

# Salt and water transport across alveolar and distal airway epithelia in the adult lung

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**Matthay, Michael A., Hans G. Folkesson, and A. S. Verkman.** Salt and water transport across alveolar and distal airway epithelia in the adult lung. *Am. J. Physiol.* 270 (*Lung Cell. Mol. Physiol.* 14): L487–L503, 1996.—Substantial progress has been made in understanding the role of the distal airway and alveolar epithelial barriers in regulating lung fluid balance. Molecular, cellular, and whole animal studies have demonstrated that reabsorption of fluid from the distal air spaces of the lung is driven by active sodium transport. Several different *in vivo*, *in situ*, and isolated lung preparations have been used to study the mechanisms that regulate fluid transport in the normal and injured lung. Catecholamine-dependent and -independent regulatory mechanisms have been identified that modulate fluid transport, probably by acting on apical sodium channel uptake or the activity of the Na,K-ATPase pumps. Recently, a family of molecular water channels (aquaporins) has been identified that are small (~30 kDa) integral membrane proteins expressed widely in fluid-transporting epithelia and endothelia. At present, four different water channels have been identified in trachea and lung. Measurements of osmotic water permeability in *in situ* perfused lung and isolated perfused airways suggest a significant contribution of these molecular water channels to measured water permeability. However, further studies are required to determine the role of these water channels in normal pulmonary physiology and disease. Recent studies have provided new insights into the role of the alveolar epithelial barrier in clinical and experimental acute lung injury. Unlike the lung endothelium, the alveolar epithelium is resistant to several clinically relevant types of injury, including endotoxemia and bacteremia as well as aspiration of hyperosmolar solutions. In addition, even when the alveolar barrier has been injured, its capacity to transport edema fluid from the distal air spaces of the lung recovers rapidly. Future studies need to integrate new insights into the molecular mechanisms of alveolar epithelial sodium and water transport with functional studies in the normal and injured lung.

aquaporins; pulmonary edema; acute lung injury; adult respiratory distress syndrome; alveolar epithelium;  $\beta$ -adrenergic agonist

WITH FEW EXCEPTIONS, the general paradigm for transepithelial fluid movement is that active salt transport drives osmotic water transport. This paradigm is probably valid for fluid clearance from the distal air spaces of the lung (5, 11, 24–26, 70). The results of several *in vivo* studies have demonstrated that changes in hydrostatic or protein osmotic pressures cannot account for the removal of excess fluid from the distal air spaces (5, 9, 11, 26, 64, 66, 67, 69, 70). Furthermore, specific inhibitors of sodium transport have been shown to inhibit alveolar fluid clearance in the lungs of different species, including humans (11, 55, 65, 82, 104, 116). Until recently, the pathways for water clearance across

alveolar and distal airway epithelium were unknown. However, recent studies suggest that a significant fraction of the osmotically driven water transport in the lung across alveolar and distal airway epithelium involves transcellular movement facilitated by plasma membrane water channels (30, 31). In light of an improved understanding of the role of the lung epithelial barrier in regulating lung fluid balance, it has now been possible to study the transport properties of the alveolar barrier under pathological conditions.

This review focuses on the mechanisms of salt and water transport across alveolar and distal airway epithelium of the adult lung. The first section will review

briefly important structure-function relationships in distal airway and alveolar epithelium. The second section describes and evaluates several different preparations that have been used to study fluid transport in the distal air spaces of the lung. Evidence for active sodium transport as a mechanism for regulating *in vivo* alveolar fluid clearance is presented in the third section, including a discussion of catecholamine- and non-catecholamine-dependent mechanisms for stimulating fluid transport. The fourth section reviews new evidence for involvement of transcellular water channels in alveolar and distal airway fluid transport, and the final section describes how the normal capacity of the alveolar epithelial barrier to transport salt and water is altered by exposure to endotoxin, bacteria, acid, and hyperosmolar solutions.

#### STRUCTURE-FUNCTION RELATIONSHIPS IN PULMONARY EPITHELIUM

The human lung consists of a series of highly branched hollow tubes that end blindly in the alveoli, with the conducting airways (the cartilaginous trachea, bronchi, and the membranous bronchioles) occupying the first 16 generations of airways (85). Gas exchange primarily occurs in the branches that make up the last seven generations, including respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli (118). The airways and alveoli constitute the interface between lung parenchyma and the external environment and are lined by a continuous epithelium. The thin alveolar epithelium (0.1–0.2  $\mu\text{m}$ ) covers more than 95% of the air space surface area and contains thin, squamous type I cells and cuboidal type II cells (118) (Fig. 1). The close

apposition between the alveolar epithelium and the vascular endothelium facilitates efficient exchange of gases but forms a tight barrier to leakage of liquid and proteins from the interstitial and vascular spaces, thus assisting in maintaining relatively dry alveoli (136).

The tight junctions are the critical structures for the barrier function of the alveolar epithelium. Tight junctions connect adjacent epithelial cells near their apical surfaces, thereby maintaining apical and basolateral cell polarity (109). Ion transporters and other membrane proteins are asymmetrically distributed on opposing cell surfaces, conferring vectorial transport properties to the epithelium. In addition, the tight junction itself might contain discrete ion-selective pores (45). In the past, it was thought that tight junctions were rigid structures that physically restrict passage of larger molecules; however, it has been shown recently that the permeability of tight junctions is dynamic and is regulated, in part, by cytoskeletal proteins and intracellular calcium concentration (109). Physiological studies of the barrier properties of tight junctions in the alveolar epithelium indicate that diffusion of water soluble solutes between alveolar epithelial cells is much slower than through the intercellular junctions of the adjacent lung capillaries (46, 84, 108, 110, 117). Based on tracer fluxes of small water-soluble solutes across the air-blood barrier of the distal air spaces, the effective pore radii were 0.5–0.9 nm in the distal respiratory epithelium and 6.5–7.5 nm in the capillary endothelium (120). In addition, studies of protein flux across both the endothelial and epithelial barriers of the sheep lung indicate that 92% of the resistance to albumin flux

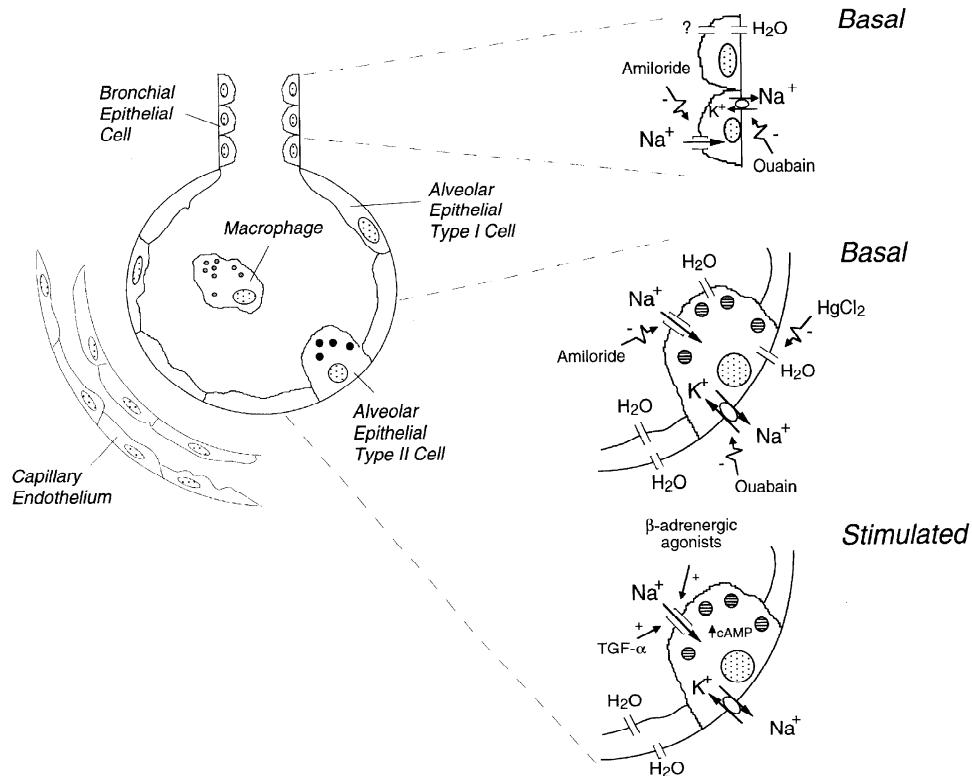


Fig. 1. Schematic diagram of the major pathways for ion and water transport across alveolar and distal airway epithelium under basal and stimulated conditions. Other known membrane transporters that are not involved directly in vectorial fluid transport, such as  $\text{Na}^+/\text{H}^+$  exchange and  $\text{K}^+$  conductance, are not depicted. See text for further explanations.

across the alveolar barrier resides in the epithelium (44). Removal of large quantities of protein from the air spaces occurs primarily by restricted diffusion (51, 52).

