻¹ was injected i.v.

After instrumentation, the pigs were allowed to stabilize for 30 min. Blood gasses were sampled every 15 min and the hemodynamic parameters measured continu-
ously. For the analyses of the arterial and venous blood gasses, samples of 1 ml were taken for a total of 15 measurements per experiment. The μPO$_2$ was measured every 30 s by calculation of the decay time of 50 flashes of the excitation lamp.

_Hemorrhagic shock_

Moderate hemorrhagic shock was induced in 11 pigs by withdrawal of 25 ml kg$^{-1}$ blood in four steps. Each step took 5 min, followed by a 10 min stabilization period. After this a set of measurements was carried out. Blood volumes, expressed as blood volume per kilogram body weight, amounted to 10, 7, 5 and 3 ml kg$^{-1}$, respectively. In four pigs, 1 h of shock was followed by donation of the withdrawn blood (autologous blood group). In the remaining seven pigs, resuscitation was applied by infusion of three times the withdrawn volume of Ringer lactate solution (N.P.B.I., The Netherlands) (crystalloid group). This resuscitation protocol was performed according to the recommendations of the American College of Surgeons Committee on Trauma. To investigate the effect of hyperoxia on the different oxygen parameters under baseline conditions, during shock, immediately after resuscitation and 60 min after resuscitation, the inspired O$_2$ fraction (FiO$_2$) was elevated from 0.3 to 1, for a period of 10 min. At the end of these FiO$_2$ steps, a complete set of measurements was made after which FiO$_2$ was brought back to 0.3. Experiments were terminated by injection of a lethal dose of 10 mM KCl. In five pigs of the crystalloid group, measurement of PO$_2$ by the surface electrode and Pd-porphyrin techniques and blood gasses was continued after the blood flow had stopped.

Simultaneous phosphorescence and oxygen electrode measurements were done on the mucosa before the haemorrhagic shock protocol was started ($n=5$). For these measurements 4 cm of the ileum was opened along the antimesenteric border to make the mucosa accessible. In these experiments, the mucosal and serosal PO$_2$ were measured simultaneously, using both the surface electrode and Pd-porphyrin phosphorescence quenching.

In a group of three pigs, severe hemorrhagic shock was induced by withdrawal of 50 % of the blood volume. Blood was withdrawn in four steps: 15, 12, 8 and 5 ml kg$^{-1}$, respectively. The changes in the oxygenation of the ileum were observed for 4 h without any form of resuscitation, other than the maintenance fluids (25 ml kg$^{-1}$ h$^{-1}$ 0.9 % NaCl solution). Every 15 min, a full set of measurements was made. The pigs were killed after 4 hrs.

_Data analysis_

Statistical significance was determined by Student’s $t$ test in which the null hypothesis was rejected for $P < 0.05$. The simulations on the propagation of light in tissue were
Results

The depth of measurement using the phosphorescence technique

The penetration depth of the excitation light in the tissue and the collection efficiency of the phosphorescence light determine the depth of measurement of the phosphorescence technique. The penetration depth of both excitation and emission light was calculated using a Monte-Carlo simulation of the light paths of emission and excitation. The calculations were done using the optical parameters reported for the intestines by Marchesini et al.\textsuperscript{30} The attenuation of the phosphorescence light at 700 nm is much smaller (Figure 1), therefore the measurement depth will be determined predominantly by the attenuation of the excitation light. The results of the simulation (Figure 1) together with the geometry of the intestine predict that 20% of the excitation light reaches the mucosa when illumination is from the serosa. Figure 1 shows that the catchment depth for the quenching of Pd-porphyrin phosphorescence was of the order of 0.5 mm. This in contrast to the surface oxygen electrode which only measures a layer 15 μm thick.\textsuperscript{37} To experimentally verify the differences in catchment depth of the electrode and phosphorescence techniques, simultaneous measurements using both techniques were done on both serosa and mucosa.

