
Low Volume Isolated *IN SITU* Lung Perfusion: Results of Studies of Metabolic Stability

Annette Basile-Borgia, D. Eugene Rannels and Dennis R. Williams

The Milton S. Hershey Medical Center
The Pennsylvania State University
Hershey, Pennsylvania

Abstract

Non-ventilatory lung functions involve uptake and release of compounds across the pulmonary vascular bed. Alterations in these functions may be readily assessed in an isolated, ventilated, small mammal lung perfusion preparation. A previously documented preparation is known to maintain metabolic stability of *in situ* rat lungs for at least 240 minutes. That model, developed by Watkins and Rannels, requires a rotating drum oxygenator which necessitates the use of 100 ml of perfusate buffer. The effects of 100 ml dilution limit the ability to measure uptake and release of metabolites because increased sensitivity of assay procedures or more prolonged experiments are required. A recently developed preparation requires only 25 ml of recirculating perfusate, clearly reducing substrate and metabolite dilution, and enhancing the sensitivity for detection of changes in pulmonary metabolism.

The intent of this study was to test the vascular permeability and metabolic stability of this low volume lung perfusion model. The low volume, *in situ* lung perfusion model remained stable for as long as 3 hours. Metabolic stability of the model was demonstrated by measurements of water balance, glucose uptake, lactate production, and protein synthesis. The clinical relevance of isolated lung perfusion is discussed with specific reference to metabolic functions of the lung.

Introduction

Lung tissue has both ventilatory and non-ventilatory functions. The well known "ventilatory" functions generally refer to exchange of oxygen and carbon dioxide content across the alveolar wall. The lesser

known "non-ventilatory" functions include drug metabolism, surfactant production, and active uptake and/or transformations of bioactive substances, such as angiotension I, norepinephrine, serotonin and prostaglandins.^{1,2} In an effort to further define these metabolic functions, investigators have developed isolated lung perfusion preparations.

A recently developed low volume, isolated lung perfusion model proved to exchange gas efficiently for at least 3 hours. The model provides the opportunity to investigate both ventilatory and metabolic integrity of the pulmonary tissue in a setting independent of the contribution of other organs. Furthermore, the low priming volume used in this model would facilitate studies designed to measure uptake and release of metabolites due to a reduction in dilution of these circulating molecules. The purpose of this study was to test the metabolic stability of the low volume *in situ* model.

Materials and Methods

Perfusion of Rat Lungs

Male Sprague-Dawley rats (200–300 g), allowed free access to Purina Lab Chow and water, were anesthetized, intubated and ventilated as described previously.^{3–5} After thoracotomy and heparinization, right and left ventricles of the heart were incised to provide access for cannulation of the pulmonary circulation. Cannulae were placed through the right and left ventricles into the pulmonary artery and left atrium, respectively. As shown in Figure 1, a bubble trap located proximal to the pulmonary artery cannula provided for continual removal of air from the circuit and pressure monitoring. A stationary conical centrifuge tube (50 ml) with stainless steel conduits for uptake and return of perfusate buffer served as a circuit reservoir.⁶

The Krebs-Henseleit buffer used contained 4.5% bovine serum albumin (Fraction V), 5.6 mM glucose and amino acids at the concentration found in rat

Direct communications to: Annette Basile-Borgia, Cardio-pulmonary Perfusion Resources, Ltd., 41 Gettysburg Pike, Mechanicsburg, PA 17055.