The most extensively studied cell in the distal pulmonary epithelium is the alveolar type II cell, partly because type II cells can be readily isolated from the lung and studied *in vitro*. The alveolar type II cell is responsible for the secretion of surfactant as well as the vectorial transport of sodium from the apical to the basolateral surface (42, 63). The active transport of sodium by type II cells provides the major driving force for removal of fluid from the alveolar space. Sodium uptake occurs on the apical surface, partly through amiloride-sensitive channels, and then sodium is pumped actively from the basolateral surface into the lung interstitium by the Na,K-ATPase (Fig. 1). An epithelial sodium channel that participates in sodium movement across the cell apical membrane has been recently cloned and characterized (15), and work by other investigators has provided new insight into the molecular and biochemical basis for sodium uptake in alveolar epithelial cells (81, 133, 141). A full discussion of this sodium channel and its potential implications for alveolar epithelial sodium transport will be presented in a subsequent review.

Recent studies also indicate that alveolar type II cells contain functional water channels (31). The epithelial type I cell covers most of the surface area of the alveoli, but there is no direct evidence that type I cells are involved in active sodium transport. Preliminary studies suggest that some Na,K-ATPase may be present in type I cells (119); however, since type I cells cannot yet be isolated and grown in culture, it is difficult to know for certain if Na,K-ATPase is involved in vectorial transport, as opposed to simply maintaining cell volume. Recent experiments indicate that transcellular water transport in the lung may be facilitated by specific water-transporting proteins, including CHIP28 or aquaporin (AQP)-1 (channel-forming integral membrane protein of 28 kDa), MIWC (mercury-insensitive water channel or AQP-4), and AQP-5 (31, 48, 96). In addition, it has been demonstrated that the distal airway epithelium actively transports sodium, a process that depends on amiloride-inhibitable uptake of sodium on the apical surface and extrusion of sodium through a basolateral Na,K-ATPase (2, 4) (Fig. 1). These cells also probably have specific transcellular water transport proteins that may be important in bidirectional movement of water across the airway epithelia (30, 34).

#### EXPERIMENTAL APPROACHES FOR MEASUREMENT OF ALVEOLAR FLUID TRANSPORT

There are a number of complementary experimental lung preparations that have been used to measure salt and water transport across the alveolar and distal airway epithelia. Some, such as the isolated perfused lung, are suitable only for short-term, 1- to 2-h studies. In contrast, some *in vivo* preparations permit studies in unanesthetized animals for up to 24 h. Recently, new insights into the transport properties of distal lung

epithelia have been facilitated by the development of novel preparations, including the *in situ* and *ex vivo* nonperfused lung, a new preparation to study transport across isolated perfused distal airways, and a new surface fluorescence method to quantify intra-alveolar fluid composition noninvasively. This section describes and evaluates the advantages and disadvantages of each preparation.

*Isolated perfused distal airways.* Recently, a novel approach was developed to measure osmotic and diffusional water permeability across distal isolated intact airways (30). Small airways (100- to 200- $\mu\text{m}$  diameter, 1- to 2-mm length) from guinea pig lung were microdissected and perfused *in vitro* using concentric holding and perfusion pipettes. Osmotic water permeability ( $P_f$ ) was measured by luminal perfusion with PBS (300 mosmol) containing a membrane-impermeable fluorophore, fluorescein sulfonate (FS), and the airway was bathed in solutions with different osmolalities ranging from 50 to 600 mosmol.  $P_f$  was determined from the change in FS fluorescence at the distal end of the airway resulting from transepithelial water transport. Diffusional water permeability ( $P_d$ ) was measured by  $\text{H}_2\text{O}/\text{D}_2\text{O}$  exchange using the  $\text{H}_2\text{O}/\text{D}_2\text{O}$ -sensitive fluorescent probe aminonaphthylene trisulfonic acid (ANTS) in the lumen. The airway was perfused with PBS in  $\text{H}_2\text{O}$  and bathed in isosmolar  $\text{D}_2\text{O}$ .  $P_d$  was determined from the change in fluorescence at the distal end of the airway. Prior conventional studies of osmotic and diffusional water permeability in kidney epithelia have utilized radioisotope tracers such as iodinated albumin for  $P_f$  and  $^3\text{H}_2\text{O}$  for  $P_d$ . Radiotracer studies require the technically difficult collection of luminal perfusate at the end of the tubule and thus cannot provide real time  $P_f$  and  $P_d$ . The new techniques enable the continuous real time measurement of airway  $P_f$  and  $P_d$  without the need for collection and analysis of the perfusate. In addition, the perfusion/fluorescence approach has potential applications in the measurement of transepithelial ion and solute transport when suitable ion-sensitive indicators are perfused through the lumen (130). A distinct advantage of the isolated perfused airway is that unambiguous information about the transport properties of the airway is obtained. A disadvantage is the technical difficulty of the microperfusion procedures.

*Isolated perfused lung.* Several investigators have used the isolated perfused lung preparation for short-term studies (5, 20, 26, 39, 43, 100). The lungs are prepared for study immediately after removal from the chest by cannulation of the main pulmonary artery and a sufficient portion of the left atrium to collect pulmonary venous outflow. Most of the experiments have been done in isolated rat, rabbit, or hamster lungs. The air spaces are fluid filled with an isosmolar, physiological solution that can include a radioactive solute such as  $^{22}\text{Na}$ . The lungs are usually not ventilated. The lungs are perfused with a physiological solution containing albumin using a roller pump that controls the rate of blood flow with continuous measurement of the inflow pressure in the pulmonary artery. Instillation of a test

solution into the air spaces is done via the trachea, generally with a volume of 5–7 ml in the rat lung. The appearance of radioactivity in the perfusate provides information on the unidirectional solute transport and net fluid transport. Labeled mannitol or sucrose in the air space solution can be used to measure flux across the paracellular pathway. The concentration of a labeled macromolecule in the air spaces, such as albumin or dextran, indicates the volume of the instilled fluid that has been removed from the air spaces. The change in the weight of the lung as whole indicates net fluid movement between the lung and the perfusate.

Isolated perfused lung experiments have particular value because the unidirectional flux of a specific ion can be measured directly and the effects of ion transport agonists or antagonists can be examined. Conclusions from these studies are based on the assumption that net transepithelial transport of a solute is coupled to osmotic flow of water out of the air spaces. In addition, the contribution of passive fluid movement through a paracellular route can be quantified.

There are several disadvantages of isolated lung preparations. The isolated lung does not have an intact bronchial or lymphatic circulation. The perfusion rates through the pulmonary circulation are lower than in the intact lung, partly because of an increase in pulmonary vascular resistance that develops in all isolated lung preparations. Thus pulmonary perfusion will not be uniform. The preparation is suitable for only short-term experiments because a generalized increase in lung endothelial permeability to protein develops after 60–120 min. In addition, net flux of solutes will be affected by potential alterations in both surface area and permeability, both of which are difficult to quantify or control in this preparation.

*In situ lung preparations.* Various *in situ* lung preparations have been developed. In isolated goat or sheep lungs, perfusion has been accomplished by an external pump and alveolar fluid clearance measured without removing the lungs from the thorax (5, 111). In this preparation, the animal is exsanguinated rapidly and then the shed blood (sometimes diluted with an isosmolar salt solution) can be used as the perfusate through a cannula inserted into the main pulmonary artery. The perfusate is then collected through a large cannula in the left atrium and passed through a heat exchanger to control temperature; the perfusate is then reinfused at the specified flow rate. Pulmonary vascular resistance is determined from the pulmonary arterial and left atrial pressures measured with catheters adjacent to the inflow and outflow cannulas. The *in situ* preparation has the advantage of preserving lung lymphatic drainage and avoiding the trauma of removing the lungs from the thorax. In addition, lung lymph flow can be collected during the experiment to provide an index of transvascular fluid flux (105). *In situ* lung experiments can be continued for up to 4 h. This preparation was used in an *in situ* goat lung to study the effect of temperature on alveolar fluid transport (111). A similar *in situ* preparation in sheep lungs was used to study osmotic water permeability of the alveolar epithelium

(31). These studies provided evidence for the importance of active ion transport as well as the involvement of water channels in removing excess fluid from the distal airspaces of the lung.

