A model of the isolated perfused rat small intestine

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1Department of Anesthesiology and Intensive Care Medicine, University Medical Center Schleswig-Holstein, Campus Kiel, Kiel; 2Division of Barrier Integrity, 3Division of Mucosa Immunology, 4Division of Clinical and Experimental Pathology, and 5Department of Clinical Pneumology, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Borstel; and 6Institute of Pharmacology and Toxicology, Medical Faculty, RWTH Aachen University, Aachen, Germany

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Lautenschläger I, Dombrowsky H, Frerichs I, Kuchenbecker S, Bade S, Schultz H, Zabel P, Scholz J, Weiler N, Uhlig S. A model of the isolated perfused small intestine. Am J Physiol Gastrointest Liver Physiol 298: G304–G313, 2010. First published November 12, 2009; doi:10.1152/ajpgi.00313.2009.—Intestinal edema remains a serious clinical problem, and novel approaches to study its pathophysiology are needed. It was our aim to develop a long-term stable isolated perfused rat small bowel preparation permitting analysis of vascular, luminal, interstitial, and lymphatic compartments and to demonstrate the utility of this model by studying the effects of the proinflammatory mediator platelet-activating factor (PAF). A temperature-controlled chamber with an integrated balance was designed to perfuse isolated intestines through the mesenteric artery and the gut lumen. Steroids or oxygen carriers were not needed. Functional and morphological integrity of the tissue was preserved for several hours as confirmed by oxygen consumption, venous lactate-to-pyruvate ratio, arterial and venous pH, lactose digestion and galactose uptake, intravascular and luminal pressures, maintained fluid homeostasis, gut motility, and quantitative light microscopic analysis. Administration of PAF caused typical effects such as vasoconstriction, gut atony, and loss of galactose uptake. PAF also elicited a transient loss of 20% of the perfusate liquid from the mesenteric vascular bed, two-thirds of which were transferred to the lumen. All these responses were entirely reversible. This new model provides detailed insights into the physiology of the small intestine and will allow to study fundamental processes such as fluid homeostasis, barrier functions, transport mechanisms, and immune responses in this organ. Using this model, here we show a dramatic and yet reversible response of the rat small bowel to PAF, suggesting luminal water clearance as a novel safety factor in the intestine that may be of clinical relevance.

INTESTINAL ENDOTHELIAL and epithelial barrier dysfunction resulting in edema formation is a hallmark of intestinal failure in sepsis (8, 9). In such patients and also after abdominal surgery intestinal edema formation may lead to disturbances in gut motility, anastomotic leak, increased intra-abdominal pressure, translocation of bacteria, liberation of proinflammatory mediators, and local and/or systemic hypoperfusion. To further understand the underlying pathophysiological processes and to develop treatment strategies that attenuate intestinal hyperpermeability, novel model systems are urgently needed.

Intestinal fluid movements and formation as well as resolution of edema are the net result of vascular endothelial permeability, hydrostatic pressure, oncotic pressure, lymphatic drainage, inflammatory cell actions, and nervous regulation. These mutually interacting factors are difficult to control and to discriminate in vivo (9, 22). In contrast, isolated perfused organs allow to account for most of these confounding factors and have greatly improved our understanding of many (patho) physiological processes in other organs such as the lung (4, 10, 27), but owing to the lack of suitable models not yet to the same extent in the intestine. In an intact isolated intestine, the four fluid compartments blood vessels, lymphatics, interstitial space, and lumen can be preserved, the lack of which is a major limitation of cell-based systems, e.g., enterocyte monolayers or Ussing chambers.

There have been several attempts to develop an isolated perfused intestine model, although mostly to study nutrient uptake rather than fluid homeostasis or other organ functions (Table 1). All of these models had important disadvantages and most seem to have been abandoned after a short time. The major shortcomings were the following: 1) Dependence on natural or artificial oxygen carriers, which are either expensive or cumbersome to use, precluding routine use. 2) Requirement of exogenous steroids, which may interfere with fluid movements, for instance by affecting vascular permeability (21). 3) Limited stability, i.e., without (artificial) oxygen carriers and/or steroids these preparations were stable for only 90 min (12).

