Ion transport across the excised bullfrog lung

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GATZY, John T. Ion transport across the excised bullfrog lung. Am. J. Physiol. 228(4): 1162-1171. 1975.—Fluxes of ions and water across the short-circuited, excised bullfrog lung were determined by radioisotope techniques. The unidirectional flows of Na⁺, K⁺, Ca²⁺, TeO₄⁻, HCO₃⁻, gluconate, p-aminohippurate, dinitrophenolate, SO₄²⁻, and water were symmetrical. Both HCO₃⁻ fluxes were reduced by acetazolamide. In contrast, Cl⁻, Br⁻, I⁻, and SCN⁻ movement from serosa to mucosa exceeded the flux in the opposite direction. Net Cl⁻ transport followed the kinetics of a saturable process and was inhibited by thiouracil and hypoxia. These results indicate an active secretion of halide anions and SCN⁻ into the lumen. Attempts to demonstrate Br⁻ anionism of Cl⁻ transport were equivocal. Cl⁻ transport accounted for 50% of the early short-circuit current but after 90 min the two measurements were equal. Incubation of the lung in bicarbonate-free Ringer revealed unequal decreases in the concentration of the bathing solutions. Net "base" addition to the serosal solution was reduced by prior removal of the blood from the pulmonary vasculature. Therefore, "base" release could not be localized to the epithelia. The Na⁺, K⁺, Ca²⁺, and Cl⁻ composition of the lung tissue was unchanged over 3 h. Since tissue and, hence, cell Cl⁻ is lower than the concentration in the bathing solution the Cl pump is probably located in the luminal border of the alveolar epithelial cell.

active Cl⁻ transport; halide anion secretion; flux of alkalai metal cations; Cl⁻ transport and short circuit current; carbonic anhydrase inhibitors; bicarbonate flux

INTEREST IN THE MAMMALIAN LUNG AS A HEMODIALYSIS INTERFACE
has led to detailed studies of solute and water exchange between the lung lavage fluid and the blood (4, 5, 22, 25). In general, the rate of permeation of small, hydrophilic solutes suggested that a barrier with properties similar to those reported for other epithelia was interposed between the blood and the lung lumen. However, the locus of the limiting barrier between air and blood is not clear. Dye-dilution studies suggest that the capillary endothelium may be the major impediment to the escape of small solutes from the blood into the air space (2). On the other hand, small solutes added to the vascular space exert a much greater osmotic effect in the fluid filled than in the air filled lung (21). These results are consistent with the notion that the alveolar epithelium is the dominant permeability barrier.

The interpretation of studies of solute and water movement between blood and the alveolar lumen has been hampered by the complex morphology of the mammalian lung. Even if indirect methods are exploited to separate the contributions of the series epithelial and capillary barriers, translocation across the alveolar epithelium cannot be distinguished from parallel flow across the bronchial mucosa. In addition, no attempt has been made to measure an electrical PD between the two fluid compartments on either side of the barrier. Therefore, ion movement against the gradient of electrochemical potential could not be determined.

Experiments in this laboratory have demonstrated that the isolated anuran lung might serve as a model system for a more direct study of solute movement across the alveolar epithelium (10). The frog alveolar epithelium is a single layer of cells that is separated from the pleural mesothelium by a connective tissue layer. Permeability coefficients have been determined for the penetration of small electrolytes across mesentery and peritoneum of mammals (12) and the peritoneum of a toad (9). These coefficients were at least an order of magnitude greater than the values calculated for solute movement between mammalian lung lumen and blood (22, 25) or for other amphibian epithelia such as skin, urinary bladder, stomach, or large intestine (3, 14, 18, 24). Therefore, the pleural covering of the amphibian lung would be expected to be very permeable to small electrolytes and it is likely that the only other continuous cell layer, the epithelium, is the major permeability barrier.

Measurements of the bioelectric properties of the bullfrog lung support this reasoning (10). Each lung lobe is a single alveolar sac and can be mounted as a near planar sheet in a Ussing-type chamber. The resistance and spontaneous transmural electrical PD across this preparation were reduced immediately by the addition of HgCl₂ or sodium edetate to the mucosal solution. Addition to the serosal solution resulted in gradual decreases after a considerable delay. The results are consistent with the slow penetration of the agents through the connective tissue layer and, hence, with the hypothesis that the epithelium is the locus of the major resistance to ion flow.

It was the objective of this study to characterize the penetration of a variety of electrolytes and to explore the relationship between the flow of bathing solution ions and the production of bioelectric current by the excised lung of the bullfrog (Rana catesbeiana).

