

# Isolated Perfused Lung Preparation for Studying Altered Gaseous Environments

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The isolated perfused lung (IPL) preparation is ideally suited to investigate lung dynamics and cellular function, and is easily adapted to investigating biochemical and physiological responses to environmental insults. The IPL offers several advantages which permit one to study endothelial/epithelial interactions that are often disrupted with other model systems (e.g., isolated cells, minces, slices, homogenates, etc.). The IPL developed in our laboratory was devised for the rat lung and allows four lungs to be perfused simultaneously in which control over ventilation, flow, pressure, pH,  $P_{O_2}$  and  $P_{CO_2}$  can be maintained. Isolated lungs perfused for 1 to 2 hr at a flow rate of 10 mL/min exhibit less than 2% weight gain, maintain normal ATP levels, and exhibit linear substrate uptake. Mechanisms leading to changes in vascular and airway resistance, lipid metabolism, vasoactive hormones, blood gases and changes in vascular permeability mediated by environmental insults can be quantified in the IPL preparation.

## Introduction

Metabolic functions of the lung have recently been recognized (1) and studies with the isolated perfused lung (IPL) have become a very important physiological model to investigate the biochemical aspects of lung function during normal and altered physiologic states. A timely aspect of lung physiology, the metabolic response to altered gaseous environments, may be studied effectively by using the IPL preparation. The lung becomes a primary target organ with altered gaseous environments. An understanding of how fundamental metabolic processes are affected provides early clues to lung injury. The IPL readily lends itself to investigating the biochemical response of the lung to altered gaseous environments and offers several distinct advantages as a model system. First, the lung remains an intact organ which permits one to study metabolic properties associated with cell-to-cell interaction which are disrupted when isolated cells and cell-free preparations are examined. Second, all other organs are removed from the circuit which otherwise alter synthetic rates and/or interfere with establishing precise metabolic function of the lung. Third, the transfer of extracellular substrates and/or nutrient transport are physiologic, because they occur via the capillary vasculature.

The IPL preparation should be as physiologic as possible and the design of the preparation should meet several criteria. First, specific aspects of ventilation, namely tidal volume, frequency, and end-expiratory

pressure should be controlled. Pulmonary hemodynamics which include pressure and flow should also be maintained. Third, perfusate composition, apart from substrate levels (glucose, palmitate, etc.), must be defined. Fourth, the IPL preparation must remain viable throughout the perfusion period. The purpose of this paper is to describe the IPL technique and to provide specific details of how the above criteria are met. A discussion will review the influence of altered gaseous environments on lipid metabolism in the isolated perfused lung.

## General Principles of Lung Perfusion

The apparatus used for artificial perfusion of the isolated lung may vary in design, but all have basic features that are common. The IPL system requires a temperature-controlled environment to house the lung and maintaining proper blood gas tension, and a mechanical pump to perfuse the pulmonary vascular bed. Modifications of the basic system can be made to meet specific demands of experimental protocol. The methodology described in this paper deals with the isolated perfused lung (Fig. 1). The system may, however, be adapted to study the lung *in situ* but isolated from the vascular beds of adjacent tissue.

## Perfusion Pump

Lung perfusion requires a continuous exchange of medium within the vascular bed, and the means by which the perfusion fluid is circulated through the pulmonary vasculature is therefore critical. The me-

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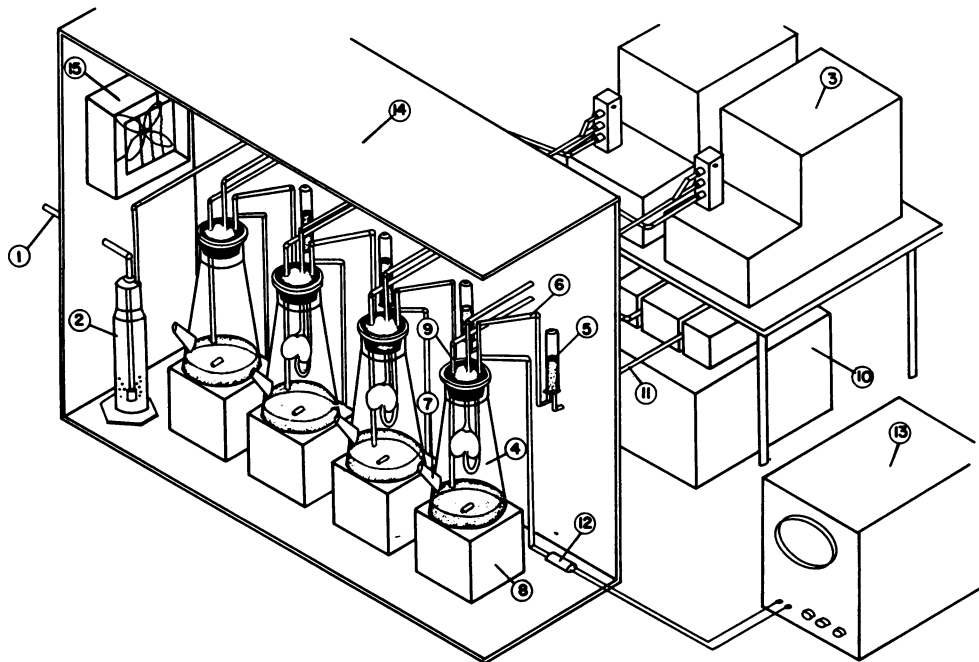