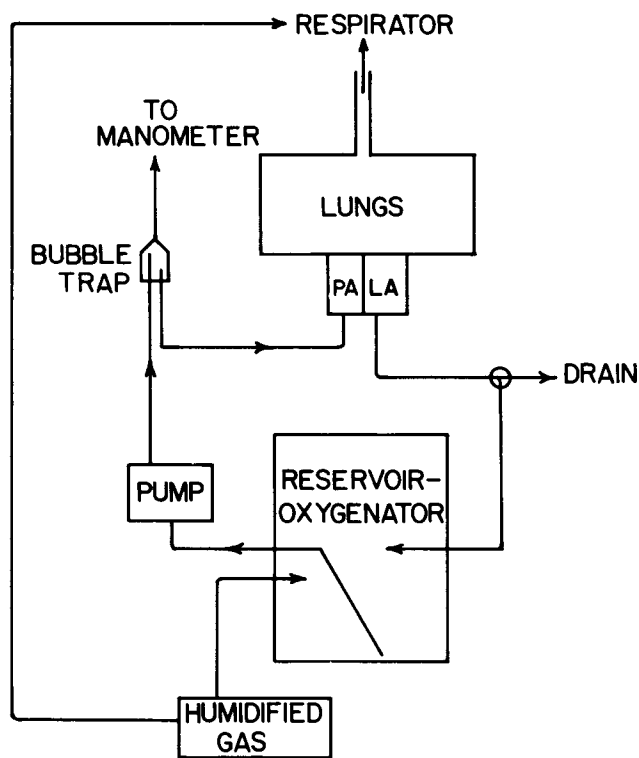


Figure 1. *Perfusion Model: Low Perfusate Volume In Situ Rat Lung Preparation.* The perfusate circuit contained 25 ml of Krebs-Henseleit buffer circulating at 20 cm of H₂O pressure. The ventilatory circuit was set at a positive inspiratory pressure of 15 cm H₂O, a positive and expiratory pressure of 2 cm H₂O, and 70 breaths per minute. The humidified gas mixture was 20% oxygen, 75% nitrogen, and 5% carbon dioxide. (PA = pulmonary artery, LA = left atrium).

plasma.³⁻⁵ This perfusate was equilibrated with warmed (37°C) O₂/N₂/CO₂ (20:75:5). The reservoir-oxygenator was filled with 45 ml of buffer. The first 20 ml of buffer that passed through the lungs were discarded (20 ml wash), after which recirculation of the remaining perfusate was begun. Using a Cole-Palmer Masterflex peristaltic pump^a with a #7014 roller head perfusion was initiated at a pressure of 10–15 cm H₂O. After application of positive and expiratory pressure, pulmonary circulatory flow was increased to obtain a perfusion pressure of 20 cm H₂O. The preparation was supported on a stainless steel tray and placed within a temperature-controlled (37°C) Plexiglas box.^b

Experimental Design

Sixteen animals were sampled at 30, 60, 120 and 180 minute intervals. The experimental time course con-

sisted of the 20 ml wash, a 1 ml baseline perfusate sample, and initiation of the experimental interval with the addition of 40 μCi [¹⁴C]phenylalanine to circulating perfusate. [¹⁴C]Phenylalanine was used to conduct studies on protein synthesis. Four μCi of [³H]sorbitol also were added 10 minutes prior to termination of the perfusion for estimates of extracellular water. Sorbitol does not enter the cell and therefore is an excellent indicator of change in extracellular water, ECW.³⁻⁵ The radioisotopes were added at the LA drain (Figure 1). After perfusion, the lungs were rinsed in ice-cold 0.9% NaCl, trimmed of large airways, and blotted on filter paper. Wet weight of the post-caval lobes was recorded. The remaining lung lobes were frozen rapidly between blocks of aluminum cooled to the temperature of liquid nitrogen and stored at -70°C. The tissue was later powdered in liquid nitrogen using a porcelain mortar and pestle.

Perfusate samples included the 1 ml baseline sample taken prior to the addition of [¹⁴C]phenylalanine and a second sample taken from buffer remaining in the perfusion circuit at the end of the perfusion interval. These samples were stored at -20°C and were later used for determination of glucose uptake and lactate production.