MATERIALS AND METHODS

A. Animals

Bullfrogs (Rana catesbeiana) were obtained from biological supply houses in California, Massachusetts, and Vermont. The animals were kept at room temperature (20-24°C) in tanks that were continuously rinsed with water and illuminated for 14 h/day. During the summer and fall the bullfrogs fed spontaneously on Rana pipiens stored in the same tanks. During the winter and spring a diet of ground liver, fish and horse meat was forced-fed about once a week.
B. Solutions

The principal salt solution used in these experiments contained 114 mM NaCl, 2.4 mM KHCO₃ and 1 mM CaCl₂ per liter. This solution had a freezing point equivalent to that of 120 mM NaCl and a pH of 8.0–8.7 after equilibration with 100% O₂. Alterations in the Cl⁻ concentration were effected by replacing CaCl₂ with Ca gluconate and NaCl with NaBr or Na gluconate or combinations of both. A similar procedure was followed when Na₂SO₄ replaced NaCl. The Na⁺ concentration was held at 114 meq/liter and approximately 60 mM of mannitol were added to each liter of solution to keep the osmolality constant. In a few studies NaSCN or NaI was substituted for NaCl. A sodium salt to the standard formula.

C. Unidirectional flux studies

After mounting a 1.8 cm² area of lung in an Ussing-type Lucite chamber the spontaneous transmural potential was monitored continuously through a pair of agar-KCl or agar-Ringer electrodes that were positioned a few millimeters from each surface of the lung. The electrodes were connected to a recording voltmeter (input impedance, 1 MΩ or greater) through calomel half-cells. Conductance was calculated from the PD change that resulted from the passage of 10 μA of current in either direction across the tissue. The short-circuit current was taken as the current required to reduce the transmural PD to zero. A small correction was made for the resistance of the Ringer solution, and weighed in tared flasks. Tissue water content was determined by 10 min for a 15-s voltage measurement. Source and sink solutions were sampled at the end of each collection period.

Bathing solution ¹H, ⁴⁰Ca, ³⁵S, ¹⁴C, ⁴⁴Tc and ³⁶Cl were determined by liquid scintillation counting in a dioxane flow. The uniformity of quenching was monitored by the channels-ratio or external-standard method. A fixed volume of Ringer solution that contained ⁴⁴Ca, ³⁵S, ³⁶Cl or ³⁶Na, or ⁴⁴K was counted with a KI crystal scintillation probe and scaler. The unidirectional flux or permeability coefficient (k) was calculated from the expression:

\[
k = \frac{\text{steady-state rate of appearance of tracer in sink (cpm/s)}}{\text{source radioactivity (cpm/cm²) \times area of lung (cm²)}}
\]

Tentative conclusions about the mode of solute movement were drawn from the unidirectional flux coefficients and the Ussing equation (23). This relationship predicts that, in the absence of net volume flow and a gradient of both electrical and chemical potential, the unidirectional fluxes (and coefficients) of a substance that diffuses between two compartments should be equal.

When H¹⁴CO₃⁻ was added to a bathing solution the gas efflux from both chambers was led through an aminoethanol solution to collect NaCl. Lungs that had been exposed to H¹⁴CO₃⁻ were dissected from the apparatus, bled briefly on filter paper that had been moistened with Ringer solution, and weighed in tared flasks. Tissue water was assumed to be 83% of the wet weight. Next, the lungs were solubilized in 3 ml of NCS (Nuclear-Chicago, Des Plaines, Ill.). Samples of this solution were counted in a dioxane fluor along with appropriate blanks and standards. The tissue HCO₃⁻ pool was calculated as:

\[
\text{tissue [HCO₃⁻] (meq/kg H₂O) = \frac{\text{steady state tissue radioactivity (cpm)}}{\text{solution [HCO₃⁻] (meq/liter)}} \times \text{solution [HCO₃⁻] (meq/liter)}}
\]

To confirm the identity of the migrating tracer 50-μl samples of the bathing solution were streaked on Whatman 3M filter paper strips. The strips were subjected to electrophoresis in a 0.5 M, pH 8.5 barbital buffer for 35 min at 50 V/cm. After drying at 60°C, the strips were cut into 1-cm sections and each section was counted in a toluene fluor. The contributions of ³⁵SCN and ³⁶Cl that were streaked at a common origin could be separated by double-label
counting. The peak of the $^{36}$Cl band was found routinely to have migrated 4 cm farther toward the anode than the $^{35}$SCN peak. Better than 90% of the radioactivity that was added to the paper was recovered.

D. Studies with Bicarbonate-Free Solution

Both surfaces of lungs in the flux chambers were exposed to bicarbonate-free Ringer solution for 1 h. During the period the solutions were bubbled with 100% O$_2$ that had been passed through alkali and acid traps. After equilibration with O$_2$ the unbuffered solution had a pH of 7.2–7.4. Incubation with the lung increased the pH of both bathing solutions. The release of "base" into mucosal or serosal solution was estimated by titrating to pH 7 with .05 N HICL under purged O$_2$. To determine the contribution of the apparatus to changes in solution pH, each experiment was preceded by a blank run in which a piece of Parafilm (Marathon Products, Neenah, Wisc.) served as the septum between the half-chambers.