FIGURE 1. Schematic of isolated perfused lung preparation with numbers indicating various components: (1)  $O_2$ - $CO_2$  mixture; (2) humidifier; (3) respirator; (4) organ and blood reservoir; (5) bubble trap and pressure dampener; (6) to pulmonary artery; (7) sample port; (8) magnetic stirrer; (9) to blood pump; (10) peristaltic blood pump; (11) to bubble trap; (12) pressure transducer; (13) recorder; (14) constant temperature box; (15) fan and heating element.

chanical pump used to circulate the medium can be utilized in two different ways: (a) to supply medium directly to the pulmonary artery or (b) to fill an elevated reservoir from which perfusion of the lung occurs by gravity under constant hydrostatic pressure. If the medium is pumped directly to the pulmonary artery, pulse and pressure characteristics of the pump are of critical importance. When the pump is required only to fill a reservoir, its pulse and other characteristics are less exacting. Most investigators have chosen to use direct perfusion to the pulmonary artery because pulmonary vascular resistance changes during the perfusion period. Therefore, when lungs are perfused with a constant hydrostatic pressure, rate of flow decreases. Maintenance of a constant flow rate is particularly advisable when rates of synthesis or degradation are being examined. A further disadvantage of perfusion by gravity is that proper gas tension of the perfusate is difficult to maintain in a large reservoir that is exposed to the atmosphere.

Two approaches have been used to supply the medium to the perfused lung; the single pass and the recirculating perfusion. Although the once through perfusion is simpler in design the usefulness of this system is limited because of the large volume of media required to carry out an experiment. In addition, rates of substrate uptake (such as glucose, fatty acids, glycerol, etc.) are much more difficult to measure in a single pass because of the high flow rates and small arterio-venous differences. Consequently, most IPL preparations employ the recirculation of the medium. Continuous recircula-

tion offers several advantages: the volume of perfusate required is much smaller and does not influence the duration of the perfusion; the slow rate of substrate and nutrient removal or addition of the solute to the perfusate are magnified several times as the perfusion period progresses; and an equilibrium is established between medium and tissue (e.g., lactate/pyruvate concentration which parallel *in vivo* situations).

## Ventilation

Proper ventilation is very critical in the perfused lung. Several approaches have been taken to ventilate the perfused lung: no ventilation, positive-pressure ventilation and negative-pressure ventilation. The advantage of ventilating the lung is that it provides the physiologic route for oxygenation. Ventilation also affects the distribution of blood flow within the lung. Consequently, studies should not be carried out without some mode of ventilation. Most laboratories have adopted positive-pressure ventilation rather than negative-pressure ventilation because of the complexity involved in maintaining a negative-pressure system. Furthermore, when negative-pressure ventilation is used, the lungs must be placed in a sealed chamber making them less accessible for many metabolic studies. Thus, negative-pressure ventilation cannot be recommended for biochemical investigation.

Inspiratory pressures for the rat lung ventilated by positive pressure range from 10 to 15 cm  $H_2O$ . A positive end expiratory pressure (PEEP) of 2 to 3 cm of

H<sub>2</sub>O is necessary to prevent the lungs from collapsing at the end of expiration. Failure to maintain PEEP will lead to edematous lobes in the perfused lung. Ventilation frequency for the isolated perfused rat lung ranges from 30 to 60 cycles/min with a tidal volume of 1 to 3 mL. Frequency and tidal volume often vary depending on the dead space air (trachea air and air in the tubing connecting the lung to the respiratory pump). Normal alveolar ventilation for the rat is approximately 40 mL/Min. However, since all of the other organs are removed from the circuit, there is no need to approximate alveolar ventilation in the IPL preparation.

## Medium

The physiochemical properties of the medium are very important toward the maintenance of a viable preparation. Temperature, pH and gas tension (O<sub>2</sub> and CO<sub>2</sub>) are all critical parameters. The initial pH and the total buffering capacity of the medium are extremely important when the standard recirculating system is used because of the accumulation of waste products (namely, lactate and CO<sub>2</sub>) in the perfusate.

