Changes in alveolar oxygen and carbon dioxide concentration and oxygen consumption during lung preservation

The maintenance of aerobic metabolism during lung preservation

The lung is the only organ to which oxygen may be supplied after its blood supply is stopped. Before this study, we were not certain whether lung cells were able to maintain aerobic metabolism with the oxygen in the alveoli during preservation. Excised rabbit lungs were used to measure changes in the concentration of oxygen and carbon dioxide in the airway and changes in glucose, glucose-6-phosphate, lactate, adenosine triphosphate, and phosphocreatine levels in the lung tissue during preservation under different conditions. Twenty-seven lungs were flushed with low-potassium dextran electrolyte solution, inflated with room air, and preserved at 1° C ($n = 8$), 10° C ($n = 8$), or 22° C ($n = 11$) for 4, 12, or 24 hours. Eight additional lungs were inflated with 100 % nitrogen and preserved at 10° C for 4 ($n = 4$) or 24 ($n = 4$) hours. Oxygen levels decreased and carbon dioxide levels increased in the airway of the lungs that were inflated with room air at rates dependent on the preservation temperature. The increase of carbon dioxide in the lungs that were inflated with 100% nitrogen was very small. When the oxygen was not available in the alveoli, lactate accumulated, and adenosine triphosphate and phosphocreatine decreased in the lung tissue. We concluded that lung cells are able to maintain aerobic metabolism with the oxygen in the alveoli during preservation and that the maintenance of aerobic metabolism may be essential to maintain the optimum viability of preserved lung tissue. (J THORAC CARDIOVASC SURG 1993;105:492-501)

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Much more difficulty has been associated with the preservation of the lung than with the preservation of solid organs in vitro because of the lung's unique structure, especially the close apposition of blood and air compartments in the alveoli. However, the lung cells may be able to maintain aerobic metabolism by using the oxygen in the alveoli during preservation, and this could be a key to successful lung preservation. With a paracorporeal circulation rabbit model, we demonstrated that lungs that are preserved with nitrogen inflation for 24 hours at 10° C rapidly become edematous on reperfusion, whereas preservation with 100% oxygen inflation appears superior to inflation with room air during the preservation period.[1]

We undertook the present experiments, therefore, to study the change in oxygen and carbon dioxide concentrations in the airway and the changes in metabolites and the energy state in preserved lung tissue at three different preservation temperatures (1° C, 10° C, and 22° C) and with different preservation gases (room air and 100% nitrogen). Adenosine triphosphate (ATP) and phosphocreatine (PCr) levels were used to monitor the chang-

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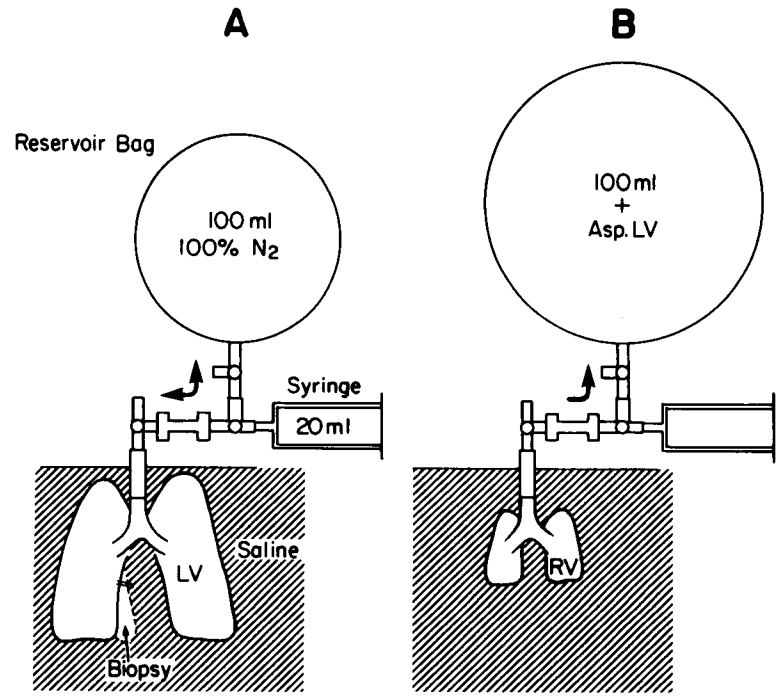

Fig. 1. A, Inflated lung connected to a reservoir bag containing 100 ml of 100% nitrogen. Gas in lung and gas in reservoir bag were mixed to equilibrate gas concentration. Twenty milliliters was removed from the lung with the syringe and placed in the reservoir bag. Twenty milliliters was then removed from the reservoir bag and returned to the lung. This process was repeated 10 times. **B,** The aspiration LV was measured after deflation of the lung with the syringe and placement of all the aspirated gas in the reservoir bag.

Table I. *Study groups*

Groups	*Inflation gas*	*Solution*	*Temperature (°C)*	*Ischemic period (hr)*
I	RA	LPD	1	4 ($n = 4$) and 24 ($n = 4$)
II	RA	LPD	10	4 ($n = 4$) and 24 ($n = 4$)
III	RA	LPD	22	4 ($n = 4$), 12 ($n = 3$), and 24 ($n = 4$)
IV	100% N_2	LPD	10	4 ($n = 4$) and 24 ($n = 4$)

RA, Room air; N_2, nitrogen; *LPD*, low-potassium dextran solution.

ing energy status of the tissue. Glucose, glucose-6-phosphate (G-6-P), and lactate were used to assess the persistence of carbohydrate metabolism during the aerobic and anaerobic preservation periods.

