Contrasting Effects of Colloid and Crystalloid Resuscitation Fluids on Cardiac Vascular Permeability

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Background: Fluid extravasation may lead to myocardial edema and consequent reduction in ventricular function. Albumin is presumed to interact with the endothelial glycocalyx. The authors’ objective was to compare the impact of different resuscitation fluids (human albumin, hydroxyethyl starch, saline) on vascular integrity.

Methods: In an isolated perfused heart model (guinea pig), Krebs-Henseleit buffer was augmented with colloids (one third volume 5% albumin or 6% hydroxyethyl starch 130/0.4) or crystalloid (0.9% saline). Perfusion pressure and vascular fluid filtration (epicardial transude formation) were assessed at different flow rates. After global, stopped-flow ischemia (37°C, 20 min), hearts were reperfused with the same resuscitation fluid additives. In a second series, the authors applied the respective perfusates after enzymatic digestion of the endothelial glycocalyx (heparinase, 10 U over 15 min).

Results: Both 5% albumin and 6% hydroxyethyl starch decreased fluid extravasation versus saline (68.4 ± 5.9, 134.8 ± 20.5, and 436.8 ± 14.7 µl/min, respectively, at 60 cm H2O perfusion pressure; P < 0.05), the corresponding colloid osmotic pressures being 2.95, 5.45, and 0.00 mmHg. Digestion of the endothelial glycocalyx decreased coronary integrity in both colloid groups. After ischemia, a transient increase in vascular leak occurred with Krebs-Henseleit buffer containing hydroxyethyl starch and saline, but not with albumin. The authors observed no difference between intravascular and bulk interstitial colloid concentration in the steady state. Notwithstanding, electron microscopy revealed an intact endothelial glycocalyx and no interstitial edema in the albumin group.

Conclusion: Ex vivo, albumin more effectively prevented fluid extravasation in the heart than crystalloid or artificial colloid. This effect was partly independent of colloid osmotic pressure and may be attributable to an interaction of albumin with the endothelial glycocalyx.

A CHIEF aim of clinical fluid management in critically ill patients is to preserve major organ function. Although little attention has been given to myocardial fluid balance in the clinical setting, fluid extravasation may result in tissue edema, adversely affecting cardiac function.

The commonly used resuscitation fluids are crystalloid, the natural colloid albumin, and artificial colloids such as hydroxyethyl starch (HES). Although inexpensive, crystallloid rapidly exits the intravascular space, and some evidence suggests that this induces myocardial fluid accumulation. Compared with crystalloids, colloids promote retention of fluid in the intravascular compartment. This effect is believed to depend predominantly on colloid osmotic pressure (COP). Although important safety differences exist between colloids, their ability to maintain COP and the circulating blood volume is presumed to be largely equivalent. Consequently, in terms of efficacy, colloids have typically been seen as a class of interchangeable fluids. Nevertheless, vascular permeability and, hence, fluid homeostasis may be influenced by nononcotic properties of colloids, especially of albumin.

A recent investigation in our laboratory suggested that the endothelial glycocalyx (EG) acts as a competent barrier against passage of water and colloids in addition to the endothelial cells themselves. Other investigators found that artificial colloids probably differ significantly in the behavior at the EG in comparison with human albumin: The latter penetrated the EG within a short period of time, whereas dextran did not enter this structure. The behavior and functional impact of other artificial colloids at the endothelial surface versus a physiologic environment remained unclear. We pursued the hypothesis that the specific interaction of colloids with the EG should influence vascular integrity.

In view of the clinical relevance, the current study was designed to determine whether resuscitation fluids of various colloid compositions differ in their capacity to prevent fluid extravasation from a vascular system, both during unperturbed flow conditions and after tissue ischemia. Of particular interest was whether divergent fluid effects depended on the presence of an intact EG.

The vascular system investigated was the intact coronary bed of isolated heart preparations (guinea pig), perfused with Krebs-Henseleit buffer (KHB) supplemented with human albumin, HES, or 0.9% saline. All hearts were subjected to 20 min of global ischemia and reperfused. Similar experiments were performed after pretreatment of hearts with heparinase, an enzyme known to strip the glycocalyx of heparan sulfate groups. The measured vascular leak was related to the COP of all applied solutions. In addition, we assessed the integrity of the endothelial surface layer (ESL) and the tissue edema at the end of the different perfusion protocols by means of electron microscopy.