Investigators have used various types of perfusion media. In earlier studies, a desire to maintain a situation similar to the state that existed *in vivo* resulted in the use of whole blood or at least the use of red cells as a component of the medium (3-6). A major difficulty encountered in using homologous, or heterologous blood or serum for biochemical studies is the undefined composition and the uncertainty concerning the donor's state (dietary status, circulating hormones, etc.). Additional problems encountered with the use of whole blood is an increase in vascular resistance due to embolus formation and release of vasoactive hormones which can lead to an unstable preparation (Fig. 2). Daley (3) has shown that defibrinated blood contains vasoactive hormones (histamine, serotonin) which cause serious pulmonary vasoconstriction. Also, mechanical pumping results in damaged platelets which also releases serotonin (7). Another problem that is often encountered with mechanical pumping is hemolysis due to red cell damage. These problems as well as the need for defined and reproducible composition of the medium have led to the increased popularity of artificial media.

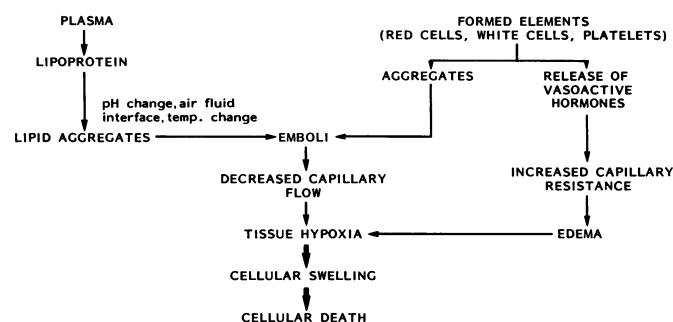


FIGURE 2. Etiological factors in lung swelling during isolated perfusion.

An artificial medium which is widely used and recommended for lung perfusion is Krebs-Henseleit bicarbonate buffer (8). This medium contains physiologic concentrations of ions, and a physiological pH (usually 7.4) with an adequate buffer capacity containing a bicarbonate (pK 6.1). In the absence of blood, the colloidal oncotic pressure must be maintained to balance hydrostatic and tissue pressures. Bovine serum albumin and dextran are two of the most common plasma expanders which are used to maintain colloidal osmotic pressure. The recommended choice is bovine serum albumin (BSA). Several commercial preparations of BSA are available. One which is highly recommended is Pentex fraction V crystalline BSA (Miles Laboratory). Concentrations of BSA in Krebs-Henseleit bicarbonate buffer range from 3 to 5 g/dL in the perfused media. Precautions should be taken when albumin is used. The albumin should always be dialyzed before use to remove possible contaminants and small molecular weight ions. If Krebs-Henseleit buffer is used for dialysis, a sealed vessel is required. Since long chain fatty acids are known to influence lung metabolism (1,9,10), it is advisable to defat the BSA in order to reduce the amount of fatty acids added initially to the medium. The charcoal method has been used for defatting albumin (11). The final Krebs-Henseleit bicarbonate buffer-albumin media should be filtered twice by Millipore filtration (0.45 and 0.2  $\mu$ m). A final precaution regarding albumin is the displacement of bicarbonate when albumin is added to the buffer which influences the final pH of the medium in an unpredictable manner. When the final media is equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub>, the pH is usually acidic and must be adjusted to 7.4.

Air emboli are particularly troublesome in lung perfusions and constitute a major cause of uneven perfusion and/or edema. Air emboli lead to a chain of events similar to that seen in Figure 2. In addition, emboli have been shown to cause prostaglandin release in the lung (12). Precautions can be taken to avoid emboli formation. A simple device which consists of a glass tube fitted with a side arm (Fig. 3) can be incorporated on the arterial side just proximal to the pulmonary artery. This device not only dampens the pulmonary pressure wave but effectively removes all emboli from the medium before they can enter the pulmonary artery.

## Tests of Lung Viability

Any physical or metabolic parameter which can be determined serially or continuously during lung perfusion may be used to assess tissue viability and determine lung stability during the course of the experiment. The test can be applied to the perfusion medium or to the lung itself. Physical parameters such as perfusion pressure, flow rate, dry/wet weight ratio of lung are commonly determined and are important indicators for normal lung function. None of these, however, are in themselves sufficiently critical to be a sensitive indica-

