Macromolecule transfer through mesothelium and connective tissue

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Bodega, Francesca, Luciano Zocchi, and Emilio Agostoni. Macromolecule transfer through mesothelium and connective tissue. J Appl Physiol 89: 2165–2173, 2000.—Diffusional permeability (P) to inulin (P_in), albumin (P_alb), and dextran [70 (P_dx 70), 150 (P_dx 150), 550 (P_dx 550), and 2,000 (P_dx 2,000)] was determined in specimens of parietal pericardium of rabbits, which may be obtained with less damage than pleura. P_in, P_alb, P_dx 70, P_dx 150, P_dx 550, and P_dx 2,000 were 0.51 ± 0.03, 0.097 ± 0.021, 0.47 ± 0.011, 0.025 ± 0.004, and 0.021 ± 0.005 × 10^{-5} cm/s, respectively. P_alb and P_dx 70 of connective tissue, obtained after removal of mesothelium from specimens, were 10.3 ± 1.42, 2.97 ± 0.38, and 2.31 ± 0.16 × 10^{-5} cm/s, respectively. Hence, P_alb, P_dx 70, and P_dx 2,000 of mesothelium were 0.54, 0.20, and 0.10 × 10^{-5} cm/s, respectively. Inulin (like small solutes) fitted the relationship P-solute radius for restricted diffusion with a 6-nm “pore” radius, whereas macromolecules were much above it. Hence, macromolecule transfer mainly occurs through “large pores” and/or transcytosis. In line with this, the addition of phospholipids on the luminal side (which decreases pore radius to ~1.5 nm) halved P_in but did not change P_alb, and P_dx 70 is roughly similar in mesothelium and capillary endothelium, whereas P to macromolecules is greater in mesothelium. The albumin diffusion coefficient through connective tissue was 17% of that in water. Mesothelium provides 92% of resistance to albumin diffusion through the pericardium.

albumin; dextran; diffusional permeability; inulin; phospholipids

STARLING FORCES, LYMPHATIC drainage, and cellular mechanisms are involved in setting the volume and composition of the pleural liquid, which, in turn, are essential to ensure an efficient mechanical coupling between lung and chest wall (2). For a better understanding of the exchange of liquid and solutes through the pleurae, it is important to determine the permeability of the mesothelium and of the underlying connective tissue. Measurements on pleural specimens appear, however, to be little reliable, because of damage of the mesothelium during sample collection. Hence, specimens of the retrosternal part of the parietal pericardium of rabbits have been used because they can be obtained with less damage (44), and the intercellular junctions of these serosae are similar (for literature, see Ref. 44). The diffusional permeability (P) of the parietal pericardium (P_pep) of rabbits to sucrose, mannitol, Na^+, Cl^−, and water has previously been determined by our laboratory (1, 44). After the mesothelium was scraped away from the specimen, P of the connective tissue (P_con) to the above molecules was also measured. P of the mesothelium (P_mes) was then computed from P of intact and scraped specimens (1, 44). P to the solutes was smaller in the mesothelium than in the connective tissue, although the latter is 35 times thicker, whereas P to water was greater in the mesothelium, suggesting a marked diffusion of water through the cell membrane. P to the small solutes of the mesothelium was of the same order of magnitude as that of the capillary endothelium. The addition of phospholipids to the solution facing the luminal side of the mesothelium, where they should be adsorbed (17, 18), markedly decreased P_mes to the small solutes, except for Cl^-'. The equivalent radius of the “small pores” of the mesothelium was found to be ~6 nm without phospholipids and ~1.5 nm with phospholipids (1).

In the present research, we measured P_mes and P of the underlying connective tissue to inulin (P_in), albumin (P_alb), and large dextrans to extend our previous studies and, particularly, to investigate the following points: 1) whether macromolecule transfer mainly occurs through “large pores” and/or transcytosis: in this case, P to macromolecules should be markedly greater than predicted by the relationship between P and solute radius (a) for restricted diffusion through small pores with an equivalent radius of 6 nm; moreover, because macromolecule diffusion through these “pores” should be negligible, the above-mentioned effect of phospholipids should vanish with macromolecules and persist with inulin; 2) whether the slope of the P-a relationship tends to become flat with the largest macromolecules: this would be a hint for transcytosis (34); 3) whether P to macromolecules of the mesothelium is similar to that of the capillary endothelium as is the case for small solutes; and, finally, 4) the scraped specimens provide the opportunity of measuring P_alb of the connective tissue.

METHODS

Specimen collection and preparation. Specimens of parietal pericardium were obtained from 98 giant rabbits (body

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wt 5–7 kg, age 7–11 mo). The animals were anesthetized with a 2 ml/kg iv solution containing pentobarbital sodium (10 mg/ml; Sigma Chemical) and urethane (350 mg/ml; Sigma Chemical) and were placed supine on a tilting board 20° head up. The trachea was cannulated to assure adequate ventilation during the preliminary surgical procedure, and air flow and tidal volume were recorded on a 7418 Hewlett-Packard thermopaper oscillograph. Collection and preparation of the specimens of the retrosternal parietal pericardium were performed with the procedure previously described, which minimizes manipulation and air exposure of the mesothelium (44). Briefly, after the rabbit was killed by an overdose of anesthetic, a segment of sternum was removed, leaving undamaged the underlying parietal pericardium. While albumin-Ringer solution was being poured on the pericardium to prevent air exposure of the mesothelium, a roughly rectangular specimen of pericardium (~3 × 2 cm) was hooked and excised. The specimen was never stretched during removal, and the whole procedure was completed within 4 min after the death of the animal. The specimen, covering the entire Ringer solution, was pinned with its interstitial side facing upwards, at its in situ length and width, to a layer of Sylgard (Dow Corning) that was adherent to the bottom of a petri dish. The solution was bubbled continuously with a 95% O₂-5% CO₂ gas mixture (30). Small vessels, fat patches, and, when present, blood clots were removed from the interstitial side of the specimen until a transparent area of ~1 × 1.5 cm was obtained; the mesothelium of the central part of the specimen was never touched. The cleaning procedure took 20–25 min. In 24 experiments (to assess connective tissue permeability, see P measurements), after the above procedure the specimen was turned and pinned with its luminal side facing upwards, the albumin-Ringer solution was removed, and the mesothelium was gently scraped away with the blade of a scalpel (1, 43, 44).

