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An Isolated Perfused Rat Lung Preparation

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An isolated perfused rat lung preparation (IPL) is described and its physiologic status is evaluated. The evaluation includes light and electron microscopy after perfusion and estimations of substrate utilization, ATP content, lactate production, and incorporation of glucose carbons into lipids and CO_2. It is concluded that the IPL is useful for short-term metabolic and physiologic experiments and offers some unique advantages in evaluating effects of reactive gases upon lung function.

The isolated perfused rat lung (IPL) preparation to be described was developed by O'Neil and Tierney (*1*). I have used their techniques to study rat lung metabolism during pulmonary edema induced by NO_2.

The advantages of an IPL for the study of lung physiology and metabolism include the relative metabolic simplicity of any isolated organ. For some purposes the IPL may be the best method to explore the interactions between pulmonary mechanics and pulmonary metabolism or to study acute metabolic effects of highly reactive gases such as NO_2 or O_3. The mechanical and metabolic effects of pulmonary edema can be studied simultaneously using an IPL. For some problems, other techniques may be more appropriate. Tissue slice methods are less time-consuming than the IPL and are useful techniques to investigate metabolic pathways, but give limited information about the relationship between metabolism and lung mechanics. Cell separation techniques may be required to investigate the metabolism of specific cell types.

For some tasks the first consideration should be the choice of an experimental animal. We have used rats for our studies and we recognize that chronic murine pneumonitis is pandemic, unless great care is used to avoid pulmonary infection. We obtain specific pathogen-free cesarean derived animals (Hilltop) weekly and we use only those lungs which are grossly normal. Light microscopy has shown few round cells near the airways. Other experimental animals should be carefully screened in order to assure normal lungs at the beginning of any experiment. We also chose the rat because of its convenient size. The 1-g rat lung is large enough to produce easily measurable metabolic products and to utilize substrates at reasonable rates but does not require large equipment or perfusate volumes for its maintenance. Regional differences in blood flow and ventilation are probably smaller than might be encountered in the lungs of larger animals.

The following factors must be considered for any IPL preparation: surgery, ventilation, perfu-

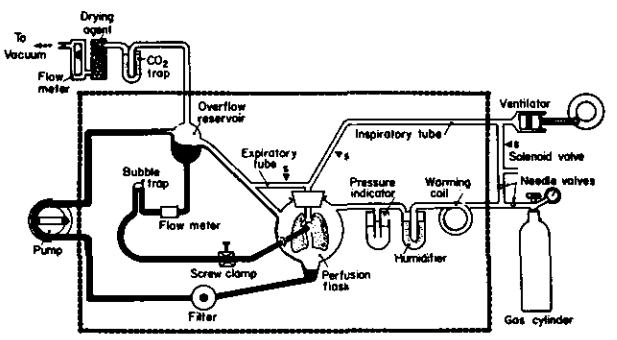

FIGURE 1. Schematic diagram of apparatus used to perfuse isolated lungs. From O'Neil and Tierney (*1*), with permission of the authors and publisher.

