Diffusional permeability of rabbit mesothelium

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Diffusional permeability of rabbit mesothelium. J. Appl. Physiol. 85(2): 471–477, 1998.—Diffusional permeability (P) to sucrose (P_{suc}) and Na^+ (P_{Na^+}) was determined in specimens of rabbit sternal parietal pericardium, which may be obtained without stripping. Specimens were mounted in an Ussing apparatus with ^3H-labeled sucrose and ^22Na^+ in a luminal (L) or interstitial (I) chamber. P_{suc} was 2.16 ± 0.44 for L–I and 2.63 ± 0.45 (SE) × 10^{-5} cm/s for I–L, i.e., ~10 times smaller than that previously obtained in stripped specimens of pleura despite the similarity of intercellular junctions in pericardium and plural mesothelium of various species. These findings suggest that previous P_{suc} was overestimated because stripping damages the mesothelium. P_{Na^+} (×10^{-5} cm/s) was 0.5 ± 0.1 cm/s for L–I and 1.37 ± 0.69 × 10^{-5} cm/s for I–L. Measurements were also done with phospholipids, which are adsorbed on the luminal side of mesothelium in vivo. With phospholipids in L, P_{I–L} was 0.75 ± 0.10 and 0.65 ± 0.08 and P_{Na^+} was 3.80 ± 0.32 and 3.76 ± 0.15 × 10^{-5} cm/s for L–I and I–L, respectively, i.e., smaller than without phospholipids. With phospholipids in I (where they are not adsorbed), P_{suc} (2.23 ± 4.02 × 10^{-5} cm/s) and P_{Na^+} (7.01 ± 4.45 × 10^{-5} cm/s) were similar to those values without phospholipids. Hence, adsorbed phospholipids decrease P of mesothelium. If the mesothelium were scraped away from the specimen, P_{suc} of the connective tissue would be 13.2 ± 0.76 × 10^{-5} cm/s. P_{suc} of the mesothelium, computed from P_{suc} of the unscraped and scraped specimens, corrected for the effect of unstirred layers (2.54 and 19.4 ± 10^{-5} cm/s, respectively), was 2.92 and 0.74 × 10^{-5} cm/s without and with phospholipids, respectively. Hence, most of the resistance to diffusion of the pericardium is provided by the mesothelium.

connective tissue; diffusional permeability to sucrose; passive sodium flux; pericardium; phospholipids

THE MORPHOLOGICAL FEATURES of the intercellular junctions of the mesothelium appear to be similar in the pleura (2, 29) and the pericardium (8, 15, 17) of various species (human, ox, sheep, pig, rabbit, and rat). On this basis, the diffusional permeability (P) to small hydrophilic solutes of the mesothelium is likely to be similar in the two tissues. P to small solutes of the pericardium is not known, whereas P to sucrose (P_{suc}) of stripped specimens of sheep visceral pleura has been found to be 12.8 ± 10^{-5} cm/s by Kim et al. (16). Values nearly three times greater have been found in stripped specimens of canine visceral and parietal pleura by Payne et al. (21). These values are one order of magnitude greater than P_{suc} of the endothelium of lung, muscle, and skin capillaries determined with the indicator-diffusion technique, ~10^{-8} cm/s (5). On the other hand, mesothelial cells may lose mutual contact when they are irritated (6), are easily detached during handling of the tissue (8), and their intercellular junctions widen on simple exposure to air (23, 27); moreover, shear stress has been found to increase the permeability to solutes of mesothelial cells in culture (30). Pleura stripping involves considerable stretching, manipulation, and exposure to air: it seems, therefore, probable that P found in stripped specimens of pleura is greater than that under physiological conditions. To the end of assessing P of the mesothelium in conditions closer to the physiological ones, we looked for a place where the pleura or the pericardium is loosely connected to the underlying tissues to get specimens without stripping, little handling, and short exposure to air. When the chest wall of rabbits is opened by sternotomy, the sternal part of the parietal pericardium appears free, except for some fat patches. This suggests that specimens of the sternal part of the pericardium may be obtained in conditions closer to the physiological ones than specimens of pleura, provided fat patches may be easily removed.