In view of the great clinical relevance of disturbances in intestinal barrier functions, we aimed to develop a reliable, stable, and reproducible organ model with full control over intestinal physiology that would permit study in detail of intestinal barrier functions, fluid homeostasis, and transport mechanisms. Here, we describe a model of a vascularly and luminally perfused isolated rat intestine with online monitoring of four intestinal fluid compartments (vessel, lymphatics, interstitial space, lumen) and characterize it with respect to fluid balance, viability, essential bowel functions, and tissue integrity. We show that the preparation is stable for 4 h. The utility of this model was further illustrated by showing that platelet-activating factor (PAF), an archetypical mediator of inflammation, causes functional changes rapidly (within minutes) accompanied by a dramatic fluid loss from the vascular bed, all of which was completely reversible. In summary, the present setup provides information on macromolecule distribution (i.e., albumin or dextran), gut motility, and vascular and luminal resistance as well as nutrient uptake and will greatly facilitate studies on the role of the gut as a barrier organ in physiological and pathophysiological conditions such as sepsis and multiple organ failure.
MATERIALS AND METHODS

Animals and Study Groups

Female Wistar rats weighing between 220 and 250 g were used as donors. All experiments were approved by the local authorities (Ministry of Agriculture, Environment and Rural Areas the State of Schleswig-Holstein, Kiel, Germany). One series of experiments was performed to confirm the long-term stability of the model for 240 min \((n = 6)\). In a second series we studied the effects of platelet-activating factor with \(n = 6\) animals in the control and the PAF group, respectively.

Dissection Technique

Nonfasted rats were anesthetized by inhalation of sevoflurane. The abdomen was opened by midline incision with bilateral subcostal extensions, and the jejunum and ileum were isolated with the vascular pedicle as described in detail elsewhere with slight modifications (17).

Chamber Fabrication

A custom-made perfusion chamber was built by Hugo Sachs Elektronik-Harvard Apparatus (March-Hugstetten, Germany) (Fig. 1). It consists of a moisturized chamber with an integrated weighing system. A moveable cannulating block and height-adjustable reservoirs were mounted at the chamber back. The block included all the heating coils, bubble trap, and tubing connectors and acted as a holder for the cannulae with their perfusion and pressure tubes.

Cannulation

Mounted on a tripod, this cannulating block was placed above the animal’s head during the in situ preparation. The cannulae were inserted into the proximal and distal small intestine as well as into the aorta close to the superior mesenteric artery and hepatic portal vein. The pressures in the various compartments were directly measured via pressure transducers connected to side ports of these cannulae.

Perfusates and Chemicals

The vascular perfusate was a modified Krebs-Henseleit solution containing 2 mM lactobionatic acid, 7.4 mM HEPES, 3% BSA (PAA, Pasching, Austria, and Sigma-Aldrich, Munich, Germany); the osmolality was adjusted to 310–320 mosmol/l. The luminal perfusate contained 114 mM NaCl, 5 mM KCl, 26 mM NaHCO\(_3\), 30 mM lactose, 5.55 mM glucose, 10 mM mannitol, and 0.8 mM glutamine; the osmolality was adjusted to 310–320 mosmol/l.