Figure 1. Simulated light paths of the used excitation (■; 520 nm) and phosphorescence light (▲; 700 nm) in the ileum. Simulations were done by a Monte-Carlo simulation using the scattering and absorbing properties of human colon tissue.\textsuperscript{30} Calculated penetration depths were related to the actual thickness of the muscle, submucosal and mucosal layers as indicated by the arrows. The thickness of the layers was measured from histological sections: muscle (Muscularis externa), 0.7 ± 0.3
The large difference between serosal versus mucosal PO$_2$ readings as measured with the surface oxygen electrode shown in Table 1 are in agreement with the findings of others. Table 1 shows that PO$_2$ values measured by serosal oxygen electrodes were higher than μPO$_2$ values obtained when measurements were made on the serosal side. When both measurements were made on the mucosal side, the μPO$_2$ was higher than PO$_2$ measured by surface electrode. These measurements confirm that the larger measurement volume of the Pd-phosphorescence technique results in a PO$_2$ value from a compartment in-between the serosa and mucosa and can be regarded as a mean intestine μPO$_2$.

**Table 1. PO$_2$ measured in the serosa and mucosa with both the Pd-porphyrin phosphorescence and surface oxygen electrode methods for 5 pigs.**

<table>
<thead>
<tr>
<th></th>
<th>Serosa</th>
<th>Mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>μPO$_2$ (mmHg)</td>
<td>63 ± 3</td>
<td>42 ± 8</td>
</tr>
<tr>
<td>Electrode PO$_2$ (mmHg)</td>
<td>87 ± 21</td>
<td>20 ± 6</td>
</tr>
</tbody>
</table>

Data are means ± S.D. In both serosa and mucosa, values obtained using the two methods of measurement were significantly different (P < 0.05).

**Functional compartment measured by Pd-porphyrin**

To test whether oxygen from the atmosphere contributes to the surface electrode and μPO$_2$ measurements, values of these parameters were monitored during circulatory arrest induced by injection of 10 mM KCl. Figure 2 shows that the surface electrode as well as the phosphorescence measurements fell to 0 mmHg as the available oxygen was consumed by the tissue. This is in contrast to the mesenteric venous PO$_2$ values, which were not significantly changed after the cardiac arrest. This experiment shows that the contribution of atmospheric oxygen can be ignored since both oxygen electrode and Pd-porphyrin PO$_2$ measurements reached 0 mmHg.

To investigate the sensitivity of the different circulatory compartments to changes in arterial PO$_2$, measurements were made at different fractions of inspired oxygen. The responses of μPO$_2$, arterial PO$_2$, mesenteric venous PO$_2$, mixed venous PO$_2$ and oxygen electrode PO$_2$ to an FiO$_2$ increase from 0.3 to 1 are shown in Table 2. The results show a ranking in the sensitivity of the different parameters to changes in the FiO$_2$. In response to increased FiO$_2$, the arterial PO$_2$ and the oxygen electrode PO$_2$ showed the largest response followed by the venous PO$_2$ and μPO$_2$. This ranking shows that the oxygen electrode measurement is more sensitive to changes in the arterial PO$_2$, whereas the μPO$_2$ is a measure of a compartment closer to the venous system. This ranking was independent of the hemodynamic changes related to shock and resuscitation.
Microvascular $\text{PO}_2$ during shock and resuscitation

Hemorrhagic shock induced by stepwise blood withdrawal resulted in a concomitant stepwise fall in the mesenteric blood flow (Figure 3B). Each step was followed by partial restoration. The continuous measurement of superior mesenteric artery flow and $\mu\text{PO}_2$ (Figures 3A and B) shows that changes in the mesenteric artery flow resulted in concomitant changes in $\mu\text{PO}_2$ values.

The oxygen-related and hemodynamic parameters were all significantly depressed during shock (Table 3). The $\text{PCO}_2$ measured tonometrically in the ileum showed a significant increase. The microvascular and mesenteric venous pre-shock $\text{PO}_2$ values were similar. During shock, however, $\mu\text{PO}_2$ dropped to a value that was lower than the venous $\text{PO}_2$ (Table 3 and Figure 3A). This $\text{PO}_2$ gap between the $\mu\text{PO}_2$ and the mesenteric venous $\text{PO}_2$ can be quantified by calculating the ratio between the two values. In Table 3 this ratio is given for the moderate shock group and shows that it was significantly lower during shock compared with the pre-shock state ($P < 0.05$, Student’s $t$ test).