The major limitations of *in situ* preparations are the absence of a systemic (bronchial) circulation and the limited time that the preparation is usable, ~4 h. Interestingly, this *in situ* preparation provided the first evidence that the alveolar epithelium remains intact for several hours without any blood flow or ventilation (105). These studies established the basis for studies of alveolar epithelial fluid clearance in an isolated, human lung preparation (104) described below.

*A surface fluorescence method to measure transalveolar transport of water and solutes in an *in situ* perfused lung.* A quantitative approach was developed recently to measure transalveolar transport processes in lungs perfused either *in situ* or *ex vivo* (17). The strategy is to fill the air space via the trachea with a physiological solution containing a membrane-impermeant fluorescent dye. Confocal microscopy demonstrated that intra-alveolar dye fluorescence can be detected selectively by front surface epi-illumination. Initial studies of water permeability were carried out in mouse lung. Osmotic water permeability was measured from the time course of fluorescence of air space fluorescein isothiocyanate-dextran in response to a vascular-to-airspace osmotic gradient. Changes in perfusate osmolality produced prompt changes in alveolar dye concentration as a consequence of osmotic water movement, giving a high and weakly temperature-dependent  $P_f$  (>0.02 cm/s). Diffusional water permeability ( $P_d$ ) was measured from the time course of  $H_2O/D_2O$  exchange, using ANTS in the air space. Surface fluorescence changed very fast ( $t_{1/2} \sim 20$  s) in response to addition of 50%  $D_2O$  to the perfusate, giving a  $P_d$  of ~0.002 cm/s. This surface fluorescence method is potentially applicable to permeability studies in lungs of any species and with the use of pH or ion/solute-sensitive fluorophores (130) for the measurement of acid and solute permeabilities. The use of fluorescence to quantify intra-alveolar water/solute content permits the continuous monitoring of transport processes and does not require fluid sampling from the distal air spaces.

*Nonperfused lung preparations.* Based on the finding that alveolar fluid clearance can be studied in lungs without perfusion or ventilation for up to 4 h (105), a new preparation was recently developed that has been particularly useful for studying alveolar fluid clearance in excised human lungs (104). The human lung tissue is usually a surgically resected lobe of one lung, often from a patient with a bronchogenic carcinoma. The resected lobe is inflated with 5–7 cm $H_2O$  end-expiratory pressure and 100% oxygen and is maintained at 37°C with an external incubator. The experimental test solution, usually 40–50 ml of isosmolar 5% albumin, is instilled into the distal bronchus of one segment of the lobe and then advanced into the distal air spaces with a single inflation. Alveolar fluid clearance is measured by the concentration of albumin in the air spaces over 1, 2, or 4 h. Because the instilled solution contains albumin

alone, it is possible to measure accumulation of immunoglobulins from the vascular or interstitial space of the lung as an index of altered epithelial permeability. Despite the lack of perfusion, only trace quantities of immunoglobulin appear in the air spaces, indicating that the lung epithelial barrier maintains its normal relative impermeability to protein during the 4-h time period of the experiment. Also, the rate of sodium transport and alveolar fluid clearance is constant for 4 h.

Recently, the preparation has been adapted to measure alveolar fluid clearance in isolated nonperfused lungs of rats and mice for 1–4 h (102). The nonperfused lung is also useful to measure alveolar epithelial barrier fluid transport in lungs that have altered endothelial permeability because of prior lung injury (99). The lack of perfusion eliminates the confounding effect of increased transvascular fluid and protein flux that may occur in injured lungs and permits the independent assessment of the permeability and transport properties of the alveolar epithelium.

The lack of perfusion constitutes the principal disadvantage of this preparation. The preparation is viable for only a few hours, and the lung does not have normal lymphatic drainage. Also, the presence of unstirred layers because of the lack of perfusion makes this model inappropriate for measuring high solute permeabilities and water permeability generated by osmotic gradients.

**In vivo lung studies.** Protein concentration in the distal air spaces provides an index of alveolar fluid clearance in vivo because fluid is removed from the alveoli much more rapidly than protein (8, 67). In most in vivo preparations, the test solution (serum, plasma, or 5% albumin) is instilled into distal air spaces of one lung and the concentration of the instilled labeled (such as  $^{125}\text{I}$ -albumin) and unlabeled protein (albumin or total protein) is measured over time to estimate alveolar liquid clearance. With this approach, it was demonstrated that alveolar protein concentration increases progressively over 4, 12, and 24 h after instillation of an isosmolar protein solution into the distal air spaces of sheep lung (Fig. 2) (67). Similar studies have been carried out in other large animals (goats and dogs) (9, 67, 70, 111) as well as in smaller animals (rabbits and rats) (55, 116). In these larger animals, lung lymph flow can be measured to provide a quantitative index of fluid and protein flux across the lung endothelial barrier. This makes it possible to study animals in which lung endothelial permeability has been increased by a pathologically relevant stimulus, such as endotoxin or bacteria, in the same experiment in which net alveolar transport of fluid and protein is measured (89, 137). It is also possible in large animal studies to restrict surgically the pulmonary circulation to one lung to examine the effect of reductions in blood flow on alveolar fluid transport (56). Because sheep and goats are relatively cooperative and docile, it has been possible to study unanesthetized animals over 24–48 h (8). Anesthetized, ventilated rabbits and rats are suitable for 4- to 8-h studies. These in vivo animal studies have established the validity for studying alveolar fluid

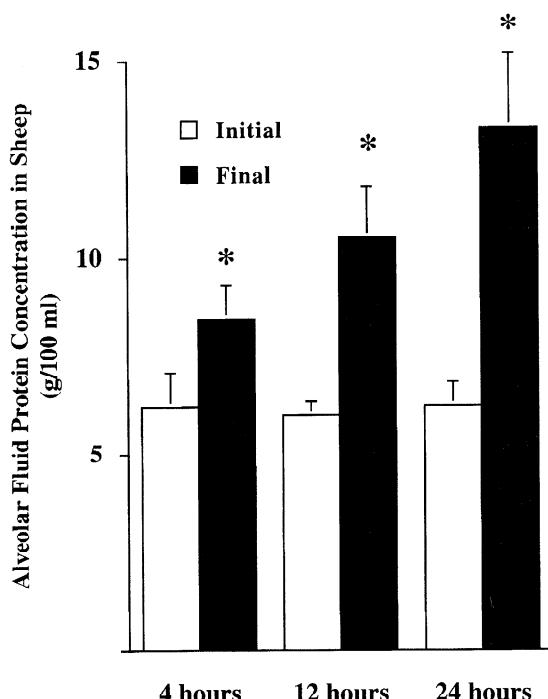


Fig. 2. Data from experiments in unanesthetized sheep in which 100 ml of autologous serum were instilled into one lower lobe. After 4, 12, or 24 h, an alveolar sample was obtained for total protein concentration. There was a progressive concentration of alveolar protein at each time period, indicating a continuous removal of alveolar fluid. By 24 h, ~80% of the alveolar fluid had been removed. Data expressed as means  $\pm$  SD; \* $P < 0.05$  compared with initial protein concentration. Data from Ref. 67.

clearance in humans by measuring protein concentration in sequential alveolar fluid samples obtained from patients with pulmonary edema (51, 73).

In contrast to the isolated perfused lung preparation in which extravascular fluid gain or loss can be continuously measured, fluid balance in the in vivo studies can only be measured at the conclusion of the experiment. The gravimetric method is the gold standard for measuring extravascular lung water; it has a low coefficient of variation, even in small animal studies (70, 116). After instillation of a known volume of fluid into the air spaces of one lung, the removal of the fluid from the lung as a whole can be determined by measuring the total water content of the instilled lung and then subtracting the water content of the contralateral noninstilled control lung. To calculate extravascular lung water, the blood volume of each lung is measured for subtraction of the water content of the blood from the total water content of the lung. To make these gravimetric calculations more accurate, corrections have been made for the dry weight that is added to the lung, either in the form of protein that is instilled into the air spaces in the instillate (serum, plasma, or 5% albumin) or in the form of plasma protein that may accumulate in the extravascular space when there is altered lung endothelial permeability (11, 116, 137).

