

# Electrolyte and fluid transport across the mature alveolar epithelium

GEORGES SAUMON AND GUY BASSET

*Institut National de la Santé et de la Recherche Médicale Unité 82, Faculté Xavier Bichat,  
75018 Paris, France*

SAUMON, GEORGES, AND GUY BASSET. *Electrolyte and fluid transport across the mature alveolar epithelium.* J. Appl. Physiol. 74(1): 1–15, 1993.—The lungs must be kept “dry” for efficient gas exchange. The mechanisms that contribute to clear alveoli from fetal lung fluid at birth are still present during adult life and allow recovery from alveolar flooding. It has recently been shown with the use of different approaches *in vitro*, as well as *in vivo*, that alveolar epithelium performs solute-coupled fluid transport. Fluid absorption from alveoli occurs chiefly as a result of active transepithelial  $\text{Na}^+$  transport. The mechanisms of  $\text{Na}^+$  transport have been partly elucidated;  $\text{Na}^+$  enters alveolar cells through apical  $\text{Na}^+$  channels and  $\text{Na}^+$ -coupled solute transporters and is pumped out at the basolateral membrane by a  $\text{Na}^+-\text{K}^+$ -adenosinetriphosphatase (ATPase). Transepithelial  $\text{Na}^+$  transport and fluid absorption are stimulated by  $\beta$ -adrenergic agonists, with adenosine 3',5'-cyclic monophosphate being the likely intracellular second messenger.  $\text{K}^+$  is probably secreted into alveoli because its concentration in the epithelial lining fluid is larger than expected for passive distribution.  $\text{K}^+$  channels have been described that, in conjunction with  $\text{Na}^+-\text{K}^+$ -ATPase, might provide pathways for active transport. Active proton secretion or bicarbonate absorption have been reported, which may explain the low pH of the alveolar epithelial lining fluid. It is probable that active solute transports are the main determinants of epithelial lining fluid depth and composition. A challenge for the future is to understand how this homeostasis is achieved.

pulmonary epithelial cells; ion channels; sodium channels; potassium channels; chloride channels; sodium-glucose symport; sodium-hydrogen antiport; sodium-coupled transports; sodium-potassium-adenosinetriphosphatase; pulmonary edema; epithelial lining fluid

DURING THE PAST DECADE, concepts concerning the mechanisms that keep alveoli free of fluid in adult lungs have been almost completely revised. The role of the alveolar epithelium is now considered prominent in performing this task. There is accumulating evidence that one of the distinctive features of epithelia, i.e., active ion and fluid transport, is shared by mature alveolar epithelial cells. The contribution of passive forces (63) to the regulation of fluid movement is probably much less important than originally thought. This concept emerged from observations *in vivo* (91) and in cultured alveolar epithelial cells (58, 87).

The alveolar epithelium forms a continuous layer that lines the alveoli. The epithelial surface is separated from the gas phase by a thin aqueous layer over which lies the

surfactant that acts to reduce the surface tension of the fluid at the air-surface interface (142). Alveolar epithelium is thought to be the main factor for maintaining the volume and composition of the epithelial lining fluid. The epithelium is composed of two main cell types: type I cells, which represent one 35–50% epithelial cell population depending on the species, and type II cells (37). In addition, a few type III cells (brush cells) that were described for the first time in rat alveoli (94) are present in most species, although at a different location within the respiratory tract (40). Type I cells are large (5,000–7,000  $\mu\text{m}^2$  depending on the species) flat thin cells that cover ~95% of the alveolar wall surface. They probably originate from type II cells that have been found to proliferate after injury (1, 49) and that preexist in fetal lungs (70).

Type II cells that are smaller ( $100\text{--}200 \mu\text{m}^2$ ) than type I cells and have a cuboidal shape are found in alveolar "corners." Type II cells have been extensively studied because they secrete surfactant. They share several features with bronchiolar nonciliated (Clara) cells (95).

#### METHODS USED TO EXPLORE ALVEOLAR TRANSPORT

The geometric complexity of mammalian lungs long precluded quantitative studies of alveolar epithelial transport. The simplest way to investigate epithelial transport properties is to fill the lungs with fluid (either *in vivo* or using isolated lungs) and to determine the rate at which water and solutes escape from the alveoli (or appear in the perfusate). This model has serious limitations. Because the composition of alveolar instillate is arbitrary, normal epithelial function may be altered. The molecular species studied must travel across successive barriers composed of epithelial and endothelial cell layers before reaching the perfusate and being cleared. They may accumulate in the interstitium, which may form an additional compartment. Also of possible importance is the lack of homogeneity; the lungs may be only incompletely filled by the instillate, perfusion may be unevenly distributed, or epithelial transport may vary from one site to another. Under such conditions, it is difficult to know with precision how large a fraction of epithelial surface contributes to solute transport. In addition, it is impossible to determine which cell type is responsible for active transport and, in particular, what is the contribution of the epithelium that lines the small airways. Because the rate of active ion transport is low compared with passive leakage, subtle variations are not easily demonstrated. *In vivo* models are much closer to normal physiological conditions because bronchial perfusion and lymphatic drainage are maintained. However, perfusate composition cannot be set, and use of some ion transport inhibitors is limited by their cardiac effects. It is therefore remarkable that most of the recent studies with fluid-filled lungs agree so well, suggesting that these flaws are not of major significance or that the conditions of possible bias are highly reproducible.

To overcome the difficulties related to the complexity of the lungs, epithelial transport has been explored with the use of epithelium grown *in vitro* from the only cells in which isolation does not raise overwhelming difficulties, namely type II cells. Membrane transport systems have also been characterized using freshly isolated cells or plasma membrane vesicles. The use of isolated cells raises its own potential difficulties: the isolation procedure may select subpopulations, purity may produce qualitative as well as quantitative variations, and seeding density and culture conditions may affect transport just as they affect phenotype and metabolism (34, 95, 112). In addition, transport processes may be altered because of enzymatic digestion during the isolation procedure. These processes may not be restored subsequently, even after several days of culture.

Other approaches that have proved fruitful include the use of amphibian lungs, the simpler shape of which allows them to be placed in Ussing chambers (53). Depending on the species, such lungs either secrete  $\text{Cl}^-$  or absorb

TABLE 1. *Ion transport mechanisms in the plasma membrane of mammalian alveolar epithelial cells*

Apical membrane	
$\text{Na}^+$ channels	
$\text{Na}^+$ -glucose cotransport*	*
$\text{Ba}^{2+}$ -sensitive $\text{K}^+$ channels*	*
Ouabain-sensitive $\text{K}^+$ pump*	*
Basolateral membrane	
$\text{Na}^+$ - $\text{K}^+$ -ATPase	
Undetermined localization	
$\text{Na}^+$ - $\text{H}^+$ exchange	
$\text{Na}^+$ -amino acid cotransports	
$\text{Na}^+$ -phosphate cotransport	
$\text{Na}^+$ - $\text{HCO}_3^-$ cotransport	
$\text{Cl}^-$ - $\text{HCO}_3^-$ exchange	
Delayed rectifier $\text{K}^+$ channels	
Low threshold $\text{K}^+$ channels ( $\text{Ca}^{2+}$ -activated $\text{K}^+$ channels?)	
$\text{H}^+$ -ATPase	
$\text{Na}^+$ - $\text{K}^+$ - $2\text{Cl}^-$ cotransport	
$\text{Cl}^-$ channels	

\* Transport processes that have not been demonstrated in isolated type II cells.

$\text{Na}^+$  and thus exhibit more or less resemblance with mammalian fetal respiratory epithelium or adult alveolar epithelium. Our present knowledge of the mechanisms of ion transport present in adult alveolar cells is presented in Table 1.

#### PASSIVE TRANSPORT PROPERTIES

One of the first well-recognized functions of alveolar epithelium is that of a tight barrier between the gas and blood phase. This barrier provides considerably more resistance to hydrophilic solute movements than the contiguous endothelium (27). The physical structures that form the barrier are the cell membranes (nearly impermeable to polar solutes in the absence of specialized pathways) and intercellular junctions.