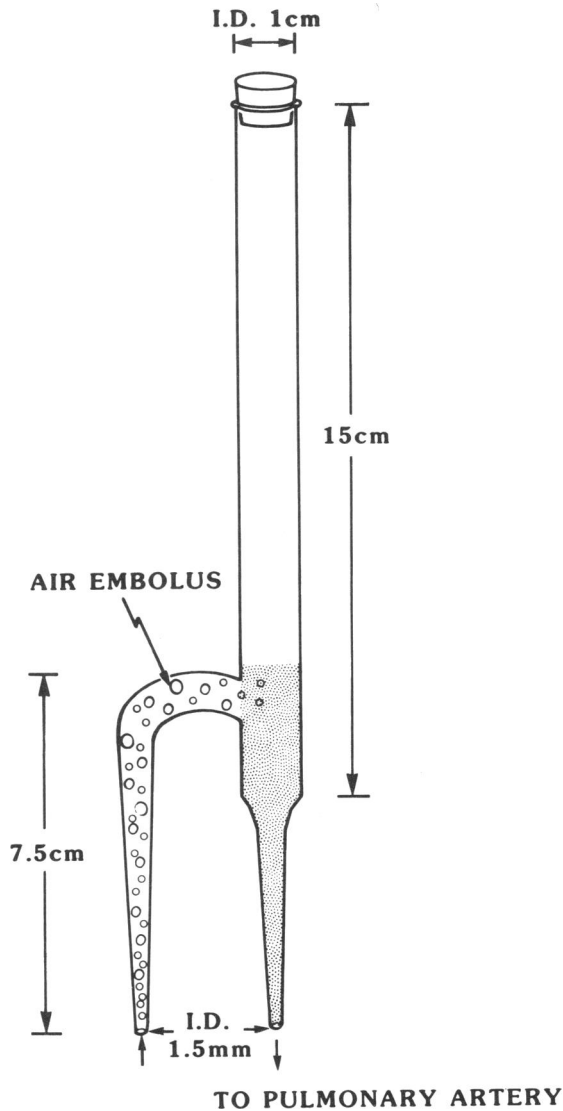


FIGURE 3. Schematic of bubble trap.

tor of the state of the perfused lung. Figure 4 shows pulmonary pressure at various time intervals during the perfusion period: Figure 4A depicts a normal pulmonary pressure curve from a stable preparation with a mean pressure of 13.0 mm Hg at a flow rate of 10 mL/min; Figure 4B, at the same flow rate, shows pulmonary pressure from an unstable preparation which subsequently became edematous. Lung dry/wet weight ratio is an important parameter to measure because pulmonary edema is a characteristic feature of an unstable preparation. Dry/wet weight ratios of 0.16 or less are unacceptable.

Metabolic parameters of lung function are more sensitive than physical parameters and are appropriate indicators of viability for biochemical experimentation. A variety of tests can be suggested. First a metabolic reaction should be linear and thus a test can be devised to determine if rates of reactions in the IPL are linear.

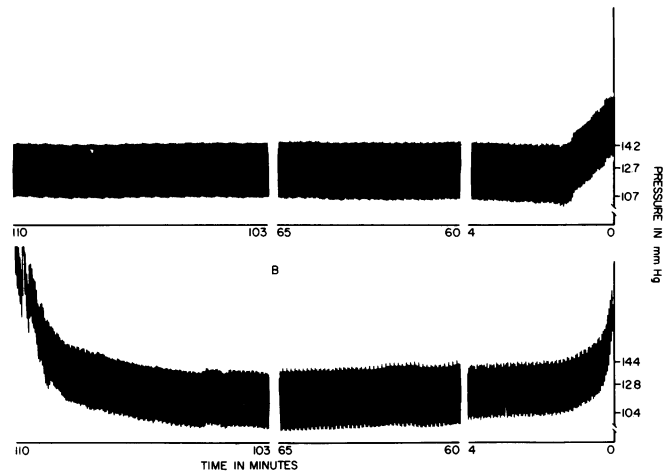


FIGURE 4. Pulmonary pressure from (A) a normal perfused lung and (B) an unstable perfused lung. Lungs were ventilated with 95% O<sub>2</sub>-5% CO<sub>2</sub> and were perfused with Krebs bicarbonate buffer at a flow rate of 10 mL/min.

