In vivo measurement of lung preservation solution efficacy: comparison of LPD, UW, EC and low K⁺-EC following short and extended ischemia

Bernard Hausen a,*, Maike Beuke a, Frank Schroeder a, Christian F. Poets b, Charles Hewitt c, Anthony J. DelRossi c, Hans-Joachim Schäfers d

a Division of Thoracic and Cardiovascular Surgery, Surgical Center, Hannover Medical School, D-30623 Hannover, Germany
b Department of Pediatric Pulmonology, Hannover Medical School, D-30623 Hannover, Germany
c Division of Surgical Research, Department of Surgery, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Camden, New Jersey 08103, USA
d Division of Thoracic and Cardiovascular Surgery, University Homburg/Saar, Homburg, Germany

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Abstract

Objective: The impact of storage solution composition on graft performance was evaluated following perfusion with either Euro-Collins (EC), low potassium Euro-Collins (rEC), low potassium dextran (LPD) or University of Wisconsin solution (UW) after brief (2 h) and extended ischemia (16 h) in an acute double lung transplantation model in the rat. Methods: Following flush perfusion and ischemia the lungs were implanted in recipient rats allowing serial assessment of graft pulmonary vascular resistance (PVR) and alveolar arterial oxygen difference (AaDO₂) during 120 min of reperfusion. Graft dynamic lung compliance (DLC) was determined by separate ventilation. Final evaluation included weight gain and histology. Results: After extended ischemia LPD provided superior graft function in respect to DLC (repeated measures ANOVA; LPD versus rEC \(P < 0.05\); versus EC \(P < 0.03\); versus UW \(P < 0.05\)) and AaDO₂ (LPD versus rEC \(P < 0.04\); versus EC \(P < 0.006\)). The PVR was significantly lower in LPD versus UW (\(P < 0.05\)). At the end of reperfusion the weight increase amounted to 229 ± 49% in rEC, 207 ± 22% in EC, 115 ± 22% in UW and 87 ± 17% in LPD (LPD versus rEC \(P < 0.01\), LPD versus EC \(P < 0.001\)). The type of preservation solution used had little impact on graft function after 2 h ischemia. Conclusions: Low potassium dextran provides superior graft function after extended ischemia. After short ischemia the type of preservation solution used in this study had little impact on global lung function. © 1997 Elsevier Science B.V.

Keywords: Reperfusion injury; Lung preservation; Lung transplantation; Rats

1. Introduction

The composition of lung preservation solutions has been the center of research for more than 30 years. The introduction of a solution with an intracellular electrolyte concentration (Collins solution) for experimental preservation of lung allografts by Grosjean [1] and later Starkey [2] have been considered major advances in clinical lung transplantation. Simple hypothermic crystalloid flush has later replaced the more cumbersome procedures involving autoperfusion or donor core-cooling on cardiopulmonary bypass [3]. Despite far more than 40 studies since 1986 showing superior graft function of various preservation solu-
tions over Euro Collins solution (EC) in in-vitro and in-vivo experiments, the latter has remained the gold standard in clinical lung preservation. In the last years the majority of research related to preservation solution composition has focused on two major solutions: University of Wisconsin solution (UW; Belzer) and low potassium dextran solution (LPD; Toronto). Each of these individual solutions has been compared with EC in a number of animal studies [4–6]. To date only two in-vitro [7,8], and one cell culture study [9] have compared UW, LPD and EC for preservation of graft function. In both in vitro experiments LPD provided superior graft function in comparison to UW and EC.

The intent of this study was to test the hypothesis, that storage solution composition is important for preservation of global lung function in vivo only for extended ischemic storage (16 h) and not for short ischemia (2 h). This is, to our knowledge, the first in-vivo comparison of these most commonly cited pulmonary preservation solutions in one comparative study.

2. Materials and methods

The animals received humane care in compliance with the ‘Principals of Laboratory Animal Care’ formulated by the National Society for Medical Research and the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No.80–123, revised 1985). Approval was granted by the State Ethics Committee of Lower Saxony, Germany. The male Lewis rats (320–380 g) were obtained from Charles River, Salzfeld, Germany.