Epithelial permeability to hydrophilic solutes that are not actively transported allows characterization of the pathways that account for passive transport. Wangensteen et al. (138) and Goodman and Wangensteen (61) evaluated the permeability of alveolar epithelium to lipid-insoluble small solutes in rabbit lungs. They found that solutes escaped from the alveoli at rates proportional to their respective diffusion coefficients in free solution, suggesting nonselective leakage. In contrast, other studies concluded that permeability is restricted depending on solute size, as if water-filled channels of finite size extend across the epithelium. This restriction property can be described in terms of equivalent aqueous pore populations. Since the work by Taylor and Garr (130), who identified a single population of pores of  $\sim 1$  nm radius in dog lungs, most studies suggest the presence of a large population of small pores and of one or two smaller populations of large pores. Berg et al. (10) determined the permeability to various small hydrophilic solutes ranging from water (0.15 nm radius) to raffinose (0.57 nm radius) in isolated fluid-filled rat lungs. They calculated that small solute permeabilities are consistent with the presence of a population of small pores (0.5 nm radius), accounting for 98.7% of the total pore population, and of a small population of pores of 3.4 nm radius. Others (31, 131), using solutes of larger sizes and parti-

cles up to  $\sim 100$  nm radius, concluded that very large pores are present in small numbers, allowing permeation of large solutes such as proteins, in agreement with the demonstration that albumin is detectable in the alveolar lining layer (16).

Because ions are actively transported, the rate of unidirectional transepithelial transport (apparent permeability) is the result of both active and passive components. Passive permeability to  $\text{Na}^+$  and  $\text{Cl}^-$  has been estimated in rat lungs by inhibiting active transport with drugs or by using a hypothetical model that allows for the separation of active and passive  $\text{Na}^+$  transport (7). Passive  $\text{Na}^+$  permeability is  $\sim 7 \times 10^{-8}$  cm/s in rat lungs, assuming an exchange surface area of 5,000 cm<sup>2</sup>. Passive  $\text{Cl}^-$  permeability is 50% larger (7). These permeabilities were found to correlate with the value simultaneously determined for mannitol (which does not cross the cell membrane but leaks through the paracellular pathway), indicating that passive transport of ions and mannitol occurs through the same pathway. The slopes of the relationships between passive  $\text{Na}^+$  and  $\text{Cl}^-$  permeabilities vs. mannitol permeability were slightly larger than expected for free diffusion in an aqueous medium (2.5 vs. 2 for  $\text{Na}^+$ -mannitol). A possible explanation is that mannitol diffusion in the paracellular pathway is more limited than ion diffusion because of the small radius of the more numerous pores (10). The electrical resistance deduced from  $\text{Na}^+$  and  $\text{Cl}^-$  permeability values is 18,000  $\Omega \cdot \text{cm}^2$  in rat lungs (8), a number that places the alveolar epithelium among the tight epithelia. It is possible that this large resistance is at least in part the consequence of the low density of intercellular junctions because most of epithelial surface is covered by the wide type I cells.

The permeability of amphibian lungs is greater than that of mammalian lungs. The electrical resistance of these epithelia is 700–1,400  $\Omega \cdot \text{cm}^2$  (52, 54, 73, 140). This difference may be the consequence of underestimation of the actual exchange surface area in amphibian lungs (52). Bullfrog alveolar epithelium has a heteroporous structure that resembles that of rat lungs. A large population of small pores (0.5 nm radius, 96% of available pore area) coexists with a small population of large (5.15 nm radius) pores (76).

The size of alveolar epithelium aqueous pores is 10 times smaller than that reported for pulmonary endothelium (110). This tightness explains why transepithelial ion transport may create an osmotic pressure difference in contrast to what happens in the capillaries. The oncotic pressure difference that is key to fluid equilibrium across capillary walls is only important because the reflection coefficient of the proteins in most capillaries is close to unity, whereas that of ions is  $<0.1$ . On the contrary, the reflection coefficient of the alveolar epithelium to small solutes like electrolytes is high (130), probably close to unity (46). Indeed, perfusion of isolated fluid-filled rat lungs with a solution hypertonic to the alveolar instillate did not remove significant amounts of  $\text{Na}^+$ ,  $\text{K}^+$ , or  $\text{Cl}^-$  from air spaces but did extract water (46). With such large reflection coefficients, a transepithelial small solute concentration difference of 1 mM, which is nearly undetectable, would correspond to a hydrostatic pressure difference of  $\sim 25$  cmH<sub>2</sub>O. Transepithelial transport of

solute would represent a powerful force driving water flow because water permeability is several orders of magnitude larger than small solute permeability (10, 36, 129). It has been demonstrated in other epithelia that active solute transport may overcome even larger forces. For example, fluid absorption against osmotic pressure differences of  $\sim 800$  mosmol/kgH<sub>2</sub>O has been described in the sheep colon (93). It follows that 1) only solutes that are actively transported across the epithelium are able to produce sustained fluid absorption, and 2) transepithelial concentration differences in macromolecules within the physiological range are too low to counteract concentration differences in small solutes resulting from transepithelial transport and, thus, cannot affect fluid absorption rate.

## SODIUM TRANSPORT AND FLUID TRANSPORT

Resorption of alveolar edema fluid has long been explained by the action of hydrostatic and, especially, by oncotic pressure differences that operate across the alveolus-airway barrier. Fluid removed from air spaces was thought to be cleared from the interstitium mainly by lymphatic pumping and by circulation. In high-permeability edema, the fluid that leaks from microvessels and fills the peribronchovascular cuffs and alveoli has a high protein content, and oncotic pressures do not differ between these compartments (135). However, alveolar edema resolves. To elucidate the mechanisms of alveolar edema fluid resorption, Matthay et al. (91) filled sheep lung lobes *in vivo* with autologous serum. They observed that water was removed faster than proteins and that protein concentration in air spaces increased with time as a result of water removal. Fluid absorption continued for hours despite a large ( $>50$  cmH<sub>2</sub>O) unfavorable pressure difference that was apparently not counterbalanced by any known physical interstitial or plasma force. Matthay et al. proposed that active transport by the epithelium lining the alveolus-airway barrier may contribute to alveolar fluid clearance. Indeed, such an active ion and fluid transport contributes to resorption of the fluid present in lungs at birth (17, 103). During fetal life, the lungs are filled with fluid that is produced as a result of  $\text{Cl}^-$  secretion by the pulmonary epithelium (108). However, before birth the rate of secretion declines. The decline has been shown to occur because of fluid absorption by the respiratory epithelium, a process that was enhanced by epinephrine and slowed by amiloride, a potent inhibitor of  $\text{Na}^+$  transport in epithelia (107). Is it possible that this process is still present in adults?

Directional transport of fluid is a process driven by transepithelial ion transport. Fluid absorption (movement from the apical to the basolateral side) usually follows  $\text{Na}^+$  transport, which is an active process that requires energy. The mechanism of active  $\text{Na}^+$  absorption conforms to the model proposed by Koefoed-Johnsen and Ussing (81):  $\text{Na}^+$  ions that enter epithelial cells at the apical membrane are pumped out of the cells at the basolateral membrane by the enzyme  $\text{Na}^+-\text{K}^+$ -adenosine triphosphatase (ATPase). Because of this continuous pumping,  $\text{Na}^+$  chemical potential is lower inside the cell. The entry step is passive;  $\text{Na}^+$  flows down the chemi-

cal potential gradient through specialized pathways, whereas basolateral transport requires energy to move ions against the gradient. Because of the pump activity,  $K^+$  electrochemical potential is larger inside the cell, and  $K^+$  leaks through the basolateral membrane and is then recycled by the  $Na^+-K^+$ -ATPase. The existence of pathways for  $Na^+$  entry into alveolar cells has been suggested by Jones et al. (68), who observed a high turnover rate of intracellular  $Na^+$  in isolated type II cells. Alveolar transepithelial  $Na^+$  transport is electrogenic, i.e., it produces a transepithelial electrical current and potential difference (3, 99, 100). The net current resulting from the sum of all the electrogenic transports is estimated as the transepithelial potential and is brought to zero in vitro in Ussing chambers. This current is thus called the short-circuit current ( $I_{sc}$ ).

Studies using type II cell cultures have demonstrated that alveolar cells from mature lungs are able to actively transport  $Na^+$  (59, 87). When type II cells are cultured on nonporous surfaces such as plastic, they adhere, spread, flatten, and form a continuous layer with distinct apical and basolateral plasma membrane domains. After 4–5 days, the monolayer is detached here and there from the substratum by small collections of fluid called domes (58, 59, 87). Domes are thought to be the consequence of active ion transport from the apical to the basolateral side with water following passively. Mason et al. (87) showed that monolayers of alveolar type II cells cultured on porous supports, such as filters that allow separation of apical and basolateral media, developed a small (1 mV, apical negative) potential difference that disappeared in the presence of inhibitors of  $Na^+$  transport. With the use of  $^{22}Na$  to trace  $Na^+$  movement, it has been shown that the unidirectional flux of  $Na^+$  in the apical to basolateral direction across such monolayers exceeds the flux in the reverse direction (25), keeping with findings in isolated lungs (7). The difference between the two unidirectional fluxes entirely accounts for  $I_{sc}$ , indicating that  $Na^+$  transport is the only factor responsible for the current flow observed in vitro (25). Active transport manifests as soon as cultured cells are confluent and persists for days (32, 58).