Perfusion

For perfusion we used a dialyzer (FX paed, Fresenius Medical Care, Bad Homburg, Germany) and a roller pump for a single-pass flow-controlled perfusion through Tygon tubes. For flow rate detection three high-precision balances (Kern 8200–1N, Kern & Sohn, Balingen-Frommern, Germany) were connected to an online monitoring system. Vascular and luminal outflow tubes (also used for sampling) led to height adjustable reservoirs that allowed clamping the afterload of the mesenteric vascular bed and the lumen to zero. This protected the sensitive tissue from structural damages and edema formation by congestion. The entire gut preparation as well as all intestinal effluents including the lymph from the opened lymphatics were continuously weighed. Thus venous, luminal, and lymphatic flow rates, bowel weight as well as arterial, venous, and luminal pressures were continuously recorded, permitting online quality control from the onset of perfusion. Arterial inflow and venous and luminal outflow were sampled every 15 min and analyzed for \(O_2\) and \(CO_2\) partial pressures, \(pH\), electrolytes, glucose, and lactate. Oxygen consumption \((V\dot{O}_2)\) was calculated by the formula \(V\dot{O}_2 [ml\cdot min^{-1}\cdot g^{-1} (dry\ tissue\ wt)] = [arterial\ Po_2 (mmHg) - venous\ Po_2 (mmHg)] \times 0.03 (mlO_2\cdot l^{-1}\cdot mmHg^{-1}) \times 0.0075 (l/min)/dry\ tissue\ wt\ (g)\).

Analysis of Peristalsis

For the offline video analysis of peristalsis, a standard digital miniature camera mounted on the lid of the chamber continuously filmed the gut. Overall motility was monitored in all sections of the isolated organ and times of first segmental appearance of atony, complete atony, first segmental reappearance of motility and complete regeneration of peristalsis were noted. In general, luminal pressure fluctuations correlated well with the visual analysis of peristalsis, but some small contractions did not result in increasing pressure owing to the free drainage from the aboral end.

Postperfusion Tissue Processing

We cut three 7-cm gut sections between the aboral and oral ends of the preparation. The first and the second sample were depleted of intestinal juice and the mesentery was resected. From the first (i.e., most distal) sample, wet weight was determined before dehumidifying to constant dry weight at 55°C for 96 h. The second sample was shock

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Table 1. Isolated and vascularly perfused rodent small intestine models

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Minutes</th>
<th>Perfusion modes</th>
<th>Vascular perfusate</th>
<th>Drugs in perfusate to prevent</th>
<th>Experimental Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>1b</td>
<td>r</td>
<td>60</td>
<td>Vascular</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>7</td>
<td>r</td>
<td>60</td>
<td>Luminal</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>6a</td>
<td>r</td>
<td>60</td>
<td>Vascular</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>12</td>
<td>r</td>
<td>90</td>
<td>Vascular</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>14b</td>
<td>r</td>
<td>60–120</td>
<td>Luminal</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>2</td>
<td>r</td>
<td>90</td>
<td>Vascular</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>23</td>
<td>r</td>
<td>120</td>
<td>Luminal</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>11</td>
<td>r</td>
<td>120</td>
<td>Luminal</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>14a</td>
<td>r</td>
<td>120</td>
<td>Luminal</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>7r</td>
<td>r</td>
<td>120</td>
<td>Luminal</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>22a</td>
<td>r</td>
<td>180</td>
<td>Luminal</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>14b</td>
<td>r</td>
<td>180</td>
<td>Luminal</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>29</td>
<td>r</td>
<td>300</td>
<td>Luminal</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
<tr>
<td>This study</td>
<td>r</td>
<td>240</td>
<td>Luminal</td>
<td>E</td>
<td>Hypersecretion</td>
<td>Hyperperistals</td>
</tr>
</tbody>
</table>

a. Anticholinergic; c, corticoid; in, net water influx into lumen; out, fluid absorption from lumen; r, rat; rab, rabbit; s, sympathomimetic; x, parameter was measured; (x), in part; A, albumin; D, dextran; E, erythrocytes; FC, perfluorocarbon; FL, flow constant perfusion; HES, hydroxyethylstarch; P, plasma; PR, pressure perfusion. Inclusion criteria for this table were oxygenation via vascular bed, minimal run time of 60 min and a minimal bowel length of 15 cm.
Histological Examinations

Hematoxylin and eosin- and periodic acid-Schiff-stained sections, for goblet cell mucus identification, were examined by a pathologist in a blinded fashion. The histological stability score was assessed as follows: 1) All villi present at the mesenteric and the antimesenteric side of the longitudinally cut slice, as well as those present in the whole circular cut slice, were counted; 2) subsequently, all fully intact villi (with intact mucosal surface) of these three sites were counted; 3) the ratio of all the three sites was calculated by the equation stability score = number of fully intact villi/overall number of villi; 4) the mesenteric and antimesenteric ratio was combined (1:1) to one value; 5) finally, this combined value was combined again with the ratio of the circular slice (1:1) to an overall ratio representing the stability score of the entire isolated perfused organ.