E. Tissue Ion Analysis

Portions of the lung that were clamped in the flux chamber or incubated in flasks with Ringer solution were removed and blotted on premoistened filter paper. The tissues were placed in tarred flasks, weighed, dried overnight at 95°C and reweighed to obtain the weight of the tissue water. The flasks with the dried lungs were placed on a shaker and extracted with .1 N HNO$_3$ for 48 h. Preliminary experiments had shown that subsequent wetashing of the extracted tissues with concentrated HNO$_3$ and H$_2$O$_2$ did not yield additional electrolyte. Samples of this extract were used to determine Na$^+$, K$^+$, and Ca$^{++}$ by flame photometry and Cl$^-$ by amperometric titration. Appropriate blanks and standard solutions were carried through the entire protocol.

RESULTS

A. Unidirectional Fluxes

1) Water and all ions except halides and SCN$^-$. Unidirectional fluxes of electrolytes across the short-circuited excised lung were measured in order to obtain preliminary information about the mode of translocation. An analysis based on the Ussing equation fits the data of Table 1 into two patterns. The coefficients for each of a number of species were similar regardless of the direction of tracer flow. Included in this group are all the cations in the bathing solution, water and all anions that were tested with the exception of the halides and SCN$^-$. It is interesting that the k's for TeO$_4^{3-}$, a pseudo-halide that competes with I$^-$ for transport into the thyroid (26), were also symmetrical. The symmetry of the unidirectional flux coefficients is compatible with passive movement of each of these species across the lung.

For sulfate the evidence for uncomplicated passive flow is even stronger. The concentrations of chemical carrier were increased over a thousand-fold but the flux coefficients were not altered significantly. Similar results were obtained with glucuronate concentrations of 0.4 and 116 meq/liter. Flux coefficients for two lung pairs at each concentration ranged from 0.7 to 2.3 $\times$ 10$^{-7}$ cm/s and did not appear to be influenced by the concentration of the chemical carrier. In addition, unidirectional fluxes of p-aminohippurate, an anion that is actively transported across a number of epithelia, were measured for two pairs of lung lobes. At .01 meq/liter, a concentration well below the level that saturates the transport mechanism in mammalian proximal tubule, both flux coefficients were between 1 and 2 $\times$ 10$^{-7}$ cm/s.

The measurement of $^4$K fluxes posed an additional problem. Preliminary experiments demonstrated that, even after a 3-h exposure, excised lung tissue continued to take up tracer at a nearly linear rate. This trend is reflected by the coefficients reported in Table 1. When samples were collected every 30 min over 3 h each of the coefficients for the last collection period (150–180 min) was significantly greater than the values for flow in the opposite direction the differences between the two were not significantly different ($P > .05$) for both collection periods. The probable magni-

<table>
<thead>
<tr>
<th>Tracer</th>
<th>Source [Carrier] meq/liter</th>
<th>$K \times 10^3$, cm s$^{-1}$</th>
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<tr>
<td></td>
<td></td>
<td>M $\rightarrow$ S</td>
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<tr>
<td>Cations</td>
<td></td>
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<tr>
<td>$^{36}$Na or $^{39}$Na</td>
<td>114t</td>
<td>6</td>
</tr>
<tr>
<td>$^{40}$K</td>
<td>2.4–3.4t</td>
<td>5t</td>
</tr>
<tr>
<td>$^{42}$Ca</td>
<td>2t</td>
<td>3t</td>
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<tr>
<td>Anions</td>
<td></td>
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<tr>
<td>$^{35}$Cl</td>
<td>116t</td>
<td>12t</td>
</tr>
<tr>
<td>$^{37}$Br</td>
<td>0.9–4t</td>
<td>3</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>$&lt;10^{-4}$t</td>
<td>5t</td>
</tr>
<tr>
<td>$^{35}$SCN or $^{35}$CN</td>
<td>0.5</td>
<td>8t</td>
</tr>
<tr>
<td>$^{45}$TeO$_4^{5-}$</td>
<td>0.2</td>
<td>3t</td>
</tr>
<tr>
<td>$^{15}$H$^+$CO$_3$</td>
<td>2.4</td>
<td>8</td>
</tr>
<tr>
<td>Dimethylophenolate$^{4+}$</td>
<td>0.15</td>
<td>3t</td>
</tr>
<tr>
<td>$^{35}$SO$_4$</td>
<td>$&lt;10^{-3}$</td>
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<tr>
<td>$^{35}$SO$_4$</td>
<td>114t</td>
<td>4t</td>
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<tr>
<td>Water</td>
<td>HTO</td>
<td>54,600t</td>
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Flux coefficients are means $\pm$ SE. 1 Concentration of chemical carrier in the sink also. 2 Estimate based on counting efficiency and source activity after addition of carrier free isotope. 3 Paired lobe experiments, i.e., flux in each direction measured in separate lobes from the same animal. 4 Significantly greater ($P < .05$) than the M $\rightarrow$ S coefficient. 5 Reflects flow that took place between 30 and 60 min after addition of isotope to the source. 6 Reflects flow that took place between 150 and 180 min after addition of isotope to the source.
tudes of the flux coefficients at specific activity equilibrium will be discussed later.

