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SIGNIFICANCE OF ACTIVE ION TRANSPORT IN TRANSALVEOLAR WATER ABSORPTION: A STUDY ON ISOLATED RAT LUNG

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SUMMARY

1. Experiments were performed on isolated rat lungs perfused with Ringer solutions containing red cells. The goal was to clarify the role of active transport of Na^+ for the absorption of fluid across the alveolar membrane, and to characterize active and passive pathways.

2. Partially degassed lungs were filled with 5 ml of an isotonic Ringer solution containing ^{125}I-labelled albumin in order to calculate the fluid movement, and $^{22}Na^+$ or $^{36}Cl^-$ for measurement of ion fluxes. Passive non-electrolyte permeability was determined in all experiments using [^3H]mannitol.

3. The average rate of fluid absorption in phosphate-buffered instillates was 134 nl/s (S.E., 18·5; $n = 14$). With ouabain (10^{-4} M) in the perfusate the fluid absorption rate fell to 57 nl/s (S.E., 8·2; $n = 18$). Amiloride (10^{-3}–10^{-4} M) in the instillate reduced the absorption to 75 nl/s (S.E., 8·6; $n = 16$). These results show that fluid absorption depends on transcellular transport of Na^+ and that alveolar epithelial cells have a Na^+ entry system in the luminal membrane and a Na^+–K^+ pump in the abluminal membrane.

4. The transcellular ion transport operates in parallel with a paracellular, passive leak that allows mannitol to pass with a permeability surface area product of $1{\cdot}2 \times 10^{-4}$ ml/s, corresponding to a permeability coefficient of $2{\cdot}4 \times 10^{-8}$ cm/s, assuming an alveolar surface area of 5000 cm^2.

5. The passive fluxes of Na^+ were 9·4 pmol/(cm^2 s) (S.E., 1·3; $n = 25$) in the direction from alveoli to perfusate and 8·0 pmol/(cm^2 s) (S.E., 0·86; $n = 6$) from perfusate to plasma. The passive fluxes of Cl^- in the two directions were not significantly different either. Thus the transalveolar electrical potential difference is too small to affect ion movements measurably.

6. The passive permeability to Na^+ was $6{\cdot}7 \times 10^{-8}$ cm/s and to Cl^- was $10{\cdot}2 \times 10^{-8}$ cm/s (alveolar surface area assumed to be 5000 cm^2). The ratio of the permeabilities is close to the ratio of the diffusion coefficients in free solution, suggesting a neutral or weakly charged paracellular channel.

7. We conclude that the alveolar epithelium performs solute-coupled fluid transport from alveoli to plasma, and that it shows many features that are common to other fluid-transporting epithelia; with an approximate surface area of 100 m^2 in humans it constitutes one of the largest epithelial surfaces in the body. If the rate

of fluid transport in the rat is converted to an equivalent rate in human lungs, about 1·5 l of fluid would be absorbed per twenty-four hours.

INTRODUCTION

Proper functioning of the lungs as gas exchange organs requires that only a thin film of fluid covers the inner surface of the alveoli. Despite the importance of the mechanisms that keep the alveoli 'dry', it is rare to find accounts of this subject in current textbooks of physiology; this reflects the fact that satisfactory explanations have not been provided. There are, generally speaking, two main ways of interpreting alveolar film homeostasis. Either it is the result of a balance of mechanical and colloid–osmotic forces across the alveolar wall (Guyton, Taylor, Drake & Parker, 1976; Guyton, Moffatt & Adair, 1984) or it is the result of solute-coupled trans-epithelial fluid transport.

That fetal lung epithelium is capable of secreting ions and water was definitely demonstrated by Olver & Strang (1974), who paved the way for a rigorous analysis of the alveolar epithelium as an ion-transporting membrane. Recently, the idea that the adult alveolar epithelium absorbs fluid by cellular processes has slowly taken form. Olver (1983) observed amiloride-blockable Na^+ absorption in the mature fetal lung of lamb and initiated modern views on the mechanism of fluid transport in adult lung epithelium *in vivo*. Experiments on lung lobes *in situ* have stressed the importance of Na^+ transport (Matthay, Landolt & Staub, 1982; Matthay, Berthiaume & Staub, 1985). Studies *in vitro* on cultured type II pneumocytes (Mason, Williams, Widdicombe, Sanders, Misfeldt & Berry, 1982; Goodman, Fleischer & Crandall, 1983; Goodman, Brown & Crandall, 1984) have demonstrated dome formation due to solute-coupled water transport.

Whilst the studies on fluid secretion in fetal lungs concluded that fluid transport was secondary to active ion transport, the same clear attitude has not been taken regarding fluid absorption in the adult lung. In this communication we show that an analysis similar to that of Olver & Strang (1974) can be applied to the adult lung. The conclusion we arrive at is similar to theirs, with the difference that in adult lung the direction of fluid transport is reversed.