***Photometric Assays***

**FITC-labeled macromolecules.** FITC dextran 150 kDa (long-term perfusion) or FITC albumin (Sigma-Aldrich, Munich, Germany) (40 mg/l) was supplied with the vascular perfusion medium. Every 30 min (long-term perfusion) or 15 min, samples of vascular in- and outflow, lymphatic outflow, and luminal outflow were analyzed in an ELISA fluorescence reader (FL 600 microplate fluorescence Reader, MWG-Biotech, Ebersberg, Germany, excitation 485 nm, emission 530 nm). After PAF stimulation, luminal and lymphatic outflow were sampled every 3 min for 15 min.

**Galactose resorption.** As a measure of metabolic competence, 30 mM of lactose were supplied with the luminal perfusion medium. Vascular galactose (derived from the luminal lactose) was determined by a commercially available assay kit (Megazyme, Bray, Ireland) with slight modifications.

**Vascular pyruvate.** As a measure of aerobic metabolism, pyruvate was determined in the venous outflow by a standard photometric method.

***Statistical Analysis***

The GraphPad Prism Computer Program (Version 5.01 GraphPad Software, San Diego, CA) was used for statistical evaluations. All data are expressed as mean values with standard deviation (SD) in each group unless otherwise stated. A P value ≤ 0.05 was considered as statistically significant.

***RESULTS***

**Model of the Isolated Perfused Small Intestine***

Isolated perfused small intestines were prepared from rats under stable general anesthesia by the following procedure: 1) Leakproof resection of the entire organ from the surrounding tissue. 2) Rapid in situ cannulation of the mesenteric artery (interruption of perfusion: 20 ± 4 s), the portal vein, and the bowel lumen at the oral as well as the aboral end. 3) Immediate start of the dual-flow-controlled perfusion. 4) Longitudinal incision of the major lymphatic duct for afterload-free drainage of the lymph. 5) En bloc transfer of the isolated perfused organ to the moisturized chamber with an integrated balance.

This setup allowed to obtain the following measurements: 1) Online monitoring of arterial, venous, and intraluminal pressures. 2) Simultaneous online analysis of vascular, luminal, and lymphatic flows as well as intestinal weight gain. 3) Measurement of electrolytes, blood gases, and pH values, glucose, galactose, lactate, and pyruvate as well as lactate dehydrogenase activity in all effluents. 4) Offline video analysis of gut motility and its quantification. 5) Histological analysis (light microscopic analysis, immunohistochemistry). 6) Distribution of FITC-labeled macromolecules (albumin or dextran) originating from the vascular perfusion indicating alterations in intestinal barrier integrity (see Effects of Platelet-Activating Factor below).

The perfusion apparatus consisted of the oxygenator and the novel chamber with various measuring units (Fig. 1). The buffer was oxygenated by a heated hollow fiber dialyzer operated at 36°C while flushed gently with carbogen gas (95% O2, 5% CO2), resulting in an oxygen pressure of 679 ± 37 mmHg (mean ± SD, n = 18).