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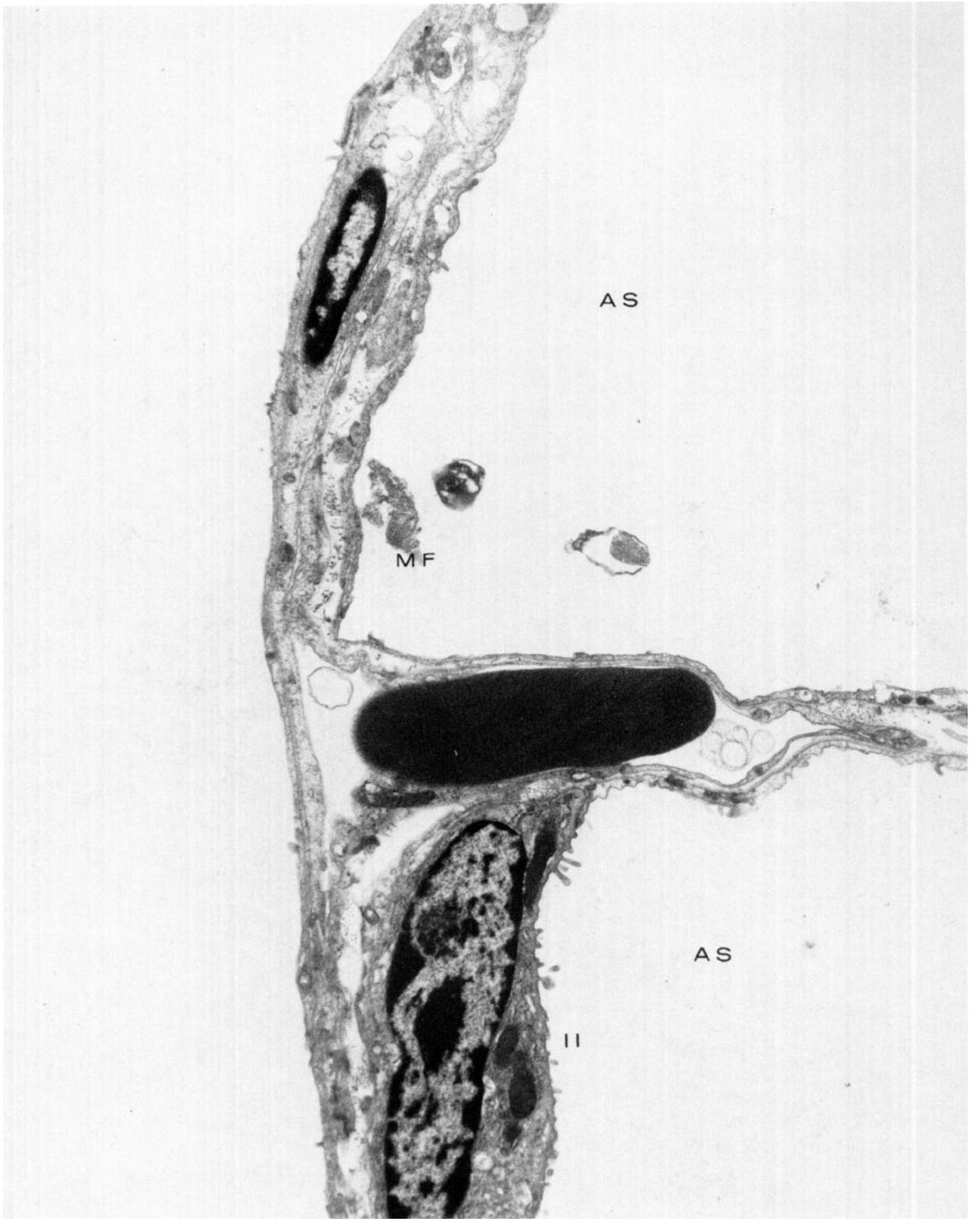

FIGURE 2. IPL after 90 min of perfusion and fixed by tracheal instillation of 2.5% glutaraldehyde. The intersection of three alveolar septae is seen. An erythrocyte is present within the capillary space and myeline figures (MF) are within an alveolar space (AS). A type II epithelial cell (II) is seen in the lower alveolar space. 2226 ×.

sion, lymphatic flow, the heterogeneity of the lung, and anesthetics.

In the surgical procedure; we are careful to minimize the animal's blood loss or trauma to the lung from handling. The animals are continuously ventilated with oxygen after the chest is opened. Perfusion of the lung begins *in situ* after cannulation of the pulmonary artery and is interrupted for less than 1 min during the entire isolation procedure. The surgical time is about 10 min.

Ventilation includes distending pressure, respiratory frequency, tidal volume and negative versus positive airway inflation pressures. O'Neil and Tierney reported results obtained with an airway pressure of 10-15 cm H_2O above atmospheric at a volume of 2.5 ml and a frequency of 13. We have used a distending pressure of 15 cm H_2O, a tidal volume of 2.5 ml, and a frequency of 50 with similar results. We used a positive end expiratory pressure of 2-3 cm H_2O. By comparison, a resting 250-g rat has a ventilatory frequency of 100-110 and tidal volumes of 2-3 ml (*2*). Higher inflation pressures lead to the early development of pulmonary edema. An artificial perfusion medium was chosen to simplify the metabolic studies we have been most interested in, although other investigators have used anticoagulated blood. A disadvantage of blood is that it has significant metabolic activity which may affect the accuracy of estimating pulmonary metabolism even when corrections are made for the activity of the medium. Aged blood is less active but retains its O_2-carrying capacity. We used Kreb's bicarbonate buffer, pH 7.4 at 37°C, for the electrolyte content of our perfusate. To each 100 ml we added 5 g bovine serum albumin (FrV fatty acid poor), 75 mg glucose, 12 mg palmitate, 50 mg mixed amino acids (Stuart-hydrolyzate of casein) and 100 μl of saline washed rat erythocytes. Flow rates averaged about 12 ml/g lung/min. This is