The principal disadvantage of the in vivo lung preparations relates to uncertainty regarding the precise alveolar and distal airway epithelial surface area available for reabsorption of fluid from the distal air spaces

of the lung. The same limitation applies, of course, to any intact or isolated lung preparation. Some efforts have been made to control for surface area by specifying the degree of lung inflation or by using different degrees of fluid filling of the lung. One group of investigators fluid filled one lung in anesthetized, ventilated rabbits and measured alveolar fluid clearance from the fluid-filled lung at different inflation pressures; the decline in the level of the fluid meniscus in a bronchial cannula was used as the index of fluid absorption (128). In other species, smaller volumes of fluid have been instilled into the distal air spaces of the lung (1–3 ml/kg), but the absolute rates of fluid clearance from the non-fluid-filled lung were similar regardless of the volume instilled (55, 67, 70, 116), suggesting that the absolute surface area is not a major limiting factor. Intact lung preparations do not distinguish between absorption across alveolar and airway epithelium; however, the alveolar epithelium makes up 99% of the available absorptive surface of the lung, so quantitatively the distal airways probably contribute minimally to net fluid absorption. Once alveolar fluid is absorbed from the air spaces, it then must cross the lung interstitium into the circulation. The precise vascular surface that is available for reabsorption of interstitial fluid can only be estimated. Pulmonary blood flow to the fluid-instilled portions of the lung can be measured with radioactive or fluorescently labeled microspheres (11, 70), but the exact perfusion pressure in the microcirculation of the lung can only be estimated from the difference between pulmonary arterial and left atrial pressures. However, this may not be a major issue, since several studies have shown that isosmolar absorption of fluid in the lung is not limited by blood flow (11, 27, 105).

#### EVIDENCE FOR ACTIVE SODIUM TRANSPORT AS A MECHANISM FOR IN VIVO ALVEOLAR FLUID CLEARANCE

For many years, it was generally believed that differences in hydrostatic and protein osmotic pressures (Starling forces) accounted for removal of excess fluid from the air spaces of the lung (117). This misconception persisted in part because experiments that were designed to measure solute flux across the epithelial and endothelial barriers of the lung were done at room temperature (121). Also, these studies were done in dogs, a species that has a very low rate of alveolar epithelial sodium and fluid transport (9). However, in the early 1980s, experimental work from both in vivo and in vitro studies provided direct evidence that active sodium transport drives alveolar fluid transport across the alveolar barrier. The principal findings of the in vivo studies are summarized below.

*In vivo studies.* Experiments in anesthetized or unanesthetized sheep over 4, 12, and 24 h indicated that spontaneous alveolar fluid clearance occurs in the face of a rising alveolar protein concentration (67, 70). The final alveolar protein concentration exceeded plasma protein concentration by 3–6 g/100 ml (Fig. 2). The same pattern was documented subsequently in hu-

mans in the resolution phase of pulmonary edema (73). The final alveolar protein concentration in some patients exceeded 10 g/100 ml, with a simultaneous plasma protein concentration of 5–6 g/100 ml. These observations indicated that an active ion transport mechanism was responsible for the removal of alveolar fluid.

If active ion transport were responsible for alveolar fluid clearance, then alveolar fluid clearance should be temperature dependent. In an *in situ* goat lung preparation, the rate of alveolar fluid clearance progressively declined as temperature was lowered from 37°C to 18°C (111). Similar results were obtained in an isolated rat lung preparation in which hypothermia inhibited alveolar sodium transport (100). In the isolated human lung, alveolar fluid clearance ceased when temperature was lowered to 20°C (104). In addition, if active ion transport were primarily responsible for alveolar fluid clearance, then the elimination of transpulmonary hydrostatic pressure generated by ventilation should not alter the rate of alveolar fluid clearance. Studies in rabbits and sheep indicated that rate of alveolar fluid clearance was unchanged in the absence of ventilation (56).

Additional evidence for active fluid transport was obtained in intact animals with the use of amiloride, an inhibitor of sodium uptake by the apical membrane of alveolar epithelium and distal airway epithelium. Amiloride inhibited 40–70% of basal alveolar fluid clearance in sheep, rabbits, rats, and in the human lung (55, 67, 104, 116), similar to the data obtained in isolated rat lung preparations (5, 20, 26). Amiloride also inhibits sodium uptake in distal airway epithelium from sheep and pigs (2, 4). To further explore the role of active sodium transport, experiments were designed to inhibit the Na,K-ATPase in alveolar type II cells. It is difficult to study the effect of ouabain in intact animals because of cardiac toxicity. However, in the isolated rat lung, ouabain was shown to inhibit >90% of clearance (6). Subsequently, with the development of an *in situ* sheep preparation for measuring alveolar fluid clearance in the absence of blood flow, it was demonstrated that ouabain inhibited 90% of alveolar fluid clearance over a 4-h period (105).

Important species differences in the basal rates of alveolar fluid clearance have been identified. To normalize for differences in lung size or the available surface area, different instilled volumes were used, ranging from 1.5 to 6.0 ml/kg. The slowest alveolar fluid clearance was measured in dogs (9), intermediate rates of alveolar fluid clearance were measured in sheep and goats (11, 67, 70, 111), and the highest basal alveolar fluid clearance rates were measured in rabbits and rats (55, 116). The basal rate of alveolar fluid clearance in the intact human lung has been difficult to estimate. The clearance in the *ex vivo* human lung is approximately half of the rate in the *ex vivo* rat lung (102). However, recent unpublished observations from our laboratory suggest that maximal alveolar fluid clearance in patients may be much higher than in the *ex vivo* human lung, a finding that agrees with earlier esti-

mates in the resolution phase of human alveolar edema (73). The explanation for the species differences is not apparent, although it may be related to the number or activity of sodium channels or the density of Na,K-ATPase pumps in alveolar epithelium in different species. However, morphometric studies (21, 86) show no significant difference in the number of alveolar type II cells in different species.

**Catecholamine-dependent regulation of alveolar fluid clearance.** Studies in newborn lambs suggested that endogenous release of catecholamines, particularly epinephrine, may stimulate reabsorption of fetal lung fluid from the air spaces of the lung (13, 134). In addition, studies of isolated alveolar type II cells indicated that sodium transport could be augmented with  $\beta$ -adrenergic agonists, probably by adenosine 3',5'-cyclic monophosphate (cAMP)-dependent mechanisms (41, 63, 64). The enhancement of alveolar fluid clearance by catecholamines was confirmed in short-term isolated perfused lung studies in which terbutaline increased alveolar fluid clearance and the effect was inhibited by propranolol (20, 27, 43). Subsequent experiments in isolated lungs (40, 107) and in vivo studies provided further evidence that cAMP is the second messenger for the  $\beta$ -adrenergic effects, whereas activation of protein kinase C does not appear to be involved (7, 10).

In vivo studies over 4 h were carried out in anesthetized, ventilated sheep to examine the potential physiological factors that might influence alveolar fluid clearance, including systemic and pulmonary hemodynamics, pulmonary blood flow, and lung lymph flow. Terbutaline ( $10^{-5}$  M) was instilled with autologous serum into the distal air spaces of the lung (11). Terbutaline nearly doubled alveolar fluid clearance over 4 h in sheep, and the increase was 90% prevented by coadministration of amiloride in the instilled solution. Although terbutaline increased pulmonary blood flow, this factor was not important, since studies with nitroprusside, an agent that increased pulmonary blood flow to an equivalent degree, did not increase alveolar fluid clearance. There was an increase in lung lymph flow, a finding that reflected removal of some of the alveolar fluid volume to the interstitium of the lung. All of the  $\beta$ -adrenergic agonist effects were prevented by coadministration of propranolol into the air spaces. Subsequent studies have demonstrated that alveolar fluid clearance is markedly increased in intact rat and dog lungs by  $\beta$ -adrenergic agonists (9, 55). Interestingly,  $\beta$ -adrenergic agonist therapy does not increase alveolar fluid clearance in rabbits and hamsters (39, 116). The explanation for this lack of effect is unclear, particularly since there are  $\beta$ -receptors in rabbit type II cells that stimulate surfactant secretion (74).

Do  $\beta$ -adrenergic agonists increase alveolar fluid clearance in the human lung? Based on studies of the resolution of alveolar edema in humans, it has been difficult to quantify the contribution of endogenous catecholamines to the basal alveolar fluid clearance rate (73). However, recent studies of alveolar fluid clearance in the isolated human lung have demon-

strated that  $\beta$ -adrenergic agonist therapy increases alveolar fluid clearance, and the increased clearance can be inhibited with propranolol or amiloride (Fig. 3) (103, 104). The magnitude of the effect is similar to that observed in other species, with a  $\beta$ -agonist-dependent doubling of alveolar fluid clearance over baseline levels. This data is particularly important based on recent evidence indicating that aerosolized  $\beta$ -agonist therapy with salmeterol, a long-acting lipid soluble  $\beta$ -agonist, markedly increased the rate of alveolar fluid clearance in sheep (14). Also, there is recent data that the long-acting lipid-soluble  $\beta$ -agonists are more potent than hydrophilic  $\beta$ -agonists in the ex vivo human lung (102). Thus aerosolized  $\beta$ -agonist treatment in some patients with pulmonary edema might accelerate the resolution of alveolar edema.