Validation

Viability. The values of several parameters obtained at 60, 135, and 240 min of extracorporeal perfusion are given in

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### Table 1.—Continued

<table>
<thead>
<tr>
<th>Viability Criteria</th>
<th>Energy</th>
<th>Fluid balance and barrier functions</th>
<th>Measurements of Organ Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hematocrit</td>
<td>Wet-to-dry weight ratio</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fluid distribution</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Luminal fluid balance</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transfer of macromolecules</td>
</tr>
<tr>
<td>Or supply/uptake</td>
<td>Lactate</td>
<td>Glucose</td>
<td>Systematic scoring</td>
</tr>
<tr>
<td>consumption</td>
<td>pyruvate</td>
<td>consumption</td>
<td>Blinded inspection</td>
</tr>
</tbody>
</table>

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frozen in liquid nitrogen for later analysis. The third sample (from the middle part of the intestine) was divided into two and both were used for histological analysis as described below. The remaining tissue was also depleted of intestinal juice and the mesentery was resected for the whole wet weight measurement of the preparation.
Table 2. Perfusion pressures and bowel weight remained constant (Fig. 2, A and B). Critical parameters such as \( \dot{V}O_2 \), the lactate-to-pyruvate ratio, the \( CO_2 \) partial pressure, and the pH were within their normal physiological ranges at all times (Table 2, Fig. 2C). Lactate dehydrogenase activity in the venous effluent as a measure of cellular necrosis remained below the detection level at all time points. As for specific gut functions, we found a transfer of fluid and galactose (from digestion of luminal lactose) to the venous compartment (Table 2, Fig. 2D). In untreated intestines gut motility (pattern and intensity) appeared normal for 240 min as illustrated in the first part of Supplementary Movie S1. (The online version of this article contains supplemental data.)

Fluid balance. A major incentive for the development of this model was to study fluid movements in critical compartments in the intestine: vessel, interstitial space, lymphatics, and gut lumen. This goal was achieved by continuously weighing the vascular and luminal effluents, the entire organ, and the fluid emanating from the intestine with the opened lymph duct. Under control conditions, the fluid distribution between these compartments (including net absorption, Table 2) and the pressures within the compartments (Fig. 2A) remained stable during the experiments. The wet-to-dry weight ratio of \( \sim 4 \) was similar at 0, 140, and 240 min (Fig. 3B). As a marker of endothelial and epithelial permeability we assessed the distribution of vascularly administered FITC albumin. Under control conditions, no FITC albumin was found in the luminal space (Fig. 4C), whereas the amount of FITC albumin in the lymphatic fluid that was drained from the intestine remained stable for 90 min and slightly increased thereafter. The fact that with respect to FITC albumin this drained fluid differed in concentration and time course from that in the vascular compartment suggests that this fluid was largely derived from the incised lymph duct.

Tissue integrity. The mucosal surface including the enterocytes’ brush border remained intact after 140 min and 240 min of perfusion (Fig. 3). The structure of the tissue was entirely preserved. Sometimes, tiny subepithelial fluid accumulations within the intestinal epithelial layer were noted [similar to observations in transport-stressed rats (28)]. Only minor mucosal lesions at some villi tips, particularly on the antimesenteric side, were present (Fig. 3, D, F, H, and J). In the intestine the enterocyte cell turnover is known to be a fast process [migration of the enterocytes from the crypt to finally be sloughed at the villus tip lasts \( \sim 2 \) days in the rat and is a continuous process (26)] and we found shed and rounded epithelial cells with their detritus intraluminally after 240
Effects of Platelet-Activating Factor

Administration of a 0.5 nmol PAF bolus into the mesenteric artery caused vasoconstriction (Fig. 5A) and a remarkable shift of fluid (Fig. 4, A and B). Immediately after PAF administration bowel weight (including tissue weight, vascular, and luminal filling) steeply rose (maximum weight gain was 3.25 ± 0.99 g at 130 ± 38 s) and returned to near baseline within a similar time interval. From the mesenteric vascular bed, 19.1 ± 2.6% (mean ± SD, n = 6) of its volume content was lost within 10 min after stimulation accompanied by a concomitant increase in luminal and lymphatic outflow. However, only a slight net bowel weight gain (Fig. 4, A and B) occurred, indicating a shift of fluid from the vessels to the luminal (65.6 ± 7.8% of the fluid) and the lymphatic (24.0 ± 5.4%) compartment via the interstitial space (4.4 ± 5.5% of the fluid). Additionally, the villus height was shortened a little after 4 h of isolated perfusion (Fig. 3, G–J).