**Solutions and labeled molecules.** The composition of the Ringer solution used during specimen collection and preparation, as well as during the measurements (see below), was (in mM): 139 Na⁺, 5 K⁺, 1.25 Ca²⁺, 0.75 Mg²⁺, 119 Cl⁻, 29 HCO₃⁻, and 5.6 d-glucose. Bovine albumin (0.5 g%; Sigma Chemical) was added to maintain normal permeability (11). The solution was preheated at 37°C. In part of the experiments (see below) a mixture of phospholipids was added to the solution facing the luminal side of the specimen (44): 50% dipalmitoyl phosphatidylcholine (367 μg/ml), 32% dipalmitoyl phosphatidylethanolamine (235 μg/ml), and 18% sphingomyelin (132 μg/ml). These proportions are those occurring in sheep pleural extract (18), whereas concentrations are one order of magnitude lower than those of the pleural extracts, but a little higher than those occurring in human amniotic liquid near term (15), i.e., in a liquid in contact with the layer of phospholipids spread on the alveolar epithelium. Phospholipids were not used in the experiments on scraped specimens because they affect P only when they are added to the solution facing the mesothelium (44), where they are adsorbed (17, 18). The labeled molecules used were [¹⁴C]inulin (ICN, specific activity 1–3 μCi/mg), bovine ¹₂⁵I-labeled albumin (ICN, specific activity ~1 μCi/mg), or one of the following dextrans labeled with FITC: 70, 150, 550, and 2,000 kDa. The Stokes-Einstein radius of the tracers used and their diffusion coefficient in water at 37°C (D) are reported in Table 1. The degree of substitution of dextrans ranged from 0.003 to 0.013 mol FITC/mol glucose. The labeled molecules were added to the solution facing the mesothelium at the following concentrations: [¹⁴C]inulin, 2.2 × 10⁻⁵ μmol/ml (providing an activity of 0.5 μCi/ml); ¹₂⁵I-albumin, 1.85 × 10⁻⁵ μmol/ml (activity 1 μCi/ml); FITC-dextrans, 0.24–15 × 10⁻⁵ μmol/ml (3.7–9.1 × 10⁻⁵ mol FITC/ml). Unlabeled molecules of the species tested were added at the same concentration in the recipient chamber. To minimize the concentration of free FITC, solutions containing FITC-dextrans were dialyzed for ~16 h at ambient temperature before the experiments.

**P measurements.** P to the various solutes was measured from the unidirectional fluxes of labeled molecules through 74 intact specimens (52 without and 22 with phospholipids added to the luminal solution) and 24 scraped specimens. Specimens were mounted as planar sheets between the frames of a Ussing apparatus (rectangular window, 0.5 cm²; chambers volume, 4 ml). Both chambers were immediately and simultaneously filled with albumin-Ringer solution without or with the addition of phospholipids to the solution facing the luminal side. The solution contained in the chambers was oxygenated and stirred throughout the experiment by bubbling 95% O₂-5% CO₂ (30) through ports opening near the bottom of the frame in each chamber; the apparatus was water jacketed to maintain temperature at 37°C. After a 30-min incubation period, both chambers of the Ussing apparatus were simultaneously emptied and refilled with labeled solution in the luminal (donor) and with unlabeled solution in the interstitial (recipient) chamber. A second incubation period was allowed to attain equilibrium of the tracer with the specimen and to continue phospholipid adsorption when scheduled. The duration of this period ranged from 30 min (inulin) to 1 h (dextran 550 and 2,000), according to the time required to reach steady-state flux. At the end of this period, a 50-μl sample was withdrawn from the donor chamber while the recipient chamber was emptied and immediately refilled with 3.95 ml of fresh, unlabeled solution. At the end of this procedure, which required 3–4 s, the first measurement period started. The duration of the measurement period was different according to the molecule tested and the specimen used (intact or scraped), to ensure that the labeled molecule concentration in the recipient chamber attained a readable value while remaining negligible (see below) relative to that in the donor chamber. Measurement period duration was 20 min in all experiments on scraped specimens. In experiments on intact specimens, it was 20 min with inulin; 30 min with ¹₂⁵I-albumin and dextrans 70 and 150; and 40 min with dextrans 550 and 2,000. At the end of this period, a second measurement period of equal duration was performed after the above procedure was repeated. The samples of liquid withdrawn from each chamber at the end of the measurement periods were treated as previously
described (44). In the experiments with radioisotopes, β-activity was determined as counts per minute in a liquid scintillation spectrometer (Minaxi βTri-Carb 4000, Packard Instrument) and expressed as counts per minute per milliliter to provide values proportional to isotope concentration in a given chamber. In the experiments with FITC-dextrans, fluorescence intensity (proportional to FITC concentration) of the samples was measured in a fluorescence spectrometer (LS50 Perkin-Elmer; excitation 494 nm; emission 525 nm). In both kinds of experiments, the values were corrected for background radioactivity or fluorescence measured in samples of liquid recovered from both chambers before the addition of the labeled solutions. Checks for constant concentration of labeled molecules in the donor chamber and for their negligible concentration in the recipient relative to the donor chamber (<2%, allowing measurement of unidirectional, rather than net, fluxes) at the end of each measurement period were performed as previously described (44). In the experiments with $^{125}$I-albumin, a correction for unbound tracer present in the samples was performed by subtracting the value due to unbound $^{125}$I from values of counts per minute measured in each sample; this was obtained by measuring, in corresponding samples, the radioactivity remaining in the supernatant after protein precipitation with trichloroacetic acid and centrifugation. If albumin digestion occurred in the luminal chamber during the experiment, due to proteases released from damaged cells of the specimens, the diffusion of labeled fragments would lead to an overestimation of $P_{\text{alt}}$. To assess whether albumin digestion occurred, the following tests were done with five specimens. One-milliliter samples of the liquid withdrawn from the luminal chamber at the end of the first incubation period (containing 0.5 g% unlabelled albumin, see Solutions and labeled molecules) were filtered by centrifugation at 5,000 g for 30 min at 4°C through low-binding cellulose ultrafiltration membranes with 30-kDa nominal molecular mass limit (PLTK, Millipore). Protein concentration was measured by colorimetry (Lowry micromethod) in the filtrate and in a sample of the solution before any contact with tissue specimens. Protein concentration in the filtrate was <1.5% of that in the control solution. Therefore, it seems likely that negligible, if any, albumin breakage occurs in the Ussing apparatus during the experiments.