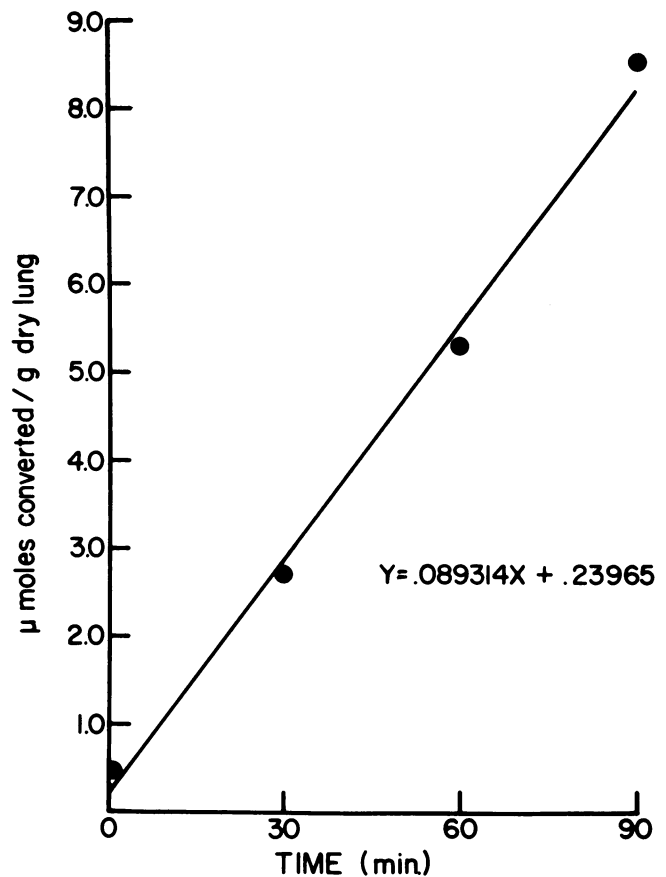


FIGURE 5. [<sup>14</sup>C]glucose incorporation into lung lipid with time.

Figure 5 demonstrates one example of a reaction which can be used and shows that glucose incorporation into lung lipids during a 1.5 hr perfusion is linear with time. Similarly, palmitate incorporation can be used to indi-

cate tissue viability (9). Enzyme leakage (such as LDH) from damaged lung can also be used as a test of viability. However, enzyme release is usually a late event resulting from extensive cell damage and, therefore, is not a sensitive indicator of viability during the early period of perfusion. Glycogen breakdown has been used as an indicator of cellular anoxia (13). However, lung glycogen in the adult is fixed rather than free glycogen and is not easily mobilized. Only during extreme anoxia does lung glycogen seem to be depleted (2,10). Concentrations of ATP, ADP, creatine phosphate, and creatine have been used to detect cellular changes in the energy state (14) and are very sensitive indicators of lung viability. Recent studies (14) have shown that lungs which are ventilated with 95% O<sub>2</sub>-5% CO<sub>2</sub> and perfused for 1.5 hr with a Krebs-Henseleit bicarbonate buffer (containing 5 g/dL BSA and 6 mM glucose) show no significant change in the energy state (Table 1). Similar findings have been reported by others (15) and show that a viable energy state can be maintained for up to a 2 hr perfusion.

Measurements of blood gases provide immediate information about the environment of the medium and also provide indirect information about tissue viability as well. Table 2 shows normal blood gas chemistry of lungs ventilated with 95% O<sub>2</sub>-5% CO<sub>2</sub> and perfused with a Krebs-Henseleit bicarbonate buffer. Blood gases become extremely important when lungs are ventilated with altered gaseous environments.

One of the striking features of carbohydrate metabolism in the perfused lung is the high rate of lactate production. Table 3 provides some comparative data on

glucose uptake and lactate production of the IPL. About 40 to 60% of the glucose taken up by lung is converted to lactate (16-18), and the lactate produced by the lung can account for up to 10% of the total body lactate. The functional importance of such a high glycolytic flux of glucose to lactate in an organ that has one of the highest P<sub>O<sub>2</sub></sub> in the body is not fully understood. Glucose utilization and lactate production are affected by circulating glucose levels (16,19) and may account for some of the discrepancies in the literature regarding glucose uptake and lactate production. Also, it is important to point out that earlier studies, in which red cells were used as part of the perfusion medium, show abnormally high values for glucose uptake and lactate production (5). This is due to interference from hemolysis and the difficulty in quantitatively separating red cell contribution from that of the perfused lung.

## Isolated Perfused Lung Technique

No single method of lung perfusion covers all possible metabolic and toxicologic applications. This section presents the methodology of an IPL preparation we have found to be appropriate for a variety of studies (10,14,18,19). In this method lungs are positive ventilated and perfused with an artificial medium which is recirculated at a constant flow. The perfusion medium enters the pulmonary artery and drains from the cut left atrium into a reservoir. It is the author's opinion that positive ventilation and recirculation of artificial medium at constant flow is best characterized and most readily applicable to biochemical studies. Although other species can be used, our technique is devised for the laboratory rat; partly because much of the data on tissue metabolism is derived from the rat. In particular, the rat lung has been studied in detail (2,5,9,10,14,15,17-27).

