# Evaluation of lung metabolism during successful twenty-four–hour canine lung preservation

**We used a canine left lung allotransplantation model to evaluate 24-hour lung preservation with two different electrolyte solutions, low-potassium dextran and low-potassium dextran with 1 % glucose. To investigate changes in the energy status during preservation, we analyzed the lungs for adenosine triphosphate, phosphocreatine, and several metabolites of the glycolysis pathway and the citric acid cycle: glucose, glucose-6-phosphate, lactate, citrate, and malate. We also devised and evaluated a pulmonary cooling jacket to prevent rewarming of the lung during implantation. The lungs were divided into four groups. Groups I ($n = 10$) and II ($n = 6$) were flushed with low-potassium dextran and groups III ($n = 6$) and IV ($n = 6$) were flushed with low-potassium dextran solution with 1% glucose. The cooling jacket was used for groups II and IV only. After 24-hour preservation at 10° C, the left lungs were implanted into the recipient animals. Function of the transplanted left lung was assessed during temporary (10 minutes) occlusion of the contralateral pulmonary artery while both lungs were ventilated with 100 % oxygen. This assessment was performed at 1 hour and at 3, 8, and 22 days after transplantation. Immediately after transplantation the arterial oxygen tension was 279 ± 70 mm Hg in group I, 376 ± 56 mm Hg in group II, 523 ± 41 mm Hg in group III, and 518 ± 50 mm Hg in group IV. The arterial oxygen tension in groups III and IV were significantly greater than in group I ($p < 0.05$). Of the lungs preserved with low-potassium dextran solution with 1% glucose solution, 11 of 12 (92%) showed excellent lung function (arterial oxygen tension >300 mm Hg) at 3 days; only 10 of 16 lungs preserved with low-potassium dextran achieved this level of function. Glucose, glucose-6-phosphate, lactate, citrate and malate levels decreased significantly during 24-hour preservation with low-potassium dextran solution; they were stable with low-potassium dextran solution with 1% glucose. Adenosine triphosphate and phosphocreatine were stable for 24 hours with both low-potassium dextran and low-potassium dextran solution with 1% glucose. The cooling jacket provided uniform cooling of the lung parenchyma during implantation, and significant increase in temperature was observed in its absence, with topical cooling by cold saline solution. These results lead us to conclude the following: (1) lung preservation at 10° C with low-potassium dextran solution with 1% glucose solution is associated with continuation of normal glucose metabolism via the glycolytic pathway and aerobic metabolism through the citric acid cycle; (2) low-potassium dextran solution with 1% glucose solution provides safe preservation for up to 24 hours; (3) the pulmonary cooling jacket prevents rewarming of the lung during implantation. (J THORAC CARDIOVASC SURG 1993;105:480-91)**

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Our previous work[1, 2] strongly suggested that the lung might be able to maintain aerobic metabolism during preservation by using oxygen in the alveoli. Preservation by means of inflation with 100% oxygen appeared superior to both inflation with room air and inflation with 100% nitrogen in an isolated rabbit lung model.[1] We also found that canine lungs that were flushed with low-potassium dextran (LPD) solution, inflated with 100% oxygen, and preserved at 4° or 10° C maintained adenosine triphosphate (ATP) levels for a minimum of 18 hours.[2] If maintenance of aerobic metabolism is important for lung preservation, glucose in the preservation solution might be important as an energy source. We therefore modified the preservation solution by adding 1% glucose to LPD[3] solution, referred to as low-potassium dextran glucose (LPDG) solution. In this study, we evaluated 24-hour lung preservation with LPD and LPDG solutions in a canine left lung allotransplantation model. The evaluation was based on lung function after transplantation and the metabolic state during preservation. We also devised a pulmonary cooling jacket to prevent rewarming of the lung during implantation and compared it with topical cooling by cold saline solution.

## Materials and methods

**Lung function study.** We performed 28 left lung allotransplantation procedures in weight-matched pairs of mongrel dogs (20.0 to 27.2 kg). The weights were not significantly different among the four groups, and the dogs were assigned randomly to one of the four study groups. In group I ($n = 10$), lungs were flushed with LPD and implanted with topical cooling by cold saline solution. In group II ($n = 6$), lungs were flushed with LPD and implanted with the cooling jacket. In group III ($n = 6$), lungs were flushed with LPDG and implanted with topical cooling by cold saline solution. In group IV ($n = 6$), lungs were flushed with LPDG and implanted with the cooling jacket. Otherwise, the animals were treated identically. Preservation solutions were blinded in eight experiments from group I ($n = 4$) and group III ($n = 4$) and in six experiments from group II ($n = 3$) and group IV ($n = 3$). The composition of the LPD and LPDG solutions was analyzed before the experiments, and the average values are shown in Table I. The addition of 1% glucose increased the osmolarity from 287 mOsm/L to 328 mOsm/L.

The handmade pulmonary cooling jacket (Fig. 1) is made of two layers of silicone sheeting (Silastic 500-5; Dow Corning Corp., Midland, Mich.), attached with silicone glue (medical adhesive type A; Dow Corning Corp.). The jacket encloses three continuous paths for circulation of cold fluid. The shell-like shape of the jacket is designed so that a canine left lung may be wrapped completely in it for uniform cooling. Hilar structures can be exposed through an opening in the jacket (5 × 3.5 cm) for anastomoses. Cold fluid is perfused through the jacket from a small inlet tube (6.5 mm diameter) with a roller pump. Perfused fluid is collected in a reservoir through an outlet tube. The jacket allows cold fluid to perfuse safely at a flow rate of up to 600 ml/min.

The donor lung was excised and implanted into the recipient by previously described techniques.[2, 3] Both donor lungs were flushed in situ with either LPD or LPDG solution (4° C, 50 ml/kg) by means of a cannula placed in the main pulmonary artery, while the flushing pressure was continuously monitored from a side arm of the cannula. Topical cold saline solution (1° to 4° C) was poured over the lungs during the flush for additional cooling. At the completion of the flush, the trachea was stapled with the lung inflated with 100% oxygen. The donor heart-lung block was then excised, immersed in the same solution that was used for flushing, and preserved for 24 hours at 10° C.

The recipient animals were given oral cyclosporine (15 mg/kg) and azathioprine (1.5 mg/kg) before anesthesia, and 500 mg of methylprednisolone was given intravenously on induction. We performed a left pneumonectomy and placed an inflatable cuff[4] around the right pulmonary artery for measurement of left lung function after implantation.