The first purpose of this research is, therefore, that of determining P_{suc} and P to Na^+ (P_{Na^+}) of specimens of the sternal part of rabbit parietal pericardium. It is not known whether an active Na^+ transport occurs in the pericardium. On the other hand, if it does occur, active Na^+ flux would be very small relative to passive flux; indeed, the active Na^+ flux that seems to occur in the pleura of rabbits, 0.1 μeq·h^{-1}·cm^{-2} (1), is two orders of magnitude smaller than the passive Na^+ flux of leaky epithelia of rabbits (9, 18). Hence, active Na^+ transport should not affect the measurement of P. The second purpose of this research is that of repeating the above experiments after addition of phospholipids to the solution facing the luminal or the interstitial side of the specimen. This was planned because it has been found that 1) in vivo phospholipids are adsorbed on the luminal side of the mesothelium of pleura (11, 13) and pericardium (12); and 2) adsorbed phospholipids decrease the permeability of epithelia (10). The third purpose of this research is that of determining P_{suc} in specimens in which the mesothelium was scraped away. This was done to measure P of the connective tissue of the pericardium and thus to compute P of the mesothelium alone.

METHODS

The experiments were performed in 60 giant rabbits, most of which were female (body wt 3–7 kg, age 7–11 mo). The animals were anesthetized with a solution (2 ml/kg iv) containing pentobarbital sodium (10 mg/ml, Sigma Chemical) and urethane (250 mg/ml, Sigma Chemical).

Specimen collection and preparation. Each rabbit was placed supine on a tilting board, 20° head up; the trachea was cannulated to ensure adequate ventilation during the preliminary surgical procedure; and airflow and tidal volume were recorded on a Hewlett-Packard 7418 thermopaper oscillograph. The sternum, for its whole length, and both parasternal regions were exposed by removing skin and superficial muscles down to the intercostal muscles. The rabbit was then
killed by an overdose of anesthetic. The sternum was first sectioned along its transversal axis ~1 cm cephalad of the xyphoid process (in this region the underlying pericardium slopes dorsally, and the risk of damaging it is minimal). The cranial stump of the sternum was then lifted, and the ribs (6th to 2nd) were cut along their sternal ends so that the parietal pleura on both sides remained undamaged. After a second transversal section was made near the manubrium, a segment of sternum 5–6 cm long was removed. Because the pericardial sac is only loosely connected to the sternum by connective and adipose tissue, the removal of the sternum leaves the parietal pericardium undamaged. The parts thus exposed consisted of the interstitial faces of the pleurae on either side and the interstitial face of the sternal pericardium in the middle; the sternal part of the pericardium was on a lower plane because of the downward pull of the weight of the heart. The larger fat patches projecting from the exposed pericardium were removed in situ. Then, the cranial and caudal regions of the exposed pericardium were hooked by a second operator with two devices built for this purpose. Each device consisted of a pair of stainless steel hooks fastened ~1 cm apart by a small handle that was kept between the thumb and index finger. While these devices were gently lifted by the second operator and albumin-Ringer solution (see Solutions) was being poured on the pericardium to prevent air exposure of the mesothelium, the first operator pierced the pericardial sac and cut a roughly rectangular specimen of pericardium (~3 cm long and ~2 cm wide). The specimen was never stretched during removal, and the whole procedure of specimen collection was completed within 4 min of the death of the animal. The specimen, held by the hooks, was immediately placed in a petri dish containing albumin-Ringer solution and rinsed. Thereafter, it was moved to another petri dish, with a layer of Sylgard (Dow Corning) adherent to the bottom, and covered by the albumin-Ringer solution, which was bubbled continuously with a 95% O2-5% CO2 gas mixture (21). The specimen was pinned to the layer of Sylgard with its interstitial side facing upward and at its in situ length and width. The specimen (always immersed in the solution) was carefully cleaned by removing small vessels, fat patches, and, when present, blood clots with fine scissors and blunt tweezers. Cleaning was considered satisfactory when a transparent layer of Sylgard (~1 × 1.5 cm) was obtained. The mesothelium was never touched during the cleaning procedure, which took 20–25 min. In eight experiments (series 6; see Measurement of T) after this procedure, the specimen was turned and pinned with its luminal side facing upward. The albumin-Ringer solution was removed, and the mesothelium was gently scraped away with the edge of a glass slide (31) or the blade of a scalpel. The tissue removed was weighed to check that it did not markedly exceed the amount computed from the area scraped times the thickness of the mesothelium (2 μm; Refs. 26, 29). Finally, in seven experiments (series 7; see Measurement of T) exposure to air was not prevented during the process of collecting and cleaning the specimen (see above). Moreover, Ringer solution, rather than albumin-Ringer solution, was used in rinsing the specimen and in the Ussing apparatus.