2) Rate of tracer from \(^{14}\text{CO}_2\); effect of acetazolamide on \(^{14}\text{C}\) flux. When \(^{14}\text{CO}_2\) was added to the source the chemical species that crossed the lung was never identified. It is apparent from the information in Table 2 that the participation of \(^{14}\text{CO}_2\) in the total flow of tracer cannot be disregarded in spite of the absence of exogenous \(^{14}\text{CO}_2\). Even when the tissue was replaced by Parafilm the rate of tracer loss from the source into the gas phase was more than twice the flow across control tissues. Furthermore, in the presence of the tissue the rates of label volatilization from the sink were about \(\frac{1}{3}\) of the transmural fluxes that were calculated from the rate of appearance of \(^{14}\text{C}\) in the sink solution. Although the lung appears to increase to rate of tracer volatilization from the source, there were too few measurements with a Parafilm septum to establish this difference. Since \(^{14}\text{CO}_2\) is likely to be close to equilibrium with \(^{14}\text{HCO}_3^-\) in the tissue compartment but not in the source or sink solution, it is difficult to estimate the contributions of \(^{14}\text{CO}_2\) and \(^{14}\text{HCO}_3^-\) to the transmural flux of radioactivity.

The measurements in the last column of the table demonstrate that both transmural fluxes were reduced equally by a high concentration of the carbonic anhydrase inhibitor, acetazolamide. At the same time the drug increased the steady-state levels of the tracer in the tissue regardless of the direction of flow. These effects are probably not the consequence of an overall change in tissue ion permeability because exposure of three lungs to the drug for an hour led to a mean spontaneous transmural PD and conductance that were 101 ± 5 SE % and 95 ± 3 SE % of the values for paired, untreated lobes.

Finally, it is reasonably certain that accurate estimates of changes in transmural flux and gas efflux of label can be made because recoveries of added isotope were nearly complete. All recoveries were between 92 and 96 % but only the recovery of tracer added to the mucosal solution in the presence of acetazolamide was significantly less than 100 %. The fact that a similar recovery was obtained when no tissue was present suggests that losses from any phase of the collection were proportional throughout all experiments.

A potential source of ion flow was the secretion or consumption of \(^{14}\text{H}\). Lungs were exposed to bicarbonate-free Ringer solution for 1 h. The transmural PD and conductance of six lobes in bicarbonate-free solution were \(96 \pm 4\) SE and \(99 \pm 6\) SE % of the values for paired controls demonstrating that the absence of bicarbonate did not affect the bioelectric properties of the lung. Table 3 lists the release of "base" (mg of HCl required to return the pH to 7) into bathing solutions that were exposed to lungs under open or short-circuit conditions or to a Parafilm septum. The pH of both compartments of the chamber tended to increase in the presence of the tissue but the increase in the pH of the serosal compartment exceeded that of the mucosal bath. However, this difference was not significant for those tissues that were perfused through the pulmonary vasculature before they were mounted in the chamber. Furthermore, perfusion decreased the apparent contribution of net efflux to the short-circuit current. These observations suggest that the source of some or all of the base may lie outside of the epithelial barrier. Because of this uncertainty no further attempts were made to identify the chemical species associated with the asymmetric pH changes.

3) Halides and SCN. The difference in the flux coefficients for the halides and SCN may be taken as presumptive evidence for their secretion into the lung lumen. However, a number of other criteria should be satisfied before the evidence for active movement can be considered to be adequate. Reduction of the net flux by metabolic inhibitors and by certain chemically similar species, a tendency of the net flux to reach a maximum as the concentration of the chemical species is increased and an inability to identify a coupling of the active flow to the net flux of any other

<table>
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<tr>
<th>Table 9. (^{14}\text{C}) transmural flux, volatilization and tissue levels in lungs exposed to solutions with Na(^{14}\text{CO}_2)</th>
<th>Chamber Septum</th>
<th>Lung</th>
</tr>
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<tbody>
<tr>
<td>Bathing Solution with Tracer (source)</td>
<td>Parafilm (n = 3)</td>
<td>Tissue, meq/kg H(_2)O</td>
</tr>
<tr>
<td><strong>Mucosal</strong></td>
<td></td>
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<tr>
<td>J, (\mu\text{eq}/1.8 \text{cm}^2\text{h})</td>
<td></td>
<td></td>
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<tr>
<td>M → S</td>
<td>.14 ± .02</td>
<td>.09 ± .01*</td>
</tr>
<tr>
<td>Gas, M</td>
<td>.67 ± .08</td>
<td>1.01 ± .18</td>
</tr>
<tr>
<td>Gas, S</td>
<td>.006 ± .002</td>
<td>.003 ± .0007</td>
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<tr>
<td>Tissue, meq/kg H(_2)O</td>
<td>2.40 ± .07</td>
<td>14.7 ± 2.8*</td>
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<tr>
<td>Recovery, % initial source</td>
<td>95</td>
<td>93.4 ± 2.9</td>
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<tr>
<td><strong>Serosal</strong></td>
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<tr>
<td>J, (\mu\text{eq}/1.8 \text{cm}^2\text{h})</td>
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</tr>
<tr>
<td>S → M</td>
<td>.14 ± .02</td>
<td>.09 ± .01*</td>
</tr>
<tr>
<td>Gas, M</td>
<td>.006 ± .002</td>
<td>.003 ± .0004</td>
</tr>
<tr>
<td>Gas, S</td>
<td>.38</td>
<td>.97 ± .15</td>
</tr>
<tr>
<td>Tissue, meq/kg H(_2)O</td>
<td>1.75 ± .58</td>
<td>12.7 ± 3.9*</td>
</tr>
<tr>
<td>Recovery, % initial source</td>
<td>95</td>
<td>93.5 ± 3.1</td>
</tr>
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Tissue values are means ± SE. * Significantly different (P < .05) from corresponding values for the untreated lung.
bathing solution constituent are additional properties that are associated with active transport systems (20). Experiments were designed to determine how many of these characteristics were described by the translocation of the halides and SCN$^-$.  