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Materials and Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the National Research Council. The protocols were approved by the officially installed, independent ethics committee of the State of Bavaria (Munich, Bavaria, Germany; an equivalent of the Institutional Animal Care and Use Committee). Licensure of the investigator was obtained from the Government of Upper Bavaria (File No. 209.1/211-2531.3-3/99).

Heart Preparation

Guinea pig hearts were isolated and perfused in a modified Langendorff mode. The animals (male; weight, 200–250 g) were stunned by neck dislocation with an especially designed instrument, and immediately after opening of the thorax, the hearts were arrested with ice-cold isotonic saline. After aortic cannulation, hearts were perfused in situ at a constant flow rate of 6 ml/min (37°C, pH 7.40 ± 0.05) and then removed and prepared as described previously. The effluent perfusate from the coronary sinus drains into the right atrium and leaves the heart via the right ventricle and the cannulated pulmonary artery. The right ventricle performs no pressure–volume work in transporting coronary effluent, because the height of egress is below the level of the cardiac apex. The initial perfusate, KHB (116 mEq/l NaCl, 23 mEq/l NaHCO₃, 3.6 mEq/l KCl, 3.48 mEq/l KH₂PO₄, 2.5 mEq/l CaCl₂, 1.16 mEq/l MgSO₄, 5.4 mm/l glucose, 0.3 mm/l pyruvate, and 2.8 U/l insulin, gassed with 94.5% O₂ and 5.5% CO₂), was administered by a peristaltic pump. The chosen rate of flow (6 ml/min) represents approximately the mean coronary flow we observed in previous experiments during perfusion of hearts at a constant pressure of 80 cm H₂O. The perfusion pressure was continuously recorded in the aortic feed line with a pressure transducer (FMI GmbH, Engelsbach, Germany). We derived and registered the heart rate via the pulsatile pressure amplitude. Coronary venous effluent was collected from the cannulated pulmonary artery. Net filtered fluid, comprising interstitial and lymphatic fluid, appeared at the epicardial surface. This so-called transudate was collected from the apex of the heart into preweighed vials.

Experimental Protocols

**Protocol A.** We investigated the impact of four different types of infusion solution on transudate formation, colloid extravasation, and the resulting perfusion pressure at various rates of coronary flow (fig. 1). The solutions chosen as additives to KHB were 0.9% saline (NaCl; Braun Melsungen, Melsungen, Germany), 5% albumin (Humanalbin; ZLB Behring, Marburg, Germany), 2.5% albumin (5% solution diluted 1:1 with 0.9% saline), and 6% HES 130/0.4 (Voluven; Fresenius Kabi Deutschland, Bad Homburg, Germany).

After an equilibration interval of 15 min at a perfusion rate of 6 ml/min (phase I: equilibration), we first measured aortic pressure (cm H₂O) and transudate formation (l/min) during perfusion with KHB at four flow rates (3, 4, 6, and 8 ml/min; phase II: basal). Because the time required to establish steady state conditions of perfusion was approximately 3–4 min, each rate was maintained for 5 min, taking pressure measurements and collecting samples of transudate and effluent in the fifth minute, i.e., at times 5, 10, 15, and 20 min after equilibration. In the next step, KHB was replaced to one third by infusion of 0.9% saline, 5% albumin, 2.5% albumin, or 6% HES 130/0.4 solution (groups NaCl, albumin 5%, albumin 2.5%, and HES 6%; n = 5 each) under the conditions of constant flow at 3, 4, 6, and 8 ml/min (phase III). Again, we took samples of transudate and effluent and measured the aortic pressure after 5 min of perfusion at the different rates of perfusion, i.e., at times 25, 30, 35, and 40 min.