Analysis of Glucose and Lactate in Perfusate

Glucose uptake was calculated by measuring changes in the level of glucose in a known volume of perfusate. Values were determined by using the glucose oxidase method.⁷ Triplicate tubes representing 0, 30, 60, 120 and 180 minutes samples were labeled, filled with 200 μl of the sample (initial and final samples were diluted 5-fold and 4-fold, respectively) and mixed with 3.0 ml of enzyme dye. Standards containing 0-100 mg glucose/0.2 ml distilled H₂O were prepared similarly. The enzyme-dye reagent mixture consisted of purified glucose oxidase (62.5 mg), horseradish peroxidase (25 mg in 500 ml 0.1 N PO₄ buffer, pH 7.0) and 2.5 ml glucose dye (3-3' dimethoxybenzidine, 10 mg/ml abs. methanol).⁷ Samples were mixed and incubated for 1 hour at 37°C after which 0.5 ml 2N HCl was added to each tube to stop the reaction. Tubes were vortexed and placed immediately in ice water. Absorbance readings were taken at 405 nm and recorded. Values of samples were compared to that of standards and corrected for dilution. Enzymatic spectrophotometric studies were also used to determine lactate production. This procedure involved lactate dehydrogenase analysis as described by Hohorst.⁸ Triplicate test tubes of standards and samples containing 0.4 ml of 1:10 dilution of perfusate samples and 3.6 ml of assay buffer were assayed. The assay buffer was made by adding 3.8 gm glycine, 0.8 gm hydrazine, 0.1 gm EDTA and 0.2 gm

a Cole-Palmer Instruments, Chicago, IL

b Model V-E Liver Perfusion Apparatus, Vanderbilt University Instrument Shop, Nashville, TN

NAD⁺ to 100 ml of H₂O. Ten micrograms of lactate dehydrogenase (10.3 mg/ml) were added to each sample and the tubes were incubated at 37°C for 90 minutes.⁸ Absorbance readings were recorded at 340 nm. A standard curve was generated and lactate production (μmol/g lung) was charted for each of the perfusion intervals.

Measurement of [¹⁴C]phenylalanine and [³H]Sorbitol

Radioisotopes (Amersham) were used to determine incorporation of [¹⁴C]phenylalanine (PHE), as a measure of protein synthesis and [³H]sorbitol space, as an index of extracellular water.³⁻⁵ Frozen lung tissue was placed in 3 ml of 5% perchloric acid (PCA) homogenized with a Polytron (Brinkman PT 10) for 10 seconds and centrifuged for 20 minutes at 10,000 xg. Aliquots (1 ml) of supernatant and ACS II (10 ml, Formula 949 scintillant, New England Nuclear) were placed in scintillation vials and counted in a liquid scintillation counter (Beckman LS 250). A duplicate set of samples was processed.

The pellet portion of the homogenized, centrifuged lung tissue was resuspended in 5% PCA, placed in a boiling water bath for 15 minutes, cooled, centrifuged for 10 minutes, and subsequently washed by centrifugation.³⁻⁵ The protein pellets were placed under a hood, in a hot water bath to drive off ether from the final wash step, then dissolved in 5 ml of 0.3 M NaOH. Dissolved protein samples were counted for [¹⁴C]phenylalanine-associated radioactivity and assayed for protein content. The Lowry protein assay used a bovine serum albumin (BSA) standard.⁹ [¹⁴C]Phenylalanine incorporation into protein and vascular permeability parameters were calculated as previously outlined by Watkins and Rannels.³⁻⁵

Results

Water Balance

Values reflecting water balance within the perfused lungs are presented in Table 1. The percent dry weight of lung tissue measured at 30, 60, 120, and 180 minutes remained unchanged (17% dry weight). In addition to this maintenance of constant total tissue water content, the absence of pulmonary edema is further demonstrated by measurements of extracellular and intracellular water (ICW). As shown in Table 1, these values also remained constant as a function of perfusion time. Calculations of [¹⁴C]phenylalanine space and the intracellular phenylalanine concentration indicate that these values also do not vary during three hours of perfusion. These results provide additional evidence for stability of the preparation, particularly with regard to membrane permeability.