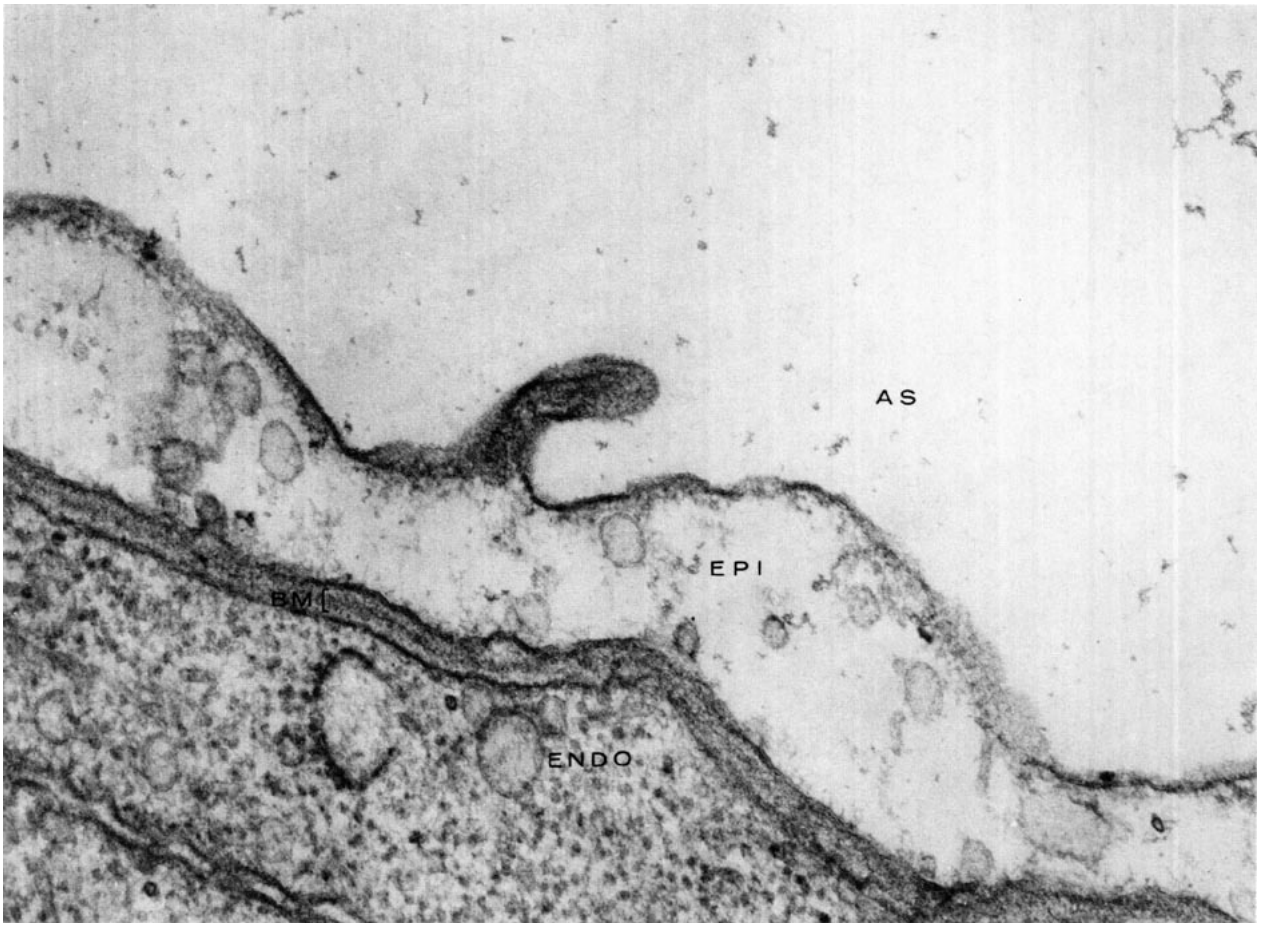

FIGURE 3. Swelling of type I epithelial cell (EPI) with decrease in electron density of cytoplasm. IPL after 90 min. The basement membrane (BM) and capillary endothelial cell (ENDO) appear normal. 28,322×.

about one-half that estimated for *in vivo* pulmonary blood flow in the 250-g rat. The bronchial circulation is not perfused. The effects of this lack of perfusion are unknown.