A) CL$^-$ FLOW AND CONDUCTANCE. Since bidirectional flux studies were not carried out on the same lung it was important to establish that the apparent asymmetry in fluxes was not the result of a greater ion leakiness in the tissues that exhibited a greater flow from serosa to mucosa. The conductance of each tissue can be used to monitor the overall permeability of the tissue to the major electrolytes in the bathing solution. The conversion of the unidirectional M $\rightarrow$ S fluxes for Cl$^-$ and Na$^+$ to partial ionic conductances indicated that most of an applied current should be carried across the tissue by these ions. Fig. 1 illustrates the relationship between the dc conductance of the lung and the flux coefficients for Cl$^-$ and Na$^+$. It is clear that the same linear relationship is described by the coefficients for Na$^+$, whereas most of the S $\rightarrow$ M coefficients for Cl$^-$ fall to the right of the line drawn through the S $\rightarrow$ M $k's$. This relationship indicates that the high S $\rightarrow$ M coefficients for Cl$^-$ were not obtained from a population of lungs with an unusually high ion permeability.

B) CL$^-$ AND SCN$^-$: IDENTITY OF TRANSPORTED SPECIES. It is equally important to ascertain the authenticity of the chemical species that undergo apparent transport. In four flux experiments $^{36}$Cl or $^{35}$SCN samples from both bathing solutions were subjected to electrophoresis. A single peak indicates that most of an applied current should be carried across the tissue by these ions. Fig. 1 illustrates the relationship between the dc conductance of the lung and the flux coefficients for Cl$^-$ and Na$^+$. It is clear that the same linear relationship is described by the coefficients for Na$^+$, whereas most of the S $\rightarrow$ M coefficients for Cl$^-$ fall to the right of the line drawn through the S $\rightarrow$ M $k's$. This relationship indicates that the high S $\rightarrow$ M coefficients for Cl$^-$ were not obtained from a population of lungs with an unusually high ion permeability.

C) EFFECT OF METABOLIC INHIBITORS ON CL$^-$ FLOW. To determine the effect of metabolic inhibitors on Cl$^-$ flow, lungs were exposed to dinitrophenol or low oxygen tension. These conditions had been shown previously to affect the bioelectric properties of the lung (10). The results of the study are reported in Table 4. Since flux measurements were initiated after the effects of the inhibitors on the short-circuit current had reached a steady-state, the coefficients must be compared with the control observations for Cl$^-$ in Table 1. Dinitrophenol reduced the short-circuit current to about one-half of the value that was recorded just before the introduction of the drug. This decrease was accompanied by a difference between the unidirectional flux coefficients that was significantly smaller than the control value of 10.6 $\pm$ 2.2 SE from Table 1. The decrease resulted from a selective inhibition of the S $\rightarrow$ M flux. Neither the M $\rightarrow$ S coefficient nor the dc conductance were affected by the drug.

In contrast, bubbling the bathing solutions with 100% N$_2$ reduced the short-circuit current to about one-half of the value that was recorded just before the introduction of the drug. This decrease was accompanied by a difference between the unidirectional flux coefficients that was significantly smaller than the control value of 10.6 $\pm$ 2.2 SE from Table 1. The decrease resulted from a selective inhibition of the S $\rightarrow$ M flux. Neither the M $\rightarrow$ S coefficient nor the dc conductance were affected by the drug.