Measurement of T. The specimen was mounted as a planar sheet between the frames of a Ussing apparatus (rectangular window: 0.5 cm²). The equal-volume chambers were immediately and simultaneously filled with 4 ml of albumin-Ringer solution without or with the addition of phospholipids, according to the type of experiment (see below). Unidirectional fluxes of sucrose and Na+ through the specimen were determined in the following series of experiments: 1) radioactive markers (3H-labeled sucrose and 22Na; see below) in the solution facing the luminal side; 2) radioactive markers in the solution facing the interstitial side; 3) phospholipids and radioactive markers in the solution facing the luminal side; 4) phospholipids in the solution facing the luminal side and radioactive markers in the solution facing the interstitial side; 5) phospholipids and radioactive markers in the solution facing the interstitial side; 6) [3H]sucrose in the solution facing the luminal side, in experiments with scraped mesothelium; and 7) [3H]sucrose in the solution facing the luminal side, in experiments in which exposure to air was not prevented and Ringer solution, rather than albumin-Ringer solution, was used. A first incubation period of 30 min was allowed for tissue recovery, temperature equilibration, and initial phospholipid adsorption when scheduled. Solutions were preheated at 37°C, and the apparatus was water jacketed to maintain this temperature in both chambers throughout the experiment. The solution in both chambers was oxygenated and stirred throughout the experiment by bubbling the 95% O2-5% CO2 gas mixture (21) through ports opening near the bottom of the frame in each chamber. Bubbling was performed at the highest rate (−500 bubbles/min) that did not result in foam reaching the upper edges of the chambers of the Ussing apparatus. At the end of the first incubation period, both chambers were simultaneously emptied, and the recovered liquid was stored for determination of background radioactivity (see below). Simultaneous refilling of the chambers was immediately made with 4 ml of labeled solution in the donor chamber and 4 ml of unlabeled solution in the recipient chamber. A second 30-min incubation period was allowed to attain equilibrium of tracers between the donor chamber and the specimen and to continue phospholipid adsorption when scheduled. At the end of this period, a 50-μl sample was withdrawn from the donor chamber, whereas all the liquid in the recipient chamber was removed. The recipient chamber was immediately refilled with a volume of fresh unlabeled solution equal to that present in the donor chamber after removal of the 50-μl sample (3.95 ml). The time required for liquid withdrawal and replacement was 3–4 s. After 20 min (end of first experimental period), the above procedure was repeated to have a second experimental period (20 min). The procedure of total liquid withdrawal from the recipient chamber (at various times from this chamber) to ensure that the concentration of isotope in the recipient chamber remained negligible relative to that in the donor chamber. This prevents isotope back-diffusion and thus allows measurement of unidirectional (rather than net) fluxes.

Radioactivity was determined as counts per minute (cpm) in samples of the liquid collected from each chamber at the end of the first and second experimental periods. Three vials were prepared for each sample and assayed for β-activity in a liquid scintillation spectrometer (Minaxi β Tri-Carb 4000, Packard Instruments). Appropriate corrections were made for spillover between channels and for background radioactivity. The average cpm value was expressed as cpm per milliliter to provide a value proportional to isotope concentration in a given chamber. The isotope concentration in the donor chamber (C0) in the first experimental period never differed from that in the second by >5%, and this difference was not systematic with respect to time, indicating that donor isotope concentration was essentially constant. The isotope concentration in the recipient chamber at the end of an experimental period (C1) never exceeded 2% of C0, confirming that isotope concentration in this chamber remained negligible relative to that in the donor chamber. In 36 of 45 experiments, the difference between C1 in the first and second experimental periods was not systematic with respect...
to time and was within ±10%. In the remaining nine rabbits, the concentration in the recipient chamber was nil at the beginning of each period, the unidirectional flux (Φ) of sucrose or of Na\(^+\) is given by \(\Phi = (C_1 \cdot V_{\text{in}})/(C_0 \cdot A_t)\), where \(C_0\) is the overall concentration of the solute (i.e., labeled plus unlabelled) in the donor chamber (4.5 \(\times\) 10\(^{-8}\) mmol/ml for sucrose and 0.139 mmol/ml for Na\(^+\)). \(V_t\) is the volume of the solution in the recipient chamber. \(A_t\) is the area of the surface of the window. Hence, the depth of focus was relatively large (\(~\times\)10 µm); therefore, the positions of the lens were taken when the tantalum particle on each side of the specimen was first focused on during the displacement of the lens in one direction. The thickness of a particle was then subtracted from the distance between the focusing positions of the lens on either side of the specimen. The smallest fine-focusing micrometer scale was 2 µm. The measurement was repeated in 10 different sites of the specimen. The mean of these measurements was taken as the average thickness of the specimen.