Materials and methods

Lung preparation. Forty-four New Zealand white rabbits that weighed from 2.7 to 5.0 kg and were free of respiratory infections were used for the study. The rabbits were premedicated with subcutaneous atropine sulfate (0.25 mg/kg), ketamine (35 mg/kg), and acepromazine maleate (0.6 mg/kg) and anesthetized with intravenous thiopental sodium (25 mg/kg). Heparin (700 IU/kg) was administered intravenously at the same time. An endotracheal tube was introduced through a cervical tracheostomy, and the animals' lungs were ventilated with room air with a tidal volume of 35 ml, a positive end-expiratory pressure of 0.5 cm H_2O, and a respiratory rate of 25 breaths/min, with the use of a volume-cycled respirator (model 671; Harvard Apparatus, Inc., Natick, Mass.). After median sternotomy, both superior venae cavae and the sole inferior vena cava were isolated and the pericardium was opened. The ascending aorta and the main pulmonary artery were also isolated. A 14-gauge cannula was introduced into the main pulmonary artery through the right ventricular outflow tract for pulmonary flushing. Cardiac inflow occlusion was accomplished by ligation of the venae cavae, and the pulmonary artery was ligated around the cannula. The left atrial appendage was amputated, and the pulmonary artery was flushed with low-potassium dextran (LPD) electrolyte solution[2] (200 ml, 10° C) from a bag hung 60 cm above the chest. After completion of the flush, the heart-lung block was excised with utmost care to prevent lung injury from atelectasis, disruption of lung surface, or any compression or manipulation of lung tissue. The lungs were continuously ventilated during this procedure. A tissue biopsy

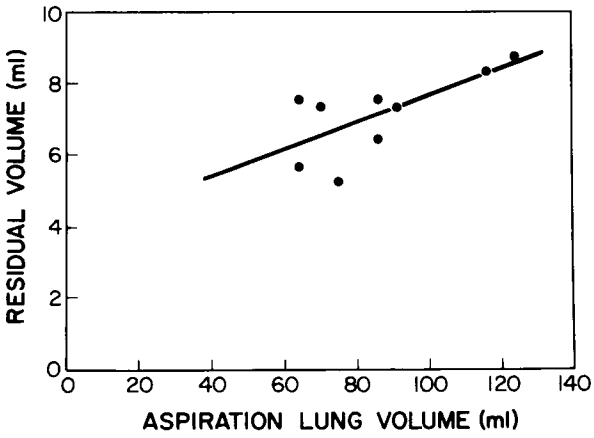

Fig. 2. Relationship between aspiration LV and RV. A positive correlation was demonstrated between the RV and the aspiration LV ($Y = 0.038X + 3.89$; $r = 0.70$, $p < 0.05$).

specimen (0.1 to 0.2 gm) was removed from the cardiac lobe distal to a ligature for metabolic study, and the lungs were inflated under water to confirm that no air leak existed.

Study groups. We studied four different groups (Table I). Lungs were inflated with room air and preserved at 1° C (group I), 10° C (group II), and 22° C (group III). Group IV lungs were inflated with 100% nitrogen and preserved at 10° C. Lungs were preserved for 4 and 24 hours in groups I, II, and IV and for 4, 12, and 24 hours in group III. In group IV, ventilation with 100% nitrogen was started after cardiac inflow occlusion.

Measurement of gas concentrations. The oxygen and carbon dioxide concentrations in the lung before and after preservation were measured. This measurement procedure was designed to transfer as much of the lung gas as possible into an attached reservoir of nitrogen for subsequent gas analysis. The reason for measuring the gas concentrations in the lung before preservation was to determine the accuracy of our method.

The tracheal tube was clamped, and the lung was inflated at end-tidal volume, completely immersed in saline solution, and covered with a plastic sheet. The temperature of the saline solution was the same as the preservation temperature. The tracheal tube was connected to an air-tight plastic reservoir bag that contained 100 ml of 100% nitrogen, and an empty 20 ml syringe was placed in the connection line via a stopcock (Fig. 1). The reservoir bag was used to minimize the effect of residual volume (RV) for the subsequent calculation of oxygen consumption ($\dot{Q}O_2$). We aspirated 20 ml of gas from the lung to the syringe and injected it into the reservoir bag by changing the direction of the stopcock in the connection line. We returned 20 ml of gas from the reservoir bag to the lung with the syringe in the same way. This pumping of gas back and forth between the lung and the reservoir was repeated 10 times to equilibrate the gas concentration. The lung was then deflated by aspiration of the air in the lung as much as possible, and all the gas was collected in the reservoir bag. The volume of gas aspirated from the lung (aspiration lung volume) was recorded. From this reservoir bag, oxygen concentration was determined with a zirconia solid electrolyte oxygen analyzer[3] and carbon dioxide concentration with an infrared carbon dioxide analyzer[4] (medical gas analyzer MG-360; Minato Medical Science Co., Ltd, Osaka, Japan).