Following shock, a group of seven pigs were resuscitated with crystalloid solution (Table 3). This procedure initially restored most parameters with the exception of the [Hb], which decreased due to hemodilution. It is noteworthy that the $\text{PO}_2$ gap be-
between microvascular and mesenteric venous PO$_2$ was narrowed compared with shock values (Table 3). These effects were only temporal and 1 h post-resuscitation (Table 3) most parameters had reverted to shock levels.

In a further group of four pigs, autologous blood (1:1) was given following shock. All values except the [Hb] improved significantly (Table 3). Values of μPO$_2$, mixed venous PO$_2$ and mean arterial blood pressure (MABP) were still significantly higher 1 h after resuscitation (Post-resuscitation) than those during shock, but were still lower than pre-shock values.

**Figure 3.** A, time course of μPO$_2$ and mesenteric venous PO$_2$ during moderate shock and resuscitation with autologous blood. B, the concomitant changes in the mesenteric artery (SMA) flow.
Table 2. The relative increases of the different oxygen measurements calculated as the ratio of the value just before switching to 100% oxygen and 10 min after the switch was made.

<table>
<thead>
<tr>
<th></th>
<th>Pre-shock (n=11)</th>
<th>Shock (n=11)</th>
<th>Resuscitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Crystalloid (n=7)</td>
</tr>
<tr>
<td>μPO₂ (mmHg)</td>
<td>1.2 ± 0.2</td>
<td>1.3 ± 0.4</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Mesenteric venous PO₂ (mmHg)</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Mixed venous PO₂ (mmHg)</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Electrode PO₂ (mmHg)</td>
<td>2.8 ± 0.9</td>
<td>2.0 ± 1.3</td>
<td>2.1 ± 1.2</td>
</tr>
<tr>
<td>Arterial PO₂ (mmHg)</td>
<td>3.2 ± 0.4</td>
<td>3.3 ± 0.4</td>
<td>3.6 ± 0.4</td>
</tr>
</tbody>
</table>

Data are means ± S.D. FiO₂ steps were made before blood withdrawal (Pre-shock), after blood withdrawal (Shock) and 1 h after resuscitation. All increases are significant (P < 0.05). The increases in the oxygen electrode PO₂ readings and arterial PO₂ are significantly larger than the increases in venous PO₂ and μPO₂ (P < 0.05).

Table 3. Global and regional oxygenation and hemodynamic parameters of the moderate shock group before (Pre-shock) and after 40% blood withdrawal (Shock), after resuscitation with crystalloid solution, and after resuscitation with the shed blood.

<table>
<thead>
<tr>
<th></th>
<th>Pre-shock (n=11)</th>
<th>Shock (n=11)</th>
<th>Resuscitation</th>
<th>Post Resuscitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Crystalloid (n=7)</td>
<td>Blood (n=4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Crystalloid (n=7)</td>
<td>Blood (n=4)</td>
</tr>
<tr>
<td>μPO₂ (mmHg)</td>
<td>60 ± 11</td>
<td>26 ± 10</td>
<td>44 ± 9’</td>
<td>29 ± 9</td>
</tr>
<tr>
<td>Mes ven PO₂ (mmHg)</td>
<td>60 ± 9</td>
<td>35 ± 8</td>
<td>44 ± 6’</td>
<td>35 ± 11</td>
</tr>
<tr>
<td>Electrode PO₂ (mmHg)</td>
<td>87 ± 21</td>
<td>56 ± 26</td>
<td>69 ± 28’</td>
<td>46 ± 19</td>
</tr>
<tr>
<td>SMA flow (ml min⁻¹)</td>
<td>547±168</td>
<td>235±111</td>
<td>609 ± 245’</td>
<td>226 ± 91</td>
</tr>
<tr>
<td>μPO₂/mes ven PO₂ (a.u.)</td>
<td>1.0 ± 0.2</td>
<td>0.8 ± 0.3</td>
<td>1.0 ± 0.3’</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>PCO₂, ileum (mmHg)</td>
<td>50 ± 6</td>
<td>64 ± 7</td>
<td>61 ± 5</td>
<td>57 ± 9.4’</td>
</tr>
<tr>
<td>Mixed ven PO₂ (mmHg)</td>
<td>53 ± 6</td>
<td>27 ± 6</td>
<td>35 ± 5’</td>
<td>33 ± 12</td>
</tr>
<tr>
<td>Cardiac output (l min⁻¹)</td>
<td>1.9 ± 1</td>
<td>1.0 ± 0.2</td>
<td>1.6 ± 0.3’</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>104 ± 15</td>
<td>58 ± 15</td>
<td>92 ± 15’</td>
<td>74 ± 13’</td>
</tr>
<tr>
<td>[Hb] (g dl⁻¹)</td>
<td>9.6 ± 1.2</td>
<td>8.4 ± 1.0</td>
<td>7.3 ± 1.2</td>
<td>7.7 ± 0.6</td>
</tr>
</tbody>
</table>