The unidirectional flux of a solute was computed from the concentration of the labeled molecule in the recipient chamber at the end of a measurement period, the overall concentration of the solute (labeled plus unlabeled) in the donor chamber, the volume of the solution in the recipient chamber, the surface area of the window, and the duration of each measurement period, as previously described (44). In the experiments with radioisotopes, the values obtained from experiments in intact specimens were corrected for the effect of liquid unstriated layers (USL) close to the membrane were obtained from the following equation (8): $1/P_{\text{alt}} = (1/P_{\text{meas}}) - (d_{\text{USL}}/D)$, where $P_{\text{alt}}$ is the corrected $P$, $P_{\text{meas}}$ is measured $P$, and $d_{\text{USL}}$ is the overall USL thickness. The overall USL thickness was 170 and 200 μm for intact and scraped specimens, respectively (1). The $P$ values obtained from experiments in intact specimens provided the $P_{\text{per}}$, whereas those obtained from experiments in scraped specimens provided the $P_{\text{con}}$. Because the mesothelium and the connective tissue are placed in series, their resistances to diffusion $(1/P)$ add up; therefore, $P_{\text{meas}}$ was computed by using the formula of series resistances: $(1/P_{\text{meas}}) = (1/P_{\text{per}}) - (1/P_{\text{con}})$.

Connective tissue hydration. Connective tissue kept in Ringer solution swells because the gel phase of the interstitium absorbs some water (9, 16). Because of this absorption, the hydration (water weight per dry tissue weight) of cat and rat mesentery has been found to increase by ~50% (7, 9). In turn, this increase in hydration increases $P$ of the connective tissue to macromolecules (16). Therefore, in 34 experiments we measured the hydration of a piece of the pericardium specimen (free of fat and of visible vessels) kept in Ringer solution plus 0.5% albumin for ~1 h, as occurs during $P$ measurements. The piece was then quickly blotted, weighed, dried, and weighed again. The water content of the specimen was obtained by the difference between the wet and the dry tissue weight. Moreover, in eight experiments, before starting the procedures required for pericardium sampling (hence, with the rabbit alive and the chest intact), we opened the abdomen, sampled a mesenteric specimen (free of fat and of visible vessels), and cut it into two pieces. The hydration of one piece was measured immediately; that of the other one, after immersion in the solution for ~1 h. This enabled us to determine the increase in hydration of the specimen caused by the procedure required by $P$ measurement, without interfering with the pericardium sampling for $P$ measurements.

The knowledge of the change in hydration of the connective tissue caused by the experimental conditions plus some considerations enable a rough correction of the experimental value of $P_{\text{alt}}$ by means of the data of Granger et al. (16). Although the connective tissue of the serosa consists of various layers (19, 41), with respect to diffusion it may be considered fairly homogeneous, provided it is fat free and one disregards the small inhomogeneities caused by microvessels. Therefore, $P_{\text{alt}}$, of the connective tissue of the pericardium multiplied by its thickness provides a macroscopic average of the apparent $D$ of albumin through this tissue ($D_{\text{alt}}$). Granger et al. (16) found that $D_{\text{alt}}$ through the connective tissue of the umbilical cord at physiological hydration was 25% of the $D$ in water ($D_{\text{alt}}$) and that, over a wide range of hydration, $D_{\text{alt}}/D_{\text{alt}}$ increases by ~0.03 per unit increase in hydration. Unfortunately, no information on the experimental approach is provided. Although the slope of the relationship between $D_{\text{alt}}/D_{\text{alt}}$ and hydration of the connective tissue of the pericardium may be different from that of the connective tissue of the umbilical cord, the error involved by using the slope provided by Granger et al. should not be large, because the change in hydration caused by the experimental condition should be ~50% (see above).