Oxygen concentration in the lung was calculated as follows:

$$\text{Total } O_2 \text{ in the lung} = LV \times \%O_2 = (LV + 100) \times \%O_2(R) \ldots (1)$$

in which LV is lung volume, $\%O_2(R)$ is oxygen concentration in the reservoir bag measured by the gas analyzer.

$$LV = \text{Asp. } LV + RV \ldots (2)$$

in which Asp. LV is aspiration lung volume (volume of gas aspirated from the lung) and RV is residual volume. From (1) and (2)

$$\%O_2 = (\text{Asp. } LV + RV + 100) \times \%O_2(R) / (\text{Asp. } LV + RV)$$

Carbon dioxide concentration was calculated in the same way.

To estimate the RV of the rabbit lung, the relationship between aspiration LV and RV was measured in nine rabbits. The lungs of these rabbits were ventilated with 100% oxygen during the preparation for excision as described. The tracheal tube was then clamped to allow the animal's own circulation to remove residual lung oxygen until the lung became completely atelectatic. The heart-lung block was excised, and the heart, esophagus, and all mediastinal fatty tissue were removed. The degassed lung was completely immersed in a graduated cylinder, which was filled with saline solution, and the volume of the degassed lung was measured by the displacement of saline solution. The degassed lung was then ventilated again with room air and with the same settings on the respirator for 1 minute. The tracheal tube was clamped, the lung was inflated at end-tidal volume, and the aspiration LV was measured after deflation of the lung with a 20 ml syringe. For measurement of the volume of the deflated lung, the lungs were immersed in saline solution and the displacement volume was recorded. The right and left main bronchi close to the lung parenchyma were divided and filled with saline solution for measurement of the volume of the dead space, which included the main bronchi, the trachea, the tracheal tube, and the connection line. The RV was calculated as follows:

$$RV = \text{Deflated } LV - \text{Degassed } LV + \text{Dead space}$$

The RV correlated well with the aspiration LV as shown in Fig. 2 ($Y = 0.038X + 3.89$; $r = 0.70$, $p < 0.05$). Therefore the RV could be estimated from the aspiration LV measured before preservation.

After measurement of initial gas concentrations, the lung was ventilated again with room air (groups I, II, and III) or with 100% nitrogen (group IV) for 1 minute, after which the tracheal tube was clamped and the lungs were inflated at end-tidal volume. The end of the tracheal tube was closed with a stopcock, and the lung was placed in a plastic bag that contained 100 ml of LPD solution. The plastic bag was tightly closed without air and immersed in saline solution covered with another plastic sheet. Flushing, immersion, and saline solution were exposed to the same gas that was used for inflation of the lung for at least 2 hours before the study. In group IV, the lung was preserved in a plastic storage bag with the preservation solution, and this bag was enclosed in two other airtight plastic bags that were filled with 100% nitrogen.

After preservation, the same measurement of gas concentrations from the lung was performed. Then both lungs were quickly separated from the heart-lung block and weighed. A biopsy specimen was then removed from the left lower lobe for metabolic study.

Table II. *Oxygen and carbon dioxide concentrations and oxygen consumption in groups I, II, and III*

Group	*0 hr*	*4 hr*	*12 hr*	*24 hr*
Oxygen concentration				
Group I (1° C)	21.9 ± 0.1	20.6 ± 0.2		14.7 ± 0.5
Group II (10° C)	21.5 ± 0.2	17.5 ± 0.4		5.9 ± 1.2
Group III (22° C)	21.3 ± 0.2	12.6 ± 1.1	0.9 ± 0.9	0.2 ± 0.1
Carbon dioxide concentration				
Group I (1° C)	0.08 ± 0.01	0.39 ± 0.06		0.98 ± 0.09
Group II (10° C)	0.08 ± 0.01	1.37 ± 0.13		2.47 ± 0.21
Group III (22° C)	0.14 ± 0.01	2.55 ± 0.08	3.71 ± 0.16	3.21 ± 0.25
Oxygen consumption (ml/gm wet weight per hour)				
Group I (1° C)		0.022 ± 0.004		0.019 ± 0.002
Group II (10° C)		0.060 ± 0.004		0.042 ± 0.003
Group III (22° C)		0.162 ± 0.017	0.108 ± 0.011	0.051 ± 0.005

*$p < 0.05$.
‡$p < 0.005$.
§$p < 0.001$.

We calculated oxygen consumption with the knowledge of the initial and final concentrations of oxygen, carbon dioxide, nitrogen, and the final lung volume and based on the assumption that the volume of N_2 was stable during the preservation period.

Metabolic study. Tissue samples for metabolic study were quick-frozen immediately by immersion in dichlorodifluoromethane (CCl_2F_2), which was precooled to the freezing point in liquid nitrogen and stored at −70° C or below until analyzed. The metabolic study included measurement of ATP, PCr, glucose, G-6-P, and lactate. Samples weighing 0.1 gm were extracted in perchloric acid as described previously.[5] After centrifugation and neutralization, the supernatant was analyzed 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.

The results are presented as the mean ± standard error of the mean. Statistical analysis was performed with analysis of variance and the Tukey honest significant difference multiple comparisons procedure. Statistical significance was accepted at the 95% confidence level, $p < 0.05$.