**Non-catecholamine-dependent mechanisms that stimulate alveolar fluid clearance.** In addition to the well-studied effects of  $\beta$ -adrenergic agonists, there is recent evidence that several non-catecholamine-dependent pathways can increase the rate of alveolar fluid clearance. Epidermal growth factor is a well-known epithelial cell mitogenic and motogenic factor (72). New work indicates that this growth factor can upregulate alveolar epithelial sodium transport. Incubation of isolated alveolar type II cells with epidermal growth factor for 24–48 h increases their capacity to transport sodium (12, 54). In addition, transforming growth factor- $\alpha$  (TGF- $\alpha$ ) recently has been reported to increase alveolar fluid clearance in anesthetized, ventilated rats (33). The addition of 50 ng/ml of TGF- $\alpha$  in the instilled

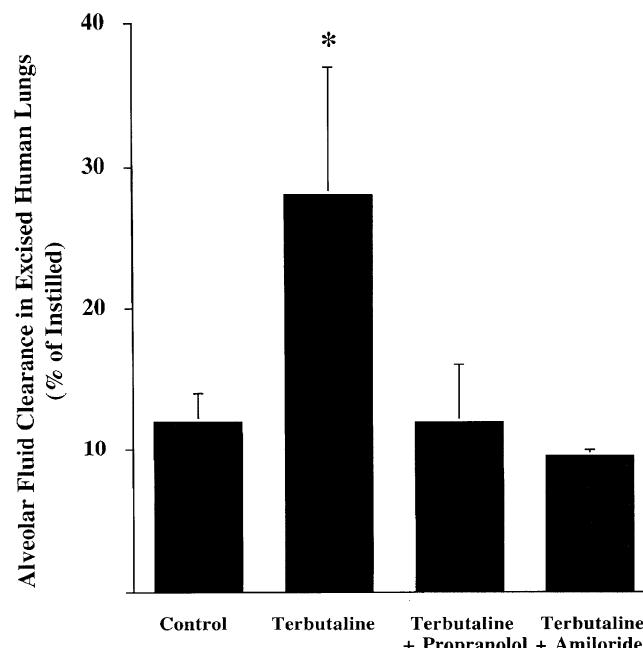


Fig. 3. Clearance of alveolar fluid in excised human lungs instilled with an isosmolar albumin solution over 4 h. Alveolar fluid clearance was calculated from the concentration of the instilled albumin in the air spaces. Terbutaline ( $10^{-4}$  M), a  $\beta$ -adrenergic agonist, more than doubled alveolar fluid clearance, an effect that was inhibited with propranolol ( $10^{-4}$  M) or by amiloride ( $10^{-4}$  M). Data are expressed as means  $\pm$  SD; \* $P < 0.05$  compared with other experimental conditions. Data from Ref. 104.

fluid increased alveolar liquid clearance by 45% over 1 h and by 53% over 4 h. This increase was similar to the 50% increase in alveolar fluid clearance in rats treated with a  $\beta$ -agonist (33). Interestingly, since cAMP was only minimally increased in isolated alveolar type II cells exposed to TGF- $\alpha$ , it is likely that the TGF- $\alpha$  effect is mediated by an alternative signal transduction pathway.

New evidence suggests that cytokines may stimulate sodium uptake and alveolar fluid clearance. It is well known that alveolar instillation of endotoxin or exotoxin releases several proinflammatory cytokines from alveolar macrophages. For example, exotoxin A from *Pseudomonas aeruginosa* can stimulate alveolar fluid clearance in rats by a non-catecholamine-dependent pathway (90). In a recent study, instillation of endotoxin from *Escherichia coli* into the distal air spaces of rats upregulated epithelial sodium transport and alveolar fluid clearance for up to 48 h by catecholamine-independent mechanisms (38). The mechanisms for the endotoxin effect may depend on release of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), since a monoclonal antibody against TNF- $\alpha$  inhibited the increase in alveolar fluid clearance that occurred 24 h after instillation of bacteria into the distal air spaces of the rat lung (98).

Proliferation of alveolar epithelial type II cells may provide another non-catecholamine-dependent mechanism for increasing net sodium and water transport across the alveolar epithelial barrier. Recent work with bleomycin-injured rat lungs indicates that hyperplasia of alveolar type II cells contributes to increased alveolar fluid clearance, especially in the subacute phase after acute lung injury (80). In addition to an increase in the number of alveolar type II cells, there may also be an oxidant-dependent mechanism that increases the sodium transport capacity of individual type II cells exposed to hyperoxia for several days (50, 77, 83, 141), although not all studies of hyperoxia demonstrate this effect (16, 119).

#### WATER TRANSPORT IN AIRWAYS AND LUNG

**A family of molecular water channels.** The existence of specialized water-transporting proteins had been proposed for many years based on biophysical measurements showing that osmotic water permeability in erythrocytes and certain kidney tubules was high and

weakly temperature dependent (for review see Ref. 131). Evidence from radiation inactivation (123) and expression of heterologous mRNAs in *Xenopus* oocytes (145) suggested that the putative water channel was an ~30-kDa protein encoded by a single mRNA. A family of related water-transporting proteins (aquaporins) was subsequently identified over the past 4 years (1, 127, 132). Each member of the family is a small (~30 kDa) integral membrane protein with 30–50% amino acid sequence identity to the major intrinsic protein of lens fiber (MIP) and related proteins from plants, bacteria, and yeast (97). Hydropathy plots of these proteins are similar, suggesting up to six transmembrane helical segments. Homology in amino acid sequence between the first and second halves of each protein suggests genesis from tandem, intragenic duplication of a three-transmembrane segment.

Table 1 summarizes the properties of various mammalian water channel family members (aquaporins; AQP) cloned to date. MIP is expressed only in mammalian lens fiber (87). AQP-CD (AQP-2) is the vasopressin-sensitive water channel expressed exclusively in kidney collecting duct (37), and thus far it is the only water channel associated with human disease: non-X-linked congenital nephrogenic diabetes insipidus (22). cDNA WCH-3 (60) is also expressed only in kidney. The first localization of a water channel in lung was an *in situ* hybridization study showing diffuse expression of CHIP28 (AQP-1) transcript in the perialveolar region (49). Subsequently, a mercurial-insensitive water channel (MIWC, AQP-4) was cloned from a lung cDNA library (48), and two other proteins, glycerol intrinsic protein (GLIP; AQP-3) (53, 59) and AQP-5 (96), were cloned from other sources and then found to be expressed in trachea and/or lung.

**Structure and function of CHIP28.** The majority of molecular-level information comes from studies on CHIP28, an integral membrane protein identified initially in the plasma membrane of erythrocytes (91). CHIP28 is easily purified in milligram quantities from blood cell membranes and forms mercurial-insensitive water-selective channels when reconstituted into liposomes (124, 142) or when expressed in *Xenopus* oocytes (92, 143) or mammalian cells (61). Residue cysteine 189 has been shown to be the site at which mercurials inhibit CHIP28 water permeability (94, 144). Fifty

Table 1. Properties of mammalian water channel family members

Original Names Alternate Names	MIP26	CHIP28 AQP-1	WCH-CD AQP-2	WCH-3	MIWC AQP-4	GLIP AQP-3	AQP-5
Protein size (amino acids)	263	268	265	271	301	285	282
mRNA size (kb)	1.4	2.8	1.9	2.2	5.5	5.5	1.6
N-linked glycosylation	–	+	+	–	–	+	+
Mercurial inhibition	–	+	+	?	–	+/-	+
Transport function	?	water	water	?	water	glycerol ? urea/water	water
Human gene locus	12q13	7p14	12q13	12q13	18q22	9p13	?
Tissue expression	lens	wide	kidney	kidney	wide	wide	wide

Major intrinsic protein (MIP) family members expressed in mammalian tissues are listed (left to right) in the order in which they were identified and cloned. Data on mRNA and protein are given for rat and chromosomal locus for human. Data are derived from Refs. 37, 48, 59, 60, 87, 96, 143, 144.

percent of CHIP28 monomers are modified by NH<sub>2</sub>-linked glycosylation with a polylactosamine sugar at residue Asn42 (126). The single-channel water permeability of CHIP28 is low ( $5 \times 10^{-14}$  cm<sup>3</sup>/s) (124) so that the membrane density of CHIP28 must be quite high ( $>10^3$   $\mu\text{m}^{-2}$ ) to increase water permeability above the background level related to water diffusion across membrane lipids. CHIP28 is expressed in many fluid-transporting tissues, including kidney proximal tubule and thin descending limb of Henle, choroid plexus, iris, ciliary body, placental syncytiotrophoblast, gallbladder, colonic crypt, and several reproductive tissues (47, 49, 78, 79, 101).