In order to analyse the transport processes *in vivo* it is necessary to instil a fluid of known composition into the alveoli and sequentially follow the modifications of this fluid. Net fluid movement was assessed by the increase in the concentration of a non-permeable reference substance ([^{125}I]albumin), and ion movements were determined by use of appropriate isotopes ($^{22}Na^+$ and $^{36}Cl^-$). The paracellular pathway (shunt path) was characterized by its permeability to a small hydrophilic solute, [^{3}H]mannitol.

The experiments were performed on pump-perfused rat lungs. A main purpose was to localize the Na^+–K^+ pump, requiring experiments with high doses of ouabain. This excludes the possibility of using the more satisfactory cross-circulation technique that was applied in another series of experiments reported in an accompanying paper (Basset, Crone & Saumon, 1987) which broadens and corroborates the conclusions reached on the isolated lung preparation. An advantage of experiments on pump-perfused lungs is the possibility of determining ion fluxes both in the direction from alveoli to perfusate and from perfusate to alveoli.

The conclusion of the present study is unambiguous: active transport of Na^+ is responsible for the fluid movement from alveoli to interstitium with mechanical and colloid–osmotic forces being of secondary importance.

METHODS

Perfusion technique

The experiments were performed on perfused isolated rat lungs (male Wistar rats, 300 g). The perfusion system contained a disk oxygenator consisting of two rotating Perspex disks in a glass chamber into which either air or 5% CO_2–95% O_2 (depending on the perfusate) was constantly supplied after having been saturated with water vapour at 37 °C.

When the perfusion system was ready and filled with perfusate the thorax of the rat (anaesthetized with Nembutal; 50 mg/kg administered intraperitoneally) was opened whilst the lungs were ventilated with a mechanical respiratory pump (Braun, Melsungen, F.R.G; tidal volume 1·5 ml, frequency about 70/min). The two lungs were carefully dissected free from the thoracic cavity. Whilst still in the thorax, a catheter connected to the perfusion system was inserted into the pulmonary artery. Another catheter was introduced into the left atrium thus closing the perfusion circle. The fluid was circulated by means of a roller pump delivering a minute volume of about 10 ml. The perfusate was heated to 37 °C by means of water-jackets. The perfusion pressure at the inlet to the lungs was continuously measured with a pressure transducer. It varied generally between 0·7–1·4 kPa. The lungs were freed from their connexions and cautiously transferred to a thermostatted glass chamber, where they were ventilated for about 10 min before instilling the test solution.

The volume of the perfusate was about 30 ml. This means that the tracers in the instillate were essentially moving into an 'infinite' compartment, as confirmed by the low isotope concentration in the perfusate at the end of the experiment (less than 2% for the small tracers).

Instillation and sampling technique

Before instilling the test solution about 2 ml of air were aspirated from the lungs by means of a syringe. A three-way stopcock allowed subsequent instillation of 5 ml of the test solution followed by 0·5 ml of air for dead-space washing. The liquid pressure after instillation was between 0·3–0·8 kPa. After an equilibrium period of 10 min the first sample was taken. First, the air in the upper airways was withdrawn. Then a sample of about 0·5 ml instillate was taken. The exact volume was determined by weighing. Then the manipulations were repeated in reverse order. This time-point marked the start of the experimental periods which each lasted 30 min. Generally, three consecutive 30 min periods were studied. The air pressure in the closed glass chamber that contained the lungs was monitored as a rough indication of accumulation of excess fluid.

Composition of solutions

Phosphate-buffered medium contained (in mequiv/l): Na^+, 142; K^+, 5·5; Ca^{2+}, 2·5; Mg^{2+}, 1·8; Cl^-, 146; HCO_3^-, 4·2; HPO_4^{2-}, 0·3; $H_2PO_4^-$, 0·4; SO_4^{2-}, 0·8. The majority of the experiments were carried out with phosphate-buffered media. Bicarbonate-buffered medium contained (in mequiv/l): Na^+, 145; K^+, 5; Ca^{2+}, 2; Mg^{2+}, 2; Cl^-, 129; HCO_3^-, 25. Glucose (10 mM) was present in both media.

The bicarbonate-buffered medium was equilibrated with 5% CO_2 for half an hour before use. The pH of the solution was controlled (PHM 82, Radiometer, Copenhagen, Denmark). Perfusate: blood drawn from the experimental rat was added to the buffered medium to provide a haematocrit of about 4%. The small amount of plasma proteins present helped to preserve a normal capillary permeability. Focoll 70 (Pharmacia, Upsala, Sweden) was added in a concentration of 4% (w/w), providing a colloid–osmotic pressure of about 2·5 kPa. Instillate: in some experiments Ficoll in the same concentration as in the perfusate was present in this instillate, but in the majority of experiments the instillate was without macromolecules.