### Table 1

| Composition of solutions used to flush pulmonary artery and store lungs |
|----------------|----------------|----------------|----------------|----------------|
|                | EC             | rEC            | UW             | LPD            |
| Na⁺ (mmol/l)   | 30             | 115            | 115            | 10             |
| K⁺ (mmol/l)    | 168            | 10             | 125            | 4              |
| Mg²⁺ (mmol/l)  | —              | —              | 5              | —              |
| Cl⁻ (mmol/l)   | 15             | 15             | —              | 103            |
| SO₄⁻ (mmol/l)  | —              | —              | 5              | —              |
| PO₄³⁻ (mmol/l) | 57.5           | 57.5           | 25             | 36.7           |
| HCO₃⁻ (mmol/l) | 10             | 10             | —              | —              |
| Hydroxyethylstarch (%) | — | — | 5 | — |
| Glucose (%)    | 3.5            | 3.5            | —              | —              |
| Lactobionate (mmol/l) | — | — | 100 | — |
| Raffinose (mmol/l) | — | — | 30 | — |
| Glutathione (mmol/l) | — | — | 1 | — |
| Adenosine (mmol/l) | — | — | 5 | — |
| Osmolarity (mOsmol/l) | 355 | 380 | 327 | 285 |

### Table 2

<table>
<thead>
<tr>
<th>Ischemia (h)</th>
<th>rEC</th>
<th>LPD</th>
<th>Statistics</th>
<th>EC</th>
<th>rEC</th>
<th>UW</th>
<th>LPD</th>
<th>Statistics</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>A nimals per group</td>
<td>2</td>
<td>2</td>
<td>—</td>
<td>6</td>
<td>16</td>
<td>6</td>
<td>16</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Donor weight (g)</td>
<td>364 ± 7</td>
<td>358 ± 6</td>
<td>n.s.</td>
<td>371 ± 8</td>
<td>358 ± 10</td>
<td>374 ± 17</td>
<td>377 ± 6</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Recipient Weight (g)</td>
<td>374 ± 7</td>
<td>370 ± 5</td>
<td>n.s.</td>
<td>390 ± 17</td>
<td>379 ± 7</td>
<td>395 ± 18</td>
<td>371 ± 7</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lung weight gain p.R. (%)</td>
<td>44 ± 11</td>
<td>46 ± 8</td>
<td>n.s.</td>
<td>207 ± 22*</td>
<td>222 ± 49†</td>
<td>115 ± 22*</td>
<td>87 ± 17*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Survival (min)</td>
<td>120 ± 0</td>
<td>120 ± 0</td>
<td>n.s.</td>
<td>97 ± 6</td>
<td>83 ± 11*</td>
<td>110 ± 7*</td>
<td>117 ± 3*</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Both weight gain and survival were adversely effected in lungs flushed with rEC.

EC, Euro-Collins solution; LPD, low potassium dextran solution; rEC, low-potassium Euro-Collins solution; UW, University of Wisconsin solution.

2.1. Experimental groups

The impact of solution composition was compared after a 16 h ischemic interval in cold storage using either the Euro-Collins solution (group EC₁₆h), a low-potassium Euro-Collins solution (group rEC₁₆h), the University of Wisconsin solution (group UW₁₆h) or low-potassium dextran solution (group LPD₁₆h; Pervadex™). The composition of the individual preservation solutions is depicted in Table 1. The number of animals per group is listed in Table 2.

In a second part of this study, the solution providing the best preservation after 16 h of ischemia was compared with the solution providing the poorest results after 16 h ischemia. Graft function was compared using...
these two solutions after an 2 h ischemic interval. The groups studied were the low-potassium Euro-Collins solution (group rEC2h) and the low potassium dextran solution (group LPD2h).

2.2. Experimental procedure

The model of double lung transplantation in the rat has been described in detail in a previous publication [10]. Following heparin administration (1000 IU/kg) the donor lungs were flushed in-situ with the individual preservation solution. The volume of the perfusate was 60 ml/kg, given with a perfusion pressure of 20 mmHg at 4°C during constant ventilation. The main pulmonary trunk was then dissected and the mitral valve closed with an 8-0 Prolene suture. The grafts were stored with static inflation using 100% oxygen at an intratracheal pressure of 26 cmH2O in the group specific preservation solution at 4°C [11].