Measurements of thickness. The thickness of the pericardium specimens was determined at the end of the experiments by focusing on two tantalum dust particles (particle size ≤5 μm; Sigma Chemical) situated approximately along the same axial line on either side of the specimen, as previously described (44). The measurement was repeated on 10 different sites of the specimens, and the mean of these measurements was taken as the average thickness of the specimen. Thickness measurement was only performed in 40 intact and 10 scraped specimens. The values (73.1 ± 2.2 and 67.2 ± 3.2 μm, respectively) were similar to those previously obtained (73.4 ± 1.6 and 67.3 ± 1.3 μm, respectively; Ref. 1).

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

RESULTS

The mean values of $P_{\text{in}}$, $P_{\text{alt}}$, and $P$ to dextran 70 ($I_{\text{dx}, 70}$) of the sternal part of the parietal pericardium, measured in experiments without and with phospho-
lipids in the solution facing the luminal side of the specimen, are reported in Table 2. These values, corrected for the effect of USL (see METHODS), are also reported in Table 2, although this correction is nearly negligible. In the experiments with phospholipids, $P_{in}$ decreased by $50\%$ ($P < 0.01$), whereas $P_{alb}$ and $P_{dx70}$ did not decrease significantly. The mean values of $P$ to dextrans 150 ($P_{dx150}$), 550 ($P_{dx550}$), and 2,000 ($P_{dx2000}$) (which have been obtained only from experiments without phospholipids) are also reported in Table 2, along with their values corrected for the effect of USL.

The mean values of $P_{in}$, $P_{alb}$, and $P_{dx70}$ measured in the experiments in which the mesothelium was scraped away from the specimen are reported in Table 3, along with the values corrected for the effect of USL. The mean values of $P_{in}$, $P_{alb}$, and $P_{dx70}$ of the mesothelium alone (computed from the values of $P_{per}$ and $P$ of connective tissue, both corrected for the effect of USL, see METHODS) are also reported in Table 3. As it appears from the data in Tables 2 and 3, $P_{alb}$ and $P_{dx70}$ of the mesothelium are only a little greater than those of whole fat-free pericardium. Hence, for these and greater molecules, $P_{mes}$ can be considered similar to that of the fat-free pericardium.

The mean values of $P_{mes}$ to small solutes previously obtained ($\text{Cl}^-$ to sucrose; Refs. 1, 44), as well as those to medium and large solutes measured in this research are plotted as a function of the Stokes-Einstein radius of the solutes ($a$) in Fig. 1. In the same diagram are drawn the theoretical $P-a$ lines for free diffusion (FD) and for restricted diffusion through paracellular pores with an equivalent radius ($r$) of 6 nm, which has been previously determined in the mesothelium (1). Under conditions of FD, $P = (D/A)(A_{p/l})$, where $A$ is the area of the membrane, and $A_{p/l}$ is the overall cross-sectional area per unit pathlength of the pores (26). Under conditions of FD in water, the relationship between $D$ and $a$ is provided by the Stokes-Einstein equation, $D = RT/N_a\eta$, where $R$ is the gas constant, $T$ is the absolute temperature, $N_a$ is the Avogadro number, and $\eta$ is the viscosity of water. Hence, by substituting $D$ in the above equation, one obtains the theoretical $P-a$

### Table 2. Diffusional permeability ($P$) of parietal pericardium to inulin, albumin, and some dextrans

<table>
<thead>
<tr>
<th>Solute</th>
<th>$P_{in}$, $10^{-5}$ cm/s</th>
<th>$P_{alb}$, $10^{-5}$ cm/s</th>
<th>$P_{dx70}$, $10^{-5}$ cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin</td>
<td>0.50 ± 0.06</td>
<td>0.18 ± 0.02</td>
<td>0.094 ± 0.019</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.24 ± 0.03</td>
<td>0.16 ± 0.03</td>
<td>0.075 ± 0.026</td>
</tr>
<tr>
<td>Dextran 70</td>
<td>0.25 ± 0.03</td>
<td>0.18 ± 0.03</td>
<td>0.097 ± 0.021</td>
</tr>
<tr>
<td>Dextran 150</td>
<td>0.10 ± 0.03</td>
<td>0.045 ± 0.011</td>
<td>0.025 ± 0.004</td>
</tr>
<tr>
<td>Dextran 550</td>
<td>0.03 ± 0.03</td>
<td>0.078 ± 0.027</td>
<td></td>
</tr>
<tr>
<td>Dextran 2000</td>
<td>0.02 ± 0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. No. of experiments is 8 for each molecule and condition, except for dextran 550 ($n = 10$), dextran 2000 ($n = 10$), and dextran 70 with phospholipids ($n = 6$).

### Table 3. $P$ of the connective tissue and of the mesothelium to inulin, albumin, and dextran 70

<table>
<thead>
<tr>
<th>Solute</th>
<th>$P_{in}$, $10^{-5}$ cm/s</th>
<th>$P_{alb}$, $10^{-5}$ cm/s</th>
<th>$P_{dx70}$, $10^{-5}$ cm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin</td>
<td>5.93 ± 0.49</td>
<td>1.75 ± 0.12</td>
<td>1.24 ± 0.05</td>
</tr>
<tr>
<td>Albumin</td>
<td>10.33 ± 1.42</td>
<td>2.97 ± 0.38</td>
<td>2.31 ± 0.16</td>
</tr>
<tr>
<td>Dextran 70</td>
<td>0.54 ± 0.20</td>
<td>0.20 ± 0.18</td>
<td>0.101</td>
</tr>
<tr>
<td>Mesothelium</td>
<td>0.25 ± 0.03</td>
<td>0.18 ± 0.04</td>
<td>0.080</td>
</tr>
</tbody>
</table>

Values are means ± SE. No. of experiments is 8 for each molecule.