After preservation for 24 hours, the left lung of the donor was trimmed from the preserved heart-lung block. To measure lung graft temperature during implantation, we connected three needle temperature probes to a thermometer (Nt-2 myocardial temperature probe and NTM-100 digital thermometer; Webster Labs, Inc., Baldwin Park, Calif.) and then inserted them into the graft (Fig. 2). The first temperature probe, its tip covered with a sterile plastic sheath, was advanced through the left main bronchus into the segmental bronchus of the upper lobe to measure lung core temperature. We inserted the second needle probe into the middle lobe through the pleura at a depth of 10 mm. This was used to measure lung surface temperature. We inserted the third needle probe into the left main bronchial wall to measure bronchial temperature as a representative temperature of the hilar structures. We monitored these graft temperatures continuously and recorded them every 10 minutes during implantation. The left atrium was anastomosed first, followed by the pulmonary artery, and the bronchial anastomosis was performed last. We removed the lung core and the bronchial temperature probes before the bronchial anastomosis and removed the lung surface temperature probe just before reperfusion.

For groups I and III, the left lung was implanted without the use of the cooling jacket. The left lung was covered with cold gauze during implantation and cold saline solution was poured frequently over the lung in the same manner as is done for human lung transplantation. For groups II and IV, the left lung was implanted with the cooling jacket. The jacket was prepared before implantation and 1° to 2° C cold water was perfused through the jacket at a rate of 500 ml/min. The lung graft was wrapped with cold wet gauze to avoid direct contact of the graft with the cooling jacket. The jacket was secured around the lung by ligation of the umbilical tapes, which were previously connected to the jacket edges. The hilar structures were exposed through the opening in the jacket and anastomosed (Fig. 3). The rate of circulation of cold fluid was regulated (150 to 500 ml/min) to keep the lung core temperature at 10° C. After completion of anastomoses, we removed the jacket by cutting the umbilical tapes, and then the left lung was reperfused.

The omentum was retrieved through an upper midline abdominal incision and an omentopexy was performed around the bronchial anastomosis. The reservoir that was connected to the inflatable pulmonary artery cuff was implanted subcutaneously.

After closure of the chest, with the dog in a supine position,

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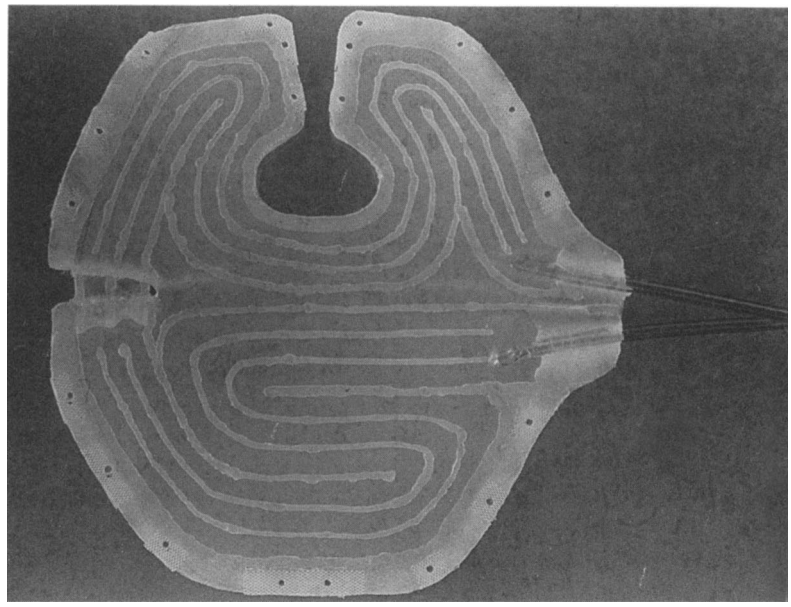

**Fig. 1.** Pulmonary cooling jacket in open position. The lung graft is placed on lower trapezoid and covered with upper trapezoid so that its hilar structures are exposed through opening. Umbilical tapes are connected to small holes surrounding jacket before implantation to close jacket quickly.

**Table I.** *Composition of flushing solutions*

| | *LPD* | *LPDG* |
|---|---|---|
| $Na^+$ (mmol/L) | 149 | 155 |
| $K^+$ | 3.7 | 3.7 |
| $Cl^-$ | 100 | 100 |
| $Mg^{2+}$ | 1.42 | 1.39 |
| $PO_4$ | 34 | 33 |
| Glucose (gm/L) | 0 | 10 |
| Dextran 40 (gm/L) | 20 | 20 |
| pH | 7.43 | 7.42 |
| Osmolarity (mOsm/L) | 287 | 328 |

*LPD*, Low-potassium dextran; *LPDG*, low-potassium dextran glucose.

systemic and pulmonary vascular hemodynamics were determined and arterial blood gas was obtained while both lungs were ventilated with 100% oxygen, with a tidal volume of 550 ml, positive end-expiratory pressure of 5 cm $H_2O$, and a respiratory rate of 12 breaths/min. We measured the function of the transplanted left lung after we occluded the right pulmonary artery for 10 minutes with the inflatable pulmonary artery cuff.

The dogs received cyclosporine (15 mg/kg), azathioprine (1.5 mg/kg), and prednisone (0.5 mg/kg) orally every day. Penicillin G (1,200,000 U) and gentamicin (40 mg) were given intramuscularly every day for 2 weeks. On postoperative days 3, 8, and 20 to 25, surviving animals were reanesthetized and attached to a ventilator, and the same assessment of lung function was repeated. Animals were killed after the final assessment. We took roentgenograms of the chest frequently, and if rejection was suspected, we administered 250 to 500 mg of intravenous methylprednisolone. When an animal that could not tolerate the right pulmonary artery occlusion, or if the roentgenogram of the chest showed complete opacification of the transplanted lung, the animal was killed and the lungs were examined macroscopically and histologically.