Isotopes

The water movement into or out of the alveoli was determined by the concentration variations of an impermeable tracer, [^{125}I]albumin, added to the instillate. A small amount of carrier albumin (rat plasma) was added in order to avoid problems with radioactive albumin sticking to the tubes or syringes. Usually 3 μCi [^{125}I]albumin (CNTS, Paris, France) was added to the instillate. To determine the permeability of the paracellular pathway 4 μCi ^{3}H-labelled mannitol (New England

Nuclear, Frankfurt, F.R.G.) was added. Unidirectional movements of Na^+ and Cl^- were followed with $^{22}Na^+$ and $^{36}Cl^-$ (CEA, Saclay, France). Routinely 1 μCi $^{22}Na^+$ or 5 μCi $^{36}Cl^-$ was added to the instillate. In some experiments the isotopes were added to the *perfusate* in order to determine the ion fluxes from perfusate towards the alveoli. In these experiments 12 μCi $^{22}Na^+$ and 40 μCi $^{36}Cl^-$ were added to the perfusate in order to obtain suitable counting statistics.

Counting was performed in a scintillation counter and in a γ-counter (Intertechnique, France). Appropriate corrections for spill-over between channels were made with standards containing only one isotope. The counting was continued until at least 10000 counts were obtained ensuring counting statistics with a S.D. below 1%.

Other analytical techniques

The instillate and perfusate concentration of Na^+ and K^+ was determined in a flame photometer (Eppendorf, Hamburg, F.R.G.). Cl^- was analysed with coulometric technique in a Cl^- titrator (Radiometer, Copenhagen, Denmark). The Na^+ analyses were carried out in triplicate, whilst double determinations were made for K^+ and Cl^-. Glucose was analysed with the glucose oxidase method (Sigma, St. Louis, MO, U.S.A.). The O_2 and CO_2 partial pressures and pH in the perfusate were controlled two to three times during an experiment (Radiometer). The osmotic activity of the Ringer solutions used for instillation and perfusion was checked with a freezing-point osmometer (Fiske Associates, Needham Heights, MA, U.S.A.) on 0·5 ml samples.

Blocking agents

Various chemicals were used to interfere with epithelial transport processes: ouabain (Sigma), bumetanide (Leo Pharmaceutical Products, Copenhagen, Denmark) and amiloride (Coger, Paris, France).

Calculations

The following variables were characterized: (1) the net transport of water from alveoli to perfusate, (2) the net fluxes of Na^+ and Cl^- from alveoli to perfusate, (3) the unidirectional ion fluxes in both directions, and (4) the flux of mannitol. These data allowed calculation of the passive permeability of the paracellular pathway to Na^+, Cl^- and mannitol. Albumin was assumed to remain quantitatively in the instillate.

In order to analyse the primary data a simple model was formulated in which two compartments (alveoli and perfusate) were separated by a membrane composed of a pathway performing active transport of Na^+ (cellular part) and a pathway in which ions and mannitol moved according to passive diffusion laws (paracellular pathway).

The model is basically identical to the one formulated by Olver & Strang (1974) with modifications as required by the slightly different experimental conditions.

According to mass concentration, for any solute in the instillate

$$\frac{dM_o}{dt} = \frac{d(V_o C_o)}{dt} = V_o \frac{dC_o}{dt} + C_o \frac{dV_o}{dt}. \quad (1)$$

In this and subsequent equations the subscript o refers to alveolar fluid (out); the subscript i to the perfusate (in). M is mass, V is volume of instillate, C is concentration, t is time. This equation expresses that volume as well as concentration may vary during an experimental period. Also

$$\frac{dM_o}{dt} = J_{act} + J_{pass}, \quad (2)$$

where J_{act} is the active ion flux due to cellular activity and J_{pass} is the flux through the paracellular pathway.

For albumin, it is supposed that

$$\frac{dM_o}{dt} = 0.$$

Then, the variation of albumin concentration is proportional to the variation of the volume of alveolar fluid

$$\frac{dC_o}{dt}(Alb) = -\frac{C_o}{V_o}(Alb)\frac{dV_o}{dt}. \quad (3)$$

The passive ion flux is supposed to obey the conditions expressed in the integrated Nernst–Planck equation describing passive ion movements

$$J_{\text{pass}} = \frac{PAz\,F\Delta\psi}{RT}\left(\frac{C_i - C_o\,e^{-zF\Delta\psi/RT}}{1-e^{-zF\Delta\psi/RT}}\right) + J_v(1-\sigma)\,\bar{C}. \tag{4}$$

J_v is the volume flow through the alveolar membrane consequent upon osmotic or hydrostatic pressure differences, σ is the reflexion coefficient of the diffusing species, $\bar{C}$ is the mean concentration in the membrane, P is the permeability of the solute and A is the total surface area of the alveolar membrane; z, F, R and T have their usual significance. $\Delta\psi$ is the electrical potential difference between the alveolar instillate and the interstitial fluid.

We define

$$J_{\text{act}} = J_w\,C_o, \tag{5}$$

where J_w is the flow of pure water following the net movement of NaCl, assuming that the absorbed fluid is isosmotic.