*Computed from values of $P$ of pericardium and of the connective tissue; both corrected for effect of unstirred layers (see METHODS).
relationship for FD: \( P = (A_p/\alpha)(RT/AN\pi\eta a) \). Because \((A_p/\alpha)(RT/AN\pi\eta)\) is a constant for a given membrane, using the logarithmic form, the \( P-a \) relationship for FD becomes a straight line with a slope of \(-1\) (Fig. 1). Under conditions of restricted diffusion, the \( P-a \) relationship is given by that for FD multiplied by the Renkin function (33), which accounts for steric exclusion and friction between the diffusing solute and the pore walls. Setting \( a/r = \alpha \), Renkin function is given by \((1 - \alpha^2)(1 - 2.1 \alpha + 2.09 \alpha^3 - 0.95 \alpha^5)\). As shown by Fig. 1, \( P \) to the small solutes and \( P_{in} \) fit the line for restricted diffusion with \( r = 6 \) nm, whereas \( P_{alb} \) and \( P \) to dextrans fall much above the line. Moreover, the slope of the \( P-a \) relationship tends to become flat with \( P_{dx, 550} \) and \( P_{dx, 2000} \) (\( P_{dx, 2000} \) is not significantly lower than \( P_{dx, 550} \), Table 2).

The mean values of \( P_{mes} \) to the various solutes in the experiments with phospholipids of this (Table 2) and the previous researches (1, 44) are plotted as a function of \( a \) in Fig. 2. With phospholipids, \( P_{in} \) (besides \( P_{alb} \) and \( P_{dx, 70} \)) also falls much above the line for restricted diffusion with \( r = 1.5 \) nm, which has been determined previously for the mesothelium with phospholipids (1).

The mean values of \( P \) of the connective tissue to the small solutes previously obtained (1, 44), as well as \( P_{in} \), \( P_{alb} \) and \( P_{dx, 70} \) are plotted vs. the Stokes-Einstein radius in Fig. 3. \( P \) to small and large solutes fit the FD line. The hydration (water weight per dry tissue weight) of the connective tissue of the pericardium specimens kept in the solution for \( \sim 1 \) h (i.e., a period similar to that required for \( P \) measurements; see METHODS) was 4.4 \( \pm \) 0.3 (\( n = 34 \)). Moreover, the hydration of the connective tissue of mesenteric specimens, determined immediately after sampling or after immersion in the solution for \( \sim 1 \) h (see METHODS), was 3.3 \( \pm \) 0.2 (\( n = 8 \)) and 5.1 \( \pm \) 0.6 (\( n = 8 \)), respectively. Hence, the increase in hydration caused by the experimental conditions is 55\%, which is in line with previous findings (7, 9). Assuming a similar change in the connective tissue of the pericardium specimens, its physiological hydration should be 2.8. To correct \( P_{alb} \) for the overhydration with the data of Granger et al. (Ref. 16; see METHODS), one has to determine the ratio between \( D_{alb} \) and \( D_{alb} \) (\( D_{alb} = 0.090 \times 10^{-5} \) cm²/s). \( D_{alb} \) is given by \( F_{alb} \) times the thickness of the connective tissue (67 \( \mu \)m; see METHODS and Ref. 1) and is equal to 0.020 \( \times \) 10\(^{-5} \) cm²/s. Therefore, \( D_{alb}/D_{alb} \) under our experimental conditions is 0.22. Because the increase in hydration undergone by the connective tissue of the pericardium under experimental conditions is 1.6 units (4.4 – 2.8) and \( D_{alb}/D_{alb} \) changes by 0.03 per unit change in hydration (Ref. 16; see METHODS), \( D_{alb}/D_{alb} \) under physiological conditions should be 0.22 – (1.6 \( \times \) 0.03) = 0.17. Hence, \( P_{alb} \) of the connective tissue of the pericardium with physiological hydration should be 2.28 \( \times \) 10\(^{-5} \) cm/s. This value is indicated by the triangle in Fig. 3. The value of \( P_{alb} \) of the mesothelium (Figs. 1 and 2; Table 3) is not appreciably affected by the correction of the value of \( P_{alb} \) of the connective tissue for overhydration (i.e., by using in the computation 2.28 instead of 2.97 \( \times \) 10\(^{-5} \) cm/s).

**DISCUSSION**

**Diffusion of inulin, albumin, and dextrans through mesothelium.** Our findings show that the addition of phospholipids to the solution facing the luminal side of the pericardium specimens markedly decreases \( P_{mes} \) to inulin (like sucrose, mannitol, and Na\(^+\); Ref. 1), but it

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Fig. 2. \( P \) of mesothelium of parietal pericardium to various solutes vs. their Stokes-Einstein radius in experiments with phospholipids added on luminal side. Values for small solutes (Cl\(^-\), Na\(^+\), mannitol, and sucrose) are taken from our laboratory’s previous researches (1, 44). Theoretical \( P-a \) relationships for FD and for restricted diffusion with 1.5-nm pore \( r \) are drawn (see text). Values are corrected for the effect of unstirred layers.