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

Gas concentrations and oxygen consumption. Oxygen concentration in the lung that was inflated with room air decreased during preservation at rates dependent on the temperature (Table II, Fig. 3). The oxygen consumption per hour over the first 4 hours was 0.022, 0.060, and 0.162 ml/gm wet weight per hour at 1°, 10°, and 22° C, respectively (Table II). This represents a 12% per degree increase between 1° C and 10° C, and an 11% per degree increase between 10° C and 22° C. The decrease in oxygen concentration in the lung was nearly linear over a 24-hour period at 1° C and 10° C but fell off sharply at 22° C because 96% of the oxygen had been used up in the first 12 hours (Fig. 3). Carbon dioxide concentration in the lung that was inflated with room air increased at rates that were also dependent on temperature but the volume ratio of carbon dioxide increase to oxygen decrease (RQ) varied from 0.12 to 0.32. This undoubtedly indicates a large loss of carbon dioxide from the lung surface since the RQ for oxidation of glucose to carbon dioxide is 1.0 and for fatty acid to carbon dioxide is about 0.7. The carbon dioxide production at 22° C ceased at 12 hours when the lungs had essentially run out of oxygen.

Oxygen concentration in the lung that was inflated with 100% nitrogen was only 0.9% before preservation, 0.6% after 4 hours, and 0.1% after 24 hours (Table III, Fig. 4). The increase in carbon dioxide in the lung that was inflated with 100% nitrogen was very small compared with that in the lung inflated with room air.

Metabolic study. ATP levels in lungs that were inflated with room air were stable for 24 hours at 10° C, fell 14% at 1° C, but at 22° C decreased 49% by 12 hours and 92% by 24 hours (Table IV, Fig. 5). With nitrogen at 10°

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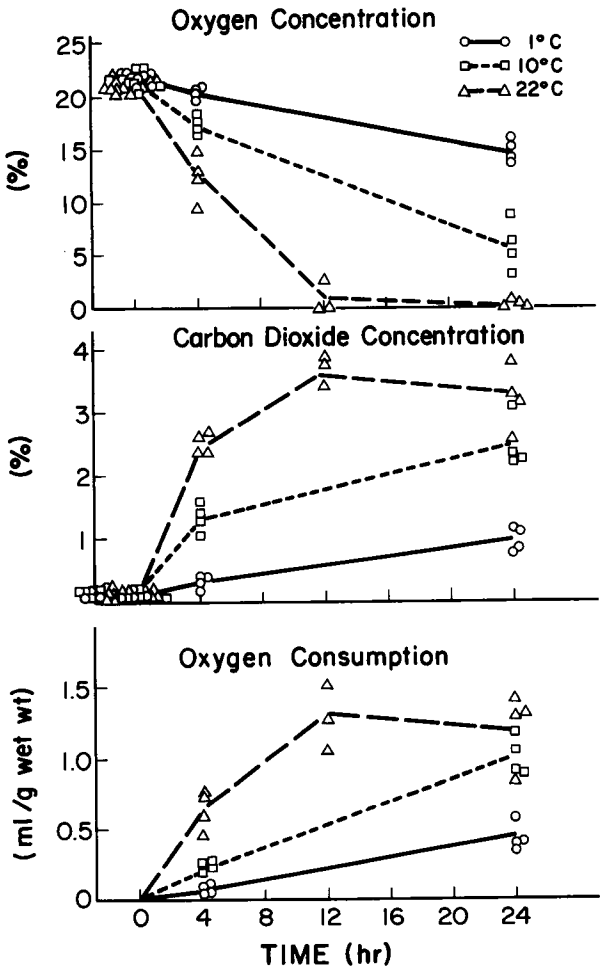

Fig. 3. Oxygen concentration and carbon dioxide concentration in the lungs and oxygen consumption of preserved lung cells, as a function of time, in group I (1° C), group II (10° C), and group III (22° C). Each *point* represents one experiment.

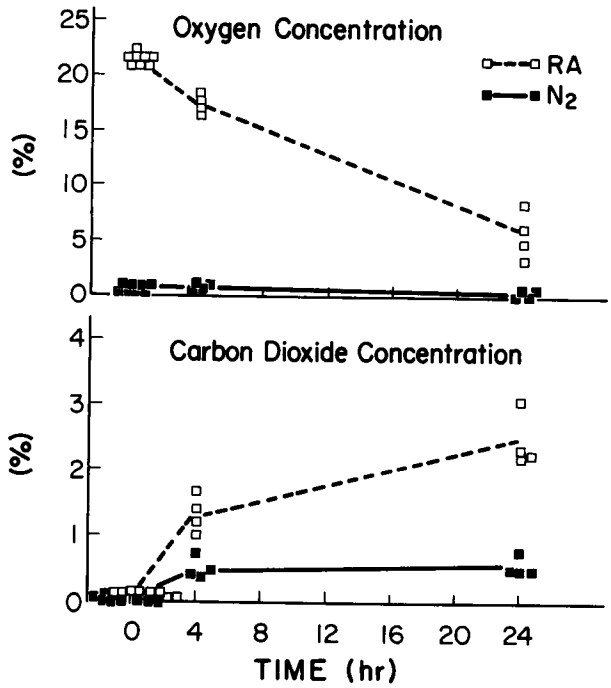

Fig. 4. Oxygen concentration and carbon dioxide concentration in the airway, as a function of time, in group II (*RA*, room air) and group IV (N_2, 100% nitrogen). Each *point* represents one experiment.