**Metabolic study.** We removed sequential biopsy specimens from the right lung to evaluate lung metabolism during the preservation interval. We reserved the left lung for subsequent transplantation. Immediately after excision, we removed a biopsy specimen from the right upper lobe as a control. The right middle, lower, or cardiac lobe was randomly chosen, separated from the heart-lung block, and preserved at 10° C in a separate plastic bag, although our preliminary data suggested no difference in metabolites between lobes. We performed sequential biopsies (0.1 to 0.5 gm) 4 and 24 hours after preservation from this lobe, and the biopsy sites were carefully ligated with silk ties to prevent air leak. Biopsy specimens of lung tissue were quick-frozen immediately by immersion in dichlorodifluoromethane ($CCl_2F_2$), precooled to the freezing point, and stored at −70° C or below until they were analyzed. Metabolic study included assessment of high-energy compounds (ATP and phosphocreatine [PCr]), metabolites of glycolysis (glucose, glucose-6-phosphate [G-6-P], lactate) and of the citric acid cycle (citrate, malate). Samples weighing 0.1 gm were extracted in perchloric acid as described previously.[5] After centrifugation and neutralization, we analyzed the supernatant for metabolites by enzymatic methods. All analytic methods have been described[5]; these methods are based on a series of enzymatic reactions that result in the oxidation or reduction of a pyridine nucleotide. Data are given as micromoles or nanomoles per gram of frozen tissue.

Statistical analysis was performed by means of analysis of variance, and multiple comparisons were made by means of Tukey's HSD technique to compare the results of the four study

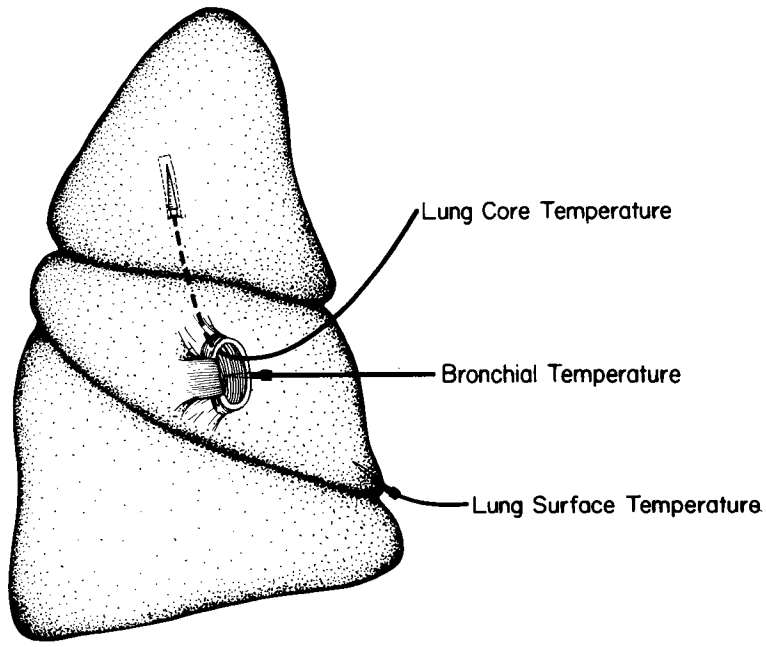


**Fig. 2.** Depiction of temperature probe locations.

**Table II.** *Characteristics of four experimental groups*

| *Group* | *Preservation solution* | *Cooling jacket* | *Flushing time (sec)* | *Flushing pressure (mm Hg)* | *Excision time (min)* | *Implantation time (min)* | *Total ischemic time* |
|---|---|---|---|---|---|---|---|
| I ($n = 10$) | LPD | − | 105 ± 5 | 15.7 ± 1.2 | 8.2 ± 0.4 | 62.9 ± 3.7 | 24 hr 58 min ±08 min |
| II ($n = 6$) | LPD | + | 89 ± 5 | 17.3 ± 3.2 | 6.4 ± 0.4 | 66.7 ± 3.4 | 25 hr 02 min ±07 min |
| III ($n = 6$) | LPDG | − | 87 ± 8 | 14.2 ± 1.3 | 7.3 ± 0.6 | 62.0 ± 6.0 | 24 hr 53 min ±11 min |
| IV ($n = 6$) | LPDG | + | 94 ± 8 | 17.7 ± 1.5 | 7.3 ± 0.4 | 64.3 ± 1.8 | 24 hr 57 min ±11 min |

*LPD*, Low-potassium dextran; *LPDG*, low-potassium dextran glucose.

groups. The unpaired and paired Student $t$ test was also used for the temperature study and the metabolic study. Statistical significance was accepted at a 95% confidence level, $p < 0.05$. All values are presented as the mean ± 1 standard error of the mean.

All animals received humane care in compliance with the "Principles 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. 86-23, revised 1985).

## Results

**Temperature during implantation.** The temperatures of the lung grafts that were implanted without the use of the cooling jacket (groups I and III) quickly rose during implantation (Fig. 4). The lung core, lung surface, and bronchial temperatures reached 25.6° ± 1.2° C, 27.1° ± 2.3° C, and 27.4° ± 1.0° C, respectively. In contrast, when the cooling jacket was used, the lung core and lung surface temperatures were maintained between 10.0° ± 0.4° C and 11.2° ± 0.3° C and between 9.5° ± 0.7° C and 11.4° ± 0.6° C, respectively ($p < 0.001$ versus implantation without the use of the cooling jacket). The bronchial temperature rose to 24.2 ± 1.8° C in spite of the use of the cooling jacket. Nevertheless, the jacket provided significantly lower bronchial temperatures for the first 30 minutes ($p < 0.01$ to 0.05) compared with the lungs without the cooling jacket. The jacket did not interfere with access to the hilar structures for anastomoses, and the implantation time was only 3 minutes longer when the jacket was used; the

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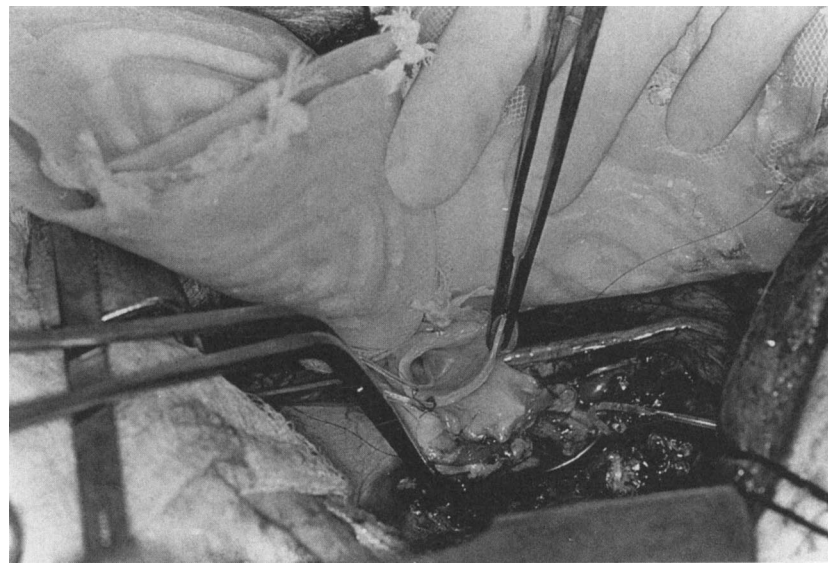

**Fig. 3.** Left lung implantation with cooling jacket with head side to viewer's right. Completed left atrial anastomosis. Lung parenchyma completely wrapped with cooling jacket.