Fig. 3. \( P \) of connective tissue of parietal pericardium to various solutes vs. their Stokes-Einstein radius. Values for small solutes (Cl\(^-\), Na\(^+\), mannitol, and sucrose) are taken from our laboratory’s previous researches (1, 44). Theoretical \( P-a \) relationships for FD are drawn (see text). Values are corrected for the effect of unstirred layers. Triangle indicates value of \( P \) to albumin with physiological hydration of connective tissue (see text).
does not decrease significantly $P_{\text{alb}}$ and $P_{\text{dx 70}}$ (Tables 1 and 2). Because phospholipids decrease pore radius from $\sim 6$ to $\sim 1.5 \text{ nm}$ (1), this finding suggests that albumin and dextran 70 do not appreciably diffuse through the small pores of the mesothelium, even without the addition of phospholipids. Indeed, as shown by the line for restricted diffusion with an equivalent pore radius ($r$) of 6 nm (Fig. 1), the diffusion of albumin through these pores should be negligible. This is at variance with the finding obtained by others with a different approach (Ref. 25; see Diffusion of albumin through serosae) and is relevant for a better assessment of the colloidosmotic pressure acting through the mesothelium.

The finding that $P_{\text{alb}}$ of the mesothelium is one order of magnitude greater than the corresponding point on the line for restricted diffusion with $r = 6 \text{ nm}$ (Fig. 1) indicates that most of albumin (and larger molecules) transfer through the mesothelium occurs through large pores and/or transcytosis. Various lines of evidence have now shown that diffusion through large pores and transcytosis occur in capillary endothelium (24, 38). As for capillary endothelium, it is not known whether the large pores of the mesothelium are real pores or are provided by transient fusion of vesicles through the cell (39). Moreover, although the sternal region of the parietal pericardium should be essentially free of lymphatic stomata (40), we cannot rule out the occurrence of a few stomata in our specimens. For this possibility and that of a few microlesions in the specimens, our values of $P$ to macromolecules are likely overestimated relative to physiological conditions.

The finding that, with large dextrans (550 and 2,000), the slope of the $P-a$ relationship tends to become flat (Fig. 1) suggests the occurrence of transcytosis in the mesothelium. Indeed, the sequence of events determining the changes in slope of the $P-a$ line for macromolecules may be summarized as follows. When the molecules are large enough to be excluded by small pores, but small enough to diffuse freely through large pores, the slope of the $P-a$ line should be $-1$. With greater molecular size, diffusion through large pores becomes restricted, and, therefore, the slope becomes progressively steeper. When molecular size becomes greater than that of the large pores, diffusion stops, and, if some molecular transfer persists, it occurs by transcytosis. At this stage, the slope would be nil, were it not for some steric exclusion of molecules from vesicles (34). Because the size of the vesicles is probably only somewhat greater than that of the large pores (19, 24, 41), the slope does not become flat but turns from steep to nearly flat when diffusion vanishes and molecular transfer occurs only by transcytosis. Transport by transcytosis would be in line with the morphological evidence of free vesicles in the cytoplasm of the mesothelial cells of pleura (41) and pericardium (19). It might be an important mechanism to remove proteins from the pleural liquid under physiological conditions.

From the values of $P_{\text{dx 70}}$ and $P_{\text{dx 150}}$, one can attempt to assess $r$ of the large pores through the following equation (31), where $F(a/r)$ is the Renkin function (33)

$$\frac{P_{\text{dx 70}}/D_{\text{dx 70}}}{P_{\text{dx 150}}/D_{\text{dx 150}}} = \frac{A_{J}/A_{L}F(a/r)_{\text{dx 70}}}{A_{J}/A_{L}F(a/r)_{\text{dx 150}}}$$

Because the term $A_{J}/A_{L}$ cancels out, the above equation contains only one unknown variable, $r$, which can be solved for by an iterative process. Neglecting transcytosis, the value of $r$ for large pores would be 39 nm. Considering transcytosis, the values of $P_{\text{dx 70}}$ and $P_{\text{dx 150}}$ should be reduced by an amount corresponding to the vesicular transfer, and, therefore, the value of $r$ would be substantially smaller.

When phospholipids are added to the solution facing the luminal side of the mesothelium, the equivalent radius of the small pores is reduced to $\sim 1.5 \text{ nm}$ (1). In this case, therefore, $P_{\text{in}}$ also falls much above the line for restricted diffusion through pores with $r = 1.5 \text{ nm}$ (Fig. 2). The finding that $P_{\text{in}}$ is one order of magnitude greater than the corresponding point on the restricted diffusion line indicates that, with phospholipids, most of inulin transfer through the mesothelium occurs through large pores and/or transcytosis.

$P_{\text{alb}}$ of the mesothelium ($0.20 \times 10^{-5} \text{ cm/s}$; Table 2) is more than one order of magnitude greater than that of the endothelium of muscle, heart, and lung capillaries ($\sim 0.005 \times 10^{-5} \text{ cm/s}$; Refs. 20, 29, 35), whereas $P$ to sucrose and $P_{\text{in}}$ of the mesothelium ($0.7-2.9 \times 10^{-5} \text{ cm/s}$, Ref. 44; and 0.25-0.54 $\times 10^{-5} \text{ cm/s}$, Table 2, respectively) are roughly similar to those of the endothelium of the above capillaries (2.0 and $\sim 0.3 \times 10^{-5} \text{ cm/s}$, respectively; Ref. 10). This difference could be due to a greater area of open intercellular junctions and to a smaller density of the glycocalyx in the mesothelium (or in our pericardium specimens) than in the endothelium, because the fiber matrix of the glycocalyx provides a further sieve to macromolecules (24). Evidence of glycocalyx on the luminal side of the mesothelium has been provided (5), but it is not known whether its fiber matrix is similar to that of the endothelium. Finally, $P_{\text{alb}}$ of the mesothelium of our specimens is smaller than $P_{\text{alb}}$ of monolayers of cultured cells of aortic endothelium ($0.56 \times 10^{-5} \text{ cm/s}$; Ref. 3). This difference likely occurs because 5–10% of these cultured cells are not tightly joined but have small gaps between them (3) and because cultured endothelial cells do not appear to be provided with glycocalyx.