Next, by clamping the aortic feed line, we imposed 20 min of global, stopped-flow ischemia (37°C), reperfusing the hearts at minute 60. Within the first 10 min after onset of reperfusion, a constant flow of 6 ml/min was maintained, and measurements were performed at minutes 1, 2, 3, 5, 7, and 10 (phase IV: early reperfusion). The four different flow rates were then again imposed with measurements taken after 5 min at each level (phase V: postischemia). At the end of this protocol, the hearts were removed from the perfusion system, and the
atria and large vessels were cut away. Excess surface and intraventricular fluid was then swabbed off, and the ventricles were weighed at once (wet weight) and after 24 h of drying at 60°C (dry weight).

**Protocol B.** To gain functional data about the EG, we replaced the basal measurements of phase II described in the previous section by an application of 10 U heparinase (Heparinase I; Sigma-Aldrich, Steinheim, Germany; application: first 15 min after equilibration; washout: from minutes 16 to 20; constant flow of 6 ml/min; fig. 1). This strips the negatively charged heparan sulfates from the glycocalyx.\(^4\,^5\) Values of pressure and rates of transudate formation were determined before and after heparinase application. After that, we continued perfusing as described in protocol A, phase III, i.e., replacing one third of the KHB perfusate with 0.9% saline, 5% albumin, or 6% HES 130/0.4 (groups NaCl + H, albumin 5% + H, and HES 6% + H; \(n = 5\) each).

A heart rate lower than 200 beats/min after rewarming (phase I), a perfusion pressure higher than 80 cm H\(_2\)O resulting from any applied flow (phases I, II, and III), or both often predicted posts ischemic heart failure. Consequently, occurrence of such led us to eliminate the respective heart (\(n = 5\) in protocols A and B, respectively). In every case, the eliminated heart was replaced by another one fulfilling the criteria.

**Evaluation of Data**

A distinct steady state perfusion pressure was established in hearts whenever the rate of coronary flow was altered. Because perfusion pressure is the driving force for net fluid filtration, the following evaluation steps were performed:

1. For every level of flow applied to a given heart under a given condition, the corresponding steady state perfusion pressure was related to the measured rate of transudate formation.
2. This always yielded a linear relation, allowing us to read off transudate flow corresponding to chosen perfusion pressures of 30, 40, 50, 60, and 70 cm H\(_2\)O.
3. The individual values for the five hearts of each group at each of these perfusion pressures were then used to calculate a group linear regression equation relating transudate flow to perfusion pressure.

**Criteria for Selecting the Experimental Setting**

The beating heart without pericardium is the only isolated organ preparation that maintains a well-defined tissue hygiene with respect to fluid transport. In all other isolated organs, tissue edema is poorly reversible. Although we expect findings comparable to the coronary system in all vascular beds, the extent of sealing of leak by albumin may vary, depending on the relative importance of the ESL versus the endothelial cell barrier in different organs.\(^4\)

To take into consideration the dilution of all electrolytes, except sodium and chloride, resulting from adding the commercial colloidal solutions to the balanced KHB, we performed a vehicle control: Replacing one third volume of the buffer with isotonic saline (groups NaCl and NaCl + H) led to a dilution comparable to that occurring in the study groups. Differences from the vehicle control should therefore reflect different physicochemical properties of the applied colloids, and not any confounding dilution effect that possibly impacts, for example, on vasomotion.

With ischemia and reperfusion both at 37°C, we were assured of highly standardized conditions and a fast return of spontaneous sinus rhythm. This is essential for monitoring transudate flow and edema without risking backup of atrial, ventricular, or interstitial fluid. Regular recovery of cell and organ function of the heart after acute warm ischemia is limited to times of ischemic challenge not exceeding approximately 30 min. Longer times are accompanied by cell death and pump failure; times shorter than 15 min induce too little damage. Performing our experimental ischemia at lower temperature than 37°C would progressively lengthen the tolerable times, but not indefinitely, and would not change the ultimate outcome. Consequently, we chose 20 min of global, warm ischemia.