Shortly before the end of the ischemic interval a left thoracotomy was performed in the syngeneic recipient. Following pneumonectomy of the left recipient lung the donor pulmonary artery and left atrial appendage were connected to the left pulmonary artery and vein of the recipient rat using two custom designed T-shaped stents. A Doppler probe (H-2R probe, Transonic Systems, Ithaca, NY) was incorporated into the venous stent. Side ports in both stents permitted blood retrieval for blood gas analysis as well as pressure measurements. The trachea of the graft was intubated with a 13 Gauge canula and the lungs were ventilated with a Harvard volume-controlled respirator (Harvard Rodent Model 683; South Natick, MS) at a tidal volume of 14 ml/kg and a positive end expiratory pressure of 3 cmH2O. The inspiratory oxygen concentration (FiO2) was 1.0. After administration of heparin to the recipient, blood was allowed to flow through the graft. The respiratory rate of the donor lung was adjusted to maintain a left pulmonary venous PCO2 of 30–40 mmHg. Fluid loss was replaced with either blood or crystalloid fluid to maintain a mean pulmonary artery pressure of 20 mmHg and a hematocrit of 30–40%. The donor lung was kept moist by intermittent topical application of warm fluid. The pH measured in the pulmonary venous blood gases was titrated with 8.5% NaHCO3 to achieve a pH of 7.25–7.5.

2.3. Measurements

Serial assessment of pulmonary venous and arterial pressures and blood gases, isolated graft blood flow and dynamic compliance as well as pulmonary resistance to airflow were performed at 20 min intervals for a total reperfusion period of 120 min. Derived parameter were pulmonary vascular resistance (PVR) and alveolar arterial oxygen difference (AaDO2). Immediately preceding each measurement the donor lungs were ventilated with high inspiratory pressure to remove atelectasis. Secretions were suctioned from the airways and quantified. The total amount of edematous secretions retrieved during the reperfusion period was averaged to length of survival. At the end of the reperfusion period final assessment included percent weight gain of the graft.

2.4. Bronchoalveolar lavage

Bronchoalveolar lavage was performed from the right lobe in all grafts at the end of the reperfusion period. The trachea was intubated with a 13 × 8 canula and 3 ml of 4°C saline was infused by gravity at a rate of 10 ml/min and carefully aspirated. This procedure was repeated total of five times. The bronchoalveolar lavage was immediately centrifuged at 270 × g and the cell free supernatant frozen at −80°C.

2.5. Phospholipid determination

From the lavage a surfactant pellet was resuspended in 154 mmol/l saline supplemented with 1.5 mmol/l calcium chloride. After separation of pellet and supernatant at 27 000 × g for 30 min, the phospholipid content was determined from a 5 μl aliquot according to the method of Bartlett et al [12].

2.6. Histology

At the end of reperfusion the left pulmonary lobe was dissected, flushed and stored in formalin, then cut and stained with hematosin-eosin. Evaluation was performed in a blinded fashion. The analysis was scored on a semiquantitative scale. The evaluation protocol was performed in a blinded fashion. The analysis was scored on a semiquantitative scale. The evaluation protocol was performed in a blinded fashion.

2.7. Statistical analysis

Data were analyzed with the Statistical Program of Social Sciences (SPSS for Windows Version 6.3, Birmingham). All data is expressed as mean ± standard error. Analysis of continuous data, such as compliance, resistance, alveolar arterial oxygen difference and pulmonary vascular resistance, was performed by repeated measures ANOVA (r.m.A) [13]. The model used incorporated a time effect, a group effect and a time by group interaction effect. The change over time in each group was evaluated by one-way repeated measures ANOVA including the random effect. Continuous data without repeated measurements, such as weight of donor and recipients animals, weight increase of graft, survival, the results of phospholipid assays as well as...
the histology results were compared with the Mann-Whitney U test.

3. Results

3.1. 16 h Ischemia

Demographic and pre-transplantation data of the four 16 h study groups is outlined in Table 2. There was no statistical difference in terms of donor or recipient weight. The average weight increase during the 2 h reperfusion period was highest in the rEC_{16 h} group (229 ± 49%) and lowest in the LPD_{16 h} group (87 ± 17%; P < 0.01). The difference in weight gain between the UW_{16 h} group (115 ± 22%) and the rEC_{16 h} group was also statistically significant (P < 0.04). Premature recipient death was related to increased pulmonary vascular resistance and subsequent right ventricular failure. The average survival was shortest in the rEC_{16 h} group (83 ± 11 min) and the longest in the LPD_{16 h} group (117 ± 3 min; P < 0.015).