The change of volume of instillate has two components

$$\frac{dV_o}{dt} = J_w + J_v. \tag{6}$$

It is assumed that the rate of variation of volume of the instillate is constant during an experimental period. This implies that

$$V_o = V_A + (J_w + J_v)\,t, \tag{7}$$

where V_A is the volume of fluid instilled in the alveoli.

This gives for the total mass flow

$$[V_A + (J_w + J_v)\,t]\frac{dC_o}{dt} + (J_w + J_v)\,C_o = PA\frac{zF\Delta\psi}{RT}\left(\frac{C_i - C_o\,e^{-zF\Delta\psi/RT}}{1-e^{-zF\Delta\psi/RT}}\right) + J_v(1-\sigma)\,\bar{C} + J_w\,C_o. \tag{8}$$

Since several observations (to be reported under Results) indicate that the electrical potential difference across the alveolar membrane is very small, eqn. (4) simplifies to the usual equation for diffusion through an uncharged membrane. Further simplifications of the flux equation are obtained assuming there is no significant osmotic concentration difference across the alveolar wall, and that the hydrostatic pressure difference is also negligible. These assumptions imply that the passive net water flux of the instillate is zero ($J_v = 0$).

Eqn. (8) then takes the form

$$(V_A + J_w\,t)\frac{dC_o}{dt} = PA(C_i - C_o), \tag{9}$$

or, for the variation in isotope concentration (marked by an asterisk),

$$\frac{d{*}C_o}{dt} = \frac{PA}{V_A + J_w\,t}\,({*}C_i - {*}C_o). \tag{10}$$

Assuming that the tracer concentration in the perfusate is constant, the equation can be integrated over a period of time x to give

$$PA = -J_w \ln\frac{({*}C_i - {*}C_o)_{t=x}}{({*}C_i - {*}C_o)_{t=0}} \Big/ \ln\frac{V_A + J_w\,x}{V_A}. \tag{11}$$

For passively transported solutes, $J_{\text{act}} = 0$. This means that the term $J_w\,C_o$ on the right-hand side of eqn. (8) disappears. Eqn. (8) then takes the form

$$(V_A + J_w\,t)\frac{dC_o}{dt} + J_w\,C_o = PA(C_i - C_o). \tag{12}$$

or for a tracer

$$\frac{d{*}C_o}{dt} = \frac{PA{*}C_i - (PA + J_w){*}C_o}{V_A + J_w\,t}. \tag{13}$$

It is seen that eqn. (10) and eqn. (13) are different. Whilst eqn. (10) applies to ions that are actively transported and coupled to net water movement, eqn. (13) applies to solutes that do not follow

this volume. In the present experiments it is assumed that only Na^+ and Cl^- are the constituents of the isotonic volume of fluid that is actively removed. The reason for the difference between the equations is due to the fact that an 'actively' removed tracer only varies in concentration due to passive diffusion and not as a consequence of the actively absorbed volume of fluid.

The withdrawal of fluid secondary to active transport of Na^+ leads to an increase in concentration of other solutes. Thus, their concentration changes not only as a consequence of passive diffusion but also because of the effects of the reduction in 'distribution' volume.

Integration of eqn. (3) gives, when ${}^*C_i = 0$,

$$PA = -J_w \left(1 + \ln \frac{{}^*C_{o,t-x}}{{}^*C_{o,t-0}}\right) \Big/ \ln \frac{V_A + J_w x}{V_A}. \quad (14)$$

It should be emphasized that although the eqns. (11) and (14) are different they both give the passive permeability of the total exposed alveolar membrane, i.e. they describe the properties of the passive (paracellular) channel for hydrophilic solutes that do not pass through the cells.

Statistical methods

Results are given as means ± S.E of the mean. All results within groups and between groups were compared by analysis of variance. Correlations were obtained by use of the unweighted least-square method.

RESULTS

The presentation of the experiments begins with an account of the rate and direction of fluid transport in the control situation and under the influence of ouabain and amiloride. Then follow studies of the transport of Na^+, divided into active and passive components. Determination of the unidirectional fluxes of $^{22}Na^+$ and $^{36}Cl^-$ from the alveoli towards the perfusate and in the opposite direction permits conclusions about the permeability of the epithelium to these ions and defines limits to the magnitude of the transalveolar potential difference. The paracellular pathway was also characterized by the permeability of mannitol.

Rate of fluid absorption

Three consecutive periods of measurement were obtained in most experiments, but it was found that the rate of fluid absorption was significantly lower in the third period (in several cases the direction of fluid movement was even reversed). Since this undoubtedly was due to deterioration of the preparation after 1 h of perfusion, only the results from the first two periods were used.

In experiments with phosphate-buffered Ringer solutions as perfusate and instillate the average rate of fluid absorption in the first two periods was 134 nl/s (S.E., 18·5; $n = 14$). The rate of absorption in the third period was 69 nl/s (S.E., 10·8, $n = 6$).