C, ATP decreased 60% in 4 hours and about 90% in 24 hours (Table V, Fig. 6).

Changes in PCr levels under room air were similar to those of ATP but somewhat greater. At 10° C the level remained constant for 24 hours. At 1° C the level declined by 32% at 24 hours, at 22° C PCr level fell 70% and 90% by 12 hours and 24 hours, respectively (Table IV, Fig. 5). With preservation by means of nitrogen inflation at 10° C, PCr was down to 28% of the initial (t_0) level by 4 hours and 11% by 24 hours (Table V, Fig. 6).

The glucose level decreased rapidly during the initial 4 hours at all temperatures under room air as well as under nitrogen; under room air, however, the level of glucose that remained at 4 hours and 24 hours was about twice as great at 1° C as at 10° C, and three times as great as at 22° C (Table IV, Fig. 6).

G-6-P increased progressively with time at 1° C and reached 228% of t_0 by 24 hours (Table IV, Fig. 5). At 10° C under room air, it increased 72% during the initial 4 hours and then decreased to about half the initial value by 24 hours. At 22° C the changes were the opposite of those at 1° C. It fell to 32% of the t_0 value in 4 hours and to 8% of t_0 at 24 hours. At 10° C under nitrogen, G-6-P rose much less than under room air during the initial 4 hours, with no further change during the next 20 hours. However, the data were too variable to analyze. (Table V, Fig. 6).

Lactate levels also changed differently at each temperature under room air (Table IV, Fig. 5). At 1° C it increased 54% during the initial 4 hours and another 38% during the next 20 hours of preservation. At 10° C it increased about the same percentage as at 1° C for the first 4 hours, but then decreased sharply to 47% of the t_0 level by 24 hours. At 22° C lactate decreased 75% during the initial 4 hours and then increased when the oxygen was gone, to 178% of t_0 at 12 hours and 248% of t_0 at 24 hours. At 10° C under nitrogen lactate rose to very high levels, 256% of t_0 by 4 hours and 368% of t_0 by 24 hours (Table V, Fig. 6).

Under nitrogen most of glycosyl units from either glycogen or preformed glucose would be expected to end up

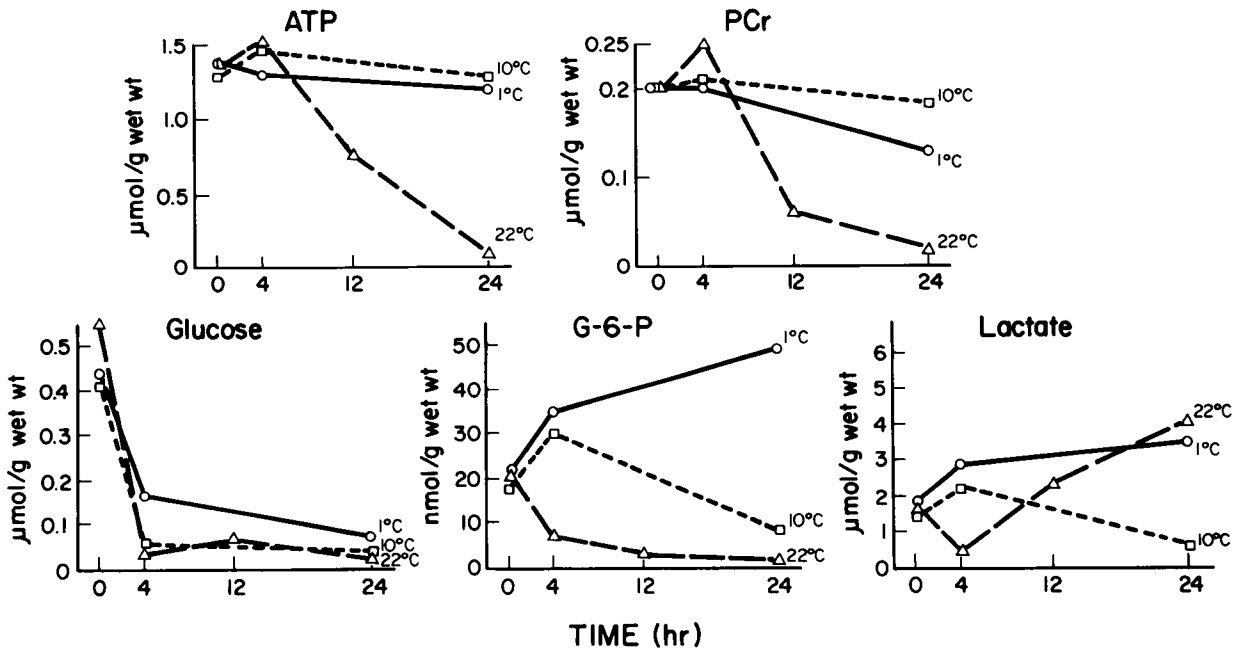

Fig. 5. ATP, PCr, glucose, G-6-P and lactate concentrations in preserved lung tissues, as a function of time, in group I (1° C), group II (10° C), and group III (22° C). The results are averages of all experiments. *ATP*, Adenosine triphosphate; *PCr*, phosphocreatine; *G-6-P*, glucose-6-phosphate.

as lactate. The sum of glucose plus half the lactate in group IV is 1.42, 2.50, and 3.57 µmol/gm wet weight at 0, 4, and 24 hours (Table V). The increase over the 24 hours, 2.15 µmol/gm wet weight, must have come mainly from glycogen. Whether any glycogen remained at 24 hours will have to be determined by a future study.