Data are means ± S.D. All shock values are significantly different from pre-shock values (P < 0.05). Data were obtained immediately after resuscitation had taken place (Resuscitation) and 1 h later (Post-resuscitation). ‘Significant difference (P < 0.05) from shock values. MABP, mean arterial blood pressure; a.u., arbitrary units.
Relation between microvascular $PO_2$ and mesenteric venous $PO_2$ in severe shock

The previous section showed that, during an episode of shock, $\mu PO_2$ dropped below mesenteric venous $PO_2$ levels. This led to the question whether this divergence of $\mu PO_2$ and mesenteric venous $PO_2$ can become more severe in deeper and extended shock. To investigate this, severe hemorrhagic shock was induced and the microvascular and mesenteric venous $PO_2$ were measured without resuscitation (Figure 4). Figure 4 shows that the mesenteric venous $PO_2$ reached a plateau at 27 mmHg below which $\mu PO_2$ continued to fall. The difference between $\mu PO_2$ and venous $PO_2$ during this severe hemorrhage was larger than during the milder hemorrhage.

**Figure 4.** $\mu PO_2$ (●) and mesenteric venous $PO_2$ (■) for the severe shock group. This figure shows that at 27 mmHg the mesenteric venous blood $gasses$ level out whereas the $\mu PO_2$ continues to decrease. Data are means ±S.D. (n= 3).

**Discussion and conclusions**

This study has shown that the quenching of Pd-porphyrin phosphorescence can be applied to study the $\mu PO_2$ of the pig ileum. We found that the $PO_2$ measured in this manner reflects the $PO_2$ of the submucosa, follows the venous $PO_2$ during changes in the inspired oxygen fraction, and becomes lower than the mesenteric venous $PO_2$ during hemorrhagic shock. Resuscitation by blood or crystalloid solution restored this $PO_2$ gap to baseline values.

The first part of this study was performed to determine which compartment of the microcirculation of the intestine was measured by Pd-porphyrin phosphorescence. Comparison of penetration depth of the excitation light with the geometry of the ilial
wall predicted that a significant portion of the excitation light reaches the mucosa when illumination is from the serosa (approximately 20 %; Figure 1). In the ileum a large difference in the tissue PO$_2$ of the serosa compared with the mucosa has been observed (Table 1). As stated before, the oxygen electrode measures a layer that is only 15 μm thick, whereas the measurement volume of Pd-porphyrin is determined by the penetration depth of the excitation light (Fig. 1), which is approximately 0.5 mm. Comparison of oxygen electrode values with the Pd-porphyrin measurements on serosa and mucosa (Table 1) showed that the difference in the μPO$_2$ was much smaller than that for the oxygen electrode readings, confirming this prediction.

Bohlen in a study on the small intestine of rats using oxygen electrodes showed that, under control circumstances, the PO$_2$ at the tips of the villi was about half the value measured at the base of the villi. The surface oxygen electrode with a penetration depth of only 15 μm will only measure the PO$_2$ in the tips of the villi whereas the Pd-Porphyrin technique with the much larger penetration depth will measure (when placed on the mucosa) the PO$_2$ of villi base and submucosa. This explains why the mucosal μPO$_2$ values were larger than the surface electrode readings.