To investigate the importance of active Na^+ transport for the fluid movement ouabain was added to the perfusate, since it was most likely that a Na^+–K^+ pump would be situated at the abluminal membrane of the epithelium (DiBona & Mills, 1979). Ouabain was used in various concentrations in order to find an upper limit of tolerance. The preparations tolerated a ouabain concentration of 2×10^{-4} M rather well, but sometimes pulmonary oedema (reversal of fluid transport) developed during the second period. These experiments were discarded. Ouabain reduced the fluid absorption rate to 57 nl/s (S.E., 8·2; $n = 18$). Rat tissues are known to be resistant to ouabain effects (Stekhoven & Bonting, 1981) which probably explains why the Na^+ transport was only partially blocked (cf. Fig. 1). Another possibility is that not all alveoli received the perfusate due to heterogeneous perfusion.

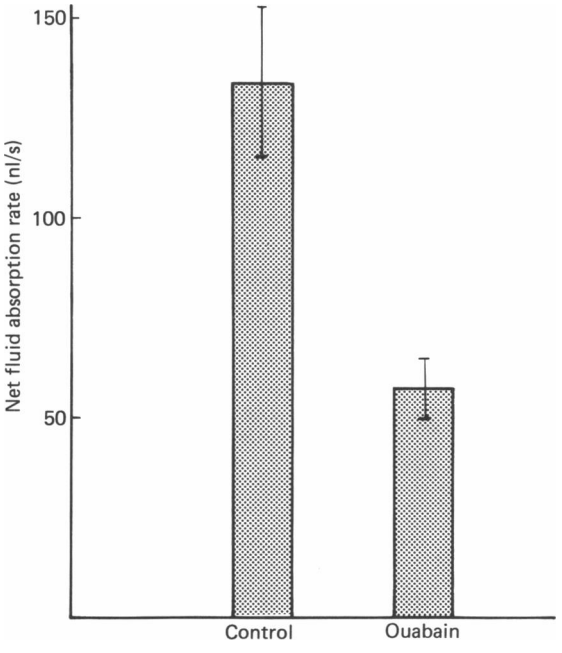

Fig. 1. Net fluid absorption rate in isolated, perfused rat lungs, in the undisturbed state and under the influence of ouabain (10^{-4} M) in the perfusate. The error bars show the S.E. of mean (number of periods given in the text).

The aminopyrazine–guanidine compound amiloride is known to inhibit entry of Na^+ across the luminal membrane of tight epithelia (Bentley, 1968; Macknight, DiBona & Leaf, 1980; Benos, 1982). In experiments in which amiloride was present in the instillate in concentrations between 10^{-3}–10^{-4} M it reduced fluid absorption to 74 nl/s (S.E., 8·6; $n = 16$). Both the ouabain and the amiloride effects were significant at the 1 % level.

In order to study the effect of colloids in the instillate, experiments were carried out with 4 % (w/w) Ficoll added to the Ringer solution, both in experiments with phosphate and bicarbonate buffers. The experiments were not entirely successful because Ficoll appeared to affect the alveolar epithelium, as reflected by a steady decrease in the rate of absorption during consecutive periods, often ending with pulmonary oedema. The over-all rate of absorption (based on the first two periods after the period of equilibration) was 106 nl/s (S.E., 7·7; $n = 21$). This value does not differ significantly ($0{\cdot}1 < P < 0{\cdot}05$) from the earlier value found in experiments with colloid-free instillates. It is, however, likely that the apparent difference in reabsorption rates is due to an effect of Ficoll on the lung rather than to the colloid pressure *per se* since, in experiments reported in an accompanying paper (Basset *et al.* 1987), it proved possible to arrest fluid absorption completely by removal of Na^+ from the instillate. In those experiments a colloid–osmotic gradient was present favouring fluid absorption, but it appeared to be without importance. This was further demonstrated in investigations where plasma was instilled and no diminution of rate of fluid absorption was seen (Basset *et al.* 1987).

Since Ficoll did not unequivocally reduce fluid absorption, results from experiments

with and without Ficoll in the instillate were treated as one population in the following presentation of active and passive ion fluxes and determinations of mannitol permeabilities. Ficoll was mostly used in experiments without pharmacological interference. This means that inclusion of the Ficoll experiments rather resulted in underestimation of the various effects elicited experimentally.

The effect of buffer on fluid absorption was studied in the Ficoll series of experiments. With bicarbonate as buffer the rate of absorption was 110 nl/s (S.E., 11·2; $n = 8$); with phosphate as buffer the rate was 103 nl/s (S.E., 10·6; $n = 13$). Thus, under these conditions the buffer medium seemed not to influence absorption.

Na^+ movements across the epithelium

It was assumed that Na^+ moved across the alveolar epithelium by two routes, one transcellular and one paracellular. The transcellular route supposedly represents the active transport pathway. The rate of active transport was determined from the rate of fluid transport on the assumption that the absorbate is similar in composition to the instillate (isotonic absorption). The osmotic activity of the instillate was not checked during the experiments, the aliquots being too small to permit such analysis.