To check whether fat removal, as judged by the naked eye or through use of the dissecting microscope, was complete, in two initial experiments histological checks were performed on the peripheral parts of the specimen, which were not used to measure \(P\). To this end, the sectioned parts were transferred in 3% glutaraldehyde (in HCO\(_3\)\(_{-}\) buffer, at room temperature). Further processing included postfixation in 1% osmium tetroxide in phosphate buffer, dehydration in alcohol, and embedding in Durcopan (Fluka). Sections ~2 µm thick were cut with a Suckcut microtome and stained with methylene blue and Azur II for light microscopy. From the same specimens, thin (60-nm) sections were further cut with an Ultracut ultramicrotome, mounted on copper grids, stained with 2% uranyl acetate and 0.2% lead citrate, and examined with a Philips CM10 electron microscope. Photographs of both light- and electron-microscopic preparations showed that fat removal, as judged by the naked eye, was complete. The thickness of the few specimens measured in the histological preparations was ~65% of that measured directly and reported in RESULTS. Most of the difference is likely due to the hydration caused by histological processing. This has to be considered if our direct measurements of specimen thickness are compared with those previously obtained on histological preparations.

Statistics. Data are expressed as means ± SE. Statistical significance of differences among groups was assessed by analysis of variance.

**RESULTS**

The mean values of \(P_{\text{Na}^+}\) and \(P_{\text{Na}^+}\) of the sternal part of the parietal pericardium obtained in the experiments without phospholipids and in those with phospholipids in the solution facing the luminal or the interstitial side of the specimen are reported in Table 1. Because the values for the lumen-to-interstitium direction were similar to those for the opposite direction, the overall

<table>
<thead>
<tr>
<th>Protocol/Direction</th>
<th>n</th>
<th>(P_{\text{suc}}), 10(^{-5}) cm/s</th>
<th>(P_{\text{Na}^+}), 10(^{-5}) cm/s</th>
<th>(J_{\text{Na}^+}, \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{cm}^{-2})</th>
<th>(P_{\text{Na}^+}/P_{\text{suc}})</th>
</tr>
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<tbody>
<tr>
<td>Without phospholipids</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lumen → interstitium</td>
<td>9</td>
<td>2.16 ± 0.44</td>
<td>7.07 ± 0.71</td>
<td>35.41 ± 3.61</td>
<td>3.95 ± 0.53</td>
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<tr>
<td>Intersitium → lumen</td>
<td>9</td>
<td>2.63 ± 0.45</td>
<td>7.37 ± 0.69</td>
<td>37.01 ± 3.44</td>
<td>3.36 ± 0.49</td>
</tr>
<tr>
<td>Total mean</td>
<td>18</td>
<td>2.39 ± 0.31</td>
<td>7.22 ± 0.48</td>
<td>36.21 ± 2.43</td>
<td>3.66 ± 0.36</td>
</tr>
<tr>
<td>With phospholipids on luminal side</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lumen → interstitium</td>
<td>9</td>
<td>0.75 ± 0.10</td>
<td>3.80 ± 0.32</td>
<td>19.02 ± 1.61</td>
<td>5.36 ± 0.42</td>
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<tr>
<td>Intersitium → lumen</td>
<td>9</td>
<td>0.65 ± 0.08</td>
<td>3.76 ± 0.15</td>
<td>18.76 ± 0.78</td>
<td>6.43 ± 0.70</td>
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<tr>
<td>Total mean</td>
<td>18</td>
<td>0.70 ± 0.06</td>
<td>3.78 ± 0.17</td>
<td>18.89 ± 0.87</td>
<td>5.90 ± 0.42</td>
</tr>
<tr>
<td>With phospholipids on interstitial side</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Intersitium → lumen</td>
<td>9</td>
<td>2.33 ± 0.42</td>
<td>7.01 ± 0.45</td>
<td>35.22 ± 2.31</td>
<td>3.66 ± 0.54</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of specimens; \(P_{\text{suc}}\) and \(P_{\text{Na}^+}\), diffusional permeability to sucrose and Na\(^+\), respectively; \(J_{\text{Na}^+}\), Na\(^+\) flux; \(P_{\text{Na}^+}/P_{\text{suc}}, P_{\text{Na}^+}-\text{to}-P_{\text{suc}}\) ratio.
means are also reported. When phospholipids were added to the solution facing the luminal side of the specimen, $P_{suc}$ decreased by 3.4 times ($P < 0.01$) and $P_{Na^+}$ decreased by 1.9 times ($P < 0.01$, Table 1). Instead, when phospholipids were added to the solution facing the interstitial side, $P_{suc}$ and $P_{Na^+}$ did not change (Table 1). In the experiments in which air exposure was not prevented, and Ringer solution rather than albumin-Ringer solution was used (see METHODS), $P_{suc}$ was $9.28 \pm 1.63 \times 10^{-5}$ cm/s ($n = 7$), that is, about four times greater than that in the experiments of series 1 and 2 (see METHODS) ($2.39 \pm 0.31 \times 10^{-5}$ cm/s; Table 1).

