Albumin transcytosis from the pleural space

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Agostoni, Emilio, Francesca Bodega, and Luciano Zocchi. Albumin transcytosis from the pleural space. J Appl Physiol 93: 1806–1812, 2002; 10.1152/japplphysiol.00494.2002.—Occurrence of transcytosis in pleural mesothelium was verified by measuring removal of labeled macromolecules from pleural liquid in experiments without and with nocodazole. To this end, we injected 0.3 ml of Ringer-albumin with 750 μg of albumin-Texas red or with 600 μg of dextran 70-Texas red in the right pleural space of anesthetized rabbits, and after 3 h we measured pleural liquid volume, labeled macromolecule concentration, and, hence, labeled macromolecule quantity in the liquid of this space. Labeled albumin left was 318 ± 28 μg in control and 419 ± 17 μg in nocodazole experiments (means ± SE); hence, whereas ventilation was similar its removal was greater (P < 0.01) in control experiments. Labeled dextran left was 283 ± 10 μg in control and 381 ± 21 μg in nocodazole experiments; hence, whereas ventilation was similar its removal was greater (P < 0.01) in control experiments. These findings indicate occurrence of transcytosis from the pleural space. Liquid removed by transcytosis was 0.05 ml/h. This amount times unlabeled albumin concentration under physiological conditions (10 mg/ml) times lumen-vesicle partition coefficient for albumin (0.78) provides fluid-phase albumin transcytosis: ~203 μg·h⁻¹·kg⁻²/³. Transcytosis might contribute a relevant part of protein and liquid removal from the pleural space.

Dextrans; lymphatic drainage from the pleural space; pleural mesothelium; vesicular liquid flow

WE HAVE, RECENTLY, PROVIDED evidence for transcytosis from the luminal to the interstitial side of specimens of parietal pericardium of rabbit by showing that the unidirectional flux of albumin or dextran 70 from lumen to interstitium is greater than that in the opposite direction and that this difference disappears at 12°C or with a transcytosis inhibitor (8). Our findings agree with earlier morphological evidence for transcytosis of macromolecules from the luminal to the interstitial side of the mesothelium in rat parietal pericardium (18) and mouse parietal peritoneum (13, 14, 16).

In the present research, we tried to provide evidence for transcytosis from the pleural space in vivo. Under physiological conditions, protein removal from the pleural space occurs by lymphatic drainage through the stomata of the parietal mesothelium (2, 5, 20, 21, 23, 25), by solvent drag because of liquid absorption through the visceral mesothelium by Starling forces (1–3), and probably by transcytosis. On the other hand, proteins enter the pleural space by solvent drag because of liquid filtration through the parietal mesothelium by Starling forces (1, 2, 5, 21, 23, 25) and by diffusion because protein concentration in the pleural liquid (22) is smaller than that in the interstitium adjacent to the mesothelium (23). The data obtained on the parietal pericardium in vitro (8) suggest that the removal of albumin from the pleural space by transcytosis might be appreciable even if transcytosis in the pleura were smaller than in the pericardium. One may, therefore, formulate the hypothesis that, in the case of transcytosis, the quantity of labeled albumin left in the pleural liquid a few hours after injection into the pleural space of a bolus with labeled albumin plus nocodazole (a transcytosis inhibitor; Refs. 12, 17) should be greater than that left after a similar bolus injection without nocodazole. Moreover, taking into account that the albumin removed from the pleural space by transcytosis is eventually drained into blood by the lymphatics of the connective tissue of the pleura, one may formulate a second hypothesis: namely, that in case of transcytosis the quantity of labeled albumin occurring in plasma a few hours after injection into the pleural space of a bolus with labeled albumin plus nocodazole should be smaller than that found after a similar bolus injection without nocodazole. To verify these hypotheses, we injected 0.3 ml of albumin-Ringer solution with a small amount of labeled albumin (or dextran 70) into the right pleural space of anesthetized rabbits. Two kinds of experiments were performed: in one the injected bolus contained nocodazole, in the other it did not. After 3 h, we measured the volume of the pleural liquid on the right side and the concentration of labeled albumin (or dextran 70) to determine the quantity of labeled molecules left in the right pleural liquid under the two conditions. Moreover, we measured the concentration of labeled albumin (or dextran 70) in plasma, and, assuming its volume to be 4% of body weight (6, 10, 15), we determined the quantity of labeled macromolecule in plasma under both conditions.