**Diffusion of inulin, albumin, and dextran through the connective tissue.** In the connective tissue of our pericardium specimens, $P_{\text{in}}$, $P_{\text{alb}}$, and $P_{\text{dx 70}}$ fit the FD line (Fig. 3). In a loose connective tissue like the subcutaneous one, the mean hydraulic radius of the pores (which is smaller than the actual radius) has been found to be 24 nm (22). The connective tissue of the pericardium (12, 37) should be tighter than the subcutaneous tissue (22), but the hydration of the specimens under experimental conditions is $\sim 55\%$ greater than under physiological conditions (see RESULTS). This is likely the reason why $P_{\text{alb}}$ of our specimens fits the FD line. We, therefore, made a rough correction of the
experimental value of $P_{\text{alb}}$ by means of the values of Granger et al. (16) on $D_{\text{alb}}$ through the connective tissue of the umbilical cord at various hydrations (see METHODS). With physiological hydration, $P_{\text{alb}}$ through the connective tissue of the pericardium would be $2.28 \times 10^{-5}$ cm/s (triangle in Fig. 3), i.e., 77% of that measured ($2.97 \times 10^{-5}$ cm/s). This indicates that restriction to the diffusion of albumin is small, although it may be that the correction made is inadequate.

Granger et al. (16) found that $D_{\text{alb}}$ through the connective tissue of the umbilical cord with physiological hydration is 25% of $D_{\text{alb}}$. $D_{\text{alb}}$ of the connective tissue of the pericardium with physiological hydration is 17% of $D_{\text{alb}}$. A value of $D_{\text{alb}}'$/ $D_{\text{alb}}$, lower in the connective tissue of the pericardium than in that of the umbilical cord seems reasonable, because the latter is more loose (22).

On the other hand, Fox and Wayland (13) found, in the connective tissue of rat mesentery, that $D_{\text{alb}}'$ is only 7.3% of $D_{\text{alb}}$. The mesentery was exteriorized, covered with mineral oil at 37°C, and slightly squeezed to remove the thin layer of peritoneal liquid remaining between the oil and the mesothelium. Blood circulation in the mesentery was not visibly impaired. The movement of fluorescent albumin coming from the capillaries was measured by video microscopy over selected regions along the plane of the membrane. The authors considered the possible sources of error of their approach and concluded that all of them should overestimate $D_{\text{alb}}'$, except for a hypothetical dehydration caused by oil absorbing water from the tissue. Another cause of dehydration could be the pressure applied to the coverslips, which could squeeze water out of the gel of the connective tissue. This, however, could explain only part of the difference between our value of $D_{\text{alb}}'$/ $D_{\text{alb}}$ and that of Fox and Wayland. Another part of this difference could depend on an inadequate correction for the overhydration of our specimens. Finally, Fox and Wayland determined albumin diffusion along the plane of the connective tissue of the serosa, whereas we did in a direction perpendicular to it.

**Diffusion of albumin through serosae.** $P_{\text{alb}}$ through the mesothelium does not seem to have been previously measured, but data on albumin diffusion through the whole serosa are available. $P_{\text{alb}}$ has been measured in vitro through rat mesentery (32), stripped specimens of visceral pleura of sheep and dogs (21, 30), and stripped specimens of parietal pleura of dogs (30) and has been indirectly computed in vivo through the parietal pleura plus endothoracic fascia of rabbits (25). Moreover, $D_{\text{alb}}$ has been recently measured through the mesentery of rabbits (27) and the mediastinal pleura of pigs (28).

Comparisons among these data have been done, but they are confusing because the features of the serosa or the experimental conditions have not been sufficiently considered. Parameswaran et al. (28) maintained that comparisons should be done after converting $P$ to apparent diffusion coefficient (which is equal to $P$ times membrane thickness) to eliminate the marked difference in thickness among the serosae (see below). This, however, would be correct only if the membrane were fairly homogeneous along its thickness, which is not the case. Indeed, the serosa consists of a single layer of mesothelial cells, which is only 2–3 μm thick (41), and of layers of connective tissue, the thickness of which ranges from 10 to >100 μm, according to the species, the serosa, and the region (4, 12, 37, 43). As it could have been expected from the morphological features and as previously shown for small solutes (1, 44), most of the resistance to diffusion of a fat-free serosa is located in the mesothelium. More particularly, the findings of the present research show that, under our experimental conditions, 94% of the resistance to albumin diffusion through fat-free pericardium is provided by the mesothelium (92% with physiological hydration of the connective tissue, see RESULTS and preceding section). Hence, the resistance to albumin diffusion through the connective tissue is nearly negligible (relative to that of the whole specimen), although the thickness of the connective tissue provides most of the thickness of the whole specimen (see METHODS). Therefore, unless the thickness of the specimen is much greater than that of ours (see METHODS), an approximate comparison among the data of $P_{\text{alb}}$ obtained in various serosae seems feasible (Table 4). It should be remembered that, in the case of the mesentery (27, 32), two layers of mesothelium are involved.