D) NET TRANSPORT AND ION CONCENTRATION. To determine how closely the penetration of Cl$^-$ through the lung followed the kinetics of a saturable process, $^{36}$Cl fluxes were measured across lungs that were exposed to different concentrations of Cl$^-$. Cl$^-$ in both bathing solutions was replaced by the poorly permeant anion, gluconate. The
The results of these experiments are shown in Fig. 2. Both unidirectional fluxes increased with increasing extracellular Cl\(^-\) concentration but the net transfer of Cl\(^-\) (dashed line) approached a maximum. The net movements of Cl\(^-\) at the two lowest concentrations were significantly different (P < .001) from each other and from the points at higher Cl\(^-\) concentrations. None of the net flows at 25, 60, and 116 meq Cl\(^-\)/liter were different from each other. When the net transport was plotted according to one of the linear transformations that is reported to yield a more reliable graphic analysis than the popular Lineweaver-Burk plot (6) the relationship in Fig. 3 was obtained. The solid line is the result of a weighted regression analysis (13) and the experimental points satisfy the requirements for linearity (P = .6; \(\chi^2\) test). A maximum velocity of 31 \(\mu\text{Eq/cm}^2\text{h}\) and apparent \(K_m\) of 19.3 \(\mu\text{Eq/ml}\) were calculated from the slope of 3.22 \(\pm\) 0.38 \(\text{cm}^2\text{h}/\mu\text{Eq}\) and intercept of 62.1 \(\pm\) 6.3 \(\text{cm}^2\text{h/ml}\).

Br\(^-\) is thought to compete with Cl\(^-\) for transport across the gastric mucosa (15). Since the lung evaginates from a region of the gastrointestinal tract that is close to the stomach it seemed likely that some of the transport characteristics of the gastric mucosa might be shared by the lung. An examination of the effect of Br\(^-\) on Cl\(^-\) fluxes should exploit a concentration of Br\(^-\) large enough to ensure inhibition and ideally, a range of Cl\(^-\) concentrations wide enough to test for competition and surmountability.

However, the total concentration is limited by the osmolality of the bathing solution. Since the total replacement of Cl\(^-\) with Br\(^-\) failed to affect significantly the bioclectric properties of four lungs, it appeared likely that the \(K_m\) for Br\(^-\) might be close to that for Cl\(^-\). If this prediction were borne out by the flux experiments then 30 meq/liter was estimated to be the Br\(^-\) concentration that would come closest to satisfying both of the requirements stated above. The open points of Fig. 3 represent the net fluxes of Cl\(^-\) obtained in presence of a constant concentration of Br\(^-\) (see text).
meq/liter resulted in an apparent decrease in the net flux coefficient from 48.0 (± 15.8) (Table 1) to 16.2 ± 5.4. However, the change was not statistically significant. Attempts to measure fluxes in the presence of 5 and 116 meq SCN−/liter were marred by irreversible increases in transmural conductance of more than 300%. The spontaneous biopotential fell contemporaneously reaching values as low as 4% of the control with the highest SCN− concentration.

Lastly, the difference in the flux coefficients for I− at a bathing solution concentration of 1 meq/liter was 12.7 ± 4.0 SE X 10⁻⁷ cm/s in four paired-lobe experiments. This value is significantly smaller than the difference calculated from Table 1 for the flow of I− in a carrier-free solution. The results are in the direction that one would associate with a saturable process but the data are too meager to warrant a firm conclusion.

e) CHLORIDE TRANSPORT AND THE SHORT-CIRCUIT CURRENT

A number of excised amphibian epithelia such as frog skin, gastric mucosa, cornea and toad bladder exhibit a fixed relationship between the short-circuit current and the net flow of an ion or ions (14, 18, 24, 27). The net Cl− flux was calculated for each of the experiments in Table 1 and was compared with the current required to short-circuit the lobes in which the S → M flux was measured. Net Cl− transport accounted for only 31 ± 7 SE% of the short-circuit current. In addition, the approach of Ussing (24) was used to compare the sum of the partial ionic conductances for each of the bathing solution ions with the total conductance. The total passive flow of solution ions approximated 1 mmho/cm² as compared with a measured conductance of 1.42 mmho/cm². However, when collections were made between 90 and 120 min after addition of the tracer to five paired lobes coefficients of 24.6 ± 3.7 and 13.0 ± 1.5 X 10⁻⁷ cm/s were obtained. These values and the net flux coefficient, 11.6 ± 2.6, were not different from those reported in Table 1 but the short-circuit current had declined gradually so that the net Cl− flux accounted for 124 ± 19% of the short circuit current. Thus, after 90 min of short circuiting, all the current can be accounted for by the secretion of Cl−.

C. Tissue Ion Analyses

The remainder of the study focussed on an evaluation of the electrolyte and water composition of the lung. Analyses were carried out on tissues that were maintained under two conditions. In the first, lung lobes were quartered and incubated in oxygenated Ringer solution for 5-180 min. The Na+ and Cl− concentrations of lungs that were incubated for 5 min tended to be higher than the values found at 1 h. All ion concentrations remained constant over the next 2 h.

In the second protocol tissues were exposed to Ringer solution in the chamber for a minimum of 2 h. Compared to the tissues incubated outside of the chamber, lungs from the apparatus had somewhat higher K+ and Cl− concentrations. The presence or absence of a transmural electrical PD did not affect the ion composition.

DISCUSSION

The primary objective of this study was to characterize the permeability of the bullfrog lung. It was hoped that this characterization would help determine the suitability of the amphibian lung as a model for the alveolar epithelium of the more complex mammalian lung. However, any comparison must be tempered by the knowledge that the permeability barriers encountered by the solutes used in studies of the mammalian lung have not been defined precisely.