METHODS

The experiments were performed on 39 rabbits (2.45–2.9 kg body wt). The animals were anesthetized with pentobarbital sodium (Sigma Chemical, 18 mg/ml); the initial dose

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was 2 ml/kg intravenously, and small additional injections (0.2 ml/kg) were delivered when required to maintain an adequate level of anesthesia. The trachea was cannulated, and air flow and tidal volume (obtained by electronic integration of the flow signal by means of a Hewlett-Packard 8815A respiratory integrator) were recorded on a 7418 Hewlett-Packard thermapaper oscillograph throughout the experiment. The left jugular vein was exposed, and 2.5 ml of blood were sampled for background fluorescence measurement (see below). With the rabbit in the left lateral posture, the sixth right intercostal space was cleared from skin and muscles down to the intercostal muscles. A double thread loop was prepared in these muscles, and a stainless-steel cannula (1.4 mm OD; 0.9 mm ID) connected to a 1-ml glass syringe containing the bolus was inserted in the pleural cavity and tightened to the muscles by one thread loop. The bolus (0.3 ml) was then injected into the pleural space, and the cannula was removed while the second thread loop was tightened. During the procedure, a layer of Ringer solution was maintained over the site of injection to prevent air entrance into the pleural space during insertion of the cannula. The injected bolus consisted of a Ringer solution (composition, in mM: Na+ 139, K+ 5, Ca2+ 2.5, Mg2+ 1.5, Cl− 119, HCO3− 29, d-glucose 5.6) containing bovine serum albumin (8 mg/ml, Sigma Chemical). Macromolecules labeled with a fluorescent marker were added to the albumin-Ringer solution: bovine serum albumin conjugated with Texas red (2.5 mg/ml, Molecular Probes) in one series of experiments (n = 16) and dextran 70 kDa conjugated with Texas red (2.0 mg/ml, Molecular Probes) in another series (n = 16). Different concentrations were used for the two tracers because fluorescence intensity was somewhat less for the albumin conjugate. For each series, two groups of experiments (n = 8 each) were performed: one as control, and in the other one an inhibitor of transcytosis, nocodazole (Sigma Chemical; Refs. 12, 17), was added to the injectate. The quantity of nocodazole added to the injectate was such that its concentration in the pleural liquid at the beginning of the experiment was ~60 μM. This concentration should decrease substantially during the course of the experiment because of turnover of pleural liquid and diffusion of nocodazole (molecular mass 301 Da; as a rough analogy, see the time course of labeled mannitol concentration in a 2-ml hydrothorax; Ref. 28). After injection, the rabbit was turned supine. Before cannula insertion and every 30 min during the experiment, the respiratory integrator system was passively expanded with a volume two to three times the spontaneous tidal volume by means of a large syringe. Three hours after the injection, a 5-ml blood sample was withdrawn from the jugular vein or the left carotid artery; then the rabbit was killed by an overdose of anesthetic and was placed in the left lateral posture 90° head up. The right pleural space was opened, its liquid was carefully collected through a polyethylene tube connected to a 1-ml glass syringe, and its volume was measured (4, 22). The liquid of the left pleural space, where no injection was made, was also collected and its volume was measured. This volume was used as an index of the volume of liquid present in the right pleural space before injection (see RESULTS). The experiments were discarded when the liquid was macroscopically contaminated by blood, when pathological signs were evident, or when the volume of liquid collected from the left space was >0.6 ml (4, 22). To approximately assess the time course of labeled albumin concentration during the experiments, in an additional series of experiments (n = 7) with albumin Texas Red without nocodazole, the experiment was ended 1 h, instead of 3 h, after the injection. In all experiments, breathing frequency and tidal volume were measured during the initial, middle, and final part of each experiment, and minute ventilation was computed. The three values of ventilation were averaged to obtain the mean ventilation in a given experiment.