Discussion

The techniques for organ preservation generally employ vascular flushing with cold solution and static immersion in ice-cold solution surrounded by crushed ice. Because the oxygen supply is interrupted in solid organs during this preservation period, mitochondrial respiration and ATP synthesis, except from glycolysis, are necessarily arrested. As a result of anaerobic metabolism, solid organs lose ATP fairly rapidly even during cold ischemia.[6-8] With the use of phosphorus 31–nuclear magnetic resonance spectroscopy, however, we recently demonstrated that canine lungs that are flushed with LPD solution, inflated with 100% oxygen, and preserved at 4° C or 10° C maintained ATP levels for 18 hours.[9] We also found that preservation with 100% oxygen inflation appeared superior to inflation with room air and much superior to inflation with 100% nitrogen, as seen with a paracorporeal circulation rabbit model.[1] These results suggested that lung cells might be able to maintain aerobic metabolism with the oxygen in the alveoli. The present experiments were undertaken to further explore this hypothesis.

Table III. *Oxygen and carbon dioxide concentrations in groups II and IV*

Group	*0 hr*		*4 hr*		*24 hr*	
Oxygen concentration						
Group II (RA)	21.5 ± 0.2	‡	17.5 ± 0.4	‡	5.9 ± 1.2	‡
Group IV (100% N_2)	0.9 ± 0.1		0.6 ± 0.1		0.1 ± 0.1	
Carbon dioxide concentration						
Group II (RA)	0.08 ± 0.01	†	1.37 ± 0.13	‡	2.47 ± 0.21	‡
Group IV (100% N_2)	0.02 ± 0.01		0.54 ± 0.07		0.60 ± 0.05	

RA, Room air; N_2, nitrogen.
‡$p < 0.005$.
§$p < 0.001$.

The oxygen consumption of the lung has been measured by several investigators. Weber and Visscher[10] used isolated perfused canine lungs, Koga[11] used isolated perfused rabbit lungs, and Krebs[12] used sliced canine lungs. The purpose of these studies was to measure the oxygen consumption of the lung under physiologic conditions. Oxygen consumption of isolated nonperfused lung has been reported only by Faridy and Naimark.[13] They placed a nonflushed, room air–inflated canine lobe in an airtight bag for 3 hours at 37° C; they mixed the gas in

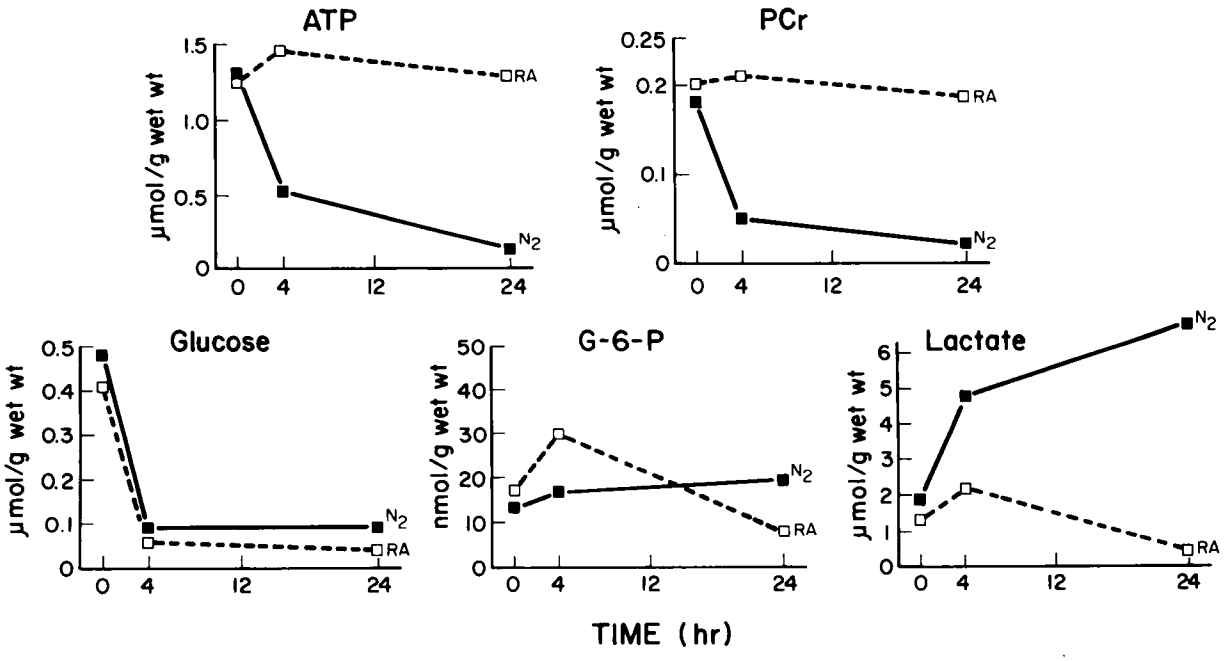

Fig. 6. ATP, PCr, glucose, G-6-P and lactate concentrations in preserved lung tissues, as a function of time, in group II (*RA*, room air) and group IV (N_2, 100% nitrogen). The results are averages of all experiments. *ATP*, Adenosine triphosphate; *PCr*, phosphocreatine; *G6P*, glucose-6-phosphate.