The average amount of edematous fluid retrieved before each measurement was insignificantly higher in the rEC_{16 h} and EC_{16 h} groups (3.5 ± 1.7, 3.4 ± 1.5 ml, respectively) compared with 2.4 ± 1.2 ml in the LPD_{16 h} group and 2.8 ± 1.7 ml in the UW_{16 h} group (Table 2).

Serial assessment of dynamic pulmonary compliance (DLC) is depicted in Fig. 1B. Preservation with LPD_{16 h} provided significantly better graft compliance than with UW_{16 h} (P < 0.002), EC_{16 h} (P < 0.0003) and rEC_{16 h} (P < 0.0001). At the onset of reperfusion the resistance to intrapulmonary airflow (Fig. 2B) was similar in all groups (range: 250–320 cmH₂O/l per s). During the remainder of reperfusion both the EC_{16 h} and the rEC_{16 h} groups showed a sharp incline in resistance (one-way ANOVA EC_{16 h} P < 0.0001; rEC_{16 h} P < 0.0001). The resistance in the UW_{16 h} group was significantly lower than in the EC_{16 h} and the rEC_{16 h} groups (repeated measures ANOVA P < 0.04, P < 0.03, respectively).
Fig. 2. (A, B) The resistance to pulmonary airflow measured in cmH₂O/l per s is significantly lower in grafts preserved with UW.

The average amount of fluid retrieved before each measurement was significantly higher following flush with rEC (1.15 ± 0.4 ml) than after preservation with LPD (0.2 ± 0.4 ml; P < 0.02).

The dynamic pulmonary compliance (Fig. 1A) and the resistance to intrapulmonary airflow (Fig. 2A) was similar in both groups with little change during the entire observation period. The alveolar arterial oxygen difference (AaDO₂) showed a trend towards lower values in the LPD₂h group in comparison to the rEC₂h group, however this did not reach statistical significance (Fig. 3A). At the onset of reperfusion the pulmonary vascular resistance (PVR) was 529 ± 133 mmHg/ml per s in the rEC₂h group versus 107 ± 26 mmHg/ml per s in LPD₂h group, however this initially significant difference vanished during the remainder of reperfusion (Fig. 4A).

3.3. Histology

The results of the semiquantitative evaluation of the specimens of all groups (2 and 16 h ischemia) are listed in Table 3. In the statistical analysis the amount of interstitial or intraalveolar edema or extravascular
Fig. 3. (A, B) Comparison of alveolar-arterial oxygen difference (AaDO\(_2\)). The use of LPD after 2 h cold ischemia did not provide better preservation with regard to AaDO\(_2\). Preservation with LPD provided improved oxygenation in comparison to both EC groups after 16 hour ischemia. While the AaDO\(_2\) was stable over the entire reperfusion period following 2 h ischemia, all four 16 h ischemia groups experienced a significant rise in the AaDO\(_2\) (one-way ANOVA).

Granulocyte infiltration was similar in all study groups. The degree of pulmonary hemorrhage was significantly higher in the rEC\(_{16}\) h group when compared with both the LPD\(_{16}\) h (P < 0.02) and the UW\(_{16}\) h groups (P < 0.03).

3.4. Phospholipid aggregates and protein content

For the analysis of phospholipids obtained from the bronchoalveolar lavage at the end of reperfusion the proportion of large (surface active) versus small (surface inactive) phospholipid aggregates was measured in the 27 000 × g pellet. In the 2 h ischemia groups there was no significant difference in the percentage of large aggregates between the rEC\(_2\) h group (31.5 ± 7%) and the LPD\(_2\) h group (40.6 ± 10%). Extended ischemia resulted in significantly lower percentage of large aggregates (5.4 ± 1.1% EC\(_{16}\) h group, 9 ± 1.8% rEC\(_{16}\) h group, 6.7 ± 1.1% UW\(_{16}\) h group, 11.4 ± 2.5% LPD\(_{16}\) h group (n.s.)). The impact of the prolonged ischemia on the percentage of large aggregates found in the BAL at the end of the reperfusion period was significant. All four 16 h study groups experienced a sharp decline in the percentage of large aggregates in comparison to the 2 h ischemia groups (P < 0.002). The protein content in the lavage was significantly lower after 2 h of ischemia with LPD (0.21 ± 0.04 mg/ml) versus rEC (0.73 ± 0.09 mg/ml; P < 0.003). Following extended ischemia the protein content of the lavage was significantly lower in LPD (0.85 ± 0.07 mg/ml) versus UW (1.25 ± 0.13 mg/ml; P < 0.03). The protein content was 1.25 ± 0.66 mg/ml in the EC\(_{16}\) h group and 1.3 ± 0.23 mg/ml in the rEC\(_{16}\) h group.