TABLE 1. Net Na^+ fluxes across rat alveolar epithelium

	Net Na^+ flux (pmol/(cm² s))	S.E.	n	Probability
Control	3·3	0·24	25	—
Ouabain	1·6	0·24	18	< 0·01
Amiloride	2·1	0·26	16	< 0·01

Based on an alveolar surface area of 5000 cm².

The total unidirectional flux of Na^+ was determined with $^{22}Na^+$, and the paracellular (passive) flux was calculated as the total unidirectional flux minus the transcellular (active) flux. The fluxes are given per square centimetre alveolar surface area assuming a total surface area of 5000 cm² in rat lungs (Weibel, 1973; Crapo, Young, Fram, Pinkerton, Barry & Crapo, 1983). The advantage of expressing the findings per square centimetre is that comparisons between experiments performed on different animals are facilitated. But the use of the total alveolar surface area to convert whole-lung data to fluxes and permeabilities may be erroneous if the entire alveolar surface area is not wetted by the instilled fluid. It cannot be excluded with present techniques that some alveoli did not receive the instillate, and permeabilities as well as fluxes are probably slightly underestimated.

The active Na^+ flux in the series of control experiments was 3·3 pmol/(cm² s) (S.E., 0·24; $n = 25$). The passive flux was 9·4 pmol/(cm² s) (S.E., 1·30; $n = 25$).

It may be asked whether this high shunt flux is artifactual and in some way related to the assumptions on which the calculations were based. An answer may be obtained from the experiments in which a major fraction of the active Na^+ flux was blocked with ouabain. With ouabain present in the perfusate the active Na^+ flux was reduced to 1·6 pmol/(cm² s) (Table 1), yet the passive flux was not significantly different from the control situation, being 8·3 pmol/(cm² s). Similarly, with amiloride in the instillate the active Na^+ flux was reduced to 2·1 pmol/(cm² s) (cf. Table 1),

TABLE 2. Passive Na^+ fluxes across alveolar epithelium

	Passive Na^+ flux (pmol/(cm² s))	S.E.	*n*	Probability
Control	9·4	1·30	25	—
Ouabain	8·3	1·05	18	n.s.
Amiloride	9·3	1·11	10	n.s.

n.s. means that the difference between the passive control flux and the fluxes under the effect of ouabain or amiloride were not statistically different. Based on an alveolar surface area of 5000 cm².

TABLE 3. Permeability (*P*) of mannitol in the paracellular pathway

	P (cm/s × 10^{-8})	S.E.	*n*	Probability
Control	2·4	0·40	32	—
Ouabain	2·0	0·56	18	n.s.
Amiloride	1·9	0·36	16	n.s.

n.s. = not significant. Based on an alveolar surface area of 5000 cm².

whilst the passive flux was 9·3 pmol/(cm² s) (Table 2). The passive fluxes with the blockers present were not statistically different from the control situation, cf. Table 2. The constancy of the calculated passive fluxes, despite variations in active fluxes, supports the applicability of the formal analysis.

The fact that the shunt flux was large relative to the net flux implies that the calculated flux ratio for Na^+ is small. It was 1·53 (S.E., 0·090; $n = 25$) in the control experiments. This value corresponds closely to the value calculated for the actively transported Cl^- in the fetal lung, which was 1·62 (Olver & Strang, 1974).

Mannitol permeability

It is unlikely that mannitol permeates through the cellular moiety of the epithelium and therefore this non-electrolyte was included in all experiments to give information about the paracellular permeability. Furthermore, the mannitol permeability provides a way of checking whether the values obtained for the paracellular Na^+ fluxes are reasonable, since the ratio of the free diffusion coefficients for Na^+ and mannitol is 2·0 (Crone & Levitt, 1984). The average permeability of mannitol was $2{\cdot}4 \times 10^{-8}$ cm/s calculated from eqn. (14). This value was calculated from a permeability surface area product of $1{\cdot}2 \times 10^{-4}$ ml/s, dividing by an alveolar surface area of 5000 cm². Table 3 shows mannitol permeabilities determined in the control situation and in experiments with ouabain or amiloride. Although the values were slightly lower with ouabain and amiloride the differences were not statistically significant. If Na^+ and Cl^- diffuse freely in an uncharged paracellular pathway the Na^+ permeability would be $4{\cdot}8 \times 10^{-8}$ cm/s and that of Cl^- would be $7{\cdot}2 \times 10^{-8}$ cm/s based on the free diffusion coefficients relative to that of mannitol.