Table IV. *Metabolic state in lung tissue during preservation in groups I, II, and III*

Group	*0 hr*		*4 hr*		*12 hr*		*24 hr*	
ATP (μmol/gm wet weight)								
Group I (1° C)	1.38 ± 0.06		1.30 ± 0.13				1.19 ± 0.04	
Group II (10° C)	1.27 ± 0.11		1.46 ± 0.12			§	1.28 ± 0.04	§
Group III (22° C)	1.38 ± 0.04		1.53 ± 0.07		0.75 ± 0.33		0.11 ± 0.05	
PCr (μmol/gm wet weight)								
Group I (1° C)	0.20 ± 0.01		0.20 ± 0.03				0.13 ± 0.01	
Group II (10° C)	0.20 ± 0.01		0.21 ± 0.01			§	0.19 ± 0.01	‡
Group III (22° C)	0.02 ± 0.01		0.25 ± 0.05		0.06 ± 0.05		0.02 ± 0.01	§
Glucose (μmol/gm wet weight)								
Group I (1° C)	0.43 ± 0.05		0.16 ± 0.04	*			0.07 ± 0.01	
Group II (10° C)	0.41 ± 0.04	*	0.06 ± 0.01				0.04 ± 0.00	
Group III (22° C)	0.55 ± 0.04		0.04 ± 0.01		0.07 ± 0.04		0.02 ± 0.00	
G-6-P (nmol/gm wet weight)								
Group I (1° C)	21.4 ± 4.0		34.7 ± 6.1				48.8 ± 3.9	‡
Group II (10° C)	17.4 ± 1.9		29.9 ± 9.3				8.1 ± 1.5	
Group III (22° C)	20.9 ± 1.2		6.8 ± 1.3		2.9 ± 1.1	§	1.6 ± 0.7	
Lactate (μmol/gm wet weight)								
Group I (1° C)	1.83 ± 0.13		2.82 ± 0.21				3.51 ± 0.44	‡
Group II (10° C)	1.37 ± 0.17	†	2.20 ± 0.12				0.53 ± 0.11	
Group III (22° C)	1.63 ± 0.10		0.41 ± 0.06		2.91 ± 1.81		4.05 ± 0.38	§

ATP, adenosine triphosphate; *PCr*, phosphocreatine; *G-6-P*, glucose-6-phosphate.

*$p < 0.05$.

†$p < 0.01$.

‡$p < 0.005$.

§$p < 0.001$.

Table V. *Metabolic state in lung tissue during preservation in groups II and IV*

Group	*0 hr*	*4 hr*	*24 hr*
ATP (μmol/gm wet weight)			
Group II (RA)	1.27 ± 0.11	1.46 ± 0.12	1.28 ± 0.04
Group IV (100% N_2)	1.31 ± 0.07	0.53 ± 0.10 §	0.14 ± 0.04 §
PCr (μmol/gm wet weight)			
Group II (RA)	0.20 ± 0.01	0.21 ± 0.01	0.19 ± 0.01
Group IV (100% N_2)	0.18 ± 0.02	0.05 ± 0.01 †	0.02 ± 0.01 §
Glucose (μmol/gm wet weight)			
Group II (RA)	0.41 ± 0.04	0.06 ± 0.01	0.04 ± 0.00
Group IV (100% N_2)	0.48 ± 0.07	0.08 ± 0.02	0.09 ± 0.03
G-6-P (nmol/gm wet weight)			
Group II (RA)	17.4 ± 1.9	29.9 ± 9.32	8.1 ± 1.5
Group IV (100% N_2)	13.2 ± 1.6	17.3 ± 8.31	19.5 ± 10.0
Lactate (μmol/gm wet weight)			
Group II (RA)	1.37 ± 0.17 *	2.20 ± 0.12	0.53 ± 0.11
Group IV (100% N_2)	1.89 ± 0.18	4.85 ± 0.82 ‡	6.96 ± 0.84 §

ATP, adenosine triphosphate; *PCr*, phosphocreatine; *G-6-P*, glucose-6-phosphate; *RA*, room air, N_2, nitrogen.
*$p < 0.05$.
†$p < 0.01$.
‡$p < 0.005$.
§$p < 0.001$.

the lobe and the gas in the bag after 3 hours and analyzed oxygen and carbon dioxide concentrations. They calculated oxygen consumption and carbon dioxide production with the knowledge of the initial and final concentrations of oxygen, carbon dioxide, nitrogen and the volume change. They demonstrated that the isolated nonperfused lung in this condition consumed oxygen in the alveoli and produced carbon dioxide, and that the oxygen consumption was directly dependent on the degree of inflation.