Lymphatics are disrupted by the isolation procedure, and their physiological status is unknown. The effects of the disruption upon lung extracellular water content or upon the flow of capillary transudate into the lymphatics have not been evaluated.

The separate metabolic contribution of airways, blood vessels, and macrophages may be significant, but are largely unknown. O'Neil lavaged free macrophages from the alveolar surface and found they contribute less than 10% of the $^{14}CO_2$ produced from 1-^{14}C glucose (*1*). Slices of the trachea and mainstem bronchi also produce little $^{14}CO_2$ from labeled glucose. Quantitative answers to questions about the contributions of specific cell populations in the lung may require other techniques such as cell separation.

As an anesthetic we used a volatile gas, halothane, to anesthetize the donor animal. Because of its high partition coefficient and the brief period of halothane exposure, we assumed the IPL was free of anesthetic during most of the experiment. Tierney has demonstrated that some anesthetics, including halothane, increase lactate production by lung tissue (*3*), and we are concerned about the effects of anesthetics upon estimations of metabolism.

Figure 1 is the schematic of the gas and perfusion tubing for this IPL. The perfusate is lifted by a rotary pump to an overflow reservoir; pulmonary flow is nonpulsatile and is measured by introducing a bubble into the tubing and timing its passage through a calibrated tube. A bubble trap and filter are incorporated into the tubing. A gas mixture of 95% O_2 and 5% CO_2 is warmed, humidified, and passed over the lung and perfu-