## Apparatus

A schematic of the isolated perfused lung apparatus is shown in Figure 1. The apparatus consists of organ chambers, magnetic stirrers and a gas humidifier, which are all housed in a temperature-controlled Lucite cabinet. A perfusion cabinet has a number of advantages: the organ chamber stands free and not restricted by a water jacket, heating by circulating warm air achieves the required temperature more rapidly than does water; the evenness of ventilation and perfusion can readily be visualized; and the simultaneous perfusion of more than one lung can be achieved. In the apparatus shown in Figure 1, the temperature is maintained at 38 to 40°C by a filament heater placed in front of a small induction fan (Rotron, Inc., Woodstock, NY). The organ chamber illustrated in Figure 1 consists of a 500-mL Erlenmeyer flask with a side arm used for sample collection. The glass organ chamber contains both the lung and the perfusion medium, and is placed

**Table 1. Concentrations of ATP, ADP, creatine phosphate and creatine in freshly excised and perfused lungs.**

| Parameter          | Concn, $\mu\text{mo/g}$ wet wt. <sup>a</sup> |                 |
|--------------------|--|-----------------|
|                    | Freshly excised                              | Perfused        |
| ATP                | 1.61 $\pm$ 0.06                              | 1.59 $\pm$ 0.12 |
| ADP                | 0.21 $\pm$ 0.02                              | 0.20 $\pm$ 0.02 |
| Creatine phosphate | 0.34 $\pm$ 0.03                              | 0.35 $\pm$ 0.02 |
| Creatine           | 0.55 $\pm$ 0.02                              | 0.56 $\pm$ 0.03 |

<sup>a</sup>Values are mean  $\pm$  SE. Lungs were rapidly excised and freeze-clamped or perfused for 1.5 hr and freeze-clamped prior to the measurement of the concentrations (22).

**Table 2. Blood gases from perfused lungs ventilated with 95% O<sub>2</sub>-5% CO<sub>2</sub>.**

| Measurement <sup>a</sup>           | Perfusion time <sup>a</sup> |                   |
|------------------------------------|-----------------------------|-------------------|
|                                    | 0                           | 90 min            |
| ph                                 | 7.396 $\pm$ 0.004           | 7.398 $\pm$ 0.021 |
| pO <sub>2</sub> , mm Hg            | 481.9 $\pm$ 24.5            | 437.0 $\pm$ 18.5  |
| pCO <sub>2</sub> , mm Hg           | 37.9 $\pm$ 0.9              | 36.9 $\pm$ 0.9    |
| HCO <sub>3</sub> <sup>-</sup> , mM | 23.3 $\pm$ 0.6              | 23.8 $\pm$ 0.8    |
| Total CO <sub>2</sub> , mM         | 24.2 $\pm$ 0.6              | 24.8 $\pm$ 0.8    |
| Base excess, mEq/L                 | -0.8 $\pm$ 0.6              | 0.01 $\pm$ 0.8    |

<sup>a</sup>Values are means  $\pm$  SE. Lungs were ventilated with 95% O<sub>2</sub>-5% CO<sub>2</sub> and were perfused with Krebs-Henseleit bicarbonate buffer for 1.5 hr at a flow rate of 10 mL/min (27).

on top of a magnetic stirrer (Curtain Scientific, Houston, TX). The lung is supported by a tracheal cannula (#15 gauge stainless steel needle) so that obstruction to blood flow and ventilation are minimal. The organ chamber is sealed with a number ten and double zero (for side arm) Neoprene stopper to prevent evaporative and gas loss from the media, and to prevent the lungs from becoming dry.

Two Harvard peristaltic pumps (Model #1201) are used to recirculate the medium (flow rate 10 mL/min) through polyethylene tubing (3 mm outside diameter, size 13, 3M Co.). Prior to entering the pulmonary artery the perfusate is passed through a glass chamber to remove air emboli and to dampen the pressure wave (Fig. 2). Lungs are ventilated with a Harvard positive-pressure pump (#680) at a frequency of 30 to 40 cycles/min. Inflation pressure for a 200 to 250 g rat should range between 10 and 15 cm H<sub>2</sub>O with a PEEP pressure of 3 cm H<sub>2</sub>O. The respiratory pumps are designed so that the respired gas can be collected for CO<sub>2</sub> production. CO<sub>2</sub> collection, shown in Figure 1, is accomplished by trapping CO<sub>2</sub> from the expired air by bubbling the respired gas through 30 mL of 3 N KOH. An increase in oxygen content of the effluent compared with the inflowing medium confirms that gas exchange occurs in the ventilated lung.