Freeze-fracture electron microscopy indicated that CHIP28 is assembled in tetramers in proteoliposomes reconstituted with purified CHIP28, transfected CHO cells, and native kidney cell membranes (129). However, target size determination (123) and expression of heterodimers containing wild-type and mutant CHIP28 subunits (113) indicated that CHIP28 monomers within tetramers function independently. Structure studies by circular dichroism (125) and analysis of epitope-tagged chimeric constructs (93, 115) suggested that CHIP28 contains multiple membrane-spanning helical domains. Two general models of CHIP28 structure have been proposed: 1) an "hour-glass" model in which regions of  $\beta$ -sheet comprise the intramembrane water pore (57), and 2) an amphipathic helix model in which water moves through an aqueous channel created by transmembrane helical domains (115). Low-resolution electron microscopy of 2-dimensional CHIP28 crystals at 12- to 20-Å resolution confirmed the tetrameric association of monomers in reconstituted proteoliposomes (76, 135). A recent study by cryoelectron crystallography visualized in each monomer six putative helical domains nearly normal to the bilayer, surrounding a central opening possibly representing the entrance to the water-selective channel (75).

**MIWC (or AQP-4).** AQP-4 was the first MIWC identified (48) and is expressed in multiple tissues, including the basolateral plasma membrane of tracheal and bronchial epithelia, kidney collecting duct, ependymal and astroglial cells in brain, iris, ciliary body, and in nuclear layer of retina in eye, gastric parietal cells, colon surface epithelium, skeletal muscle, and various secretory glands (34, 35). MIWC has greatest homology to the big brain protein of *Drosophila*. MIWC forms mercurial-insensitive water-selective channels when expressed in *Xenopus* oocytes (48). The lack of mercurial sensitivity is due to absence of cysteine residues in the vicinity of the two conserved NPA motifs (114). Analysis of MIWC biogenesis and topology indicated that MIWC contains six membrane-spanning domains with NH<sub>2</sub>- and COOH-termini in the cytoplasm (112). Recently a human homologue (hMIWC) of rat MIWC was cloned (140). Two distinct hMIWC cDNAs were found: clone hMIWC1, encoding 301 amino acids, and hMIWC2, which contains a distinct 5'-sequence upstream from bp -34 in clone hMIWC1 and two additional in-frame translation start codons. Analysis of hMIWC gene structure indicated two distinct but over-

lapping transcription units from which multiple hMIWC mRNAs are transcribed. Genomic Southern blot analysis and in situ hybridization indicated the presence of a single copy hMIWC gene at location 18q22. Recent freeze-fracture electron microscopy studies showed that MIWC forms square orthogonal arrays of particles in cell membranes (140a).

**GLIP (or AQP-3).** Another MIP family member, assigned the names GLIP and AQP-3, was cloned by three laboratories (23, 53, 59). GLIP is most homologous to the bacterial glycerol facilitator protein GlpF. Initial immunolocalization studies indicated GLIP expression in basolateral membrane of kidney collecting duct (53, 59); all groups reported that GLIP (AQP-3) transported glycerol when expressed in *Xenopus* oocytes; however, Ma et al. (59) found little or no increase in osmotic water and urea permeability. GLIP is coexpressed with MIWC in several tissues, including trachea, kidney collecting duct, brain ependymal cells, and colon surface epithelium (34). In addition, GLIP (but not MIWC) is expressed in epidermis, urinary bladder transitional epithelium, and conjunctiva (35).

**AQP-5.** Another related mercurial-sensitive water channel, AQP-5, was cloned from a rat submandibular cDNA library (96). Northern blot analysis showed transcript expression in salivary gland, lacrimal gland, eye, trachea and lung; however, no information is available to date on AQP-5 localization in lung.

Figure 4 shows immunolocalization of water channel family members in airways and lung from rat. CHIP28 (AQP-1), MIWC (AQP-4), and GLIP (AQP-3) proteins were detected by immunostaining utilizing polyclonal antibodies. MIWC and GLIP are expressed in basolateral membrane of tracheal epithelia, and MIWC (but

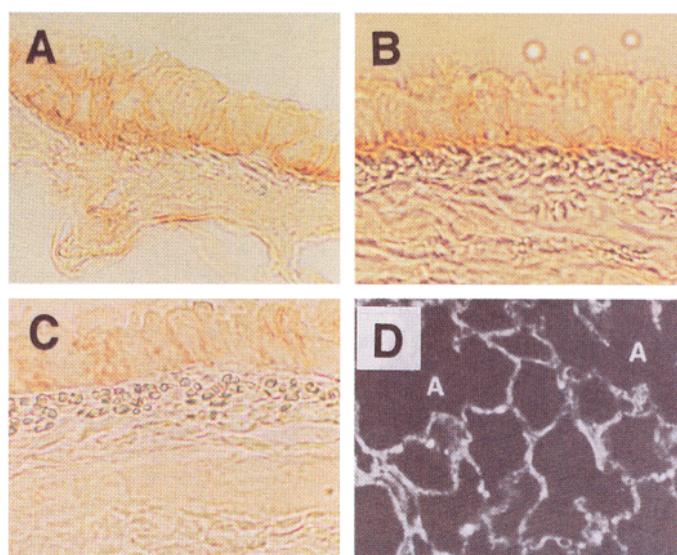


Fig. 4. Immunolocalization of water channels in rat airways and lung. Immunoperoxidase staining by a polyclonal anti-mercurial-insensitive water channel (MIWC) antibody shows localization to basolateral membrane of airways (A) and trachea (B). Glycerol intrinsic protein (GLIP) was found only in basolateral membrane of trachea (C). Positive staining is seen as the gold-colored membrane staining. Immunofluorescence of lung with anti-CHIP28 antibody shows extensive perialveolar staining (D). Data from Refs. 31, 34.

not GLIP) is expressed throughout large and small airways. CHIP28 is expressed predominantly in perialveolar capillaries and to a lesser extent on alveolar epithelium.

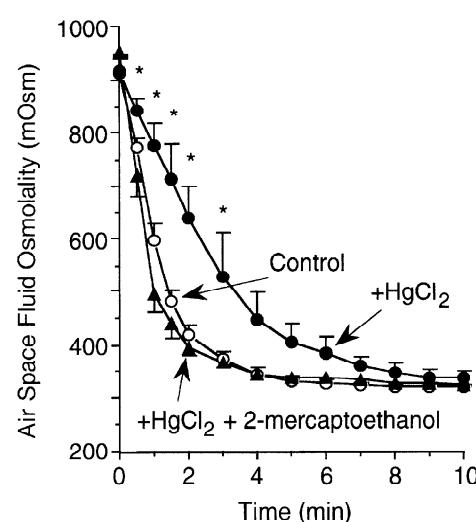
We recently obtained data on water channel transcript expression in the developing and postnatal lung using the RNase protection assay (122). Transcripts encoding CHIP28, MIWC, and AQP-5 are expressed at relatively low levels in lung before birth. CHIP28 mRNA increases strongly just after birth and remains elevated. MIWC mRNA increases strongly between days 1 and 2 after birth and decreases slightly over the first week. In contrast, AQP-5 mRNA slowly and progressively increases over the first week. Functional studies of water permeability and analysis of protein expression will be needed to define the physiological implications of these observations.

**Measurement of water permeability in lung tissue.** There have been few measurements of water permeability in lung. More than 20 years ago, Effros (24) demonstrated rapid translocation of solute-free water into the vascular space after injection of a hypertonic solution into the perfusate of isolated perfused lungs. However, direct evidence for the existence of specific transcellular water pathways in the lung was not available until recently, when a combination of molecular, cellular, isolated airway, and whole lung studies were utilized to test the hypothesis that osmotically driven water movement in the lung occurs across plasma membrane water channels (29, 31).