Passive permeabilities of Na^+ and Cl^-

The assumption that the transalveolar potential difference is insignificant (below 5 mV) is particularly important in determinations of ion permeability values. Since

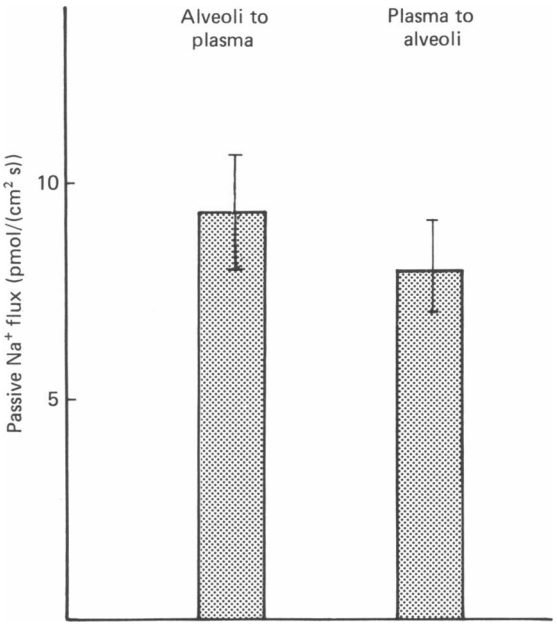

Fig. 2. Passive fluxes of Na^+ across the alveolar epithelium in isolated, perfused rat lungs. The fluxes were determined in the direction from the alveoli to perfusate and in the opposite direction (in separate experiments). The error bars show the S.E. of the mean (number of periods given in the text). The calculations were based on an alveolar surface area of 5000 cm^2.

TABLE 4. Permeabilities (P) of Na^+ and Cl^- ($cm/s \times 10^{-8}$) across the alveolar epithelium in isolated perfused lungs

	Alveoli to perfusate			Perfusate to alveoli		
Ion	P	S.E.	n	P	S.E.	n
Na^+ (control)	6·7	0·93	25	—	—	—
Na^+ (+ouabain)	5·9	0·76	18	—	—	—
Na^+ (+amiloride)	6·8	0·74	10	5·2	0·55	6
Cl^-	10·2	1·68	7	13·8	1·42	10

it may be asked whether this assumption holds true, we performed measurements of undirectional Na^+ and Cl^- fluxes in the direction from alveoli to perfusate and in the opposite direction. If an important electrical potential difference is present the two fluxes would differ significantly (Ussing, 1949).

In order to avoid the possibility that the $^{22}Na^+$ which reaches the alveoli from the perfusate returns to the perfusate via active transport through the cells (thus leading to underestimation of the unidirectional flux) the experiments were performed with the Na^+ influx into the epithelium from the alveolar side being partially blocked with amiloride. Similarly, in the case of Cl^-, in the experiments in which $^{36}Cl^-$ was added to the perfusate, bumetanide was added to the perfusate in order to block any

co-transport system for Cl^- which might be present in the abluminal membrane (Carrière & Dandanivo, 1976). The passive Cl^- flux in the direction from alveoli to perfusate was 14·7 pmol/(cm² s) (S.E., 2·4; $n = 7$) and in the opposite direction was 20·2 pmol/(cm² s) (S.E., 2·0; $n = 10$). Fig. 2 illustrates the findings in experiments with $^{22}Na^+$.

The similarity of the respective ion fluxes in the two opposite directions speaks against the presence of an important transalveolar potential difference and it is thus permissible to calculate the permeabilities directly without taking the potential term into consideration.

The results of the calculations of the passive permeability to Na^+ and Cl^- are summarized in Table 4. It is seen that the permeability values for Na^+ and Cl^- calculated from fluxes in either direction correspond quite closely to the expected figures based on the mannitol permeability (cf. p. 319).

DISCUSSION

The fluid absorption *in vivo* by adult alveolar epithelium has not formerly been analysed within the analytical framework applied in studies of transepithelial transport. It appears, however, that concepts from general epitheliology are directly applicable to liquid-filled lungs as shown by the analysis by Olver & Strang (1974) and Olver (1976, 1977) of the secretion process in the fetal lung. The analysis used in the present paper is very close to that used on the fetus. Our results show that a secretory epithelium at the time of birth is changed into an absorptive epithelium and that the adult alveolar epithelium may be listed among absorptive epithelia such as urinary bladder, intestinal epithelium, salivary duct, etc.

The common explanation for fluid absorption from the alveoli is that it depends on a combination of surface tension, interstitial negative pressure and an oncotic pressure difference. Two arguments let us think that these mechanisms are of minor importance for water absorption in liquid-filled lungs. First, the addition of a large molecular weight solute to the instillation in concentrations that match those in the perfusate did not change the rate of fluid transport appreciably. Secondly, the effects of ouabain and amiloride strongly suggest that reabsorption is dependent on Na^+–K^+-ATPase activity in the alveolar epithelium and on the presence of Na^+ channels in apical membranes of these cells. Further, the passive permeability of the epithelium to mannitol and Na^+ was unaffected by ouabain or amiloride, indicating that these substances did not exert their effect by allowing fluid entry due to augmentation of the membrane permeability. The occurrence of large leaks, following the blocker action, is also unlikely because the [^{125}I]albumin counts in the perfusate at the end of the experiments always were less than 0·2 % of those in the instillate.