## Perfusion Medium

The recommended perfusion medium is Krebs-Henseleit bicarbonate buffer (8) containing 40 to 50 g/L of dialyzed bovine serum albumin (Pentex fraction V, Miles Laboratory). If fatty acid free BSA is desired the BSA should be charcoal treated after dialysis (11). Glucose (5–6 mM) should always be included to support synthesis of cellular constituents and as an energy fuel. In some cases, amino acids (18) and palmitic acid (19) are added. When palmitate is used, binding palmitate to BSA requires some care. Palmitate is first dissolved in Hexane (0.5 mM/2 mL) and then an equal molar amount of potassium hydroxide–ethanol is added at 60°C in water bath. The two phase solution is evaporated to dryness under nitrogen. The potassium salt of palmitate is put into solution by adding 10 mL of 60°C double distilled water. The palmitate is then gently added to the KHB–BSA solution that is pretreated to 40 to 45°C. The KHB–BSA–palmitate is cooled and filtered twice by Millipore filtration (0.45 and 0.2 μm). The medium is gassed with 95% O<sub>2</sub>–5% CO<sub>2</sub> and pH adjusted to 7.4 with 0.8 M sodium carbonate.

## Operative Procedure

Well-fed rats, male or female, weighing 200 to 250 g, are anesthetized with an intraperitoneal injection of Nembutal (5–6 mg/100 g body weight). With the animal on an operating tray (Thomas Co., Philadelphia, PA) a tracheostomy is performed. An incision is made with scissors through the skin of the throat, and the trachea

is exposed. A curved pair of forceps is passed under the trachea. A ligature is then drawn under the trachea and tied loosely. The trachea is cannulated by making an incision across half of its diameter and inserting a cannula (polyethylene or stainless steel tubing, OD 3–4 mm) well into the trachea and tying it in place. The abdomen is opened and the abdominal aorta is severed. A plug is placed in the tracheal cannula to keep the lung inflated, and the chest is then rapidly opened by an anterior midline incision. The trapped air prior to the thoracotomy is critical because it prevents the lungs from collapsing and enhances uniform perfusion. A small incision is made in the right ventricle and a polyethylene tube (#240), filled with perfusion media to prevent air emboli, is inserted through the right ventricle and into the pulmonary artery. A ligature is placed at the base of the pulmonary artery using a surgical needle and thread. The end of the polyethylene tubing should be flared to hold the cannula in place when the ligature is secured. Care must be taken when the pulmonary artery is ligated so that the pulmonary veins are not occluded. After the pulmonary artery is cannulated, the left atrium is cut and the lung is perfused with KHB buffer saturated with 95% O<sub>2</sub>–5% CO<sub>2</sub>. Interruption of pulmonary flow by this procedure is less than 15 sec. While the lungs are perfused *in situ*, the trachea, lungs and great vessels are dissected free from connective tissue. All excess tissue including major portions of the heart are removed and the lung is then quickly transferred to the organ chamber and suspended by the tracheal cannula. Ventilation and flow of the medium are initiated, and lungs are perfused for 5 min to allow equilibration before the start of an experiment. The operative procedure takes approximately 10 min. During that time, essentially no anoxia occurs because of the trapped air in the lung and pulmonary flow (containing 5 mM glucose and saturated with 95% O<sub>2</sub>–5% CO<sub>2</sub>) is essentially uninterrupted.

## Characteristics of Perfusion

Lungs which are positive ventilated at 30 cycles/min with (PEEP 2–3 cm) 95% O<sub>2</sub>–5% CO<sub>2</sub> and perfused for 1 to 2 hr at a flow rate of 10 mL/min with Krebs-Henseleit bicarbonate buffer provide a viable preparation. Lungs perfused under these conditions show less than 2% weight gain, maintain gas tension (Table 2), have normal ATP levels (Table 1) and have linear glucose utilization (Fig. 5). Glucose uptake and lactate production are comparable to those values seen in Table 3.

## Effects of Altered Gaseous Environments on Lung Lipid Biosynthesis

One of the most important and extensively studied metabolic functions of the lung is the biosynthesis of pulmonary surfactant. Surfactant coats the inner sur-

**Table 3. Comparable data on the isolated perfused lung.**

| Parameter                         | Mean $\pm$ SE <sup>a</sup> |
|-----------------------------------|----------------------------|
| Glucose, $\mu$ mole/hr-g dry lung | 50-70                      |
| Lactate, $\mu$ mole/hr-g dry lung | 40-60                      |

<sup>a</sup>These data were obtained from lungs ventilated with 95% O<sub>2</sub>-5% CO<sub>2</sub> and perfused with Krebs-Henseleit bicarbonate buffer for 1 to 2 hr (2,16,17,22). Flow rates ranged from 10 to 15 mL/min and ventilation ranged between 30 and 60 cycles/min.

face of the alveolar lining and functions to reduce surface tension and imparts stability to the terminal respiratory units at low lung volumes (1). Pulmonary surfactant is a lipoprotein that is particularly rich in phospholipids. The principle agent responsible for the surface-reducing properties of surfactant is dipalmitol phosphatidylcholine (1), comprising over 40% of the total lung phospholipid. This phosphatidylcholine is unusual, in that it contains two saturated fatty acid residues, whereas phosphatidylcholine in other mammalian tissue contains an unsaturated residue at the C-2 glycerol position. Another important phospholipid species that appears in high concentrations compared to other tissues is phosphatidylglycerol (26). Phosphatidylglycerol accounts for approximately 2% of total phospholipid in most mammalian tissue, but reaches 7 to 10% in surfactant (26). Another striking feature of lung phospholipid metabolism is its high turnover rate, which is thought to be linked to the continual replacement of surfactant at the alveolar surface (28).