When both measurements were performed on the serosa the surface electrode PO$_2$ values were much larger than the μPO$_2$ values. Table 2 shows that during FiO$_2$ steps the oxygen electrode PO$_2$ followed the arterial PO$_2$ whereas the μPO$_2$ followed the venous PO$_2$. This result suggests that the electrode measures a more arterial compartment, which could explain the high values of the oxygen electrode readings compared with the Pd-porphyrin measurements shown in Table 1. These results suggest that the μPO$_2$, as measured by the quenching of Pd-porphyrin phosphorescence, represents a mean μPO$_2$ over the thickness of the intestinal wall.

The classic view based on Krogh cylinders predicts a gradual decrease in the intravascular PO$_2$ as blood flows from the arteries through the microcirculation to the venous pool. In contradiction to this view, the present study shows that under conditions of shock the μPO$_2$ can become lower than the venous PO$_2$. These results are in agreement with those of other investigators who also observed that microcirculatory oxygenation can become lower than venous PO$_2$ values. These studies have included intravital phosphorimetry and intravital measurement of hemoglobin saturation. In severe haemorrhagic shock in the skin fold of hamsters, Kerger et al. found a significantly lower capillary than venous PO$_2$.

Several mechanisms have been proposed to explain the observed differences in μPO$_2$ versus venous PO$_2$ that develop during shock. It has been observed that the red blood cell concentration and flow in the capillaries is distributed heterogeneous-
ly.\textsuperscript{43,44} Possible mechanisms to explain this heterogeneity include separation of plasma and red blood cells at arteriolar bifurcations\textsuperscript{45} and/or intracapillary mechanisms.\textsuperscript{46} This heterogeneity in flow and red blood cell concentration can cause heterogeneity in oxygenation between the capillaries. The high-oxygenated areas will contribute more oxygen to the venous oxygen content than the low-oxygenated areas. This results in areas with lower capillary then venous PO\textsubscript{2}. However, in studies on the distribution of flow in the small intestine it has been shown that during hypovolemia and hemorrhagic shock the heterogeneity is decreased.\textsuperscript{47,48} This makes the heterogeneity of flow a less probable explanation for the observed divergence between microvascular and venous PO\textsubscript{2} during shock.

A second explanation can be found in the studies of Stein et al.\textsuperscript{18} They suggested that direct diffusion of oxygen from arterioles to collecting venules could result in lower PO\textsubscript{2} values being found in the capillaries than in the veins.\textsuperscript{18} This direct diffusion is dependent on the oxygen gradient between the arterioles and the venules. In the hemorrhagic shock described in the present study the arterial PO\textsubscript{2} did not change significantly from baseline values during shock (172 ± 15 mmHg at baseline and 165 ± 23 mmHg in shock), whereas the mesenteric and mixed venous PO\textsubscript{2} decreased by almost 50 % (Table 3). This results in an increase in the arterial/venous oxygen gradient and can therefore be responsible for an increase in the diffusive oxygen shunt, which in turn would result in a gap between the venous PO\textsubscript{2} and the μPO\textsubscript{2}.

A third possibility proposed by Gutierrez in an early theoretical study concerns restrictions of the kinetics of oxygen release from the erythrocytes to the serum.\textsuperscript{17} The mathematical model proposed in that paper and the experimental data from this study can be used to calculate an end-capillary PO\textsubscript{2} that becomes lower than the measured mesenteric venous PO\textsubscript{2} during shock. These model calculations are in agreement with the observed divergence between microvascular and venous PO\textsubscript{2} during shock.

The present study demonstrates the conditions under which the microcirculation can become more hypoxic then the venous pool and illustrates the limitation of using blood gas values as indicators of tissue oxygenation. Measurement of μPO\textsubscript{2} together with venous PO\textsubscript{2}, however, can be used to demonstrate the occurrence of functional shunting of the microcirculation.