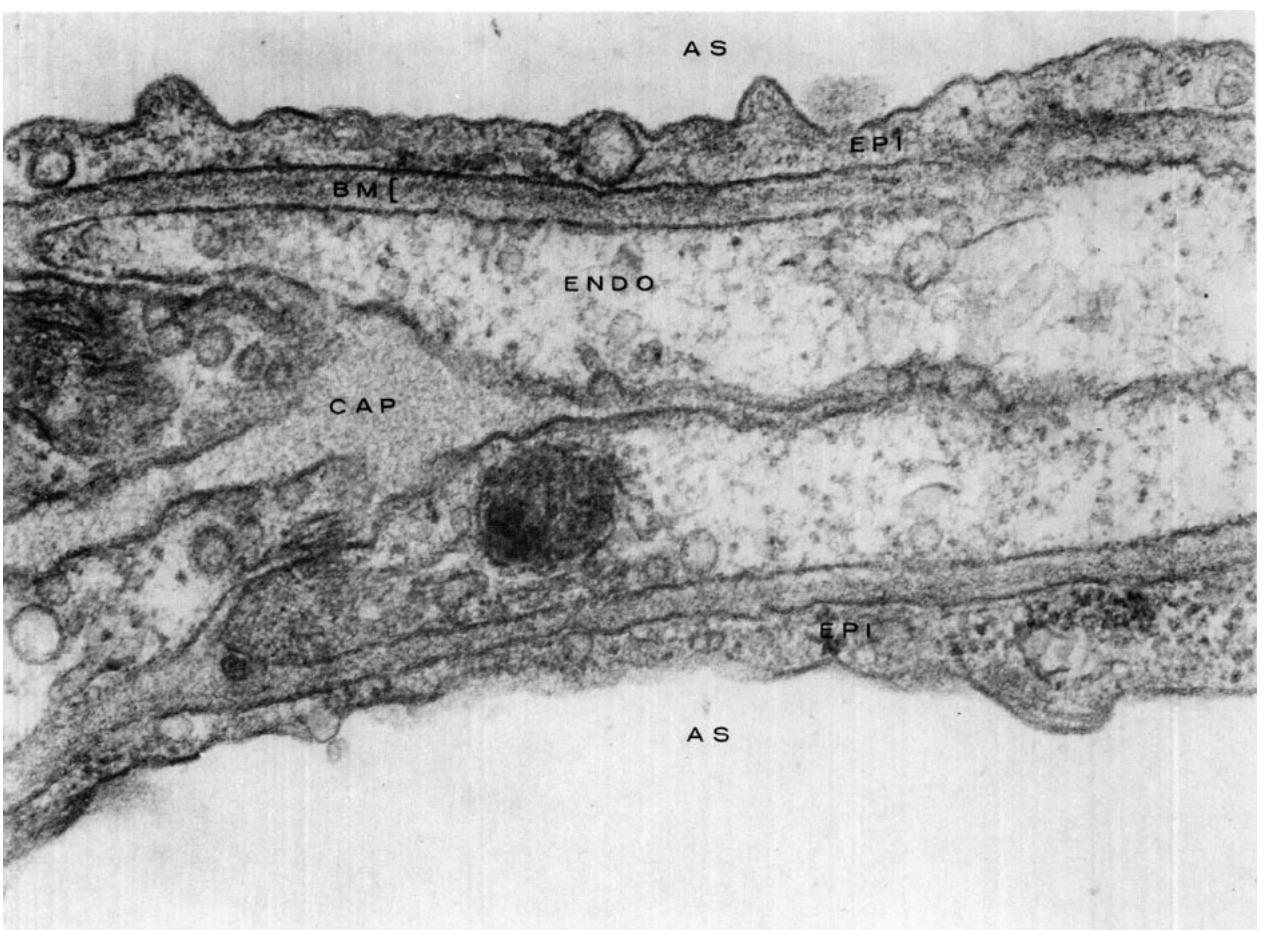

FIGURE 4. Intact type I epithelium (EPI) with swelling and decreased electron density of capillary endothelium (ENDO). IPL after 90 min. The capillary lumen (CAP) appears narrowed in this section. 28,592 ×.