A number of the ions examined in this report have been tested in the dog and turtle lung. The permeability coefficients for Na+ and SO₄²⁻ are in excellent agreement with the measurements of others (5, 22). In contrast, the values for K+ from Table 1 are about an order of magnitude smaller. However, K+ had not reached specific activity equilibrium during these measurements. The ratio of total counts in the tissue to that in the medium was 5.7 ± 0.5 after 3 h. This ratio is about one-third the equilibrium value predicted from the tissue K+ concentrations reported in Table 5. If flux coefficients are projected to increase proportionately with the increase in tissue labeling by K's of about 35 (S → M) and 14 (M → S) X 10⁻⁷ cm/s would be expected with an equilibrium tissue to medium ratio of 20. These values probably represent upper limits since equilibrium with the pool involved in transmural flow may precede that with the entire tissue. Taylor and co-workers (23) point to a high coefficient for K+ relative to that for Na+ as evidence for movement of the solutes across a barrier with the selectivity of the cell membrane. Although this statement may be reasonable when the comparison is made with skeletal muscle cells, it need not be valid for epithelia. For example, permeability coefficients calculated from the passive flow of Na+ and K+ across the excised toad bladder are small and approximately equal (11).

The coefficients for dinitrophenol and water reported in Table 1 are greater than the values for any other ion with the exception of HCO₃⁻. These relatively high K's follow the pattern described for other lungs (5, 22) but the absolute values are about an order of magnitude smaller. The discrepancy for dinitrophenol may be the consequence of the high pH of amphibian Ringer. Dinitrophenol is thought to penetrate cellular barriers rapidly because of the high lipid solubility of the unionized moiety. At pH 8.4 one
would expect the concentration of unionized dinitrophenol (pK_a = 4.0) and, hence, the rate of penetration to be 10 times less than that found at the pH of mammalian physiological salt solutions.

The difference in the coefficients for water is more difficult to explain. It is possible that the pleural covering of the excised bullfrog lung offers an additional barrier to the diffusion of water.

Based on the common charge and similar free diffusion coefficients one might expect the HCO_3^- to penetrate the lung about as rapidly as Cl^-. The data in Table 1 show that the flow of tracer added as H^14CO_3^- greatly exceeded this expectation. Since CO_2 is acknowledged to cross the pulmonary epithelium more rapidly than HCO_3^- the evidence for tracer volatilization in Table 2 points to the contribution of a H^14CO_2 flux to the total flow. The inhibition of the flux by acetazolamide is similar to that reported for the movement of the label of H^14CO_3^- out of the vasculature and across the alveolar barrier of dogs (1). The carbonic anhydrase inhibitor increased the steady-state pool of tracer in the bullfrog lung simultaneously. These results suggest a reduction in the rate of exit of HCO_3^-, CO_2, or both from the epithelial barrier into the sink.

Other investigators have alluded to the possibility of active transport across the lung of the dog (22). However, the measurements required to confirm these hypotheses have not been carried out with mammalian tissue. In this report a substantial body of evidence favors the existence of a Cl^-secretory pump in the bullfrog lung. Not only was net Cl^- movement in the absence of an electrochemical potential demonstrated but other transport characteristics such as the kinetics of a saturable process and inhibition by metabolic inhibitors have been shown. Furthermore, net Cl^- movement was not coupled with the net flow of any other constituent of the bathing solution. Since the same solution bathed both surfaces of the short-circuited lung there was no difference in electrochemical potential to drive the net movement of Na^+, K^+, Ca^{++}, HCO_3^- or water across the tissue. The symmetry of the flux coefficients for each of these species except, perhaps, K^+ indicates the absence of a net flow. Even the asymmetry in K^+ fluxes at specific activity equilibrium that was estimated above would represent a net K^+ secretion equal to about 7% of the net Cl^- flow. Thus, the projected K^+ secretion cannot account for the flow of Cl^- and would be expected to affect the relationship between Cl^- transport and short-circuit current minimally. The ion or ions that are associated with the residual 50% of the early short-circuit current have not been identified. However, the equivalence between the current and net Cl^- flux after 90 min is compatible with the loss of an ion or ions from the alveolar epithelium during the initial period of voltage clamping.

Compared with active Na^+ transport across frog skin and toad bladder, Cl^- transport across the bullfrog lung operates at a relatively low rate. The information in Table 6 is provided to put this transport into the perspective of anion transport across a variety of amphibian epithelia. When viewed in this light it is clear that, with the exception of the skin of the South American bullfrog (Leptodactylus) and the histamine stimulated frog gastric mucosa, the rate of Cl^- secretion across the lung is comparable to values that have been reported for many other epithelia.