Whether cultures of alveolar cells exhibit type I or type II cell transport properties or both is unclear. Type II cells in culture progressively lose their cuboidal shape. Plasma membrane proteins shift to the type I phenotype (39). Morphology and electrical properties are affected by culture conditions. Monolayers grown on collagen-coated filters display a low ( $\sim 200 \Omega \cdot \text{cm}^2$ ) transepithelial resistance (87). Epithelium grown on human amniotic basement membrane exhibits higher resistances [ $\sim 400 \Omega \cdot \text{cm}^2$  (32)] and maintain morphological and metabolic characteristics of type II cells (34). With increased purity and seeding density and with the use of culture-treated Nucleopore filters, monolayers with even greater resistance ( $2,000 \Omega \cdot \text{cm}^2$ ) have been obtained (25). Under such conditions, cells display even more pronounced type I cell-like morphological and morphometric features (24). Rates of ion transport are less variable than electrical resistances, and mean  $I_{sc}$  observed in these studies ranges from 3.5 to  $4.5 \mu\text{A}/\text{cm}^2$  depending on the substratum. Type II cells maintain by place their cuboidal shape

when they are seeded on collagen gels that contract one-half their original diameter in culture (32). Resistance of such monolayers is lower and  $I_{sc}$  is larger, perhaps because cell number per unit surface area is greater than with monolayers grown on filters with the same initial seeding density.  $Na^+$  transport in monolayers of late fetal rat type II cells is similar to that seen in adult type II cells (105, 113). Thus the ability to transport  $Na^+$  is a feature shared by all alveolar type II cells derived from either adult or late fetal lungs, regardless of culture conditions or cell morphology.

The basolateral and apical steps of transepithelial  $Na^+$  transport have been partly elucidated. It has been demonstrated that the  $Na^+-K^+$ -ATPase present in the basolateral membrane of alveolar cells is involved in transepithelial  $Na^+$  and fluid transport. With the use of cytochemical techniques  $Na^+-K^+$ -ATPase activity has been observed in the basolateral plasma membrane of type II but not of type I cells (120). The activity of the enzyme is probably too low to be demonstrated by this technique in type I cells. Adult type II cells display a high rate of  $Na^+$  extrusion (68), probably because the density of pumps present in the cell membrane is high, three times that seen in fetal cells (23). Evidence that active ion transport is driven by  $Na^+-K^+$ -ATPase has been obtained by inhibiting this enzyme with ouabain. Ouabain decreases dome formation (59, 87) and abolishes electrical potential difference across monolayers grown on filters when added to the basolateral medium (25, 87). The small inhibitory effect of apical ouabain (87) was probably due to diffusion across the monolayer through leaky intercellular junctions because it was not observed in tighter monolayers (25).  $Na^+$  transport in intact alveolar epithelium is similarly affected by ouabain (7, 60). Ouabain also decreases fluid absorption from alveoli when it is added to the perfusate in isolated fluid-filled rat lungs, keeping with the concept that fluid absorption results from transepithelial  $Na^+$  transport (7). On the contrary, addition of ouabain to the alveolar instillate does not alter fluid absorption (6).

The absolute requirement for  $Na^+$  for fluid absorption to occur has been suggested by the observation that replacement of  $Na^+$  by choline completely abolishes dome formation (59) and  $I_{sc}$  (33) in cultured cells, as well as fluid resorption from rat alveoli (7). The entry of  $Na^+$  across the plasma membrane of alveolar epithelial cells probably occurs through multiple pathways (Table 1). Among the apical transport systems, amiloride-inhibitable transport has a major role in  $Na^+$  absorption. The effect of the diuretic amiloride on  $Na^+$  transport has been demonstrated in cell cultures, mammalian lungs, and frog lungs. Amiloride decreases dome formation (59, 87) and the transepithelial potential, or  $I_{sc}$  (25, 32, 87), when it is added to the apical side of the monolayer but is ineffective on the basolateral side (25). In isolated rat lungs, the rate of active  $Na^+$  transport was reduced by 30–70% (7, 8, 45, 60) when amiloride was added to the alveolar instillate in concentrations of  $\geq 10^{-4} \text{ M}$ . A decrease in alveolar instillate resorption in response to similar concentrations of apical amiloride has been observed in isolated rat lungs (7, 45) and in rat, sheep, and rabbit lungs

in vivo as well (8, 15, 123). With lower amiloride concentrations,  $\text{Na}^+$  and fluid transports were less affected (45).

The amiloride-sensitive pathway may consist of  $\text{Na}^+$  channels or  $\text{Na}^+ \text{-H}^+$  antiport. Both have been found to be present in the plasma membrane of alveolar epithelial cells (89, 101). The  $\text{Na}^+ \text{-H}^+$  antiport has been evidenced in apical membrane vesicles of freshly isolated fetal sheep type II cells (122) but is probably present on the basolateral surface of cell monolayers of adult rats (85). If  $\text{Na}^+ \text{-H}^+$  exchange is located in adult as in fetal alveolar cells,  $\text{Na}^+ \text{-H}^+$  exchange may have a role in  $\text{Na}^+$  absorption, as in the case of various fluid-absorbing epithelia (28). Typically, amiloride in submicromolar concentrations blocks  $\text{Na}^+$  channels such as those in the frog skin or in the collecting tubule, whereas it inhibits  $\text{Na}^+ \text{-H}^+$  exchange in concentrations two to three orders of magnitude larger. The amiloride analogues benzamil or phenamil are more specific inhibitors of these  $\text{Na}^+$  channels, whereas the analogues ethylisopropylamiloride or dimethylamiloride are more specific of the  $\text{Na}^+ \text{-H}^+$  exchanger. Thus the sensitivity and the response of  $\text{Na}^+$  transport to amiloride and to its different analogues have been used to distinguish between  $\text{Na}^+$  channels and the  $\text{Na}^+ \text{-H}^+$  antiport. Ballard and Gatzky (3) found that amiloride is more effective in reducing the potential difference developed across subpleural alveolar walls than ethylisopropylamiloride. Russo et al. (114) showed that  $\text{Na}^+$  uptake by alveolar type II cells is inhibited more efficiently by benzamil or amiloride than by dimethylamiloride. These findings are in keeping with the observations that relatively low concentrations ( $<5 \mu\text{M}$ ) of amiloride inhibit the epinephrine-induced fetal fluid absorption (107), despite possible leakage of the drug out of alveoli, and that half-inhibition of  $I_{sc}$  in cultured type II cell monolayers is obtained with  $\sim 1 \mu\text{M}$  of amiloride (29).  $\text{Na}^+$  channels with high affinity for amiloride have also been described in the apical membrane of frog alveolar epithelial cells (52). Bullfrog lungs (54) and mudpuppy lungs (140) actively transport  $\text{Cl}^-$  from the basolateral to the apical side in a manner similar to mammalian fetal lungs. In addition, mudpuppy lungs perform  $\text{Na}^+$  transport in the opposite (absorption) direction, whereas those of the frog *Xenopus laevis* only absorb  $\text{Na}^+$  (52, 73). This transport is inhibited by low ( $1 \mu\text{M}$ ) amiloride concentration. Noise analysis showed that this apical  $\text{Na}^+$  conductance is due to the presence of high amiloride affinity  $\text{Na}^+$  channels (52). On the other hand,  $\text{Na}^+$  uptake by plasma membrane vesicles from rabbit alveolar type II cells was found to be inhibited more efficiently by ethylisopropylamiloride than by amiloride or benzamil in concentrations  $>10 \mu\text{M}$  (89). Despite these inhibition characteristics, the  $\text{Na}^+$  pathway present in membrane vesicles is probably not the  $\text{Na}^+ \text{-H}^+$  exchanger because  $\text{Na}^+$  uptake increased when vesicle potential was made more negative and decreased in the presence of an outwardly directed proton gradient that would have stimulated the exchange of  $\text{H}^+$  vs.  $\text{Na}^+$ .  $\text{Na}^+$  channels with an affinity for amiloride and its analogues different from those of the typical epithelial  $\text{Na}^+$  channel have been described in other epithelia (124). It is possible that two populations of  $\text{Na}^+$  channels coexist in mammalian alveolar cells, which may account for these inhibition char-

acteristics (103). One of these populations may be composed of cation channels that do not discriminate between  $\text{Na}^+$  and  $\text{K}^+$  and that have been described in fetal (109) and adult type II cells (50, 144).