The underestimation of $P$ caused by unstirred liquid layers close to the membrane may be computed with the formula of resistances in series (3, 24): $1/P_{corr} = (1/P_{mes}) - (d_{liq}/D)$, where $P_{mes}$ is the corrected $P$, $d_{liq}$ is the thickness of the unstirred liquid layers (which may be assumed to be 170 µm: 70 µm on the luminal side and 100 µm on the interstitial side) (3), and $D$ is the diffusion coefficient of the solute in water at 37°C ($0.70 \times 10^{-5}$ cm²/s for sucrose and $1.80 \times 10^{-5}$ cm²/s for Na⁺) (5). According to the above formula, the corrected values of $P_{suc}$ and $P_{Na^+}$ in the experiments without phospholipids are $2.54 \pm 0.33$ and $7.75 \pm 0.52 \times 10^{-5}$ cm/s, respectively. Hence, the underestimation caused by unstirred liquid layers is 6% for $P_{suc}$ and 7% for $P_{Na^+}$. In the experiments with phospholipids, the corrected values of $P_{suc}$ and $P_{Na^+}$ are $0.71 \pm 0.06$ and $3.93 \pm 0.18 \times 10^{-5}$ cm/s, respectively. Hence, the underestimation caused by unstirred liquid layers is only 1% for $P_{suc}$ and 4% for $P_{Na^+}$. Finally, in the experiments in which air exposure was not prevented and albumin was not added to Ringer solution, the corrected value of $P_{suc}$ is $12.0 \pm 2.11 \times 10^{-5}$ cm/s. Hence, the underestimation caused by unstirred liquid layers is marked (23%): indeed, the effect of unstirred layers increases progressively with the increase in $P$ (3, 24).

The average thickness of the specimens in series 1–5 (see METHODS) was $76.4 \pm 3.09$ µm ($n = 26$). There was no relationship between $P_{suc}$ or $P_{Na^+}$ and the thickness of the specimen (Fig. 1). This finding indicates that the resistance to diffusion is mainly provided by the mesothelium because the variance in the thickness of the specimen depends essentially on the difference in thickness of its connective tissue (see below). The mean value of the $P_{Na^+}$-to-$P_{suc}$ ratio ($P_{Na^+}/P_{suc}$) in the experiments without phospholipids, $3.66 \pm 0.36$ (Table 1), is higher than the corresponding free-diffusion ratio, 2.57 (5). On the other hand, in 9 of 18 specimens the individual value of $P_{Na^+}/P_{suc}$ ranged from 2.20 to 2.96 (Fig. 2, open symbols below dotted line) with a mean of $2.59 \pm 0.09$, which is similar to the free-diffusion ratio, whereas in the rest of the specimens (Fig. 2, open symbols above dotted line) the value of this ratio is higher (average $4.72 \pm 0.50$), reaching the lower or middle values obtained in the experiments with phospholipids on the luminal side (Fig. 2, filled symbols). In 9 of the former specimens, $P_{suc}$ and $P_{Na^+}$ were $3.36 \pm 0.34$ and $8.52 \pm 0.59 \times 10^{-5}$ cm/s, respectively; in the other 9 specimens, the values were $1.43 \pm 0.23$ and $5.92 \pm 0.46 \times 10^{-5}$ cm/s, respectively. The mean value for $P_{Na^+}/P_{suc}$ in the experiments with phospholipids on the interstitial side ($3.66 \pm 0.54$; Table 1 and Fig. 2, dashed symbols) is lower ($P < 0.01$) than that in the experiments with phospholipids on the luminal side ($5.90 \pm 0.42$; Table 1 and Fig. 2, filled symbols) and equal to that in the experiments without phospholipids ($3.66 \pm 0.36$; Table 1 and Fig. 2, open symbols).