4. Discussion

Since the introduction of the Collins-Sachs solution for lung preservation in 1972 [1] and its regular clinical application by the Toronto Lung Transplant Group [3], this particular solution has become the gold standard flush solution. While new solutions have been successfully introduced into organ preservation for kidney or liver transplantation, thoracic surgeons have in the past
been very reluctant to change. In one of the first retrospective analyses of clinical data related to lung preservation solutions, Keenan and colleagues in 1991 described an increased perioperative incidence of lung preservation injury by EC flush [14], pointing out, that alternatives to the EC solution should be sought. In many experimental studies comparing EC with new preservation solutions the vast majority of investigators have chosen either UW or LPD for comparison to EC. These two distinctly different solutions have proven quite successful and both have provided superior early graft function when compared with EC individually [4,5,15,16]. Based on these data some centers have recently altered their procurement procedure and are now using UW solution clinically [17] (verbal communication Dr. Novick, London Ontario). However, in the light of recent findings stressing the negative impact of intracellular ionic composition on vascular endothelial cell function [18], additional considerations may be warranted.

To date UW and LPD solutions have only been compared with one another in two in-vitro and one cell culture study. In an in-vitro experiment with closed circuit perfusion of rabbit lungs Oka and colleagues found LPD to be superior to UW and EC [7], while Xiong et al., compared all three solutions in a similar study using an in-vitro rat lung preservation model to a blood based pulmoplegia (Wallwork solution) [8]. In the latter experiment the Wallwork solution proved to offer the best preservation with only slightly better graft function with LPD versus UW. The cell culture study by Spaggiari using fibroblasts demonstrated improved viability with a special new extracellular, colloidal formulation (SPAL UP) and similar viability with either UW or LPD [9]. Both in-vitro studies used extremely short reperfusion periods of less than 15 min, a fact that could possibly limit the conclusions drawn from these studies.

According to the data provided by this study LPD solution provided superior protection following ex-
tended ischemia when compared with EC, low-potassium EC (rEC) and UW. Grafts flushed and stored in low potassium dextran solution and subjected to 16 h of cold ischemia had higher dynamic lung compliance, lower resistance to intrapulmonary airflow and lower PVR than all three other solutions evaluated. Using LPD solution the oxygenative capacity was better than using either EC or rEC, while UW and LPD provided similar AaDO₂ values. The improved global lung function is reflected in lower weight gain, lower protein content in the lung lavage and less structural damage evidenced by histology. After 2 h of ischemia the composition of the preservation solution had little impact on graft function. Comparing hemodynamic data and oxygenative capacity of the solution providing the best graft function after 16 h of ischemia (LPD) to the solution responsible for the worst function (rEC) did not reveal any statistical difference by repeated measures ANOVA.

This study shows that the impact of the preservation solution on graft function increases with increasing ischemic intervals. As had been demonstrated in the previously mentioned in vitro studies, LPD solution provided the best results. The graft function after flushing with the intracellular EC and modified extracellular EC performance was equally poor in comparison to LPD after 16 h of ischemia. Therefore it is not merely reducing the potassium content, that is responsible for the beneficial effects of a storage solution. In the past a number of studies were performed in which the ionic composition of both UW and LPD were altered. Regarding the UW solution, the results were quite variable showing improved function with low-potassium UW in one study [19], improved function with modified UW with an potassium content of 30 mEq in another [20], similar function with low-K⁺ UW and standard UW in a study performed by Sasaki et al [21] and also improved preservation using the standard UW over other modified UW solutions [15,22]. Only one study has compared the standard LPD solution with a modified, high potassium LPD solution demonstrating improved preservation with the standard LPD solution [23].