The second pathway for  $\text{Na}^+$  entry across the apical membrane of alveolar epithelium with a possible role in fluid absorption (at least in rat lungs) is  $\text{Na}^+$ -glucose cotransport. The cotransporter was believed to be present in lungs following the work by Kerr et al. (71), who showed that the glucose analogue  $\alpha$ -methylglucoside (which is cotransported with  $\text{Na}^+$  but does not enter cells by facilitated diffusion) accumulated in lung cells when placed in the perfusate of isolated ventilated lungs. Goodman and Wangensteen (61) and Wangensteen and Bartlett (137) also observed that D-glucose disappeared faster from alveoli than L-glucose and concluded that D-glucose was removed through some special process.

The possibility that pathways other than  $\text{Na}^+$  channels may be present in the luminal membrane of alveolar cells has been suggested by the observation that amiloride at a rather high concentration does not completely abolish alveolar fluid absorption and  $\text{Na}^+$  transport in rat lungs (7, 8). It has been reported that the glucose concentration in the fluid instilled in the air spaces of isolated rat lungs decreased well below that in the perfusate (8, 47). Furthermore, choline substitution for  $\text{Na}^+$  in the instillate decreased glucose disappearance from alveoli, suggesting that glucose efflux required the presence of  $\text{Na}^+$  (8). Additional evidence was provided by the finding that phlorizin inhibited glucose disappearance from alveoli, whereas phloretin, which inhibits facilitated transport, was without any effect, confirming that most of glucose removal from alveoli is obtained by cotransport with  $\text{Na}^+$  (8). Because cotransport of  $\text{Na}^+$  with glucose is electrogenic, omission of glucose from the instillate significantly decreases the alveolar potential difference (3).

The presence of the  $\text{Na}^+$ -glucose cotransport in rabbit lungs is disputed (44, 123). However, Wangensteen and Bartlett (137) found that phlorizin inhibited the incorporation of D-[<sup>14</sup>C]glucose instilled in rabbit alveoli into compounds of large molecular weight. A possible explanation may be that alveolar glucose is taken up by epithelial cells by cotransport with  $\text{Na}^+$  and leaves these cells at the basolateral side by a yet unidentified pathway (presumably by facilitated diffusion as in other epithelia performing glucose transport) to be metabolized somewhere in the lung. The cotransporter may also be present in human alveolar epithelium (133).  $\text{Na}^+$ -dependent glucose transport has not been unequivocally demonstrated in cultured type II pneumocytes. Kerr et al. (72) found that rat cells accumulated the nonmetabolizable analogue 2-deoxyglucose after 24 h in culture. This accumulation was moderately affected by phlorizin and by the absence of  $\text{Na}^+$  in the extracellular medium. However, other studies failed to demonstrate  $\text{Na}^+$ -dependent 2-deoxyglucose or  $\alpha$ -methylglucoside uptake in rat type II cells (30), after either 1 day or several days of culture, or any effect of phlorizin on potential difference developed across monolayers (87, 105). In keeping with these observations, no influence of glucose on  $\text{Na}^+$  uptake by type II cells could be demonstrated (114). The cotransporter may have been lost during the isolation procedure per-

haps because of enzymatic digestion, and its subsequent expression may depend on culture conditions. As mentioned above, these factors may influence confluence and the development of well-organized tight junctions, a prerequisite to the expression of the cotransporter in other cell types (98).

The specificity of the  $\text{Na}^+$ -glucose cotransport has been evaluated with use of various nonmetabolizable glucose analogues (9). The analogue  $\alpha$ -methylglucoside is cotransported with  $\text{Na}^+$  in rat distal lung epithelium, keeping with findings in other fluid-absorbing epithelia. The ratio of cellular vs. extracellular concentration of  $\alpha$ -methylglucoside was estimated at  $\sim 100$  as the sugar was present in tracer amounts in the alveolar instillate, a value similar to that observed in the small intestine (80). A  $\text{Na}^+$ -sugar stoichiometry of  $\geq 2$  is necessary to account for such a large cellular accumulation. Another glucose analogue, 3-O-methylglucose, is probably not cotransported in rat lungs. This is somewhat surprising because 3-O-methylglucose is cotransported with  $\text{Na}^+$  in the small intestine and proximal renal tubule epithelia, as well as in the fetal sheep lung (5). The presence of the cotransporter has also recently been demonstrated in equine (69) and rat (119) tracheal epithelium. In keeping with what has been observed in rat alveoli, 3-O-methylglucose is apparently not transported in rat large airways.

Additional  $\text{Na}^+$ -coupled transporters have been described in rat alveolar type II cells. These transports include  $\text{Na}^+$ -neutral amino acid cotransport (21, 30, 127) and  $\text{Na}^+$ -phosphate cotransport (30). Their precise localization, in the apical or basolateral membrane, has not been established. Apical  $\text{Na}^+$ -dependent lysine transport has been described in the bullfrog lung (78). The presence of apical  $\text{Na}^+$ -coupled organic solute transports in alveolar cells is puzzling. Their role in transepithelial  $\text{Na}^+$  transport has been conjectured (21) but has not been demonstrated. One may speculate that a  $\text{Na}^+$ -amino acid cotransport could help in removing protein fragments from air spaces (78). However, when albumin was instilled into sheep alveoli, alveolar clearance of the protein followed first-order kinetics and most of the albumin was removed as an intact molecule (12).

The rate of active  $\text{Na}^+$  transport by alveolar epithelium is low compared with other transporting epithelia. It accounts for only one-third of the unidirectional flux of  $\text{Na}^+$  out of alveoli (7). The potential difference developed across the intact epithelium is only 3–5 mV (3, 99) despite the high paracellular resistance. Monolayers of alveolar cells also display much lower rates of ion transport than, for example, tracheal or bronchial epithelial monolayers.  $\text{Na}^+$  absorption in bicarbonate-Ringer-filled rat lungs has been estimated at  $3\text{--}5 \text{ pmol} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$  (7, 8, 45, 118). With *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid as the buffer, fluid absorption was slightly lower (44, 47). It is not clear whether fluid absorption is affected by substituting phosphate for bicarbonate (8, 45) and, therefore, whether acid secretion or bicarbonate absorption contributes to fluid movement.

The relative rates of  $\text{Na}^+$  transport and fluid absorption suggest that the absorption process is isosmotic or nearly isosmotic (7, 10, 45). Fluid absorption was not

slowed by the presence of large solutes in the alveolar instillate in rat lungs (8, 43), although these solutes can represent a counterforce of up to 65 Torr, a finding consistent with the permeability characteristics of the epithelium. However, *in vivo*, 1 day after instillation of autologous serum in sheep lungs, alveolar fluid clearance was only 15% the initial rate for a 50% increase in protein concentration (90). An influx of neutrophils and macrophages occurred in these lungs and may have altered epithelial permeability to small solutes (125). This may explain why fluid resorption progressively stopped, whereas protein permeability remained unchanged 144 h after serum instillation (12).

Interspecies differences are probably large. Isolated rabbit lungs transport fluid at a slightly lower rate than rat lungs (44). *In vivo* instillation of lobes suggests that the rate of alveolar fluid absorption is (in increasing order) dog < sheep < rabbit  $\leq$  rat (13, 90, 91, 123). Human lungs probably absorb at a high rate close to that observed in rats (92). The transposition to humans of fluid absorption rate observed in rats yields a daily absorption of 2 liters, i.e., 100 times the amount of fluid present in the alveoli in aerated conditions.

## POTASSIUM TRANSPORT

$\text{K}^+$  concentration in the aqueous lining layer of rabbit alveoli has been estimated at twofold the plasma concentration by direct puncture of subpleural alveoli (99). High values have also been obtained by bronchoalveolar lavage in rats (41, 42) and humans (133). The alveolar transepithelial electrical potential difference is too small (3, 99, 100) to explain alone this asymmetrical distribution.  $\text{K}^+$  may thus be secreted into alveoli. However,  $\text{K}^+$  absorption rather than secretion is observed when alveoli are filled with Ringer fluid in rabbit (44) and in some rat studies (6). The rate of  $\text{K}^+$  absorption is too low to have a significant role in fluid absorption. The reason why, on the contrary,  $\text{K}^+$  secretion occurs in “dry” lungs has not been elucidated. In addition to  $\text{Na}^+-\text{K}^+$ -ATPase,  $\text{Na}^+-\text{K}^+-2\text{Cl}^-$  cotransport and  $\text{K}^+$  channels have been described in the plasma membrane of type II cells that might provide pathways for transepithelial  $\text{K}^+$  transport.