Table 2. Critical values of the isolated perfused intestine

<table>
<thead>
<tr>
<th>Vitality, Physiology, and Metabolism</th>
<th>In vivo</th>
<th>Isolated Perfused Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 min</td>
<td>135 min</td>
</tr>
<tr>
<td>Oxygen consumption, ml·min⁻¹·g⁻¹ DW</td>
<td>0.20 ± 0.07 (3)</td>
<td>0.32 ± 0.05</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.41 (24)</td>
<td>7.37 ± 0.03</td>
</tr>
<tr>
<td>Portal venous pH</td>
<td>7.35 ± 0.08 (13)</td>
<td>7.35 ± 0.03</td>
</tr>
<tr>
<td>Arterial PCO₂, mmHg</td>
<td>39.9 (24)</td>
<td>39.6 ± 2.4</td>
</tr>
<tr>
<td>Portal venous PCO₂, mmHg</td>
<td>34.2 ± 6.5 (13)</td>
<td>40.3 ± 2.2</td>
</tr>
<tr>
<td>Portal venous lactate, mmol/l</td>
<td>2.85 (5a)</td>
<td>0.91 ± 0.23</td>
</tr>
<tr>
<td>Portal venous lactate-to-pyruvate ratio</td>
<td>11.0 ± 1.7 (n = 3, own data)</td>
<td>19.9 ± 4.8</td>
</tr>
<tr>
<td>Portal venous LDH, µkat/l</td>
<td>4.4 (5a)</td>
<td>below detection level</td>
</tr>
<tr>
<td>Luminal lactate, mmol/l</td>
<td>0.11 (14)</td>
<td>1.32 ± 0.20</td>
</tr>
<tr>
<td>Galactose uptake, mg·min⁻¹·g⁻¹ DW†</td>
<td>not available</td>
<td>0.50 ± 0.16</td>
</tr>
<tr>
<td>Portal venous LDH activity, µkat/h</td>
<td>not available</td>
<td>0.08 ± 0.03</td>
</tr>
<tr>
<td>Arterial pressure, mmHg</td>
<td>89.8 ± 21.8 (13)</td>
<td>39.5 ± 2.7</td>
</tr>
<tr>
<td>Portal venous pressure, mmHg</td>
<td>10.6 ± 0.7 (13)</td>
<td>0.1 ± 0.5</td>
</tr>
<tr>
<td>Luminal pressure, mmHg</td>
<td>2–4 (6)</td>
<td>1.6 ± 0.8</td>
</tr>
</tbody>
</table>

LDH, lactate dehydrogenase. †Derived from a bolus administration of lactose at t = 30 min, afterward continuous flow; ‡luminal default – luminal measured; §after preparation; †luminal default flow perfusion per gram tissue. DW, dry weight; ND, not determined. Parenthetical numbers represent references.

Fig. 2. Smooth muscle activity, bowel weight, and metabolism of controls. A: mesenteric artery (Pₘₐₓ), mesenteric vein (Pₐₐₜ), and luminal (Pₐₐₜ) pressures in control preparations. B: weight change in bowel weight after administration of a solvent bolus at t = 60 min. C: oxygen consumption and lactate-to-pyruvate ratio. D: galactose uptake to the venous compartment. Data are shown as means ± SD (mean without SD in B) of 6 independent experiments.
fluid). About 6% of the fluid lost from the vasculature remained unaccounted for. In line with this fluid shift, FITC-labeled albumin from the vascular perfusion medium was found temporarily in the luminal and lymphatic effluent (Fig. 4C).