We are then led to conclude that most of the fluid uptake depends on specific metabolic processes.

Methodological considerations

It is impossible to study transalveolar transport processes *in vivo* unless fluid is instilled into the alveoli. This technique has, therefore, often been used to characterize the epithelium since the pioneering studies by Taylor, Guyton & Bishop (1965) and

Wangensteen, Wittmers & Johnson (1969). It is surprising that such a procedure apparently works so well since the lungs are, in fact, not totally emptied of gas. Whether all alveoli receive the instillate is unknown – if, say, half of the alveolar population participates in the absorption the results would, however, only be changed by a factor of 2, and nothing fundamental in the conclusions would be affected.

The important figure upon which all flux calculations rest is the volume of fluid absorbed per unit of time. This was determined by the steady increase in the concentration of the impermeable tracer albumin in the instillate. Small amounts of plasma were always added to the instillate to act as carrier for the radioactive albumin and thus avoid adherence problems. The appearance of ^{125}I label in the perfusate was routinely checked and the counts represented only a negligible fraction of the counts instilled into the alveoli. This figure was dramatically changed when oedema developed following blocker action or due to an alteration of the epithelium after more than 1 h of experiment.

The experiments with ouabain and amiloride together constitute evidence that Na^+ is the prime mover, and not Cl^-. The net flux of Na^+ would be equal to the active flux only if there is no backflux of absorbed fluid. This is probably correct if one considers the large perfusate flow through the lung capillaries, the small extravascular space and the large exchange surface area compared to the small rate of fluid transport. It should be stressed that the observed rate of active Na^+ transport per square centimetre is among the smallest so far determined in Na^+ transporting epithelia, only matched by the rabbit urinary bladder (Lewis & Diamond, 1976). On the other hand, the alveolar epithelium (together with the intestine) has the largest surface area of any of the body's epithelial membranes so that the total rate of transport is not negligible. A fluid transport rate of 134 nl/s in rat lungs would roughly correspond to 1·5 l/24 h in human lungs.

Relation to other studies

The view that fluid movement across the alveolar membrane in the adult lung is secondary to Na^+ has been advanced several times in recent years based on findings in the mature fetal lamb (Olver, 1983), on dome formation in cultured type II epithelial cell layers (Goodman *et al.* 1983) and amiloride effects in sheep lung (Matthay *et al.* 1982, 1985). The present experiments are in accordance with these findings. We are, however, not convinced that the active Na^+ transport *in vivo* is carried out by type II cells alone and find that the evidence on this point remains controversial.

The question cannot be answered at this stage with reference to experimental observations so any hypothesis goes by necessity beyond available evidence. We propose that the type I cells are also involved in Na^+ reabsorption and fluid transport.

The passive permeabilities of mannitol, Cl^- and Na^+ ranged between 10^{-8} and 10^{-7} cm/s. Both non-electrolyte and ion permeability in the mammalian alveoli has been determined several times. Our results comply with those of Wangensteen *et al.* (1969) and Taylor *et al.* (1965) when correction is made for the eight-times smaller lung surface assumed by Taylor *et al.* Permeability values of this size are among the lowest reported for any epithelium – they correspond to those found in notoriously tight epithelia such as the frog skin (Bruus, Kristensen & Larsen, 1976) and rabbit urinary bladder (Lewis, Eaton & Diamond, 1976).

Although the paracellular pathway probably has a high electrical resistance (Basset *et al.* 1987) it nevertheless shunts the electrical potential created by the electrogenic Na^+–K^+ pump in the abluminal membrane since the two oppositely directed passive fluxes of Na^+ and Cl^- were almost similar (Fig. 2). This is only possible if the transepithelial electrical potential difference is small (Ussing, 1949). This inference is supported by the measurements of Nielson, Goerke & Clements (1981) who determined a potential difference of 1 mV in the rabbit, the alveoli being positive. The combination of a 'tight' epithelium and a low transmembrane potential is uncommon, and it is disputable whether the alveolar epithelium should be classified as a tight epithelium, despite its assumed high electrical resistance. This stresses the fact that what counts is not so much the absolute resistance of an epithelium, as the relation between transcellular ion transport and paracellular shunting (Boulpaep, 1982).

These aspects are, however, details as compared with the main conclusion that it is active ion transport that is responsible for net fluid transport out of the alveoli. The present findings strongly indicate that passive forces do not explain fluid movement across an intact alveolar membrane. This conclusion implies that supply of oxygen by the perfusate may be more critical than hitherto assumed and we want to emphasize the fact that red blood cells were added to the perfusate in the present experiments, significantly increasing the oxygen-carrying capacity.

Basset *et al.* (1987), describing experiments on lungs perfused *in situ* with blood, using cross-circulation technique expand the present conclusions and show that it is possible by various chemical modifications completely to arrest fluid absorption despite the presence of a large difference of colloid–osmotic pressure that would favour absorption.

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