Table 1.
Vascular Permeability: Stability of Rat Lungs
Perfused *In Situ*

Parameters	Minutes of Perfusion			
	30	60	120	180
[³ H]Sorbitol space, μl/g (ECW)	434 ± 11	401 ± 1	423 ± 21	464 ± 17
ICW, μl/g	387 ± 3	416 ± 0	399 ± 16	367 ± 16
[¹⁴ C]phenylalanine space, μl/g	699 ± 5	671 ± 12	650 ± 26	681 ± 10
IC phenylalanine, nmol/ml	419 ± 23	446 ± 20	412 ± 16	408 ± 13

Values are means ± S.E. of 3-4 observations. (ECW = extracellular water, ICW = intracellular water, IC = intracellular)

Metabolic Stability

The lungs accumulated glucose during the duration of perfusion, indicating continued substrate availability. The rate of glucose uptake increased somewhat after the first hour of the experiments, but remained stable thereafter (Table 2). Nevertheless, at 180 minutes the rate of glucose uptake (0.53 μmol/g/min) was approximately double the 30 minute value (0.25 μmol/g/min). Lactate production at the 30 and 60 minute intervals was not detectable. At 120 minutes of perfusion, a small amount of lactate (0.025 μmol/g lung) was released to the perfusion buffer. This value increased after 180 minutes.

As shown in Table 3, the rate of [¹⁴C]phenylalanine incorporation into whole lung protein remained between 8.8 and 11.3 dpm/mg protein/min for all perfusion intervals. This indicates continued and stable protein synthesis. Based on the specific radioactivity of extracellular phenylalanine,⁴ these values were converted to nmol incorporated, which reflect "absolute" synthetic rates. The values observed are in good agreement with those published previously.⁴

Discussion

Interpretation of Results

To categorize a perfusion model as active and stable it must fulfill certain criteria. First, the tissue being perfused should demonstrate adequate gas exchange and stable pH. Hypoperfusion would lead to acidosis. The model described here has previously demonstrated adequate gas exchange as evidenced by measurements of perfusate pO₂, pCO₂ and pH. Secondly, to categorize a lung perfusion preparation as stable,

Table 2.

Substrate Availability and Metabolic Stability

Time (Min.)	Glucose Uptake		Lactate Production	
	Total ($\mu\text{mol/g lung}$)	Rate ($\mu\text{mol/g lung/min}$)	Total ($\mu\text{mol/g lung}$)	Rate ($\mu\text{mol/g lung/min}$)
30	7.6 \pm 14.9	0.25	*ND	*ND
60	27.5 \pm 2.61	0.45	*ND	*ND
120	55.3 \pm 8.3	0.46	0.025 \pm 0.008	0.002
180	95.4 \pm 17.6	0.53	0.161 \pm 0.012	0.009

Lungs were perfused for the period indicated in the Materials and Methods section. Data represent the mean \pm S.E. of 4 observations. (*ND = not detectable)

the perfused pulmonary tissues should maintain stable pulmonary vascular and cell membrane permeability. Significant decreases in circulating perfusate volume might indicate a leak in tubing connections. Importantly, a very small decrease in circulating perfusate could be an uptake of fluid by the perfused organ. An organ can take on fluid by shifts of intracellular and extracellular volumes. For example, due to changes in osmotic pressure, fluid can move from intravascular compartment into the interstitial space or the cells. The organ would then become edematous. This was not the case with the model presented in this study wherein the isolated lung preparation remained edema-free. All lung weights proved to be similar to previously published results.³⁻⁶ Extracellular water measured as [³H]sorbitol space was similar to results by Watkins and Rannels.³⁻⁵ Other results relevant to water distribution and cell membrane stability (Table 1) proved to be similar to previous findings.³⁻⁵ There was no evidence of alterations in ICW or ECW over intervals as long as 180 minutes.

When an organ takes on a significant amount of fluid, changes in resistance will exist in the vessels leading to the organ. For example, during pulmonary edema, pulmonary tissue pressure increases due to changes in pulmonary vascular resistance (PVR). This

was *not* the case with the perfusion model developed here. Pump flow rates did not need adjustments in order to maintain stable pulmonary vascular pressures. Therefore, PVR remained constant, characterizing vascular stability.