In this model, tissue edema can be visualized by electron microscopy and in addition quantified by the wet-to-dry weight ratio. However, even if an impressive interstitial storage of fluid occurs, this does not quantitatively impact the amount of fluid appearing on the epicardial surface and measured and interpreted as coronary transudate. A wet-to-dry weight ratio of 10.0, representing major edema, would only lead to a total amount of fluid “swallowed” by the interstitial space of far less than 200 \(\mu\)l in comparison with the conditions in an ex vivo preparation, assuming an exemplary heart wet weight of 1.5 g. This worst-case edema, developing in the course of the experimental protocol time of 50 or more min, should subtract far less than 4 \(\mu\)l/min from the transudate flow.

**Determination of Albumin and HES Concentrations**

Human albumin in the samples of coronary effluent and transudate was quantified by colorimetry of the bromocresol complex as described previously.\(^1^3\) The concentration of HES in the samples was quantified using a previously described modification\(^1^4\) of a method of Förster et al.\(^1^5\) In brief, the HES molecules are hydrolyzed in several steps and, after adding hexokinase plus glucose-6-phosphate-dehydrogenase (Glucocquant; Boehringer, Mannheim, Germany), the extinction is measured at 340 nm with a spectrophotometer (Cary 100 Bio; Varian, Melbourne, Australia) and compared with the extinction of a standard sample containing a known concentration of HES.
Electron Microscopy
Electron microscopy was performed in modification of a method described by Vogel et al., based on in situ stabilization of the glyocalyx by intracoronary application of a fixative containing lanthanum and glutaraldehyde.4,16

Measurements of COP
We determined COP by oncometry (Osmomat 050; Gonotec GmbH, Berlin, Germany).

Statistical Analysis
Data were distributed normally (tested by Kolmogorov–Smirnov tests) and are presented as mean ± SEM in the figures, with n indicating the number of experiments. Comparisons were made using the Student t test or, for multiple comparisons, analysis of variance with the Bonferroni correction. Post hoc testing was performed using the Student-Newman-Keuls method for multiple comparisons. In addition, we performed an analysis of variance for repeated measurements to detect intergroup differences. A P value less than 0.05 was considered to be significant.

Results
Transudate Formation
Transudate formation was related to the respective perfusion pressure resulting from given flow rates in each heart. A straight-line relation between transudate flow and perfusion pressure existed in every case. This net fluid filtration per pressure level did not differ between groups before intervention (phase II of fig. 1): The groups were comparable during basal conditions with slopes of 3.3 ± 0.6, 2.8 ± 0.3, 2.8 ± 0.6, and 2.8 ± 0.5 in groups NaCl, HES 6%, albumin 5%, and albumin 2.5%, respectively (values are mean ± SEM; P > 0.05; n = 5 each). The averaged relation, obeying the function y = 2.95 × 19, where y = transudate flow (µl/min) and x = perfusion pressure (cm H2O), is indicated by the dashed line in figure 2 (average of all hearts before infusion, slope = 2.93 ± 0.53 µl · min⁻¹ · cm H2O⁻¹, mean ± SD; n = 20). In fact, the rate of transudate formation in the steady state always depended directly and linearly (but not identically) on the coronary perfusion pressure within the tested range of 30–70 cm H2O, irrespective of the perfusion phase or the perfusate composition. The mean correlation coefficient for all 90 linear regressions calculated for phases II, III, and V of all hearts amounted to 0.92 (SD = 0.12). Therefore, in each separate phase, we used the pressure dependence of transudate formation of each individual heart (µl · min⁻¹ · cm H2O⁻¹) to calculate the value of transudate formation that would correspond to perfusion pressures of exactly 30, 40, 50, 60, and 70 cm H2O in that phase. These values were then averaged per group at the five levels of pressure shown to generate the mean transudate–pressure relation for each group per phase (see Materials and Methods). These lines are presented in the figures for both protocols.