Bland and Boyd (18) used  $^{86}\text{Rb}$  to trace  $\text{K}^+$  uptake by suspensions of freshly isolated type II cells from fetal or adult rabbits. They observed that a ouabain-insensitive transport mechanism that accounts for 15–20% of cellular  $^{86}\text{Rb}$  uptake is present in these cells. This transport is inhibited by loop diuretics (furosemide or bumetanide), which are specific inhibitors of  $\text{Na}^+-\text{K}^+-2\text{Cl}^-$  cotransport. When present in the apical membrane of epithelial cells, this cotransporter is involved in  $\text{NaCl}$  absorption (62), whereas when present in the basolateral membrane it has a role in  $\text{Cl}^-$  secretion [as in fetal respiratory epithelium (22) or in airway epithelium (143)] and in  $\text{K}^+$  secretion in some epithelia (64). The activity of  $\text{Na}^+-\text{K}^+-2\text{Cl}^-$  cotransporter is probably low in type II cells because Russo et al. (114) did not observe that bumetanide modifies cellular  $\text{Na}^+$  uptake. The absence of a significant contribution of  $\text{Na}^+-\text{K}^+-2\text{Cl}^-$  cotransport to transepithelial ion fluxes is corroborated by the observation that

loop diuretics do not affect  $\text{Na}^+$  or  $\text{K}^+$  transport in isolated lungs (6, 7).

$\text{K}^+$  channels have been evidenced in rat type II cell plasma membrane with use of the whole cell patch-clamp technique (38, 111). Two types of voltage-sensitive  $\text{K}^+$  currents have been described that differ by their activation potential threshold and their sensitivity to various  $\text{K}^+$  channel blockers. Most cells display  $\text{K}^+$  currents in response to a depolarizing voltage step from low ( $< -20$  mV) holding potentials; these currents are only weakly sensitive to tetraethylammonium [half-block concentration 15 mM (38)] but are strongly blocked by quinine and 4-amidopyridine (111). They resemble the currents occurring through the delayed rectifier  $\text{K}^+$  channel that is present in various cell types. The other currents that are seen only at high ( $> -20$  mV) holding potentials are blocked by low concentrations of tetraethylammonium (half-block 100  $\mu\text{M}$ ) and are less sensitive to quinine and insensitive to 4-amidopyridine. These latter channels resemble the  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels found in mammalian exocrine cells. Interestingly, these two types of  $\text{K}^+$  currents distinguish two cell populations because they never coexist in the same cell (111).

Secretion of  $\text{K}^+$  is triggered in isolated fluid-filled lungs when isotonic glucose is instilled into the alveoli with bicarbonate-Ringer as the perfusate (6).  $\text{K}^+$  concentration in the instilled fluid increases with time up to twofold perfusate concentration after 1 h. This  $\text{K}^+$  flux was slowed when  $\text{Ba}^{2+}$ , a potent blocker of various  $\text{K}^+$  channels, was added to the instillate and when the basolateral  $\text{Na}^+-\text{K}^+$ -ATPase was inhibited by the addition of ouabain to the perfusate, indicating that most of  $\text{K}^+$  originated from epithelial intracellular pools. Addition of bumetanide to the perfusate did not slow  $\text{K}^+$  transport, suggesting that a basolateral  $\text{Na}^+-\text{K}^+-2\text{Cl}^-$  cotransporter was not involved in this  $\text{K}^+$  secretion.

With Ringer instillate,  $\text{K}^+$  is, in contrast, continuously removed from rat alveoli (6).  $\text{K}^+$  absorption rate was enhanced when  $\text{Ba}^{2+}$  was added to the instillate and slowed by addition of ouabain to the same medium. Apical ouabain also decreased the unidirectional  $^{86}\text{Rb}$  (which traces  $\text{K}^+$  movement) flux in the instillate-perfusate direction by >50%. The presence of simultaneous  $\text{K}^+$  absorption and secretion has been proposed as a possible explanation for these observations. Absorption might be performed by an apical ouabain-sensitive  $\text{K}^+$  pump present in the apical membrane of some alveolar cells. Secretion might be the consequence of the activity of the basolateral  $\text{Na}^+-\text{K}^+$ -ATPase and occur through apical  $\text{K}^+$  channels. Another explanation for the effect of apical ouabain might be that the inhibitor crossed the epithelium and inhibited the basolateral  $\text{Na}^+-\text{K}^+$ -ATPase, resulting in cellular  $\text{K}^+$  leakage (88). However, fluid absorption (and thus  $\text{Na}^+$  transport) remained unaffected when ouabain was placed in the instillate. Moreover, inhibition of the basolateral  $\text{Na}^+-\text{K}^+$ -ATPase by adding ouabain to the perfusate (which resulted in a clear-cut inhibition of  $\text{Na}^+$  transport) rather increased  $\text{K}^+$  absorption and  $^{86}\text{Rb}$  efflux out of alveoli, as if some  $\text{K}^+$  secretion were inhibited (117).

The putative apical pump may be a  $\text{Na}^+-\text{K}^+$ -ATPase.

However, no  $\text{Na}^+-\text{K}^+$ -ATPase activity was found in the apical membrane of alveolar cells (120). It is possible that the pump activity (which might represent only 5% basolateral  $\text{Na}^+-\text{K}^+$ -ATPase activity considering the rate of  $\text{K}^+$  transport) cannot be detected by cytochemistry, as is probably the case for type I cell  $\text{Na}^+-\text{K}^+$ -ATPase activity. Another possibility is that instillation of fluid in isolated lungs produces tissue hypoxia because of local underperfusion. It has been shown in the kidney that ischemia resulted in epithelial tight junction disruption and rapid redistribution of basolateral plasma membrane proteins, such as  $\text{Na}^+-\text{K}^+$ -ATPase, to the apical domain (97). After only 15 min of ischemia, the activity of the enzyme in apical microvilli was sufficiently high to be detected by cytochemistry. However, it is not known whether the pump would remain sufficiently active under such conditions to convey significant amounts of  $\text{K}^+$ .

The apical pump may also be a  $\text{K}^+-\text{H}^+$ -ATPase because a variety of such pump, inhibited by ouabain, has been described in the apical membrane of guinea pig colon epithelial cells (128). Effros et al. (44) have proposed that  $\text{K}^+-\text{H}^+$ -ATPase might be present in rabbit alveolar epithelial cells, which could account for the observed  $\text{K}^+$  absorption. They did not succeed in stimulating  $\text{K}^+$  absorption or  $\text{H}^+$  secretion by histamine, a known mediator of  $\text{K}^+-\text{H}^+$ -ATPase activation in the stomach. In a preliminary report, Boyd et al. (19) described a  $\text{K}^+$ -dependent ATPase activity in apical membrane vesicles of freshly isolated guinea pig type II cells. This ATPase activity was insensitive to ouabain but was depressed by omeprazole, a known inhibitor of  $\text{K}^+-\text{H}^+$ -ATPase. Active proton secretion has been reported in cultured type II cells (86). This secretion was not affected by external  $\text{K}^+$  concentration and was not inhibited by vanadate, an inhibitor of  $\text{E}_1\text{-E}_2$  ATPases, the class to which  $\text{K}^+-\text{H}^+$ -ATPase belongs. It has been proposed that proton secretion might occur because of insertion of vacuolar  $\text{H}^+$  pumps in the plasma membrane (86). Interestingly, omeprazole has also been found to inactivate vacuolar  $\text{H}^+$ -ATPase (145). However, the presence of such an  $\text{H}^+$ -ATPase in alveolar cells has recently been challenged (20).

A model of alveolar epithelium (6, 117) has been hypothesized that could account for the features of  $\text{K}^+$  transport observed in isolated lungs. Coexistence of two different cell types that may perform  $\text{Na}^+$  and  $\text{K}^+$  absorption separately has been conjectured because the presence of  $\text{Na}^+-\text{K}^+$ -ATPase in both apical and basolateral membranes of the same cell would reduce the effectiveness of transcellular  $\text{Na}^+$  transport. The existence of two functionally different cell populations might in addition account for the distribution of  $\text{K}^+$  channels among type II cells (111). One cell type would be responsible for  $\text{Na}^+$  absorption. According to the Koefoed-Johnsen and Ussing model,  $\text{Na}^+$  enters the cells at the apical membrane through  $\text{Na}^+$  channels (or through cotransport with glucose or other organic solutes) and is pumped out by the basolateral  $\text{Na}^+-\text{K}^+$ -ATPase. The other cell type, which is pure speculation, would perform  $\text{K}^+$  absorption by means of the  $\text{K}^+$  pump present in the apical membrane.  $\text{K}^+$  must necessarily leave this cell through a basolateral pathway that might consist in  $\text{K}^+$  channels.