**Table III.** *Cause of early sacrifice or death within 22 days*

| *Group* | *Day of death* | *Cause of sacrifice or death* |
|---|---|---|
| I ($n = 10$) | Day of operation | Lung edema |
| | Day of operation | Lung edema |
| | Day of operation | Lung edema |
| | 6 | Rejection |
| | 7 | Left atrial thrombus |
| | 8 | Left atrial thrombus |
| | 21 | Anesthesia accident |
| II ($n = 6$) | 1 | Lung edema |
| | 2 | Aspiration pneumonia |
| | 10 | Rejection |
| III ($n = 6$) | 3 | Left atrial thrombus |
| | 10 | Rejection |
| IV ($n = 6$) | 8 | Left atrial thrombus |
| | 22 | Rejection |

flushing time, flushing pressure, excision time, implantation time, and total ischemic time did not differ significantly among the four groups (Table II).

**Survival.** The causes of death or sacrifice within 22 days of transplantation are shown in Table III. Acute lung edema resulting from poor preservation was the cause in three cases in group I and one case in group II. Left atrial thrombus was often the cause of death at 3 to 8 days, and rejection was often the cause at 6 to 22 days in all groups. Three of ten dogs (30%) in group I, three of six (50%) in group II, and four of six (67%) in groups III and IV survived for 22 days with functional transplanted lungs (Fig. 5, *A*).

**Lung function study.** Function of the left lung immediately and at 3 days after the operation is shown in Table IV. In group I, three dogs had copious serous tracheal secretions immediately after reperfusion and were unable to tolerate single lung perfusion after 2, 3, and 8 minutes because of significant ventricular arrhythmia and high pulmonary artery pressure. We were therefore unable to assess function of the left lung in the first two of these dogs. The remaining dog was assessed after 8 minutes of single lung perfusion, and this assessment was used to express the function of its left lung. Therefore the data for group I were obtained from eight dogs, excluding the first two dogs (in which single-lung perfusion was not successful). All dogs in groups II, III, and IV completed single-lung perfusion for 10 minutes without difficulty. Immediately after the operation, arterial oxygen tensions ($Pa_{O_2}$) in groups III and IV were significantly greater than in group I: 523 ± 41 mm Hg and 518 ± 50 mm Hg versus 279 ± 70 mm Hg; $p < 0.05$. Arterial carbon dioxide tension ($Pa_{CO_2}$) in group IV was significantly lower than in group II: 41.7 ± 2.7 mm Hg versus 59.1 ± 3.1 mm Hg; $p < 0.05$. Mean pulmonary artery pressure, pulmonary vascular resistance, and cardiac output did not differ statistically among the four groups.

Assessment at 3 days after the operation included seven of ten dogs in group I, four of six dogs in group II, five

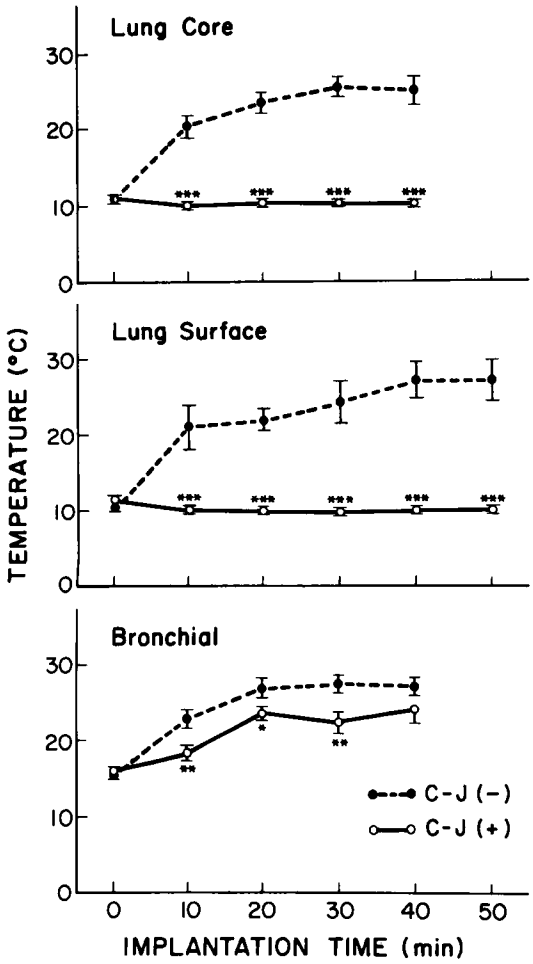


**Fig. 4.** Comparison of two methods of maintaining lung core temperature, lung surface temperature, and bronchial temperature during implantation. *C-J(−)*, Implantation with topical cooling by cold saline solution; *C-J(+)*, implantation with cooling jacket. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ [versus *C-J(−)*].

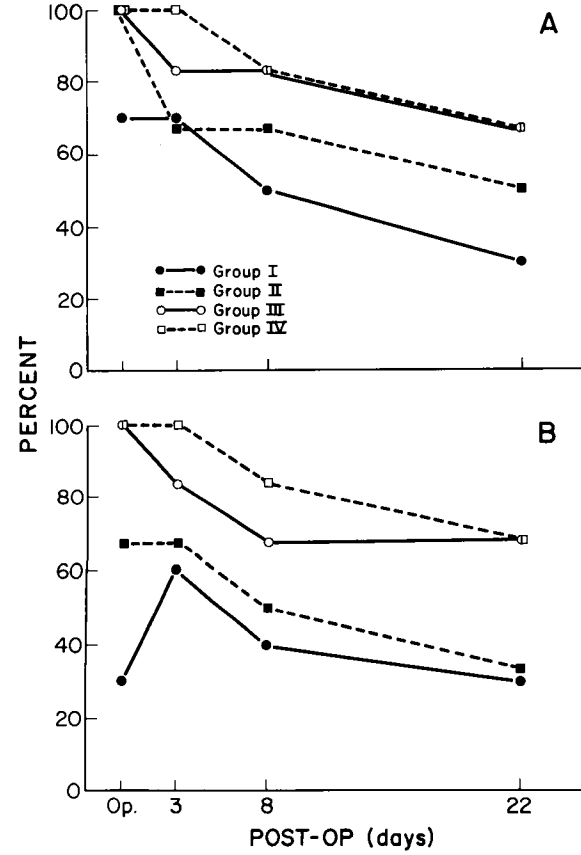


**Fig. 5. A**, Percentage of animals that successfully tolerated single lung perfusion for 10 minutes. **B**, Percentage of animals that had a $PaO_2$ greater than 300 mm Hg during single lung perfusion for 10 minutes.