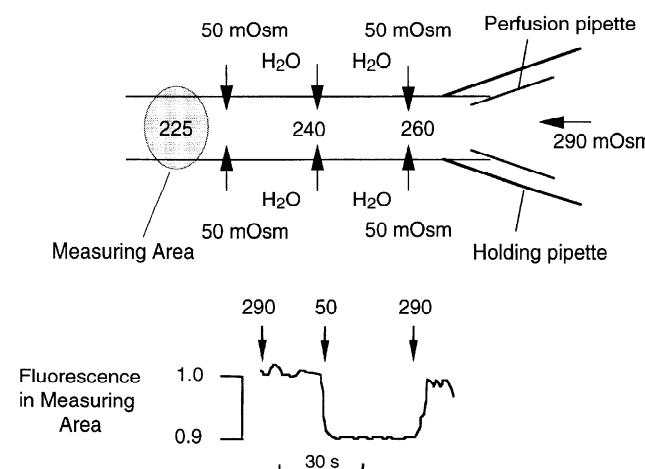
An *in situ*-perfused sheep lung model was utilized to measure transalveolar osmotic water permeability. Intact lungs were perfused continuously with an isosmolar dilute blood solution. Hypertonic fluid (900 mosmol) was instilled bronchoscopically into the air spaces, and the time course of water movement from capillary to airspace was deduced from the dilution of instilled radiolabeled albumin and from air space fluid osmolality (31). In control lungs, osmotically induced water movement was rapid (equilibration half-time  $\sim 45$  s) and had an apparent  $P_f$  of  $\sim 0.02$  cm/s, similar to that in erythrocytes (Fig. 5A). Water permeability in the contralateral lung was inhibited reversibly by  $\sim 70\%$  by  $HgCl_2$ . These results indicated that mercurial-sensitive water channels facilitated the transcellular movement of water between the airspace and capillary compartments in lung. High osmotic water permeability was as found recently in mouse lung utilizing a novel fluorescence method described earlier (17).

As described earlier in this review, we recently developed a strategy to measure osmotic and diffusional water permeability in intact airways (30). Small airways (100- to 200- $\mu m$  diameter, 1- to 2-mm length) from guinea pig lung were microdissected and perfused *in vitro* with fluorescent markers in the lumen (Fig. 5B). Osmotic water permeability ( $P_f$ ) was  $4-5 \times 10^{-3}$  cm/s at  $23^\circ C$  and independent of lumen flow rate and osmotic gradient size and direction. Temperature dependence measurements gave an activation energy of 4.3 kcal/mol, suggesting the passage of water through molecular water channels, consistent with the water

A



B



**Fig. 5.** A: transepithelial osmotic water permeability in intact sheep lung. Lungs were instilled with hyperosmotic saline (900 mosmol), and serial alveolar fluid samples were withdrawn and assayed for osmolality. Half-times for osmotic equilibration were (in min):  $0.85 \pm 0.1$  (control),  $2.7 \pm 0.4$  ( $HgCl_2$ ), and  $0.7 \pm 0.1$  ( $HgCl_2 + 2\text{-mercaptoethanol}$ ). Each data point is the mean  $\pm$  SE of measurements performed 3–6 times. \*Significant difference ( $P < 0.05$ ) compared with control values. B: measurement of transepithelial water permeability across the microperfused distal airways. *Top:* distal airway held by the concentric holding pipette with the perfusion pipette cannulating the lumen. The airway was perfused with a membrane impermeable fluorophore in isosmolar PBS and bathed in a hyposmolar solution. As water enters the airway lumen over the length of the distal airway, the osmolality and fluorescence intensity decrease. *Bottom:* representative recording of fluorescence signal. Arrows indicate change in bath solution osmolality. Calculated osmotic water permeability ( $P_f$ ) from this data was  $0.004 \pm 0.001$  cm/s. Data from Refs. 30, 31.

channel immunolocalization results described above. Osmotic water permeability has not been measured in epithelia of trachea and larger airways.

Despite the strong expression of several water channels in pulmonary tissues, the physiological relevance of these proteins remains to be proven. Mutations in water channel CHIP28 have been detected in humans (95). Genomic DNA analysis on three rare individuals who do not express CHIP28-associated Colton blood

group antigens demonstrated that two of them carried a CHIP28 pseudogene with different nonsense mutations, and another had a missense mutation encoding a nonfunctional CHIP28 molecule. Although red blood cells from these individuals had low osmotic water permeability, the subjects were phenotypically normal, raising questions about the physiological importance of CHIP28. Naturally occurring mutations in MIWC (AQP-4), GLIP (AQP-3), and AQP-5 have not as yet been identified. Definition of the physiological role of these proteins will require evaluation of knock-out transgenic animals or other suitable models and/or the identification of specific, nontoxic inhibitors of water channel function.

#### CLINICAL-PATHOLOGICAL IMPLICATIONS OF ALVEOLAR EPITHELIAL FLUID TRANSPORT

It is well established that increased permeability to protein is a hallmark of clinical acute lung injury. In a recent study, patients who died had threefold more protein lavaged from their distal air spaces than did patients who survived (18). The fluid transport capacity of the alveolar epithelial barrier under pathological conditions, particularly in patients with pulmonary edema and acute lung injury, has not been well studied. More than 10 years ago, clinical studies indicated that protein-rich pulmonary edema fluid can be collected from patients with acute lung injury, whereas patients with cardiogenic or hydrostatic pulmonary edema have a significantly lower protein concentration in the edema fluid (28, 68). However, there was no direct information in these clinical studies regarding the contribution of the epithelial barrier to the development or resolution of the alveolar edema. Until recently, pathological studies provided the only direct information regarding the status of the alveolar epithelial barrier in patients with acute lung injury. For example, post mortem studies of patients who die with acute lung injury report diffuse alveolar damage to both the endothelial and epithelial barriers of the lung with protein-rich edema fluid, inflammatory cells, and intra-alveolar exudate, pathological hallmarks of the pulmonary response to acute lung injury (36). Ultrastructural studies indicate widespread necrosis and denuding of alveolar epithelial type I cells, usually with some evidence of alveolar epithelial type II cell hyperplasia (3). However, these post mortem studies represent a biased sampling of only the most severe cases of acute lung injury. Clinical studies indicate that there is considerable heterogeneity in the fluid transport and barrier properties of the alveolar epithelial barrier of patients with acute lung injury (71, 73).

Two properties of the epithelial barrier can be assessed clinically. First, since the epithelial barrier is normally impermeable to protein, the quantity of protein that accumulates in the distal air spaces is a good index of epithelial permeability. Second, since concentration of protein in alveolar fluid reflects net clearance of alveolar fluid (Figs. 2 and 3), measurement of protein concentration in sequential alveolar edema fluid samples provides a physiological index of the ability of

the alveolar epithelial barrier to remove edema fluid. In one study, ~40% of patients were able to reabsorb some of the alveolar edema fluid within 12 h of intubation and acute lung injury (73). These patients had a more rapid recovery from respiratory failure and a lower mortality. In contrast, the patients who did not reabsorb any of the alveolar edema fluid in the first 12 h after acute lung injury had protracted respiratory failure and a higher mortality (Fig. 6). Based on clinical studies, the ability of the alveolar epithelial barrier to reabsorb alveolar edema fluid from acute lung injury within the first 12 h after acute lung injury is preserved in 30–40% of patients (Fig. 6) (73).

As illustrated in Table 2, many experimental studies have provided new insights into the function of the alveolar epithelial barrier under clinically relevant pathological conditions. In each of the studies, the primary focus was to assess the net fluid transport capacity of the alveolar and distal airway epithelium under specific physiological stresses as well as well-defined pathological insults. Interestingly, the results indicate that the alveolar and distal airway epithelium is remarkably resistant to injury, particularly compared with the adjacent lung endothelium. Even when mild-to-moderate alveolar epithelial injury occurs, the capacity of the alveolar epithelium to transport salt and water is often preserved. In addition, several

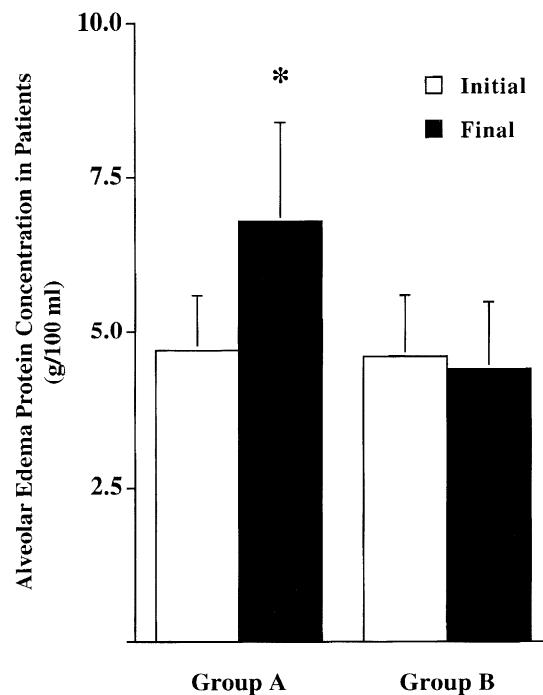


Fig. 6. Concentration (mean  $\pm$  SD) of protein in the alveolar edema fluid obtained from patients with acute lung injury. Initial sample was obtained within 0.5 h of endotracheal intubation; the subsequent sample was obtained within 12 h after intubation. Patients were classified into group A if their alveolar edema fluid protein concentration increased by 10% or more above their initial value, thus indicating net alveolar fluid clearance. Patients in group A had a more rapid recovery from respiratory failure and a lower mortality than patients in group B, thus suggesting that intact fluid alveolar fluid transport mechanisms in the early phase of acute lung injury provide a favorable prognostic indicator. \* $P < 0.05$  compared with initial protein concentration. Data from Ref. 73.