## CHLORIDE TRANSPORT

The most important counterion that follows  $\text{Na}^+$  absorption by the alveolar epithelium is  $\text{Cl}^-$ . None of the studies using type II cell monolayers or isolated lungs was able to demonstrate active  $\text{Cl}^-$  transport under basal conditions. Replacement of  $\text{Cl}^-$  by gluconate in type II cell culture medium decreases dome formation (59) but not  $I_{sc}$  during short-term replacement (33).  $\text{NO}_3^-$  substitution for  $\text{Cl}^-$  does not affect dome formation (59). Dome formation remains unaltered after addition of  $\text{Cl}^-$  transport inhibitors such as the stilbene derivative 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), loop diuretics, and the indanyloxy carboxylic acid derivative MK196 (59). Neither furosemide nor the  $\text{Cl}^-$  channel blocker diphenylamine-2-carboxylate, which is effective in blocking airway  $\text{Cl}^-$  channels (143), affects the electrical potential difference or  $I_{sc}$  in adult or late fetal type II cell monolayers (33, 87, 105). Fluid absorption in rat lungs remains unaltered in the presence of basolateral (8) or apical bumetanide (6) or DIDS (8). In frog lungs, unidirectional  $^{36}\text{Cl}$  fluxes are identical in the apical to basolateral and in the reverse direction under short-circuit conditions (73) and are unaffected by inhibition of  $\text{Na}^+$  transport, suggesting that  $\text{Cl}^-$  transport may be passive in these lungs as well.

Recently, the presence of epitopes recognized by antibodies against the epithelial  $\text{Cl}^-$  channel has been reported in fetal lung tissue but also in adult alveolar type II cells (106). Chloride channels have been described in a differentiated cell line derived from mouse type II cells (121). These channels (known as maxi-channels) open at zero transmembrane potential, are of large unitary conductance, and seem to be made of several "cochannels" arranged in parallel (82). They have been observed in both apical and basolateral membranes of cells derived from tight epithelia and are usually blocked by stilbene derivatives. Their physiological significance is not clear. However, they differ from the channels observed in epithelial tissues that are involved in transepithelial  $\text{Cl}^-$  transport such as large airway epithelium, which secretes  $\text{Cl}^-$ , or ascending limb of the loop of Henle, which performs  $\text{Cl}^-$  absorption (55). No present confirmation has been made of the presence of maxi-channels in normal type II cells.

Nord et al. (102) documented an anion-exchanging membrane system in cultured type II cells that is inhibited by stilbene derivatives. This antiport exchanges  $\text{Cl}^-$  for  $\text{HCO}_3^-$  (the presence of  $\text{Cl}^-$  is an absolute requirement) and is the dominant mechanism involved in recovery of pneumocytes from an alkaline load. It may have an important role in intracellular pH regulation at physiological pH. A similar stilbene-sensitive  $\text{Cl}^-$ -base exchanger has been described in the luminal membrane of several epithelial cell types in parallel to  $\text{Na}^+-\text{H}^+$  antiport that provides pathways for  $\text{NaCl}$  absorption. Whether such a mechanism is involved in transepithelial  $\text{NaCl}$  transport across alveolar epithelium is dubious because DIDS does not alter  $\text{Na}^+$  and fluid absorption in isolated rat lungs (8).

## PROTONS

Acid secretion (or bicarbonate absorption) almost certainly occurs in adult alveoli because the alveolar lining fluid pH is only 6.9 (100). This acidification mechanism is perhaps similar to that which acidifies fetal lung fluid (2). A slight decrease in the pH of alveolar instillate has been observed by Effros et al. (44) in isolated rabbit lungs; this decrease was equivalent to a proton secretion of 0.19 meq/h. Possible mechanisms of  $\text{H}^+$  secretion include  $\text{Na}^+-\text{H}^+$  exchange,  $\text{H}^+-\text{ATPase}$ , or  $\text{K}^+-\text{H}^+-\text{ATPase}$ . As mentioned above, firm demonstration of the presence of this latter pump is presently lacking.

An electroneutral  $\text{Na}^+$ -dependent mechanism that allows cells to recover from an acid load has been described in freshly isolated (101) as well as in cultured (20, 85) pneumocytes. Cyttoplasmic alkalinization in the presence of  $\text{Na}^+$  was inhibited by 90% after addition of amiloride, a finding that confirms that it was  $\text{Na}^+-\text{H}^+$  exchange. In a preliminary report, the antiport has been localized in the basolateral membrane of type II cell monolayers cultured on filters (85). Two different  $\text{Na}^+-\text{H}^+$  exchange subtypes are likely to exist in epithelial cells with different properties, depending on their presence on apical or basolateral membrane (28). The basolateral  $\text{Na}^+-\text{H}^+$  exchange is probably implicated in the cellular response to growth factors, in cell volume regulation, and in the regulation of intracellular pH.  $\text{Na}^+-\text{H}^+$  antiport activity was detected only at intracellular pH values <7 and therefore is not likely to be active at physiological pH (101). The  $\text{Na}^+-\text{H}^+$  exchanger thus has a role only for recovery after substantial acidification. This is not uncommon because in most cell types intracellular pH is regulated near neutrality by  $\text{Na}^+$ -coupled and  $\text{Na}^+$ -independent  $\text{Cl}^--\text{HCO}_3^-$  antiports working in opposite directions (132). The different transport mechanisms that contribute to intracellular pH regulation in alveolar epithelial cells have recently been reviewed (84).

Lubman et al. (86) have shown that type II cells actively secrete protons. Cultured type II cells were able to recover from an acid load despite the presence of extracellular amiloride in concentrations that would have inhibited  $\text{Na}^+-\text{H}^+$  exchange. Acidification recovery did not depend on the presence of  $\text{Na}^+$ ,  $\text{CO}_2$ , or  $\text{HCO}_3^-$ . Recovery was abolished by cellular ATP depletion or by *N*-ethylmaleimide, a sulphydryl reagent that preferentially inhibits epithelial plasma membrane proton pumps. This proton pump may be similar to that found in the plasma membrane of cells of the kidney medulla where it is responsible for urine acidification. An interesting suggestion is that  $\text{H}^+-\text{ATPase}$  may in fact be endosomal ATPase, which is present in lamellar body membranes and may be incorporated into plasma membrane during exocytosis. However, Brown et al. (20) failed to observe any effect of *N*-ethylmaleimide or 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, a more specific inhibitor of vacuolar  $\text{H}^+-\text{ATPase}$  on acidification recovery in cultured pneumocytes. In addition, these authors observed that recovery was strictly dependent on the presence of extracellular  $\text{Na}^+$  and was almost completely abolished by amiloride. Brown et al. concluded that recovery could be

explained by an ATP-sensitive  $\text{Na}^+ \text{-H}^+$  antiport alone. At the present time, these discrepancies have not received satisfactory explanation.

#### BICARBONATE

Another mechanism of cellular alkalinization has been described that depends on the presence of  $\text{Na}^+$  and  $\text{HCO}_3^-$  but not  $\text{Cl}^-$ . This mechanism is inhibited by DIDS but not by amiloride and is active at intracellular pH  $\sim 7$  (83). These characteristics distinguish this process from  $\text{Na}^+ \text{-H}^+$  exchange and proton secretion by  $\text{H}^+$ -ATPase. The sensitivity to stilbene derivatives and the requirement of  $\text{HCO}_3^-$  suggest that this mechanism may be  $\text{Na}^+ \text{-HCO}_3^-$  cotransport. This alkalinization mechanism alone does not suffice to allow recovery from intracellular acidification down to pH 7, because no recovery was observed when *N*-ethylmaleimide was added to inhibit  $\text{H}^+$ -ATPase. Thus,  $\text{Na}^+ \text{-HCO}_3^-$  cotransport might work together with  $\text{H}^+$ -ATPase to allow recovery from intracellular acidification and to provide a supplementary mechanism for epithelial lining fluid acidification. The role of this cotransporter in transepithelial  $\text{Na}^+$  transport remains speculative. However, it is worth noting that amiloride-sensitive  $\text{Na}^+$  transport and  $\text{Na}^+$ -glucose cotransport account for most, if not all, fluid absorption in the presence of bicarbonate (8). In addition, fluid absorption is unaffected by acetazolamide or DIDS, two agents that may potentially interfere with  $\text{HCO}_3^-$  production or transport (8).