of six dogs in group III, and six of six dogs in group IV. Function of the left lung did not differ statistically among the four groups. Only one of the animals in group I had a $PaO_2$ that was less than 300 mm Hg after the 10 minutes of perfusion of the isolated left lung, whereas the $PaO_2$ was greater than 300 mm Hg in all of the other animals assessed.

The persistence of $PaO_2$ levels greater than 300 mm Hg is shown in Fig. 5, *B*. Six of six dogs (100%) in group IV, five of six (83%) in group III, four of six (67%) in group II, and six of ten (60%) in group I satisfied this criterion at 3 days after the operation, and four of six (67%) in groups III and IV, two of six (33%) in group II, and 3 of 10 (30%) in group I at 22 days after the operation. Function of the left lung of the survivors is shown in Fig. 6. One dog in group II, which had a lung fistula at 8 days, was excluded from the results of $PaCO_2$ at 8 and 22 days. Survivors in all groups maintained good function in the transplanted lung up to 22 days.

Transplanted lungs of the 22-day survivors in all four groups looked normal on roentgenograms of the chest (Fig. 7) and in macroscopic examinations, except in one dog that had lung fistula. Histologic examination showed normal lung structures with various degrees of mononuclear infiltrates caused by rejection (Fig. 8).

**Metabolic study.** The effects on lung metabolism of the addition of glucose to the preservation solution were evaluated by comparison of the results from groups that received LPDG (groups III and IV) with those that received LPD (groups I and II) (Table V, Fig. 9).

*Energy status.* Levels of ATP and PCr were unchanged for 24 hours with both solutions, except that immediately after excision of the donor lung, ATP level

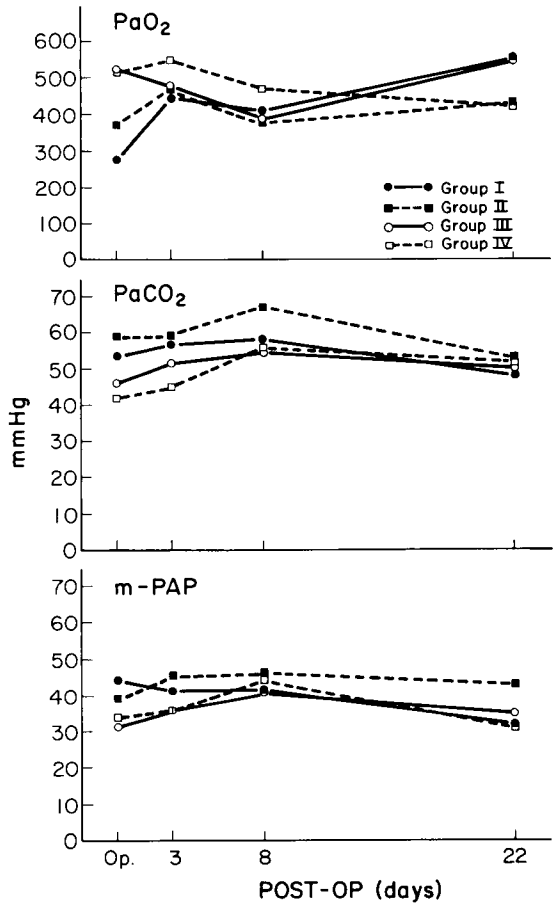


**Fig. 6.** $PaO_2$, $PaCO_2$, and m-PAP during single lung perfusion for 10 minutes. *m-PAP*, Mean pulmonary artery pressure.

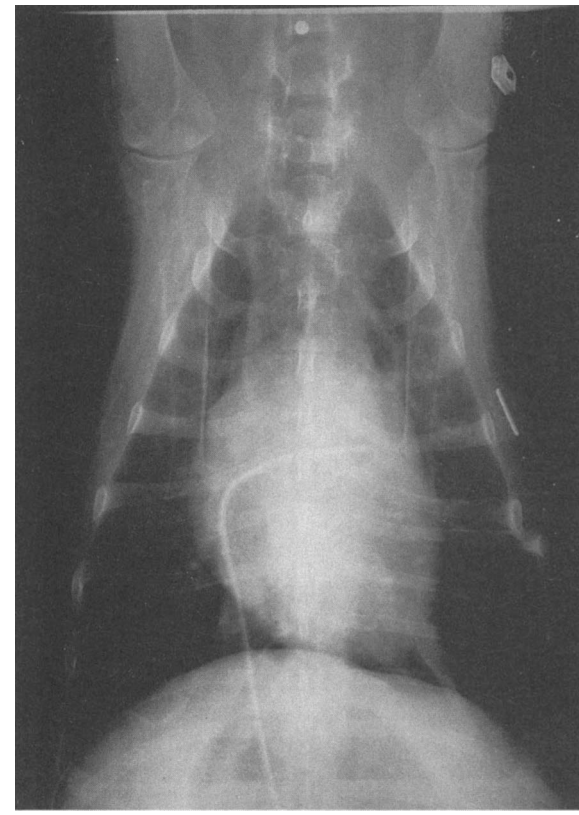

**Fig. 7.** Chest roentgenogram of a recipient (No. 46) 22 days after receiving a transplant of a left lung preserved for 24 hours with LPDG solution and implanted with pulmonary cooling jacket. No significant abnormalities are seen in lungs.

in the LPDG group was 15% higher than in the LPD group ($p < 0.05$).

*Metabolites of glycolysis.* As expected, glucose levels were significantly higher with LPDG than with LPD. The average level for the lungs treated with LPDG was five times that of normal dog blood plasma and 40% of the level in the LPDG solution itself. By 24 hours the tissue concentration had increased 34% ($p < 0.01$). In sharp contrast, the initial glucose level in the LPD lungs was only 25% of the level in normal dog plasma; that level fell 40% ($p < 0.05$) in 4 hours and 90% ($p < 0.001$) in 24 hours to a level of only 0.1 μmol/gm.