Experiments without Heparinase.
During phase III, supplementing perfusate with albumin solution (groups albumin 5% and albumin 2.5%) decreased pressure-dependent transudate formation. The slopes became significantly lower than basal (1.16 ± 0.43 and 1.24 ± 0.42 µl · min⁻¹ · cm H2O⁻¹, respectively; fig. 2). This difference led to statistically significant decreases in net transudate formation from a pressure level of 30 cm H2O upward versus all groups in phase II and those in phase III receiving any infusion other than albumin. Infusing HES (group HES 6%, slope = 2.14 ± 0.70 µl · min⁻¹ · cm H2O⁻¹) did not significantly change pressure-dependent transudate formation versus phase II. Replacing one third of KHB perfusate by 0.9% saline (group NaCl) significantly increased net transudate formation (slope = 8.70 ± 0.72 µl · min⁻¹ · cm H2O⁻¹) versus the corresponding values before infusion and versus all of the other groups in phase III at all pressures.

Within the first 1–2 min of early reperfusion, the hearts often did not beat or had arrhythmia. The values at minute 1 are consequently not shown in the figures representing the early reperfusion phase (phase IV) and are replaced in the time course by the dashed lines. From the second minute after reperfusion, the hearts included in our study beat normally without any arrhythmia, and all reached a steady state level of transudate formation, latest at minute 5. As can be seen in figure 3, hearts of group NaCl exhibited a pronounced postschismic coronary leak. This was greatest during the first 2–3 min of reperfusion. Net transudate formation at the fifth minute of early reperfusion was 4.96 ± 0.95 µl · min⁻¹ · cm
dependence of transudate formation (1.92 ± 0.5%, albumin 2.5%, and HES 6% regarding the pressure reveal a significant difference between groups albumin not significant before minute 10.

Nevertheless, in all groups receiving colloid, pressure-dependent transudate formation during early reperfusion (groups albumin 5% and albumin 2.5%) led to a significantly lower transudate formation during early reperfusion \(1 \cdot \text{cm H}_2\text{O}^{-1}\) at the fifth minute, respectively; fig. 3). Only in the latter case did a transient increase in coronary leak occur during the first 2–3 min of reperfusion. Although HES tended to decrease mean transudate formation during early reperfusion versus saline, the difference was not significant before minute 10.

The postischemic measurements of phase V did not reveal a significant difference between groups albumin 5%, albumin 2.5%, and HES 6% regarding the pressure dependence of transudate formation (1.92 ± 0.83, 2.34 ± 1.19, and 3.02 ± 0.79 \(\mu\text{L} \cdot \text{min}^{-1} \cdot \text{cm H}_2\text{O}^{-1}\), respectively), although coronary leak tended to be decreased for both albumin groups versus HES 6% (not shown). Nevertheless, in all groups receiving colloid, pressure-dependent transudate formation was significantly lower than in the NaCl group at all pressures (8.90 ± 1.43 \(\mu\text{L} \cdot \text{min}^{-1} \cdot \text{cm H}_2\text{O}^{-1}\)).

Experiments with Heparinase. As figure 4 demonstrates, pretreatment with heparinase significantly increased pressure-dependent transudate formation during infusion of albumin (group albumin 5% + H, 2.16 ± 0.42 \(\mu\text{L} \cdot \text{min}^{-1} \cdot \text{cm H}_2\text{O}^{-1}\) versus the situation with an intact glyocalyx, leak rising to a level comparable to that found for the infusion of HES without pretreatment (group HES 6%). Notably, heparinase pretreatment also led to a mean increase in transudate flow during infusion of HES (group HES 6% + H, 3.86 ± 0.59 \(\mu\text{L} \cdot \text{min}^{-1} \cdot \text{cm H}_2\text{O}^{-1}\)). Therefore, albumin still generated a better vascular barrier than the artificial colloid could.

Within the early reperfusion phase, transudate formation in the hearts of groups albumin 5% + H and HES 6% + H was significantly higher than in the untreated albumin 5% group (net transudate formation at the fifth minute of early reperfusion was 2.01 ± 0.69 and 3.44 ± 2.35 \(\mu\text{L} \cdot \text{min}^{-1} \cdot \text{cm H}_2\text{O}^{-1}\), respectively; fig. 5). Again, albumin tended to perform better than HES.

In phase V (postischemia), the ability of both colloids
to limit coronary vascular leak versus group NaCl was totally lost after pretreatment of hearts with heparinase (slopes of 7.24 ± 8.13 and 5.88 ± 6.11 for groups albumin 5% + H and HES 6% + H, respectively; data not shown).