We flushed rabbit lungs with cold electrolyte solution and preserved them by complete immersion in preservation solution as is currently done for preservation of human lungs. We developed the described technique to measure the changes in oxygen and carbon dioxide concentration in the airway and oxygen consumption in this condition. The initial gas concentration before preservation was measured to demonstrate the accuracy of this technique. Inflation with room air was used instead of inflation with 100% oxygen because it made the change in oxygen concentration easier to detect. The reservoir bag that contained 100 ml of 100% nitrogen was used to increase the total volume of gas in the closed lung-reservoir system and to thus minimize the effect of RV on the calculation of oxygen consumption. The RV was estimated by its relationship to the aspiration LV, as measured in the separate experiments with degassed lungs. This estimation would not decrease the accuracy of the measurement of gas concentrations, because an error in the estimate of RV (which ranges only between 5.3 and 8.8 ml total) makes a trivial difference in the numerator (100 + Asp. LV + RV) and a minor difference in the determination of gas concentrations. Despite potential sources of error in this technique, the analysis of oxygen and carbon dioxide concentrations in both initial and final air samples and the values for oxygen consumption were reproducible in each condition. Because some carbon dioxide diffused out through the surface of the lung into the preservation solution,[10] the true rate of carbon dioxide production could not be measured in our study. Nevertheless, it is clearly shown that when the lungs are inflated with room air, the cells are able to use oxygen and produce carbon dioxide; This indicates an active aerobic metabolism. In contrast, when the lungs were inflated with 100% nitrogen the increase in carbon dioxide was very small. Because only anaerobic metabolism could take place in the lung that was inflated with nitrogen, ATP and PCr decreased, and lactate accumulated after only 4 hours of preservation. By contrast, aerobic metabolism took place in the lung that was inflated with room air, and ATP and PCr levels were stable; lactate did not accumulate even after 24-hour preservation at 10° C. These results demonstrated that the oxygen in the alveoli permits continuous metabolism and may be a key component in maintaining the viability of preserved lung tissue.

The three preservation temperatures were chosen for

the following reasons: 1° C is the temperature currently used for human lung preservation. We recently proved with an ex vivo rabbit model[14] and a canine allotransplantation procedure[9] that the optimal preservation temperature is in the vicinity of 10° C; it is well known that 22° C is too warm to preserve the lung,[14-15] but this group was included, for the sake of completeness, to evaluate lung metabolism over a wide range of temperatures and hence over a wide range of metabolic rates.

Oxygen consumption is ordinarily a good measure of metabolic rate. The present oxygen consumption data, therefore, can be used to assess this rate at the different preservation temperatures. Weber and Visscher[10] observed oxygen consumption in perfused dog lung at 37.5° C equal to about 0.58 ml/gm wet weight per hour. On the basis of this, during the first 4 hours of preservation the metabolic rates of rabbit lungs that were inflated with room air at 1°, 10°, and 22° C were 4%, 10%, and 28%, respectively, of the metabolic rate of the perfused dog lung at 37.5° C. In their study, Weber and Visscher[10] observed an RQ value of 1.0 for dog lung, which indicated an essentially exclusive use of glucose as an energy source. The observed oxygen consumption during the first 4 hours would be sufficient to convert glucose to carbon dioxide at 1°, 10°, and 22° C at rates of 0.17, 0.45, and 1.21 μmol/gm wet weight per hour, respectively. In the present study, because we used a glucose-free preservation medium, the source of the glucose or glycosyl units would have to be glucose and glycogen from the lung itself. We found an average of only 0.46 μmol/gm of glucose per gram of wet weight in the flushed rabbit lungs, and Weber and Visscher[10] reported glycogen equivalent to 9.5 μmol/gm wet weight. Assuming this is about the same for rabbit lung, the total endogenous glycosyl units (10 μmol/gm wet weight) would be sufficient to support the lung metabolism for 60, 22, and 8 hours at 1°, 10°, and 22° C, respectively, if the rate of metabolism remained at the same level as was observed during the initial 4 hours. Additional glucose in the preservation solution may prolong this period if the glucose in the solution is metabolized during preservation.

A significantly different metabolic behavior between 1° C and 10° C was noticed. The fact that the level of PCr falls by 24 hours at 1° C but not at 10° C indicates that at 1° C ATP generation, a function primarily of mitochondrial enzymes, has not kept up with ATP consumption. The fact that the level of lactate at 24 hours is much higher in lungs preserved at 1° C than in those preserved at 10° C indicates that sluggishness of glycolysis or glycogenolysis is not at fault. A tentative hypothesis, therefore, is that some mitochondrial enzyme (or enzymes) of the citric acid cycle or electron transport system has a higher temperature coefficient than the processes that use ATP. Further study is needed to explore this area.

Lungs preserved at 22° C used up the oxygen after 12 hours of preservation, at which time anaerobic metabolism set in. As a result, metabolites thereafter changed in the same manner observed for lungs that were inflated with 100% nitrogen. Levels of ATP and PCr were drastically decreased, and lactate accumulated to very high levels.

The lung has proved to be more difficult to preserve than have solid organs, because of its delicate structure and the rapid edema that can develop because of the close apposition of the capillaries and the alveoli. However, the lung is the only organ that can be preserved with a ready supply of oxygen. We believe, therefore, that maintenance of aerobic metabolism, through use of the oxygen in the alveoli, may be a key to prolonged lung preservation.

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