The changes in G-6-P reflected the differences in glucose levels. With LPDG, lung G-6-P almost doubled in 4 hours ($p < 0.05$) and tripled in 24 hours. With LPD, G-6-P increased 73% ($p < 0.005$) in 4 hours; however, during the subsequent 20 hours the level fell 80% ($p < 0.001$) to 16% of the corresponding 24-hour level with LPDG.

Lactate levels changed less than those of G-6-P. There were increases of about 40% ($p < 0.05$) by 4 hours with LPD and LPDG and almost no further change with LPDG. With LPD, after 24 hours lactate had fallen to 20% of the level after 4 hours ($p < 0.001$).

*Citric cycle metabolites.* There were no statistically significant changes during preservation in either citrate or malate in the LPDG lungs, but in the LPD lungs citrate level decreased 52% ($p < 0.001$) between 4 and 24 hours and malate level decreased 67% ($p < 0.005$). Thus the lungs preserved in LPD not only ran out of carbohydrate, but this deficiency caused a deficit within the citric acid cycle as well.

## Discussion

Belzer and Southard[6] have reviewed the principles of solid-organ preservation in relation to their investigation

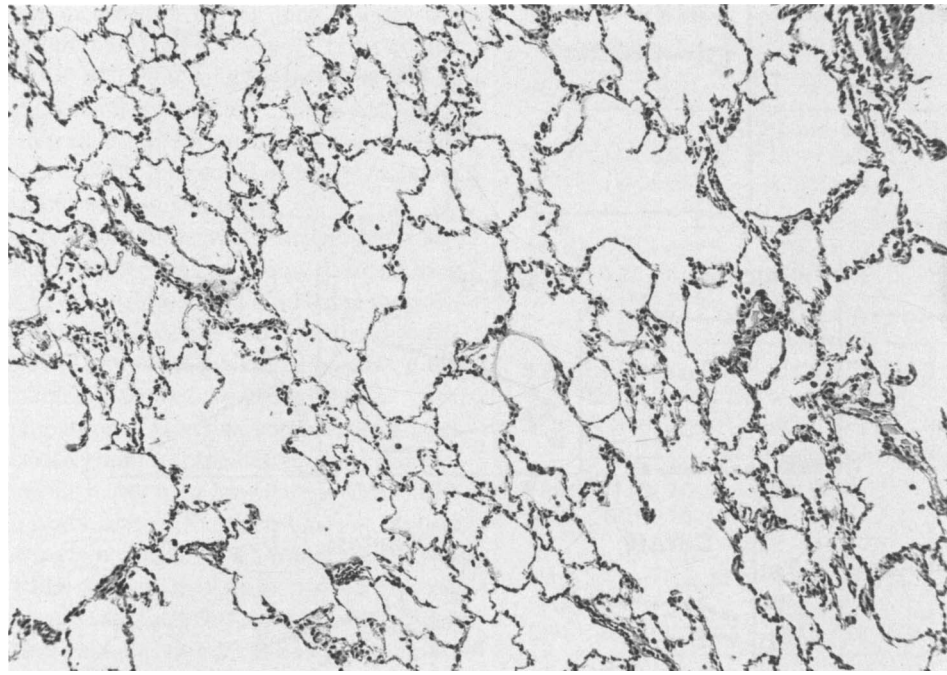

**Fig. 8.** Photomicrograph of lung allograft (No. 54) preserved for 24 hours with LPDG solution and implanted with the pulmonary cooling jacket; virtually normal morphology. Sample taken 22 days after transplantation.

**Table IV.** *Left lung function during right pulmonary artery occlusion for 10 minutes*

| | *Group I* (*n* = 10) | *Group II* (*n* = 6) | *Group III* (*n* = 6) | *Group IV* (*n* = 6) |
|---|---|---|---|---|
| *Immediately after transplantation* | | | | |
| Failure of single lung perfusion | 3/10 | 0/6 | 0/6 | 0/6 |
| $Paco_2$ (mm Hg) | 53.4 ± 5.1 | 59.1 ± 3.1 | 46.1 ± 1.0 | 41.7 ± 2.7 |
| | | | * | |
| $Pao_2$ (mm Hg) | 279 ± 70 | 376 ± 56 | 523 ± 41 | 518 ± 50 |
| | | * | | |
| | | * | | |
| m-PAP (mm Hg) | 44 ± 5 | 39 ± 5 | 31 ± 2 | 34 ± 5 |
| PVR (dyn/sec/cm$^{-5}$) | 1143 ± 439 | 683 ± 56 | 706 ± 108 | 735 ± 111 |
| CO (L/min) | 2.4 ± 0.4 | 3.1 ± 0.4 | 2.5 ± 0.2 | 2.4 ± 0.2 |
| | *Group I* (*n* = 7) | *Group II* (*n* = 4) | *Group III* (*n* = 5) | *Group IV* (*n* = 6) |
| *Three days after transplantation* | | | | |
| Failure of single lung perfusion | 0/7 | 0/4 | 0/5 | 0/6 |
| $Paco_2$ (mm Hg) | 57.1 ± 3.0 | 59.2 ± 2.5 | 51.3 ± 5.4 | 47.7 ± 3.2 |
| $Paco_2$ (mm Hg) | 446 ± 46 | 477 ± 59 | 480 ± 51 | 546 ± 20 |
| m-PAP (mm Hg) | 41 ± 2 | 45 ± 2 | 36 ± 2 | 35 ± 3 |
| PVR (dyn/sec/cm$^{-5}$) | 819 ± 107 | 801 ± 24 | 640 ± 101 | 653 ± 100 |
| CO (L/min) | 3.1 ± 0.5 | 3.4 ± 0.1 | 3.4 ± 0.5 | 2.8 ± 0.2 |

Data of group I were obtained from eight dogs and excluded two dogs in which single lung perfusion failed. *m-PAP*, Mean pulmonary artery pressure; *PVR*, pulmonary vascular resistance; *CO*, cardiac output.

*$p < 0.05$.