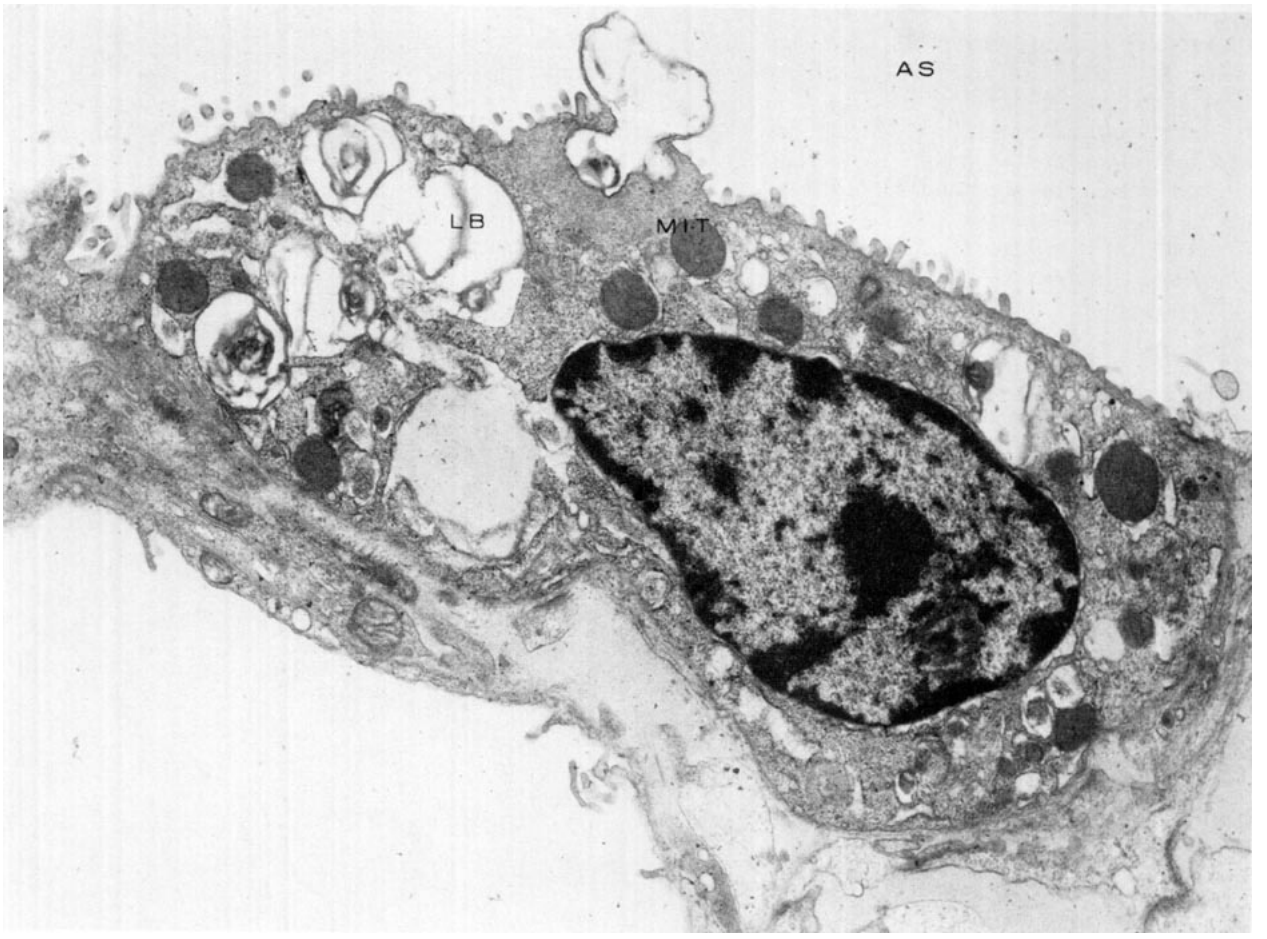

FIGURE 5. Type II cell from IPL after 90 min of perfusion. The surface bleb probably represents release of a lamellar body (LB). Mitochondria (MIT) appear normal in size and electron density. 20860 ×.

sate before being collected by bubbling through a 2*N* NaOH solution. A small animal respirator ventilates the lungs with the same mixture, and the exhaled gas is also drawn through the NaOH solution. The apparatus is enclosed in a heated box at 38°C. At the end of a 90-min experiment, the lungs have a normal static compliance and weight. The distribution of blood flow, as estimated by adding carbon black to the perfusate 1 min before ending an experiment, remains uniform. We are unable consistently to perfuse lungs longer than 2 hr without edema developing.

O'Neil and Tierney measured the lung's glucose consumption and lactate production to be 11.2 and 12.1 μmole/g lung-hr, respectively. I found that our IPL consumed 13 μmole glucose/g lung-hr and produced 14 μmole lactate/g lung-hr. Lungs which became edematous on exposure to high (20-30 ppm) levels of NO_2 demonstrated a large increase in their glucose consumption (*4*). Incorporation of ^{14}C from labeled glucose into lipids, including saturated lecithins, was similar to *in vivo* rates (*4*).

Postlethwait and I measured ATP in rat lung using the luciferase assay (*5*). We found 1.55 (SD±0.10) μmole ATP/g after anesthesia but before isolation or perfusion. Lungs which had been isolated and perfused for 90 min contained 1.95 (0.15) μmole/g. By contrast, tissue slices had a decreased ATP content of 0.80 (0.05) μmole/g immediately after slicing which returned to 1.60 (0.15) μmole/g after 90 min of incubation.

We have used light and electron microscopy to help evaluate the integrity of the IPL. Light microscopy shows the IPL to have perivascular edema after perfusion for 90 min. This frequently occurs even though the lungs had no detectable gain in weight based upon body weight or DNA estimation. The injected carbon black particles are present in all alveolar capillaries. Electron

microscopy reveals most of the sampled alveolar septae to be normal in appearance (Fig. 2). Rare areas of intracellular edema of type I cells (Fig. 3) and capillary endothelial cells (Fig. 4) were seen. We have not attempted to quantitate the alveolar capillary barrier thickness but it was generally normal in appearance and no areas of disruption were seen. Type II cell inclusions and organelles were well preserved (Fig. 5). Alveolar macrophages and interstitial cells appeared normal.

Although useful for periods of 2 hr or less and time-consuming to prepare, the IPL is one of several valuable methods for the study of pulmonary mechanics and metabolism. It may be the best preparation for those studies which require an intact ventilated lung.

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