Lung lipids are an integral part of surfactant as well as the alveolar membrane (which constitutes one of the largest membranes in the body). These lung lipids and their synthetic machinery are continually exposed to the atmosphere making them extremely vulnerable to environmental insult. Longmore and co-workers (25,26) have shown that ventilation of perfused lungs with high CO<sub>2</sub> (hypercapnia) significantly alters glucose incorporation into surfactant lipids, specifically phosphatidylcholine and phosphatidylglycerol. These data show that acute exposure to CO<sub>2</sub> may drastically alter surfactant synthesis. Acute hypercapnia also increases the lactate/pyruvate ratio (27). Acute exposure to carbon monoxide has a more striking effect on lung metabolism. Fisher and co-workers (21) have shown that ventilating isolated perfused rat lungs with carbon monoxide for 1.5 hr significantly inhibits oxidative metabolism which was reflected by a decrease in the cytoplasmic redox state. Associated with the decrease in the redox state was a corresponding 6-fold increase in the lactate/pyruvate ratio. Also, there was a concomitant increase in glycolytic activity with a 4-fold increase in lactate production. CO<sub>2</sub> production was almost totally inhibited. The elevated glycolytic rate did not maintain ATP levels as evidenced by a 56% decrease in ATP concentration. The decreased energy state of the lung from CO exposure caused a significant decrease in lipid synthesis. Acute hypoxic exposure shows similar effects, as seen with CO (20). None of these studies (CO<sub>2</sub>, CO and hypoxia) have

determined if the effects are reversible; however, these data do show that lung lipid synthesis is extremely sensitive to altered gaseous environments.

One area of extreme importance related to lung lipids is exposure to oxidants such as ozone, nitrogen dioxide, and therapeutically administered oxygen in high concentrations. Several effects of oxidant stress on biosynthesis of lung lipids have been reported. Morgan (29) showed that *N*-methyltransferase is rapidly inactivated on exposure to high oxygen. Gilder and McSherry (24) demonstrated inhibition of choline and palmitate incorporation into lung phosphatidylcholine from rabbits exposed to hyperbaric oxygen (3 atm) for 3 hr. However, Newman and Naimark (30) showed that hyperbaric oxygenation increased palmitate incorporation into lung phospholipids. In a later study, Morgan and co-workers (31) showed that oxygen-exposed dogs had increased alveolar phospholipids but that the esterification of fatty acids in surfactant phosphatidylcholine was markedly altered, with palmitate levels about one-third normal. Valimaki and co-workers (32) extended these studies by showing that oxygen exposure impairs synthesis and removal of alveolar phospholipids. More recently, Fisher (21) has shown that lungs which were pre-exposed to oxygen for 48 hr and then subsequently perfused for 80 min showed elevated lactate and lactate/pyruvate production. Isolated perfused lungs ventilated with hyperbaric oxygen (5 atm) for 80 min resulted in a similar increase (22). In addition, hyperbaric perfused lungs resulted in a decrease in lung ATP levels.

Other types of oxidant exposure, using techniques other than the IPL preparation, have resulted in similar effects on lung lipids. Acute exposure of nitrogen dioxide (5 ppm) for 14 days resulted in decreased rates of replacement of lung phosphatidylcholine (33). Chronic low level exposure to NO<sub>2</sub> resulted in decreased lung compliance, increased surface tension, and altered fatty acid composition from alveolar wash (34). Thomas' studies (35) confirm direct peroxidation of lung lipids from NO<sub>2</sub> exposure. A direct effect on lung structural proteins (collagen and elastin) has also been reported by Buell (36). Similar effects have been observed with ozone (37,38).

## Summary

As pulmonary metabolism becomes better understood at the cellular level, investigators are turning to special experimental procedures that allow them to study the integrative processes in the intact tissue. The isolated perfused lung technique offers a versatile experimental system which compliments isolated organelle preparation, tissue homogenization and the tissue slice preparation. In the perfused lung preparation, cells are maintained in a normal anatomical and physiological relationship and are not dispersed or fragmented. Moreover, intracellular cofactors are not diluted as occurs with homogenate and incubation preparations, and cellular contents do not leak into the medium from

transected cells as occurs with the tissue slice preparation. The technical information provided, hopefully, will be of benefit to new investigators who intend to use the isolated perfused lung preparation for their biochemical investigation on lung injury.

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