The net transport of Cl^-, Br^-, SCN^-, and I^- (Table 1) and maintenance of the lung's bioelectric properties after replacement of Cl^- by Br^- hinted that the alveolar epithelium and the gastric mucosa may possess similar mechanisms for transepithelial ion movement. Durbin (7) assessed the ability of various anions to support acid production by the bullfrog gastric mucosa. A K_m of 6.4 meq/liter and V_max of 2.7 peq/cm^2 h were obtained for Cl^- These values are not matched precisely by the bullfrog lung but the deviations are less than an order of magnitude. The K_m reported for SCN^- inhibition of acid production was 32 meq/liter and the data of Hogben (15) suggest that the V_max for SCN^- is considerably less than that for Cl^- The K_m and V_max estimated from the SCN^- concentrations that did not affect lung conductance were .15 meq/liter and .004 peq/cm^2 h if SCN^- transport is independent of Cl^- flow or a K_m of .02 meq/liter if Cl^- competes with SCN^- Again, these values for the bullfrog lung do not match the estimates for the stomach but the observation that both constants are much smaller than those for Cl^- is in the same direction. Additional difficulties are encountered when the transport of Br^- across the two epithelia is compared. Durbin (7) found the K_m for Br^- to be about 75% of the value for Cl^- and Hogben (15) reported the same net transport of Br^- and Cl^- across the gastric mucosa at equal concentrations. The fact that Br^- substitution does not affect the bioelectric properties of the lung is compatible with these estimates if it is accepted that the anion transport is closely linked to the short-circuit current. On the other hand, the Br^- displacement of Cl^- concentration flow relationship (Fig. 3) indicates that, if Br^- competes with Cl^- for transport, the K_m for Br^- is several fold larger than the value for Cl^-.

Further examination reveals that some transport properties of the gastric mucosa are not shared by the bullfrog lung. For example, a large fraction of the 36Cl^- movement across gastric mucosa takes place by exchange diffusion (15). The total ion conductance that was calculated from the fluxes of individual ions across the lung was slightly less than the measured conductance and, therefore, could not support a large "electrically silent" exchange of Cl^- Furthermore, the passive flux coefficient for Cl^- did not increase as the Cl^- concentration was raised.

Probably the most distinctive feature of gastric Cl^- secretion is its close coupling to the secretion of H^+. The data in

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**TABLE 6. Active anion transport across amphibian epithelia**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Epithelium</th>
<th>Conditions</th>
<th>Net Transport (peq/cm^2 h) S → M</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl^-</td>
<td>Bullfrog lung</td>
<td>Resting</td>
<td>0.3</td>
<td>This report</td>
</tr>
<tr>
<td>Cl^-</td>
<td>Bullfrog cornea</td>
<td>Resting</td>
<td>0.65</td>
<td>27</td>
</tr>
<tr>
<td>Cl^-</td>
<td>Frog and bullfrog gastric mucosa</td>
<td>Histamine stimulated</td>
<td>2.5~5.0</td>
<td>15</td>
</tr>
<tr>
<td>SO_4^-</td>
<td>Bullfrog gastric mucosa</td>
<td>Ringer</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Cl^-</td>
<td>Frog skin</td>
<td>Epinephrine stimulated</td>
<td>0.2~0.4</td>
<td>16</td>
</tr>
<tr>
<td>Cl^-</td>
<td>Toad bladder</td>
<td>K^+ depleted</td>
<td>0.2</td>
<td>8</td>
</tr>
<tr>
<td>Cl^-</td>
<td>Frog skin (Leptodactylus)</td>
<td>Resting</td>
<td>1.3</td>
<td>28</td>
</tr>
<tr>
<td>Cl^-</td>
<td>Toad retinal pigment</td>
<td>Na^+ free</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Cl^-</td>
<td></td>
<td>Resting</td>
<td>0.1~0.9</td>
<td>17</td>
</tr>
</tbody>
</table>

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Table 4 provide convincing evidence that the secretion of H⁺ is not a property of the bullfrog lung.

The information summarized in Table 5 can be used to speculate about the probable locus of the Cl⁻ pump. A tissue Cl⁻ concentration that is lower than the bathing solution level necessitates an even lower intracellular concentration for the average cell. To maintain a low intracellular concentration and effect a net S → M movement the pump would have to be located at the luminal border of the epithelial cell layer.

It is reasonable to ask why an organ that is bent on keeping its lumen nearly free of solution should possess a Cl⁻ secretory mechanism. Zadunaisky (27) points out that Cl⁻ secretion by the amphibian cornea is replaced by active Na⁺ reabsorption in the mammalian cornea. Thus, it is possible that in the mammal, the dominant active solution flow is in the reabsorptive direction. As yet no evidence of ion transport has been obtained for the mature mammalian lung.

Alternatively, one might reason that Cl⁻ and, therefore, electrolyte secretion are required for maintenance of the small amount of surfactant in the amphibian lung. This idea is supported by the observation that mammalian surfactant requires a minimal ionic strength for activity. However, preliminary studies in this laboratory have failed to demonstrate Cl⁻ secretion across the excised lungs of the toad, Bufo marinus, or the South American bullfrog, Leptodactylus pentadactylus. Thus, Cl⁻ secretion appears to be a property of the bullfrog lung that is not common to all amphibia. This fact is difficult to reconcile with the knowledge that surfactant has been found in the lungs of the toad.

REFERENCES


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