The experiments in which the mesothelium was scraped away enabled us to measure $P_{suc}$ of the connective tissue; it was $13.2 \pm 0.76 \times 10^{-5}$ cm/s ($n = 8$). Correcting for the effect of unstirred liquid layers (see above), it became $19.4 \pm 1.12 \times 10^{-5}$ cm/s (Table 2). Hence, the underestimation is marked (32%). Because the thickness of the unscraped specimens in series 1–5 (see METHODS) was $76.4$ µm, and that of the mesothelium is $2$ µm (26, 29), the average thickness of the connective tissue in these specimens is $74$ µm. The average thickness of the scraped specimens was essentially similar (69.9 ± 3.64 µm; n = 8). Because the mesothelium and the connective tissue are placed in series, their resistances to diffusion add up; hence, $1/P_{sp} = (1/P_{mes}) + (1/P_{con})$, where sp, mes, and con are unscraped specimen, mesothelium, and connective tissue, respectively. Hence, $1/P_{mes} = (1/2.54 \times 10^{-5}$ cm/s$) - (1/19.4 \times 10^{-5}$ cm/s$)$; hence, $P_{mes} = 2.92 \times 10^{-5}$ cm/s (Table 2). The resistance to diffusion of the mesothelium is, therefore, about seven times greater than that
with phospholipids the resistance to diffusion of the mesothelium is so high that the resistance of the connective tissue becomes negligible.

When diffusion through the “pores” is not restricted, \( P = (D/l) (A_p/A) \), where \( l \) is the membrane thickness and \( A_p/A \) is the area of the pores relative to the area of the membrane. Hence, \( A_p/A = (P \cdot l)/D \). The value of \( P_{\text{mes}} \) in the nine experiments in which diffusion of sucrose was not restricted (computed from \( P_{\text{sp}} \) and \( P_{\text{con}} \), both corrected for the effects of unstirred liquid layers) was \( 4.56 \times 10^{-5} \text{ cm}^2/\text{s} \). Hence, \( A_p/A \) of the mesothelium is roughly given by \( (4.56 \times 10^{-5} \text{ cm}^2/\text{s}) \cdot (2 \times 10^{-4} \text{ cm})/(0.7 \times 10^{-5} \text{ cm}^2/\text{s}) = 1.3 \times 10^{-3} \), that is, \( \sim 0.1\% \).

**DISCUSSION**

\( P_{\text{sp}} \) of the sternal part of the parietal pericardium in rabbits in the experiments with albumin-Ringer solution, \( 2.39 \pm 0.31 \times 10^{-5} \text{ cm}^2/\text{s} \), is about one order of magnitude smaller than that found in stripped specimens of sheep visceral pleura, \( 12.8 \pm 1.4 \times 10^{-5} \text{ cm}^2/\text{s} \) (17), and of dog visceral, \( 33.1 \pm 3.1 \times 10^{-5} \text{ cm}^2/\text{s} \), and parietal pleura, \( 37.1 \pm 3.5 \times 10^{-5} \text{ cm}^2/\text{s} \) (22). Because the morphological features of the intercellular junctions of the mesothelium appear to be similar in the pleura (2, 29) and the pericardium (8, 15, 17) of various species, these results support the hypothesis, based on morphological findings (see the introduction), that the stretching, handling, and air exposure involved in stripping the specimen of pleura widen the tight junctions between mesothelial cells and may even detach some of the cells. Furthermore, the solution used in the Ussing chambers in the studies on stripped specimens of pleura (16, 21) was protein free, and it has been shown that a protein-free solution increases the permeability of the capillary endothelium (7). In the experiments in which air exposure was not prevented and albumin was not added to the Ringer solution, \( P_{\text{sp}} \) of the pericardium, corrected for the effects of unstirred liquid layers (\( 12.0 \times 10^{-5} \text{ cm}^2/\text{s} \)), is about five times greater than the corrected value (\( 2.54 \times 10^{-5} \text{ cm}^2/\text{s} \)) of series 1 and 2 (the uncorrected value is \( 2.39 \times 10^{-5} \text{ cm}^2/\text{s} \), Table 1). Hence, part of the difference in \( P_{\text{sp}} \) between stripped specimens of pleura and unstripped specimens of pericardium may be explained by air exposure and lack of albumin. The rest is likely due to the stretching involved in stripping. This factor, however, cannot be tested because one does not know quantitatively the tension that has to be applied to strip the pleural specimen.