No impact of pretreatment with heparinase was observed in the hearts receiving crystalloid (group NaCl + H) versus the NaCl group during any phase of the experimental protocol. Pressure-dependent transudate formation during phases III and V was 8.70 ± 0.44 (fig. 4) and 9.10 ± 1.51 µl · min⁻¹ · cm H₂O⁻¹ (not shown), respectively. Transudate formation at the fifth minute of early reperfusion (phase IV) was 5.06 ± 0.89 µl · min⁻¹ · cm H₂O⁻¹ (fig. 5).

Heart rate did not differ significantly between groups at the end of phases II, III, and IV and was, on average, 233 ± 7, 240 ± 12, and 217 ± 7 beats/min, respectively.

Extravasation of Colloid

The concentration of colloid found in transudate lagged behind that in the coronary venous effluent during the early phase of colloid infusion (groups albumin 5% and HES 6%, phase III). The difference was significant at the 5-, 10-, and 15-min measuring points (fig. 6A). However, we saw a significantly slower approach of the albumin concentration within transudate to the level in the effluent than for HES. On the other hand, as already noted in previous studies, we observed no significant difference in colloid concentration between effluent and transudate 20 min after starting the infusion of either albumin or HES. Nevertheless, the slower kinetic of transport and the slower rate of transudate flow for albumin together demonstrate that the permeability of the intact vascular wall for albumin is lower than that for HES. This is visualized in the plot of net extravasation of colloid versus time (fig. 6B). After 20 min infusion, i.e., at the saturation level of colloid in the interstitial space, approximately five to six times as much HES was passing across the vascular barrier per unit of time as there was of albumin.

Electron Microscopy, COP, and Measured Tissue Edema

None of the resuscitation fluids applied led to electron-microscopically detectable denudation of the EG; an exemplary electron microscopic picture for a heart perfused with KHB containing albumin is shown in figure 7. However, there were differences in the interstitial spaces, with tissue of hearts perfused with albumin appearing more dense than that of hearts receiving buffer plus saline. HES perfusion led to an intermediate appearance (fig. 8).

Surprisingly, COP of 5% albumin solution is only half that of 6% HES 130/0.4 solution (11.45 and 21.65 mmHg, respectively), despite the approximately double molecular weight of the latter. The values obtained in the perfusates containing one third volume albumin or HES are listed in table 1. Again, the COP of KHB with HES was almost twice that of KHB with albumin.

The mean wet-to-dry-weight-ratio of hearts perfused with KHB plus isotonic saline (group NaCl) amounted to 8.25. As also listed in table 1, the mean ratios of 7.16 and 7.13 determined for hearts perfused with the natural colloid albumin at concentrations of 0.83 and 1.67 g%,
respectively, indicate significantly less edema formation. Supplementing perfusate with albumin further to the physiologic level of 4 g% decreased edema even more (table 1; additional experiments, n = 4). In contrast, infusion of the artificial colloid HES at an effective concentration of 2 g%, resulting in a COP of 5.45 mmHg, allowed for higher edema than 1.67 g% albumin with a COP of 2.95 mmHg (table 1).

**Discussion**

The primary hypothesis was that, because of specific interactions of colloids with the EG, colloids of different chemical nature will exert different effects on the vascular barrier. Our results confirm this suggestion: Albumin performs much better than HES in maintaining vascular fluid homeostasis. In the intact coronary system perfused with KHB, infusing this natural colloid decreased net fluid extravasation. We found it to be much more powerful in this on a weight-for-weight basis than the artificial colloid HES with a mean molecular weight of 130 kd and a substitution degree of 0.4. Nevertheless, this representative of the latest generation of HES solutions was able to attenuate the impressive increase in transudate formation (a direct measure of the coronary leak) that we observed when infusing an equal volume load of 0.9% saline. This latter phenomenon results, in our protocol, from a dilution of perfusate electrolytes, foremost calcium. Accordingly, the presence of 2% HES in the respective group was able to compensate for the dilution effect. The presence of albumin, however, even decreased transudate formation versus basal measurement (KHB alone) already at 0.83 or 1.67%. Therefore, intravascular colloid, especially albumin, has the capacity to at least partly restore the barrier properties of the vasculature lost during preparation and perfusion of hearts with a colloid-free KHB.