The high values of $P_{\text{alb}}$ found in rat mesentery ($7.1 \times 10^{-5}$ cm/s; Ref. 32), in stripped specimens of visceral (2.0 $\times 10^{-5}$ cm/s, Ref. 21; and 3.4 $\times 10^{-5}$ cm/s, Ref. 30) and parietal pleura (13.3 $\times 10^{-5}$ cm/s; Ref. 30) seem mainly due to a marked damage of the mesothelium because $P/D$ for H₂O is not higher than that for small

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**Table 4. $P$ to albumin ($P_{\text{alb}}$) of various serosae determined in various species with different approaches**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Species</th>
<th>Serosa</th>
<th>Experimental Condition</th>
<th>Temperature, °C</th>
<th>Thickness, μm</th>
<th>$P_{\text{alb}} \times 10^{-5}$ cm/s</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Mesentery*</td>
<td></td>
<td>In vitro</td>
<td>37</td>
<td>20</td>
<td>7.1</td>
<td>32</td>
</tr>
<tr>
<td>Sheep</td>
<td>Stripped visceral pleura</td>
<td></td>
<td>In vitro</td>
<td>25</td>
<td>10</td>
<td>2.0</td>
<td>21</td>
</tr>
<tr>
<td>Dog</td>
<td>Stripped visceral pleura, caudal lobes</td>
<td></td>
<td>In vitro</td>
<td>25</td>
<td>15</td>
<td>3.4</td>
<td>30</td>
</tr>
<tr>
<td>Dog</td>
<td>Stripped parietal pleura, caudal intercostal</td>
<td></td>
<td>In vitro</td>
<td>25</td>
<td>20</td>
<td>13.3</td>
<td>30</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Parietal pleura plus endothoracic fascia, intercostal</td>
<td></td>
<td>In vivo</td>
<td>30</td>
<td>0.14†</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>Mesentery*</td>
<td></td>
<td>In vitro</td>
<td>22–24</td>
<td>30</td>
<td>0.67</td>
<td>27</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Parietal pericardium, retrosternal</td>
<td></td>
<td>In vitro</td>
<td>37</td>
<td>70</td>
<td>0.18‡</td>
<td>Present study</td>
</tr>
</tbody>
</table>

*Two layers of mesothelium. †Indirectly computed from data of liquid flow and convective albumin flux. ‡Corrected for effect of unstirred layers.
solutes, as it should be, because H₂O also diffuses through the cellular membrane, whereas the small solutes tested only diffuse through the paracellular pores of the mesothelium (1). The probable causes of damage have already been pointed out (44). In two of these researches, P_{alb} of the serosa is even higher than that provided by the connective tissue in the present research (2.97 × 10⁻⁵ cm/s; Table 3). This seems to be due to the thinner connective tissue of their specimens (Table 4). The higher value in the parietal than in the visceral pleura is likely due to the occurrence of lymphatic stomas in the former (30).

The value of P_{alb} indirectly obtained from measurements across the parietal pleura plus endothoracic fascia of rabbits (0.14 × 10⁻⁵ cm/s; Ref. 25) is close to that measured in the present research (0.18 × 10⁻⁵ cm/s; Table 2). This value has been computed from in vivo measurements of liquid flow and convective albumin fluxes. To this end a small capsule was glued to the endothoracic fascia of an intercostal space, and pleural liquid was sucked into the capsule by lowering its pressure 30 cmH₂O below atmospheric. After 1–3 h, the volume of liquid collected into the capsule and its albumin concentration were measured. The solvent drag reflection coefficient for albumin was determined from the ratio between albumin concentration in the capsule and in the pleural liquid. The hydraulic conductivity was determined from liquid flow and Starling forces across the membrane. The equivalent pore radii were determined according to a graphical analysis based on the solvent drag reflection coefficient and α (36). The pore area was then computed from hydraulic conductivity and the equivalent pore radii: P_{alb} was finally calculated from the pore area, D_{alb} times the Renkin function for restricted diffusion (33), and other parameters available. In this connection one has to consider that the pressure applied to the capsule, without a rigid support to the endothoracic fascia, should distend the pleura and enlarge the pores of the mesothelium. This should lead to an overestimation of P_{alb} and may explain the substantial transfer of albumin through small pores. On the other hand, the inclusion of the endothoracic fascia in the measurement has likely involved an underestimation of P_{alb}.

If the thickness of the membrane is known, P_{alb} may be obtained from the values of D_{alb} provided by Parmewaran et al. for the mesentery of rabbits (27) and the mediastinal pleura of pigs (28). On the other hand, P_{alb} of the mediastinal pleura of pigs cannot be compared with that of other serosae (see above), because the thickness of the specimens (230 μm; Ref. 28) is much greater than that of our specimens (73 μm, see METHODS). With regard to the measurements on rabbit mesentery, the following considerations should be made. 1) The data were not corrected for the effect of unstirred layers. 2) The concentration of labeled albumin in the recipient chamber was not negligible, and, therefore, an appreciable back diffusion should have occurred. 3) Measurements were done at 25°C. These situations lead to an underestimation of the unidirectional flux of albumin and, hence, of P_{alb}. On the other hand, the damage to the mesothelium caused by air exposure, manipulation, and cyanoacrylic glue at the margins should lead to an overestimation of P_{alb}. In the mesentery of rabbits, P_{alb} was 0.67 × 10⁻⁵ cm/s (with an albumin concentration of 0.5 g%); this value is approximately three times greater than that found by us in the parietal pericardium of rabbits (0.18 × 10⁻⁵ cm/s). The occurrence of Kampmeier’s foci or “milky spots” in the mesentery (41) provides a greater permeability to this serosa. It seems unlikely, however, that this feature explains the whole difference in P_{alb} because the mesentery is lined on both sides by the mesothelium. The rest of the difference in P_{alb} should be due to damage of the mesothelium.

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