#### MODULATION OF ION AND FLUID TRANSPORT

The effects of various agents on  $\text{Na}^+$  transport and fluid absorption by alveolar epithelium are summarized in Table 2.  $\beta$ -Adrenergic agonists stimulate the potential difference or  $I_{sc}$  and dome formation in type II cell monolayers (25, 33, 57, 87). A short-term effect of insulin on ion transport was observed when insulin was added to the basolateral medium (126) in a rather large concentration. In addition, insulin in concentrations within the physiological range increases type II cell 2-deoxyglucose uptake (126) and  $\text{Na}^+$ -dependent neutral amino acid uptake (127) after a 2-h incubation period. Inconsistent effects were seen with vasopressin; vasopressin was found to increase dome formation (57) but did not affect transepithelial potential difference (33). A vasopressin-induced increase in fetal fluid absorption has been reported in late fetuses (e.g., see Ref. 136).

In agreement with findings in cultured alveolar cells,  $\beta$ -adrenergic agonists proved to be very potent stimulants of  $\text{Na}^+$  transport and fluid absorption in isolated lungs (10, 35, 47, 116) or in vivo (13, 15) in mammals, with the exception of rabbits (44, 123). The intracellular second messenger involved in the  $\beta$ -adrenergic response is likely adenosine 3',5'-cyclic monophosphate (cAMP). Agents that increase intracellular cAMP concentration such as the cAMP analogue dibutyryl cAMP (DBcAMP) in conjunction with phosphodiesterase inhibitors (11, 116) reproduce the epithelial response to adrenergic agonists, whereas the effect of DBcAMP or phosphodiesterase inhibitor alone is less consistent (11, 56). The effect

TABLE 2. Effect of various agents on  $\text{Na}^+$  transport and/or fluid absorption in type II cell monolayers and fluid-filled mammalian and amphibian lungs

Agent	Type II Cells in Culture	Fluid-Filled Mammalian Lungs	Amphibian Lungs
Insulin	+(126)		0 (52)
$\beta$ -Adrenergic agonist	+(25, 33, 57, 87)	+ (10, 15, 35, 47, 60, 116)	0 (52) +(140)
Cholera toxin	0 (87) +(33)		
cAMP analogue	+(33, 57)	+ (56) 0 (11)	0 (52)
Phosphodiesterase inhibitor	+ (33, 57)	+ (56) 0 (11)	
cAMP analogue + phosphodiesterase inhibitor		+ (11, 116)	
Vasopressin	0 (33) +(57)		0 (52)
Aldosterone	0 (33)		+(51)
Acetylcholine, carbamylcholine	0 (57)		
cGMP analogue	0 (57)		0 (52)
EGF	0 (126)		
Glucagon	0 (126)		
VIP	0 (33)		
Bombesin	0 (33)		
Substance P	0 (33)		
Histamine	0 (33)		
Bradykinin	0 (33)		
Phorbol ester	0 (87)	0 (14)	
Sodium butyrate	+(87)		

cGMP, guanosine 3',5'-cyclic monophosphate; EGF, epidermal growth factor; VIP, vasoactive intestinal peptide. +, Stimulation of transport; 0, no effect. Numbers in parentheses are reference numbers.

of  $\beta$ -adrenergic agonists and cAMP analogues on mature lung fluid transport is thus similar to that observed in fetal lungs (107, 139). Interestingly,  $\beta$ -adrenergic agonists appear equally efficient when introduced in the alveoli or in the plasma, suggesting that receptors may be present on both sides of the epithelium (15, 35). Increases in fluid absorption after  $\beta$ -adrenergic stimulation are impressive, ranging from 300 to 400% the baseline level (10, 15, 116). It has been shown that stimulation of fluid absorption was the result of increased  $\text{Na}^+$  transport through the amiloride-sensitive pathway, whereas  $\text{Na}^+$  cotransport with glucose remained unaffected or was slightly depressed (116). The response to  $\beta$ -adrenergic stimulation probably does not occur as the result of  $\text{Na}^+ \text{-H}^+$  exchange stimulation because the agonist terbutaline does not change the intracellular pH in type II cells (115). It has been observed that the response to cAMP analogue depends on the concentration of the analogue or on the concomitant presence of phosphodiesterase inhibitor (11, 56, 116). In the presence of DBcAMP (1 mM) and of a phosphodiesterase inhibitor, an increase in fluid absorption was consistently observed, whereas with DBcAMP in a concentration of only 0.1 mM or in the absence of phosphodiesterase inhibitor, the response was erratic (56, 116). It is possible that under the latter condition intracellular cAMP concentration was close to the value that elicits a complete response.

The patterns of frog lung hormonal response appear

quite different from those of mammalian lungs. Aldosterone is a potent stimulant of  $\text{Na}^+$  transport in frog lungs (51), whereas it is devoid of any effect on ion transport in rat type II cell monolayers after overnight incubation (33).  $\text{Na}^+$  transport by frog lung is apparently insensitive to epinephrine or cAMP (51). On the contrary, mud-puppy lungs respond to  $\beta$ -adrenergic agonists by an increase in  $I_{sc}$  and fluid absorption (140), likely because of stimulation of  $\text{Na}^+$  transport.

The pattern of modulation of alveolar  $\text{K}^+$  transport by  $\beta$ -adrenergic agonists appears complex (117). The response of fluid-filled rat lungs to  $\beta$ -adrenergic agonist or  $10^{-3}$  M DBcAMP stimulation is biphasic: there is immediate, short-lived  $\text{K}^+$  secretion into the alveolar lumen followed by more sustained  $\text{K}^+$  absorption. However, when the concentration of DBcAMP is 10 times smaller, only  $\text{K}^+$  secretion is observed. The transient  $\text{K}^+$  secretion obtained in response to isoproterenol or  $10^{-3}$  M DBcAMP (as well as that obtained with  $10^{-4}$  M DBcAMP) is abolished by apical  $\text{Ba}^{2+}$ , whereas subsequent stimulation of  $\text{K}^+$  absorption is abolished by apical ouabain. A possible interpretation of this phenomenon (117) is that there is a dose-dependent response to an increase in intracellular cAMP concentration. A low intracellular concentration results in opening of apical  $\text{K}^+$  channels and  $\text{K}^+$  secretion, whereas at larger concentrations both  $\text{Na}^+$ ,  $\text{K}^+$ , and fluid absorption is stimulated. Thus,  $\beta$ -adrenergic agonists may potentially elicit  $\text{K}^+$  secretion. Whether the basal level of catecholamines contributes for the large  $\text{K}^+$  concentration in the epithelial lining fluid (41, 42, 99) remains to be elucidated.

Recent evidence has been obtained in type II cell monolayers grown on filters that stimulation of ion transport with the  $\beta$ -agonist terbutaline increases  $^{36}\text{Cl}$  fluxes in both directions (74), in part because of an increase in paracellular permeability. The increase in the apical to basolateral  $^{36}\text{Cl}$  flux exceeded that in the other direction, suggesting that under such circumstances of stimulation  $\text{Cl}^-$  transport might be transcellular, perhaps through secondary active (directly or indirectly linked to  $\text{Na}^+$  transport) mechanisms. The cellular  $\text{Cl}$  transport pathway involved in this response has not been identified.

Stimulation with  $\beta$ -adrenergic agonists or DBcAMP (1 mM) together with phosphodiesterase inhibition has been found in some studies to increase paracellular permeability (116, 117) in isolated lungs, an observation that was not corroborated by others (10, 35, 56). The reason for these discrepancies is not clear, but it is worth noting that increases in paracellular permeability were observed when lungs were perfused with 4% blood cells. This may have been an indirect rather than a direct effect on alveolar epithelium.