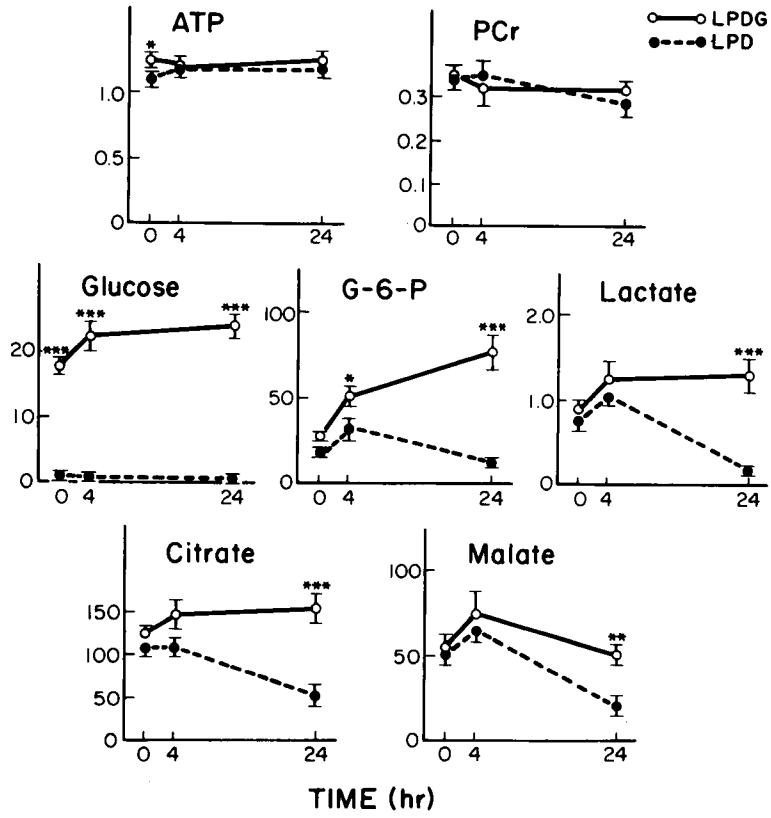


**Fig. 9.** ATP, PCr, glucose, G-6-P, lactate, citrate, and malate concentrations in lung tissue preserved with LPD and LPDG solutions, as a function of time of preservation. *ATP*, Adenosine triphosphate; *PCr*, phosphocreatine; *G-6-P*, glucose-6-phosphate. $*p < 0.05$, $**p < 0.005$, $***p < 0.001$ (versus *LPD*).

of organ-specific metabolism during anaerobic hypothermic ischemia. However, the lung may be able to maintain aerobic metabolism by using the oxygen in the alveoli.[1,2] If so, the strategies for lung preservation should be based on maintenance of aerobic metabolism, which can generate 11 and 17 times more high-energy phosphate than anaerobic metabolism of glycogen or glucose, respectively. With the LPD solution we previously demonstrated reliable and reproducible lung preservation for 12 hours at a preservation temperature of 1° to 4° C.[3] Originally glucose was not included in the LPD solution because it has been demonstrated that glucose is not an effective impermeant for liver or pancreas. It readily enters cells of these organs and is metabolized to lactic acid under anaerobic conditions.[7] Therefore, if the lung were similar, preservation solutions that contained high concentrations of glucose would have no advantage in regard to cell swelling and might produce acidosis. However, after preliminary studies showed that the oxygen-filled lung can maintain aerobic metabolism during preservation, we thought that glucose in the preservation solution might be important as an energy source. On the basis of this possibility, the LPDG solution was developed with the addition of 1% glucose to LPD solution.

Glucose metabolism in the lung has been investigated and reviewed[8] by several investigators. Most of the studies involved normal metabolism under physiologic conditions. With an isolated perfused canine lung model at 37.5° C, Weber and Visscher[9] showed that a lung can use carbohydrates almost exclusively for its energy requirement and that lactate production was not serious even when glucose in the perfusate was increased to three times the normal plasma level. Yeager and Massaro[10] used incubated slices of rabbit lung in Krebs-Ringer-bicarbonate medium at 37° C with a gas phase of 95% oxygen and 5% carbon dioxide. They demonstrated that glucose uptake was 1.83 ± 0.32 mg/gm wet weight per hour and lactic acid production was 0.55 ± 0.06 mg/gm wet weight per hour with 1% medium glucose. They also reported that after a 2-hour incubation with glucose-U-$^{14}$C, 19.0% of the radioactivity that was taken up appeared in carbon dioxide and 29.7% in lactic acid. In

contrast, glucose metabolism during lung preservation has been studied only marginally. Wichert[11] reported that an excised nonflushed rabbit lung preserved at 37° C exhausted its glucose reserves within 2 hours and that 2 hours was the tolerance limit for warm ischemia, according to the concept that the lung could no longer maintain normal metabolism without substrate.

Several questions were addressed by the present study: (1) Can the lung use glucose as an energy source during cold ischemia? (2) Will glucose that is added in the preservation solution be metabolized and contribute to the citric acid cycle? (3) Does the additional glucose in the preservation solution improve lung preservation?

From the comparison of lung function between groups III and I immediately after transplantation, it is clear that LPDG does provide better lung preservation than LPD. The metabolic study showed the importance of glucose metabolism during ischemia at 10° C. When the lung was flushed with LPD solution that contained no glucose, glucose concentration was only 1.0 μmol/gm wet weight immediately after excision; this decreased to 0.6 μmol/gm wet weight by 4 hours and was almost gone by 24 hours. As a result of the glucose depletion, metabolites of glycolysis (G-6-P, lactate) and of the citric acid cycle (citrate, malate) decreased significantly. In contrast, these metabolites did not decrease for 24 hours in the lungs flushed with LPDG, which indicates that the glucose added in the preservation solution was metabolized via the glycolytic pathway and aerobic metabolism through the citric acid cycle.

The actual increase in the tissue glucose level over time, noted in the LPDG group, likely represents diffusion of glucose from the intravascular space and from the immersion solution into the lung parenchyma.

In spite of the reduced levels of metabolites of both the glycolytic pathway and citric acid cycle in the lungs preserved with LPD solution, there was no decrease in ATP on PCr during the 24 hours of preservation. Why, then, did the LPD lungs not perform as well as those preserved with LPDG? At the present time, we cannot answer this question. However, the metabolic analyses were carried out on pieces of lung tissue that contained many different cell populations. It is possible, for example, that vascular endothelium has a different pattern of metabolism than lung parenchyma, and such a difference might not be reflected in the measurements made on the lung biopsy specimens.