A third characteristic of an active and stable perfusion model is evidence of metabolic stability. Substrates must be available in order to maintain normal metabolism. Measurements of substrate utilization and by-product output prove to be valuable in evaluating a perfusion model as metabolically active and stable. The uptake of glucose and lack of high rates of lactate production, as recorded in Table 2, indicate continued metabolic activity. The rate of glucose uptake at 180 minutes was over twice that at 30 minutes, demonstrating a time-dependent increase in uptake of glucose from the perfusate. As for lactate production, hypoperfusion and hypoxia are indicated when rates are high.³ Rates presented in this study were low. At 30 and 60 minutes, lactate production was not detectable. The lag of measurable lactate production and low rates of output at 120 and 180 minutes suggest continued aerobic metabolic activity. In addition, ongoing protein synthesis further supports conclusions of continual aerobic metabolism and metabolic stability. Measurements of [¹⁴C]phenylalanine incorporation indicate that the perfused lung tissues did indeed continue to synthesize protein for the duration of the investigation. The rate of protein synthesis at 30 minutes (8.8 dpm/mg protein/minute) did not differ substantially from that at 180 minutes of perfusion (8.6 dpm/mg protein/minute).

The intent of this study was to further validate the stability of a low buffer volume *in situ* rat lung perfusion model. The reduction in volume did *not* compromise the metabolic or gas exchange functions of the lungs, nor limit substrate availability. In conclusion, the low volume, isolated lung perfusion preparation is indeed a stable and metabolically active model. This

Table 3.

Protein Synthesis [¹⁴C]Phenylalanine Incorporation

Time	dpm/mg protein	dpm/mg protein/min	nmol/mg protein
30	265 \pm 12	8.8	1.2 \pm 0.1
60	675 \pm 82	11.3	2.3 \pm 0.3
120	1158 \pm 187	9.7	4.3 \pm 0.10
180	1552 \pm 96	8.6	6.8 \pm 0.50

Lungs were perfused for the period indicated as described in the Materials and Methods section. Data represent the mean \pm S.E. of 4 observations.

model is thus appropriate to be used in studies to detect alterations in pulmonary metabolism and non-ventilatory lung functions.

Importance of Isolated Lung Perfusion

Isolated lung perfusion provides investigators the unique opportunity to study functional aspects of pulmonary tissue in a setting free of exogenous hormonal and other influences. Evaluation of post-perfusion tissue and perfusate samples yields valuable information regarding the structural and functional integrity of the tissue, as well as assessment of the perfusion techniques employed.

With over 40 different types of cells and the largest capillary network in the body, it is not surprising that the lungs are involved in more than gas exchange.¹⁰ Setting between the venous and arterial systems, pulmonary tissue is in the ideal location to meet certain homeostatic needs of the body. For example, consider the renin-angiotensin and kallikrein-kinin systems. Briefly, one enzyme (angiotensin converting enzyme) located at the pulmonary endothelial surface removes a blood pressure-lowering-agent (bradykinin) and facilitates in the formation of a blood pressure-elevating-substance (angiotensin II). This central location allows aspects of the lungs to affect not only the pulmonary circulation, but the coronary and peripheral circulations as well. An expanding field of information has developed concerning non-ventilatory roles of the lung. Recent emphasis has been on the lung as an endocrine organ, as a metabolic organ, as a site for the formation and removal of large quantities of liquid and solute, and as a site for production and transformation of a variety of bioactive substances.¹⁰

Early works by Eiseman and his colleagues suggest the utility of isolated lung perfusion to obtain a better appreciation of basic pulmonary physiology and to gain insight into the clinical conditions that might result when the lung is diseased or excluded from the circulation.¹¹ During cardiac surgery, the heart and lungs are routinely excluded from the normal circulation due to techniques of cardiopulmonary bypass.

Prolonged bypass may lead to damage of endothelial cell transport systems.¹ Pulmonary vasoactive substances have been implicated in post-bypass phenomena, such as changes in pulmonary and systemic vascular resistances.^{1,12} Alterations in extraction or metabolism of vasoactive substances by the lung has been cited as a possible cause of the pulmonary injury sustained during cardiac surgery.¹ Together, these considerations suggest a significant role for stable isolated perfused lung preparations in experimental efforts to define the basis and physiological implications of lung injury and disease.

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