Surprisingly, we did not find any substantial difference in colloid concentration between transudate and effluent 20 min after starting the infusion. A similar finding has been reported before. Furthermore, there was a faster extravasation of HES than of albumin. This is unexpected, because the mean molecular weight of the applied HES preparation is 130 kd versus 66 kd for albumin, so that the diffusion rate of albumin in water should be 39% higher than that of HES, according to the respective diffusion coefficient. Therefore, the result demonstrates that egress of colloid was not controlled by free diffusion in water. This extravasation is not trivial, because it is known that colloids such as HES and
Table 1. Colloid Osmotic Pressure and Tissue Edema Resulting from Perfusing Hearts with the Respective Solution

<table>
<thead>
<tr>
<th>Perfusion Solution</th>
<th>COP, mmHg</th>
<th>Wet Weight/Dry Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taken from present work</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/3 KHB + 1/3 NaCl 0.9%</td>
<td>0</td>
<td>8.25 ± 0.23</td>
</tr>
<tr>
<td>2/3 KHB + 1/3 albumin 2.5%</td>
<td>1.40</td>
<td>7.16 ± 0.44</td>
</tr>
<tr>
<td>2/3 KHB + 1/3 albumin 5%</td>
<td>2.95</td>
<td>7.13 ± 0.41</td>
</tr>
<tr>
<td>2/3 KHB + 1/3 HES 6%</td>
<td>5.45</td>
<td>7.70 ± 0.38</td>
</tr>
<tr>
<td>Taken from additional experiments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex vivo†</td>
<td>25–28</td>
<td>4.76 ± 0.24</td>
</tr>
<tr>
<td>60 min 4% Albumin</td>
<td>8.60</td>
<td>5.41‡ ± 0.36</td>
</tr>
<tr>
<td>60 min KHB</td>
<td>0</td>
<td>8.33 ± 0.25</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n = 5 each (present work) or n = 4 (additional experiments).
* P < 0.05 with respect to 2/3 KHB + 1/3 NaCl 0.9% and 60 min KHB.
† Determined directly after in vivo perfusion and removal from the thorax.
‡ P < 0.05 with respect to all groups other than ex vivo.
COP = colloid osmotic pressure; KHB = Krebs-Henseleit buffer.

albumin remain within the vasculature for hours and that the Starling equation, describing the forces preventing fluid extravasation, relies on the difference between the oncotic pressure in the capillaries and the interstitial space. Because we found no apparent difference in colloid concentration between inside and outside the vascular space (in our experimental protocol at the latest during the reperfusion phase), Starling’s concept is not able to explain our findings. There must be another benefit to the endothelial barrier resulting from infusing colloids, one that seems to be greater when using the natural colloid albumin.

The first pictures of an EG showed only a thin layer of 20–30 nm because conventional fixation techniques almost completely destroyed this fragile structure before electron microscopy. In the past 15 yr, direct and indirect approaches demonstrated that there is a 0.4- to 1.2-μm-thick ESL. This exclusion zone for flowing erythrocytes contains noncircular plasma, including plasma proteins absorbed to and intercalated in the membrane-bound glyocalyx and in dynamic equilibrium with the flowing plasma. Strong binding of albumin to the EG of microvascular endothelial cells has been demonstrated previously. This may be mediated by electrostatic interaction between positive charges of the amphiphilic albumin molecule carried by arginine and lysine moieties and the negative charges carried by sulfate groups on the glyocalyx. As described in our recent work, we introduced the EG as a competent barrier for fluids and colloids, acting in addition to the barrier posed by the endothelial cells themselves. Our new findings underline this concept, but the current study particularly addresses the question of whether colloids of different chemical nature influence the quality of the glyocalyx barrier. The experiments were performed using a perfusion protocol designed to give quantitative insight into the net filtration of fluid and colloids in an intact vascular bed. Particular attention was given to the consequences of the altered filtration properties with respect to edema formation. An important finding of this study is that, without colloid, the EG seems to have no barrier properties. As can be seen in figure 4, there is no difference in transudate formation in NaCl groups with and without degradation of the EG. Accordingly, it seems necessary to extend the consideration beyond the glyocalyx and to address the ESL.