Donor pretreatment with prostaglandins has been shown to be of primary importance for good preservation as an adjunct to the EC solution [24]. The increased pulmonary vascular resistance during flush perfusion due to the high potassium content seems to impede the homogenous distribution of the perfusate in the entire lung [21,25]. In this investigation prostaglandins have not been used, as this pharmacological pretreatment has proven beneficial for all four preservation solutions used [26,27]. It was felt that the use of prostaglandins would have distorted the true effect of the preservation solution on the quality of preservation. In addition, this study shows excellent organ function without prostaglandins using UW, a solution with a similar electrolyte composition as EC. Also the reduction of the potassium content in the modified EC solution (rEC) did not improve organ function during reperfusion after extended ischemia.

The model used in this study has been validated in different experimental studies [10,11,26]. Lung preservation studies using in-vivo models have major advantages over in-vitro studies as they allow a complete interaction of the recipients' immune system within an homeostatic system. This is important for cell recruitment, cytokine release, absorption and oxygen radical neutralization. This in-vivo syngeneic rodent model avoids any possible interference of ischemia and reperfusion with an alloimmune response. Also, it has become clear from a large number of experimental studies, that the hemodynamic and oxygenative function of the implanted graft may be subject to drastic changes during early reperfusion [4,25,28,29]. This supports the requirement for extended reperfusion periods of at least 1–2 h for evaluation of lung preservation. Nevertheless, it remains unclear how much of the results obtained from rodent models concerning lung
preservation can be used for designing future clinical trials.

In conclusion, this is the first in-vivo comparison of the two most commonly used experimental preservation solutions, low potassium dextran solution and the University of Wisconsin solution, to the gold standard in clinical lung transplantation, the Euro Collins solution. Low potassium dextran provides superior graft function after extended ischemia. After short ischemia the type of preservation solution used in this study has little impact on global lung function.

References


Appendix A. Conference discussion

Dr M Attila (Helsinki, Finland): I have two discussion slides. We did a similar study in Helsinki some years ago with pigs, and we noticed that when using Fluosol-DA solution and comparing that with Euro-Collins solution...
Euro-Collins solution, the oxygenation in the pulmonary venous blood was better when Fluosol DA was used.

(Slide) Those diagrams show the oxygen saturation. After 8, 9 h, the Euro-Collins reached the level of that of artificial blood. So what we routinely use is the Euro-Collins and it seems to work, but there is a period just immediately after transplantation when there is an impairment in the lung oxygenation.

(Slide) Then we looked at the morphology and noticed that after Fluosol-DA solution, the alveolar capillary endothelium was much better preserved, as seen in this picture, compared with the next picture with the Euro-Collins, which shows that there is some loss of the endothelial cells. So there is also morphological evidence that our Euro-Collins solutions are not perfect at the moment.

Dr Hausen: I think it is true that a prolonged reperfusion period is important in the assessment of graft function and I think that even if a solution improves, or the parameters of a different solution improve after 12 h, it is exactly this early time period that causes the clinicians the most headaches. So, I think that it is important that a solution should be good at the onset of reperfusion for the next hours following lung transplantation. But this is very nice data.

Dr Odom (Hannoer, Germany): I would like to congratulate you on your success with a small animal model for looking at these things. I know how difficult these models can be to run, but the great advantage of low cost and high numbers means that you can do large numbers of experiments. Just a comment. I feel that the time has come to stop comparing X solution with Y solution. What we need to do is to analyse individual components of the solution in a scientific manner. Euro-Collins' solution has had its day. It is still being used clinically, but all the results over the last few years have shown that it is inferior to UW solution and to virtually any other solution that you can think of. The high glucose concentration as an osmotic impermeant is harmful; of that there is little doubt.

The other comment I would like to make is that, with all these tests, a relatively prolonged period of perfusion is necessary, certainly at least 30 min, and that the conditions of perfusion in the first 10 min are highly critical. You can get very different results depending on whether you perfuse at low flows or at high flows, particularly with low-potassium solutions. Congratulations once more on a fine study.

Dr Hausen: Thank you. There have been a number of studies comparing the individual components of UW in lung preservation, however, they have been quite conflicting. Some say that glutathione is important in individual components. It is very difficult to draw a conclusion on that. And the same with low-potassium dextran, although most studies show that the dextran component is the most portion of the low-potassium dextran. The model I use has the advantage that you still have a right lung, the native lung of the animals, so most of the blood flow will initially go to the right lung, with a high pulmonary vascular resistance in the left lung, the transplanted double lung, sort of protecting that graft at the onset of reperfusion and doing just what you suggested as having a low pressure, low flow on the graft at the onset of reperfusion. Thank you.