One could argue that the difference in \( P_{\text{sp}} \) between stripped specimens of pleura and unstripped specimens of pericardium is due to the smaller thickness of pleural specimens (16, 21). This, however, is not the case because there is no relationship between \( P \) and the thickness of the specimens (Fig. 1). Moreover, the findings obtained in the experiments in which the mesothelium was scraped away from the specimen show that most of the resistance to diffusion in the pericardium is provided by the mesothelium (86\% in the experiments without phospholipids and 96\% in the experiments with phospholipids on the luminal side).

**Table 2.** Diffusional permeability to sucrose of pericardium, connective tissue, and mesothelium

<table>
<thead>
<tr>
<th></th>
<th>Pericardium (unscraped specimens), ( \text{cm}^2/\text{s} )</th>
<th>Connective Tissue (scraped specimens), ( \text{cm}^2/\text{s} )</th>
<th>Mesothelium,( \dagger ) ( \text{cm}^2/\text{s} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Without phospholipids</strong></td>
<td>18 2.54 ± 0.33 ( \times 10^{-5} )</td>
<td>8 19.4 ± 1.12 ( \times 10^{-5} )</td>
<td>1.3 ( \times 10^{-3} )</td>
</tr>
<tr>
<td><strong>With phospholipids</strong></td>
<td>18 0.71 ± 0.06 ( \times 10^{-5} )</td>
<td>8 19.4 ± 1.12 ( \times 10^{-5} )</td>
<td>2.92</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( \times \) No. of specimens. \*Values are corrected for effect of liquid unstirred layers. †Computed as indicated in text. ‡Equal to without phospholipid value because phospholipids affect diffusional permeability only when added to solution facing mesothelial side of specimen (see Table 1 and text); in scraped specimens, both sides are made of connective tissue and, therefore, are not affected by phospholipids.
For this reason a twofold change in specimen thickness does not produce an appreciable change in $P$ (Fig. 1). The finding that $P_{su}$ of scraped specimens is similar to that found in stripped specimens of sheep visceral pleura (16), and smaller than that found in stripped specimens of dog visceral or parietal pleura (21), indicates that 1) the mesothelium of stripped specimens of pleura was damaged so much that its resistance to diffusion was nearly nil; and 2) $P$ of the connective tissue of the pleura is smaller in sheep than in dogs, and/or in the latter species or experiments $P$ not only of the mesothelium but also of the connective tissue was increased by stripping. In conclusion, although we cannot rule out that $P$ of the pleura to small solutes is a little higher than that of the pericardium or that there are small species differences, the above findings indicate that $P$ of the pleura should be on the same order of magnitude as that presently found in the pericardium.

In the sternal part of rabbit pericardium, cribiform zones and milky spots (Kampmeier foci) are fewer and smaller than in the parietal pleura (26). Because these areas are likely more permeable than is the rest of the mesothelium, the permeability of the sternal part of the pericardium could be smaller than that of the parietal pleura but greater than that of the visceral pleura (which lacks cribiform zones and milky spots). Considering also that our specimens of pericardium may have been damaged, although markedly less so than were the stripped specimens of pleura (16, 21), the value of $P$ obtained in the experiments with phospholipids on the luminal side ($0.70 \times 10^{-5}$ cm/s; Table 1) is likely still higher than that occurring under physiological conditions. If this is the case, our finding for $P_{su}$ of the pericardium supports the value of $P$ to mannitol of the pleura, indirectly assessed in anesthetized rabbits ($0.5 \times 10^{-5}$ cm/s) (35). The latter value was obtained from the time course of the concentration of labeled mannitol in a hydrothorax, the diffusional outflux of mannitol from the hydrothorax, an estimate of mannitol concentration in the interstitium of the area of apposition between right and left pleural space, and the area of pleural surface (35). Finally, $P_{su}$ of the pericardium in the experiments with phospholipids ($0.70 \pm 0.06 \times 10^{-5}$ cm/s), is somewhat larger than or similar to $P_{su}$ of the gallbladder, $0.38 \pm 0.14$ (25) and $0.76 \pm 0.14 \times 10^{-5}$ cm/s (28), the wall of which consisted of a leaky epithelium, connective tissue, and smooth muscles (the mesothelium was likely destroyed by the manipulations required by the experiment).