PAF also increased the mesenteric artery and luminal pressures, whereas venous pressure slightly decreased (Fig. 5A). Gut motility responded to PAF with decreasing peristalsis and atony (duration 84 ± 13 s; Fig. 5B), with a phase of recovery followed by a short transient phase of hyperperistalsis (see Supplementary Movie S1). Lactose conversion to galactose and its transfer from the lumen to the portal vein was impaired within 15 min after PAF administration and recovered thereafter (Fig. 4D). The luminal lactate concentration increased, whereas the vascular lactate and pyruvate concentrations remained unchanged, indicating some local, intramucosal anaerobic metabolism (Fig. 4E).

Despite these massive transient biochemical and physiological alterations in response to PAF, the enterocytes’ morphology and the epithelial continuity were not affected and the tissue remained perfectly intact. At the end of the experiments (80 min after PAF administration) no significant edema formation was apparent: the wet-to-dry weight ratio and the mucosal stability score were comparable to controls (Fig. 3, A and B), the fluid distribution had normalized (Fig. 4A) and the lymphatic as well as luminal FITC albumin levels returned back to baseline (Fig. 4C).

**DISCUSSION**

The present setup for the isolated perfused intestine provides several novel and unique features and offers a new approach to studying mechanisms of intestinal edema formation. For the first time fluid movements in the four compartments critical to intestinal fluid homeostasis (vessels, tissue, lymphatics, lumen) have been analyzed in real time. In combination with labeled macromolecules (e.g., FITC albumin) this permits detailed insights into pathophysiological alterations and in the intestinal fluid movements as was demonstrated for PAF. In addition, parameters such as gut motility, vascular, and luminal pressures and venous galactose derived from luminal lactose provide further critical information on vital organ functions. In principle, all effluents could be analyzed for other molecules of interest like cytokines, antigens, or bacteria.

Other models that have been used for the study of intestinal physiology are either more complex such as in vivo studies or much simpler such as cell culture systems or excised tissues (Ussing chamber and everted sac). Compared with the present setup of the isolated perfused intestine, none of these other models grants such a comprehensive overview of intestinal fluid balance and bowel functions (Table 1). Neither in cell culture nor in excised tissues nor in vivo has it yet been possible to monitor the vascular, the interstitial, the lymphatic, and the luminal space simultaneously.
Stability of the Preparation

By reviewing previous isolated rat small bowel perfusion studies (Table 1) and analyzing the data of ~200 preliminary experiments with different perfusion media (e.g., normal saline, minimal essential medium, blood-based buffers, Krebs-Henseleit buffers) and perfusion modes (pressure controlled, flow controlled), we found that apart from an absolute requirement for sufficient oxygenation, the structural and functional preservation of the isolated rat small bowel preparation depended on the following factors: 1) Fast (67 ± 6 min) surgery with rapid cannulation of the mesenteric artery (20 ± 4 s). 2) A microsurgical dissection technique (25). 3) Inhalation anesthesia with sevoflurane (in spontaneous respiration) to secure hemodynamics. 4) Flow-constant perfusion with 7.5 ml/min modified Krebs-Henseleit solution through the mesenteric artery and 0.15 ml/min carbonate-buffered saline solution enriched with glutamine, glucose, and lactose through the lumen (Fig. 1). 5) Norepinephrine in the perfusion medium to mitigate hypersecretion and hypermotility.

The gut is an extremely sensitive tissue and, in our opinion, this mandates that structural integrity be quantitatively demonstrated, in particular at the antimesenteric side of the gut where oxygen pressure is the lowest for anatomical reasons.

Fig. 4. Fluid homeostasis and metabolic responses. A: weight change in the 4 studied compartments (mean of 6 independent experiments). All tracings remained unaltered and stable until the end of the experiment at 135 min (not shown). B: volume shifts between the compartments within 10 min after solvent (−) or PAF (+) stimulation. C: amount of FITC albumin transferred from vasculature to the luminal and the lymphatic compartments. D: galactose uptake to the venous compartment. E: luminal and vascular lactate concentration. Data in B–E are shown as means ± SD of 6 independent experiments with and without PAF each. *P < 0.01 vs. control (t-test).
Most previous perfusion studies, however, only used surrogate parameters (e.g., \( \dot{V}O_2 \)) or broad qualitative assessments (Table 1). Even if histology was described (7, 29), the morphology and the information provided have not been entirely convincing and the integrity of the critical antimesenteric side has never been documented. In all our experiments the gut was a viable organ and remained stable for up to 4 h. Thus this is the first study to demonstrate the structural integrity of the isolated perfused intestine by quantitative light microscopy for up to 240 min after the onset of perfusion (Fig. 3 and Table 2). Except for minor alterations in the mucosal surface at the antimesenteric side we found that the tissue remained intact.