#### POSSIBLE ROLE OF AIRWAYS IN LUMINAL ION AND FLUID ABSORPTION

The precise localization of ion and fluid transport in fluid-filled lungs has not been definitely demonstrated.  $\text{Cl}^-$  secretion and  $\text{Na}^+$  absorption are simultaneously present in airway epithelia. Whereas large airways se-

crete  $\text{Cl}^-$ ,  $\text{Na}^+$  absorption is the general rule in bronchi under resting conditions (143). Ballard and Gatzky (3, 4) attempted to delineate the respective roles of airways and alveolar epithelia in  $\text{Na}^+$  and fluid absorption in isolated fluid-filled rat lung lobes. They measured the electrical potential difference developed across subpleural alveolar walls by impalement with a microelectrode while alveoli and airways were filled in continuity by conducting Krebs-Ringer solution or while airways were blocked by insulating fluorocarbon (3). Ballard and Gatzky observed that substitution of gluconate for  $\text{Cl}^-$  in the alveolar instillate induced hyperpolarization that was not observed when airways were blocked with fluorocarbon. Because such hyperpolarization was also noted across large airway epithelium, Ballard and Gatzky concluded that alveolar potential is more representative of airway transport than of alveolar transport in fluid-filled lungs. In a complementary series of experiments (4), they studied the rate of fluid absorption in subpleural alveoli while airways were blocked with fluorocarbon, thus eliminating the effect of large airway transport. They did not observe any significant fluid absorption in these alveoli, whereas total lung weight decreased reflecting overall volume absorption. The authors concluded that fluid absorption takes place in small airways or in more remote alveolar spaces. However, micropuncture of subpleural alveoli of dogs where the lungs were filled with fluid in vivo yielded opposite observations (13). Some fluid was probably present in airways in the latter study, but it is unlikely that airways were entirely responsible for alveolar fluid absorption considering their limited exchange surface area. These studies again emphasize that inhomogeneity may be an important factor in isolated fluid-filled lungs.

The main difference between airway and alveolar epithelia is their response to  $\beta$ -adrenergic stimulation. Bronchial epithelium responds to  $\beta$ -adrenergic agonists and cAMP by  $\text{Cl}^-$  secretion (143), whereas alveolar epithelium responds by increasing  $\text{Na}^+$  absorption. Clara cells, which represent an important subpopulation of rat bronchial epithelium, also actively absorb  $\text{Na}^+$  in a way similar to that seen in type II cells and probably have a role in the homeostasis of the small airway lining fluid. However,  $\text{Na}^+$  transport in Clara cells is not stimulated by  $\beta$ -adrenergic agonists (134). Thus, the structure responsible for the increase in  $\text{Na}^+$  transport after  $\beta$ -adrenergic or cAMP stimulation is in all likelihood the alveolar epithelium. It is worth noting that type II cells of late fetal lungs display  $\text{Na}^+$  transport properties similar to mature type II cells (105, 113). These findings suggest that the alveolar epithelium may be responsible for fluid absorption (after  $\text{Na}^+$  transport) in late fetal and mature lungs as well.

#### RESORPTION OF ALVEOLAR EDEMA

It is clear that preservation of the barrier properties of alveolar epithelium is indispensable for efficient alveolar edema clearance. Epithelial damage, such as that observed during high-permeability edema and acute respiratory distress syndrome, that leads to loss of protein

permselectivity (65) obviously impairs ion transport efficiency. The need for an intact epithelium has been supported by the observation that resolution of pulmonary edema occurs faster in patients who show an increase in edema fluid protein concentration during their disease compared with those who do not (92). However, more subtle alterations may affect  $\text{Na}^+$  and fluid transport in the absence of marked cellular damage. For example, it has been shown (the list is not restrictive) that  $\text{NO}_2$  (26), acid exposure (75), distention (48, 77), and phagocytizing neutrophils (125) increase epithelium permeability in varying degrees. What could be the effect of a moderate increase in paracellular permeability? Would fluid absorption rate decrease as a result of an increase in passive leakage, reducing the effectiveness of  $\text{Na}^+$  transport? Or, on the contrary, would accelerated removal of medium-sized solutes cause alveolar edema to clear faster? The answer to these questions is far from obvious. Let us consider the example of glucose transport. One hour after filling rat lungs with fluid (a situation comparable to alveolar flooding), the glucose concentration in the alveolar instillate is one-tenth that in the perfusate (8). Active glucose removal from air spaces results in a concentration difference that strongly promotes glucose entry into alveoli, a situation that is unique because other small solutes are close to equilibrium (42, 45). If we assume that two to four ions (i.e., 1 or 2  $\text{Na}^+$  plus their counterions, depending on  $\text{Na}^+$ -glucose stoichiometry) are removed from alveoli with each glucose molecule, two to four osmoles would leave the alveoli in addition to glucose by cotransport. Increasing glucose entry through the paracellular pathway would thus increase  $\text{Na}^+$  removal and fluid absorption unless saturation of the cotransport occurs. The kinetics of  $\text{Na}^+$ -glucose cotransport are unknown. However, if the apparent Michaelis-Menten constant ( $K_m$ ) of the cotransport is close to alveolar glucose concentration, an increase in paracellular permeability may well contribute to increased transepithelial solute transport and fluid absorption (118). The same reasoning applies for amino acids and for solutes that are not at equilibrium across the respiratory epithelium because of similar active removal. The mechanisms involved in the resorption of alveolar edema are not strictly identical to those that act to clear fetal lung fluid at birth. Because glucose is continuously removed from the lumen by cotransport with  $\text{Na}^+$ , glucose concentration in fetal lung fluid is often below detection level (<0.01 mM), well below the apparent  $K_m$  of the cotransport that has been estimated at ~0.14 mM in these lungs (5). The rate of  $\text{Na}^+$  cotransport with glucose corresponding to such a low concentration is almost negligible. It is thus not surprising that phlorizin does not modify lung fluid absorption in newborns (104). However, addition of glucose to lung fluid in late sheep fetuses decreases the rate of fluid secretion [i.e., accelerates the rate of fluid absorption (5)], which demonstrates that the cotransport is potentially effective and may also have a role in the clearance of alveolar edema (the entry of fluid with large glucose concentration into alveoli) under such circumstances.

Alveolar fluid absorption is enhanced in isolated lungs

by agents that increase intracellular cAMP, which include  $\beta$ -adrenergic agonists, cAMP analogues, and phosphodiesterase inhibitors. In vivo,  $\beta$ -adrenergic agonists increase alveolar fluid clearance when added to the fluid instilled into airways (15). The potential value of this therapeutic approach may be worth consideration if parenteral pharmacological doses of agonists or phosphodiesterase inhibitors prove efficient. It has been shown that isoproterenol or aminophylline attenuate pulmonary edema (96), an effect that has been ascribed to vasodilation. The meaning of this observation deserves reappraisal, more especially because it has been observed that interruption of perfusion does abolish alveolar fluid absorption in isolated lungs (47) or in vivo (66).

#### COMPOSITION OF THE ALVEOLAR EPITHELIAL LINING FLUID

All available studies suggest that the alveolar epithelium absorbs fluid. Thus what is the origin of epithelial lining fluid? Is it produced by active secretion or, on the contrary, by passive leakage because of the surface active force developed at the air-water interface (63)? Because of the low permeability of the alveolar epithelium and of active transport, the composition of the alveolar lining layer differs from that of interstitial fluid or plasma. Electrolyte concentrations in the alveolar epithelial lining fluid have been determined in rabbits by micropuncture of subpleural alveoli (99). Whereas  $\text{Ca}^{2+}$  and  $\text{Cl}^-$  concentrations do not significantly differ from those predicted on the basis of passive distribution considering the small luminal negative potential difference of 3.5 mV,  $\text{Na}^+$  concentration is significantly lower and  $\text{K}^+$  concentration is larger (7.3 mM) than predicted. Even higher values have been obtained in rats (41, 42) or in humans (133) by bronchoalveolar lavage, suggesting active secretion. The consequence of the presence of the  $\text{Na}^+$ -glucose cotransport in the alveoli-airway lumen is that glucose concentrations in the lining layer must be lower than in plasma because continuous removal occurs (8). Indeed, values of <1 mM have been estimated in humans (133). Total protein concentration was found similar in epithelial lining fluid and in plasma (42). This high value is somewhat surprising; however, it is possible that almost no ultrafiltration occurs across alveolar-capillary walls because alveoli are almost blind structures. In that case, solute concentrations must equilibrate on either side unless the epithelium is completely impermeable (which is apparently the case for proteins of molecular weights >200,000) or the solute is actively transported. Albumin and transferrin concentrations in the epithelial lining fluid are probably below those in the lymph and plasma (42, 133), suggesting either that interstitial fluid rather than plasma equilibrates with the epithelial lining fluid or that some removal process is active. Asymmetric albumin transport has been described in the bullfrog lung (79) and in canine bronchial epithelium (67), as well as in the ferret trachea (141), in the reverse secretion direction. In the presence of epithelial damage, active transport, barrier properties, or both may be altered. Thus, determination of epithelial lining fluid concentrations of

solutes that do not distribute passively may provide information on preservation of epithelial function.

Address reprint requests to G. Saumon.

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