The 3.4-fold decrease in $P_{su}$ and the 1.9-fold decrease in $P_{Na}$ occurring when phospholipids are added to the solution facing the luminal side of the specimen of pericardium (Table 1) are likely due to the adsorption of phospholipids on the luminal side of the mesothelial cells, according to the findings by Hills (11) and Hills et al. (13) in the pleura and Hills and Butler (12) in the pericardium. Indeed, this marked decrease in $P$ does not occur when phospholipids are added to the solution facing the interstitial side of the specimen, where they are not adsorbed (10). As a matter of fact, the layer (or layers) of phospholipids adsorbed on the luminal side of the mesothelial cells provides a further resistance to diffusion. Moreover, the greater decrease in $P_{su}$ than in $P_{Na}$ (and hence the increase in $P_{Na}/P_{su}$; see RESULTS) occurring in the experiments with phospholipids in the solution facing the luminal side of the specimen suggests that the layer of phospholipids adsorbed on the mesothelial cells restricts the diffusion of sucrose. Finally, the finding that $P_{Na}/P_{su}$ in the experiments without phospholipids is similar to that occurring in free diffusion in only one-half of the specimens, whereas in the rest it is greater (see RESULTS), suggests that the layer of phospholipids normally adsorbed on the luminal side of the mesothelium is altered to a varied extent in the process of collecting the specimens. Therefore, one should consider only the nine specimens with $P_{Na}/P_{su}$ similar to that in free diffusion to have a more precise indication of the effect of the phospholipids on $P$. If this is done, it turns out that phospholipids decrease $P_{su}$ by 4.8-fold and $P_{Na}$ by 2.3-fold.

The finding that $P_{Na}$ is similar in both directions suggests that active Na$^+$ transport in the pericardium is nil or very small relative to passive Na$^+$ flux. Passive Na$^+$ flux in the experiments with phospholipids on the luminal side, $18.9 \pm 0.9 \mu$mol·h$^{-1}$·cm$^{-2}$ (Table 1), is smaller than that in renal proximal tubule of rabbits, $55.8 \pm 9.6 \mu$mol·h$^{-1}$·cm$^{-2}$ (18), and is roughly similar to that in rabbit gallbladder, $22.1 \pm 0.6 \mu$mol·h$^{-1}$·cm$^{-2}$ (9). On the other hand, even in the experiments with phospholipids, passive Na$^+$ flux through pericardial specimens is ~200 times greater than active Na$^+$ flux that seems to occur in the pleura, according to indirect evidence in anesthetized rabbits, $0.1 \mu$mol·h$^{-1}$·cm$^{-2}$ (1). This situation makes it extremely difficult at present to detect an active Na$^+$ transport from the differences between unidirectional Na$^+$ fluxes across a specimen of pleura. Nevertheless, as already pointed out by Kim et al. (16), it is possible that active transport occurs across the pleura. The present finding that passive Na$^+$ flux through the pericardium is similar to or smaller than that through leaky epithelia displaying a marked active transport suggests that pleural permeability is compatible with a small active transport, which is in line with the various pieces of evidence indirectly obtained in vivo (1, 32–35).

In the experiments with phospholipids on the luminal side of the specimen, $P_{su}$ of the mesothelium, $0.74 \times 10^{-5}$ cm/s (Table 2), is similar to $P_{su}$ of the leaky epithelium of renal proximal tubule, $0.69 \times 10^{-5}$ cm/s (14), and roughly similar to (perhaps smaller than) $P_{su}$ of the endothelium of lung, muscle, and skin capillaries, $\sim 1 \times 10^{-5}$ cm/s (5). The relative pore area of the mesothelium (~0.1%) appears also similar to that of capillary endothelium (~0.1%) (20).

The experiments in which the mesothelium was scraped away from the specimen provided the opportunity to measure the apparent diffusion coefficient ($D'$) of sucrose through the connective tissue of the pericardium. $D'$ is lower than $D$ because 1) only part of the area of the membrane is available for diffusion; 2) the diffusion path is tortuous; and 3) the diffusion may be restricted if the ratio between solute radius and pore...
radius is $>0.01$ (22). All three factors affect $P$; hence, $D'$ can be obtained from $P$·$l$. From the above data for $P_{\text{on}}$ (corrected for the effect of unstirred liquid layers) and the corresponding values of $l$ (see Results), one gets $D'_{\text{on}} = (19.4 \times 10^{-5} \text{ cm}^2/\text{s}) = (74 \times 10^{-4} \text{ cm}) = 0.14 \times 10^{-5} \text{ cm}^2/\text{s}$. This value is 20% of that in water ($0.7 \times 10^{-5} \text{ cm}^2/\text{s}$). This difference should mainly depend on the reduction in diffusion area brought forth by the presence of many fibers in the connective tissue of the pericardium (particularly collagen) (26). $D'$ of K$^+$ through the connective tissue of frog mesentery has been found to be 37% of that in water (4). $D'$ of sucrose through the relaxed myocardium has been found to be 23% of that in water (25).

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REFERENCES