The ESL can be subdivided into the EG (consisting of membrane-bound molecules such as proteins, glycolipids, glycoproteins, and proteoglycans with many exposed, negatively charged groups) and absorbed plasma proteins such as albumin. Adamson et al. found, in an isolated vessel preparation, that the colloid osmotic forces opposing filtration across the capillaries are developed not across the vessel wall, but across the EG. The resulting model suggests an ESL acting like a sponge in the outwardly directed stream of filtered fluid, permeable for fluids but nearly impermeable for proteins. Consequently, a high protein concentration within the ESL is opposed to a low concentration directly below in the intercellular clefts of the endothelium, permanently cleared of colloid molecules by the centrifugal fluid stream, which also limits access of colloid via diffusion from the interstitial side. Such a scenario would easily provide the relevant difference in COP preventing the vasculature from losing fluids. Appropriately, degrading the glyocalyx with heparinase here strongly infringed the sealing effect of albumin.

Hydroxyethyl starch also attenuated fluid extravasation, an ability slightly reduced by pretreatment of the coronary system with heparinase. Accordingly, also the effect of this artificial colloid seems to depend on an intact EG. Presumably, HES is able to form a rudimentary surface layer, inferior to the ESL generated with albumin but superior to a completely colloid-free scaffold of proteoglycans and glycosaminoglycans. This intrinsic effect of albumin is most likely based on its electrostatic binding properties. The charges exposed by the molecules forming the EG are mainly negative (heparan-, dermatan-, and chondroitin sulfates, etc.), as is the HES molecule, whereas albumin carries not only negative (carboxylate groups) but also positive charges (arginine, lyses) at physiologic pH. We confirmed the dependence on binding to the glyocalyx in the experiments where colloid infusion took place after pretreatment of hearts with heparinase. This intervention, known to remove the heparan sulfates of the glyocalyx, leaving just a rudimentary scaffolding of syndecans, glypicans, etc., reduced the power of albumin to support vascular integrity to approximately that of HES with an intact EG.

The behavior of the albumin-perfused hearts during the period of early reperfusion indicates that, in clinical practice, providing albumin to the endothelium, before and after ischemia, should help to maintain vascular
integrity during reperfusion and alleviate development of tissue edema. Although tissue fluid accumulation will possibly be adequately tolerated by young, vigorous patients, elderly or frail patients may be seriously jeopardized because of impairment of oxygen delivery to the lungs, myocardium, and brain. In our experimental model, HES infusion only proved significantly superior to isotonic saline in the very late stage of reperfusion. This could be too late to be of much benefit.

The ESL represents a major barrier against extravasation of fluids and colloids. The EG forms this layer by sieving and binding the intravascularly available colloids from the plasma flowing outward along the hydrostatic pressure gradient. Binding albumin results in a more competent layer than binding HES. Accordingly, this natural colloid seems to be an essential part of a competent ESL, which cannot simply be replaced by an artificial alternative without altering the vascular barrier function. Although we performed our experiments in the isolated heart, it should be expected that vascular permeability is controlled by the ESL, in addition to the endothelial cell lining, not only in the heart but also in other organs, e.g., the brain, the lung, the kidney, or the liver. Based on our ex vivo data, we recommend maintaining a physiologic albumin blood concentration already from the onset of a surgical procedure, especially in the ischemia–reperfusion setting and in critically ill patients. Our findings are in good agreement with clinical data indicating hypoalbuminemia to be strongly associated with poor outcomes. However, the optimal serum level that is to be maintained in critically ill patients remains controversial at this time, and well-designed clinical trials are urgently needed.

The power of albumin to prevent tissue edema is dependent on an undamaged EG. Iatrogenic conditions suspected of decreasing the volume of the surface layer, especially hypervolemia, but also extensive normovolemic infusion of certain artificial colloids should possibly be avoided.

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