We chose 10° C for the preservation temperature; we have previously demonstrated that this is close to the optimal temperature for lung preservation and is superior to preservation at either 4° or 15° C.[2, 12] Our current hypothesis is that optimum lung preservation should take advantage of the oxygen stored in the lung to maintain a low level of glucose metabolism through aerobic metabolism. A preservation temperature that is too low, absence of adequate oxygen in the alveoli, and depletion of adequate glucose levels would then be considered adverse factors.

**Table V.** *Metabolic state in lung tissue during preservation*

| *Group* | *0 hr* | *4 hr* | *24 hr* |
|---|---|---|---|
| | ATP (μmol/gm wet weight) | | |
| LPDG | 1.24 ± 0.05 * | 1.19 ± 0.07 | 1.25 ± 0.07 |
| LPD | 1.09 ± 0.05 * | 1.18 ± 0.04 | 1.18 ± 0.06 |
| | PCr (μmol/gm wet weight) | | |
| LPDG | 0.35 ± 0.03 | 0.32 ± 0.04 | 0.32 ± 0.02 |
| LPD | 0.34 ± 0.02 | 0.35 ± 0.03 | 0.29 ± 0.02 |
| | Glucose (μmol/gm wet weight) | | |
| LPDG | 18.1 ± 1.0 ‡ | 22.7 ± 1.7 ‡ | 24.2 ± 1.5 |
| LPD | 1.0 ± 0.1 * | 0.6 ± 0.1 ‡ | 0.1 ± 0.0 |
| | G-6-P (nmol/gm wet weight) | | |
| LPDG | 27.4 ± 3.0 * | 52.1 ± 4.6 * | 77.6 ± 9.5 ‡ |
| LPD | 19.0 ± 3.6 † | 32.9 ± 5.5 ‡ | 12.1 ± 2.3 |
| | Lactate (μmol/gm wet weight) | | |
| LPDG | 0.91 ± 0.08 * | 1.28 ± 0.17 | 1.33 ± 0.19 ‡ |
| LPD | 0.76 ± 0.09 * | 1.05 ± 0.08 ‡ | 0.21 ± 0.03 |
| | Citrate (nmol/gm wet weight) | | |
| LPDG | 126.5 ± 8.6 | 147.9 ± 18.7 | 156.4 ± 16.4 ‡ |
| LPD | 107.7 ± 8.2 | 108.9 ± 9.0 ‡ | 52.7 ± 13.8 |
| | Malate (nmol/gm wet weight) | | |
| LPDG | 56.5 ± 6.0 | 75.1 ± 13.1 | 51.7 ± 6.0 † |
| LPD | 50.5 ± 6.7 | 65.4 ± 6.9 † | 21.9 ± 5.6 |

*ATP*, Adenosine triphosphate; *PCr*, phosphocreatine; *G-6-P*, glucose-6-phosphate; *LPD*, low-potassium dextran; *LPDG*, low-potassium dextran glucose. Sample numbers are as follows: LPD ($n = 11$); LPDG at 0 and 24 hours ($n = 12$), and at 4 hours ($n = 8$).

*$p < 0.05$.

†$p < 0.005$.

‡$p < 0.001$.

The addition of 1% glucose to the LPD solution increased the osmolarity from 287 mOsm/L to 328 mOsm/L. It is our opinion that this is not the primary benefit of the glucose addition, because the metabolic studies clearly demonstrate that the glucose is actively metabolized. A further benefit of the addition of glucose may be the ability of the lung to synthesize phospholipids from glucose to maintain a sufficient quantity of surfactant,[13] which might be important for optimum lung viability during preservation.

Many investigators have demonstrated that hypother-

mia is an essential component of lung preservation.[12, 14] Ideally, the lung should be maintained at the optimum preservation temperature during the entire ischemic interval between inflow occlusion of the donor and reperfusion of the transplanted lung. We have previously demonstrated that the canine lung temperature fell to 11.5° C after only 1 minute of flushing of the pulmonary artery with cold (4° C) electrolyte solution that was supplemented with topical cold saline solution (1° to 4° C).[2] During implantation, however, the measured lung graft temperature reached 28.8° C with the conventional method of prevention of rewarming in which the lung is wrapped in cold moist gauze that is intermittently soaked with topical cold saline solution or the lung is surrounded with crushed ice. Such a method may retard warming somewhat, but it cannot maintain the optimum preservation temperature. A myocardial cooling jacket was first reported in 1981 by Bonchek and Olinger,[15] and it is now widely used for cardiac surgical procedures. We devised the pulmonary cooling jacket on the same principle. With it, the lung temperature could be regulated accurately, and we were thus able to maintain donor lung temperature at 10° C during virtually the entire ischemic period. With the use of the LPDG flush solution, additional use of the cooling jacket seemed to make little difference; posttransplant function was excellent with or without the jacket. With the LPD solution, however, lungs transplanted with the use of the cooling jacket (group II) performed better than those transplanted without the cooling jacket (group I). In group I, three of ten dogs that underwent transplantation without the cooling jacket showed such severe lung edema on reperfusion that they were unable to tolerate right pulmonary artery occlusion for 10 minutes. In contrast, all six dogs in group II (the group in which the cooling jacket was used) were able to tolerate the 10 minutes of contralateral pulmonary artery occlusion.

Numerous studies of lung preservation have been published and reviewed.[16] With current clinical methods of lung preservation, 6 hours of preservation proves routinely successful, and a period of ischemia of up to 9 hours has been well tolerated.[17] Several investigators have reported preservation for periods ranging from 20 to 48 hours,[18-21] but none of these have been associated with consistent, reliable results, which would be required for clinical application. Although the lung function after reperfusion was not measured, Fujimura[21] reported that dogs could survive after transplantation of the left lung after 48 hours of preservation with $EP_3$ solution. The composition of LPDG is very similar to that of $EP_3$.[21] In our present study, 11 of 12 lungs (92%) preserved with LPDG solution showed excellent gas exchange immediately after transplantation and again at 3 days. With this single lung transplant model, the animal may be maintained on the function of the native lung even if the transplanted lung malfunctions. We considered the initial 3 days to be the most important period for evaluation of the adequacy of preservation because, beyond this time, other factors such as rejection and infection may supervene. Nonetheless, 67% of the lungs preserved with LPDG showed excellent function for 22 days ($PaO_2 > 300$ mm Hg with an inspired oxygen fraction of 1.0, Fig. 5, *B*), and none of them showed lung edema attributable to poor preservation. The consistently excellent lung function achieved with this preservation method is most encouraging.

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