Table 2. *Effect of pathological conditions on alveolar epithelial fluid clearance in several species*

Pathological Condition	Species	Severity of Lung Injury		Alveolar Epithelial Fluid Clearance	Reference
		Endothelium	Epithelium		
No pulmonary blood flow	Sheep	None	None	Normal	105
Endotoxin, intravenous	Sheep	Mild	None	Normal	137
Endotoxin, alveolar	Sheep	None	None	Normal	137
Exotoxin, alveolar	Rat	None	None	Increased	38
Bacteria, intravenous	Rat	None	None	Increased	58, 90
	Sheep	Moderate/Severe	Mild/Severe	Increased/Decreased	89
	Rat	Mild	None	Increased	88
Bacteria, alveolar	Rabbit	Mild	None	Normal	88
	Sheep	Mild	Mild	Normal	137
	Rat	Mild	Moderate/Severe	Normal/Increased	98, 99
Oleic acid, intravenous	Rabbit	Mild	Moderate/Severe	Normal/Decreased	58, 139
Acid aspiration	Sheep	Moderate	Moderate	Normal	138
Salt water aspiration	Rabbit	Severe	Severe	Decreased	32
Hyperoxia	Rabbit	Mild	Mild	Normal	29
Drug induced (Bleomycin, intratracheal)	Rat	Moderate	Moderate	Normal/Increased	50, 77, 83, 119, 141
	Rat	Moderate	Mild	Increased	80

For intravenous and alveolar bacteria, the extent of injury to the epithelial and endothelial barriers is dependent on the dose and virulence of bacteria that are administered (88, 89, 98, 99, 139). For intravenous oleic acid, initially the injury after intravenous oleic acid is severe, but the alveolar epithelium recovers sufficiently after 4 h to remove some of the excess edema fluid (138).

mechanisms may result in an upregulation of the fluid transport capacity of the distal pulmonary epithelium, even after moderate-to-severe epithelial injury.

The first evidence demonstrating the resistance of the alveolar epithelial barrier to injury evolved from studies in which large numbers of neutrophils and monocytes crossed the tight alveolar epithelial barrier without inducing a significant change in either permeability to protein or the transport capacity of the alveolar epithelium. Instillation of autologous serum or plasma into the distal air spaces of sheep was associated with an influx of neutrophils and monocytes. Despite the influx of inflammatory cells, there was no increase in epithelial permeability to plasma protein; in addition, alveolar fluid clearance was normal (67). In a subsequent study in normal human volunteers, large numbers of neutrophils were recruited to the distal air spaces by instillation of the potent neutrophil chemotactic factor, leukotriene B<sub>4</sub>, without influx of plasma protein into the air spaces (62). Instillation of a hyperosmolar solution (sea water) into rabbit lungs caused a rapid translocation of a large volume of water into the distal air spaces as well as the influx of large numbers of neutrophils (29). However, there was only a transient change in epithelial permeability to protein. Moreover, after osmotic equilibration occurred, the rate of alveolar sodium and fluid transport was normal in rabbits (29) and in one well-described clinical case (19). Finally, recent data indicate that alveolar epithelial transport mechanisms in the human lung are not altered by 6–8 h of severe hypothermia (7°C) followed by rewarming to 37°C (106).

Even when lung endothelial injury occurs, the alveolar epithelial barrier may remain normally impermeable to protein and retain its normal fluid transport capacity (Table 2). For example, intravenous endotoxin or bacteria have been used to produce lung endothelial injury in sheep (137) or rats (88), but permeability to protein across the lung epithelial barrier was not

increased. When septic shock was produced in rats, there was a marked increase in plasma epinephrine levels. Even though endothelial injury and mild interstitial pulmonary edema occurred, alveolar epithelial fluid transport was increased from 45% over 4 h in control rats to 75% over 4 h in the septic rats. The effect was inhibited with instillation of amiloride ( $10^{-4}$  M) or propranolol ( $10^{-4}$  M) into the distal air spaces, proving that the stimulated clearance depended on β-agonist stimulation of alveolar epithelial sodium transport. When more severe septic shock was produced in sheep, the alveolar epithelial barrier was resistant to injury in the majority of sheep, with confinement of the edema to the pulmonary interstitium (Table 2) (89). In some sheep, however, more severe systemic and pulmonary endothelial injury was associated with alveolar flooding, a marked increase in epithelial permeability to protein, and the inability to transport fluid from the air spaces of the lung. The inability to remove excess fluid from the air spaces in these sheep was probably related more to a marked increase in paracellular permeability from injury to the epithelial tight junctions rather than to a loss of salt and water transport capacity of alveolar epithelial cells.

In some experimental studies, such as acid aspiration-induced lung injury (32), the injury to the epithelial barrier is so severe that recovery does not occur (Table 2). In other types of severe lung injury, as occurs from intravenous oleic acid, the initial injury to the tight junctions results in severe alveolar flooding, although recovery may occur within a few hours, presumably from reestablishment of the normal tight barrier characteristics of the alveolar barrier (138). A similar pattern of severe injury may develop in animal models of pneumonia in which large numbers of virulent bacteria are used (58), although studies with less virulent bacteria are associated with less epithelial injury and a preserved capacity to transport fluid from the distal air spaces of the lung (139).

In summary, although much has been learned about the resistance of the alveolar epithelial barrier to injury and its capacity for preserved transport function after injury, more work is needed to understand the local and systemic factors that regulate sodium and water transport across distal airway and alveolar epithelium under pathological conditions.

## PERSPECTIVE AND FUTURE DIRECTIONS

Recent studies have established that transport of sodium from the air spaces to the lung interstitium is the primary mechanism driving alveolar fluid clearance. While there are significant differences among species in the basal rates of sodium and fluid transport, the basic mechanism depends on sodium uptake by channels on the apical membrane of alveolar type II cells followed by extrusion of sodium on the basolateral surface by the Na,K-ATPase. This process can be upregulated by several catecholamine-dependent and -independent mechanisms. The identification of water channels expressed in lung, together with the high water permeabilities, suggest but do not prove a role for channel-mediated water movement between the air space and capillary compartments. Direct experimental evidence utilizing transgenic knock-out and specific water channel inhibitors is required to define the roles of specific water channels in lung physiology. Functional measurements will be needed to evaluate the role of water channels during perinatal lung development. In addition, there is a need to identify other proteins that facilitate water movement across the various cell types in lung. Finally, pharmacological modulation of airway and/or alveolar water permeability may have clinical applications, as has been proposed in kidney (132), where hypothetical water channel blockers (quaretics) are predicted to increase solute-free water clearance.

The application of this new knowledge regarding salt and water transport in distal airway and alveolar epithelium to pathological conditions has been successful in clinically relevant experimental studies, as well as in a few clinical studies. The studies of exogenous and endogenous catecholamine regulation of alveolar fluid clearance are a good example of how new insights into the basic mechanisms of alveolar sodium and fluid transport can be translated to clinically relevant experimental studies. For example, it is clear that exogenous catecholamines can increase the rate of alveolar fluid clearance in several species, including the human lung, and it is also apparent that release of endogenous catecholamines can upregulate alveolar fluid clearance in animals with septic or hypovolemic shock. It is possible that therapy with  $\beta$ -adrenergic agonists might be useful in hastening the resolution of alveolar edema in some patients, but the use of exogenous  $\beta$ -adrenergic agonists in some clinical conditions may be unnecessary if endogenous catecholamines are already elevated by the clinical condition, such as shock. In some patients, the extent of injury to the alveolar epithelial barrier may be too severe for  $\beta$ -adrenergic agonists to enhance the resolution of alveolar edema, although

several experimental studies indicate that alveolar fluid clearance can be augmented in the presence of moderately severe lung injury.

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