To the best of our knowledge, functional parameters such as lactose digestion, gut motility, luminal pressure, and fluid distribution among several compartments have not been studied in isolated intestine models before (Table 1). With the help of these and other measures, the functional integrity of the preparation was demonstrated at several levels: oxygenation (\( \dot{V}O_2 \), partial pressures of \( O_2 \) and \( CO_2 \)), oxidative phosphorylation (lactate-to-pyruvate ratio), maintained fluid balance (constant compartment volumes, net fluid absorption, lack of macromolecule efflux), vessel tone (perfusion pressure), nutrient uptake (vascular galactose derived from luminal lactose), and gut motility. Particularly remarkable was the ability of the isolated intestine to fully recover from a PAF bolus.

Our findings show that, in contrast to previous beliefs, sufficient and prolonged tissue oxygenation can be achieved without extra oxygen carriers. Unlike previous small bowel setups that depended on blood or fluorocarbons (11, 23, 29), those were not required to guarantee stable oxygen uptake and a physiological lactate-to-pyruvate ratio in our model. In fact, the present preparation is unmatched in terms of vascular resistance stability and maintenance of vascular and mucosal integrity. This stability, the simple perfusion medium, and the lack of steroids make this model highly attractive for studies on the intestinal (patho)physiology.

**Effects of PAF**

PAF was chosen as an archetypical inflammatory mediator that affects numerous intestinal functions: it promotes gut atony (20), induces loss of fluid (16) and protein (19) from the vasculature, and alters vascular resistance (5). Carbohydrate resorption of, for instance, D-galactose is reduced in intestinal endotoxemia (1), and because there is a pathophysiological link from endotoxin to PAF we expected and found diminished galactose uptake in response to PAF. This evidence of a direct effect of PAF on galactose uptake was not available so far. The present model allowed continuously monitoring of all of these different aspects in one integrated approach. PAF induced gut atony and impeded lactose uptake and conversion into galactose, it increased vascular resistance and caused severe fluid accumulation that in contrast to the lungs was reversible (10; see also below). Of note, these effects occurred in the absence of circulating neutrophils, thus further clarifying their role in PAF-mediated intestinal injury (15).

The analyses of intestinal fluid homeostasis and macromolecule movements suggest that PAF caused both hydrostatic and permeability edema and that from all of the water and the protein lost from the circulation ~30% were cleared by the lymph and the remaining 70% by the lumen. This leakiness of the epithelial barrier was quite unexpected and cannot be explained by injury to the mucosal epithelium, given the histological integrity after PAF administration. Thus our findings identify a safety mechanism of the intestine that permits transfer of excess interstitial fluid to the lumen.

Most surprisingly, despite the massive fluid mobilization induced by PAF-amounting to ~3 g, a doubling of the organ weight, these effects were completely reversible within 30 min after PAF administration, leading to complete normalization of all gut functions including organ weight. This
strongly indicates that the intestinal effects of PAF are tightly controlled, that PAF assumes a well-defined role in intestinal inflammation, and that PAF is by itself not a necrotizing agent in the intestine. With such effective mechanisms of fluid and protein clearance in place, one wonders whether the clinically observed intestinal edema may result from the inhibition of these effective clearance processes, possibly aggravated by gut atony, rather than their initiation. This isolated intestine model will be valuable to examine the mechanisms of intestinal barrier dysfunction, hyperpermeability, and edema formation.

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