Fluorescence intensity in the liquid collected from the right pleural space at the end of the experiment was measured by a spectrofluorophotometer (Shimadzu RF1501) (excitation 596 nm; emission 615 nm). A calibration curve was made for each experiment by diluting the solution prepared for injection into the pleural space. The background fluorescence of albumin-Ringer solution was measured and subtracted. The calibration was linear over the range of labeled molecule concentration between 0 and 15 μg/ml. A calibration factor was computed for each curve by linear regression through the points. Samples (25 or 50 μl) of the liquid collected from the right pleural space were diluted 120 or 60 times, respectively, with albumin-Ringer solution, so that the reading on the spectrofluorophotometer fell on the linear portion of the calibration curve. Readings were also made of 1) similarly diluted samples of liquid collected from right and left pleural space of two extra rabbits, in which no bolus was injected, to obtain the background fluorescence of pleural liquid; 2) similarly diluted samples of the liquid collected from the left pleural space (where no injection was made), to determine the concentration of labeled molecules entered into this liquid during the experiment; 3) plasma obtained from the blood sampled before injection, to obtain the background fluorescence in plasma; and 4) plasma obtained from the blood sampled at the end of the experiment, to determine the concentration of labeled molecules entered in plasma during the experiment. Readings obtained in pleural liquid and plasma samples at the end of the experiment were corrected for background fluorescence. Checks for the possible occurrence of unbound tracer in the injectate were made by measuring fluorescence intensity in the dialysate (for ~16 h at ~37°C) or ultrafiltrate (by centrifuging at 5,000 g for 30 min through low-binding cellulose ultrafiltration membranes with a 10-kDa nominal molecular weight cutoff, PLTK, Millipore) of diluted solutions of the labeled molecules. The fluorescence found both in the dialysate and in the ultrafiltrate, corresponding to that due to unbound Texas Red, was <0.1% of that in the original solution, both with labeled albumin and with labeled dextran 70. To assess whether macromolecule catabolism occurred in the pleural space, in two experiments with albumin-Texas red and in two experiments with dextran 70-Texas red, diluted samples of the pleural liquid collected at the end of the experiment were ultrafiltered by the above procedure through ultrafiltration membranes with a 30-kDa nominal molecular weight cutoff (PLTK, Millipore). Fluorescence in the filtrate was <0.1% of that in the solution before filtration. Hence, an appreciable catabolism of albumin or dextran 70 does not seem to occur in the pleural space during the experiments.

The quantity of labeled macromolecule present in the pleural liquid of each space at the end of the experiment was obtained by multiplying the corresponding concentration by the overall volume of liquid in the pleural space. This is given by the volume collected plus that remaining adherent to the walls of the space when it is opened. The former was measured, and the latter was computed from the mean value found in previous ad hoc experiments (0.25 ml in 2.23-kg rabbits; Ref. 22). Because the volume of liquid adherent to the walls is related to their surface area, this volume of liquid was computed as 0.25 ml × (body wt2/3−2.32/3). The quantity of labeled macromolecule present in plasma at the end of the experiment was obtained by multiplying the corresponding concentration by the volume of plasma in that rabbit. The
volume of plasma was assumed to be 4% of the body weight (Refs. 6, 10, 15). The surface area of the parietal and of the visceral pleura was obtained from previous measurements (22) by normalizing to body weight $^{2/3}$. Because the surface area of the parietal pleura previously measured was only that facing the lung, the surface area of the costophrenic sinus was added by assuming it to be 1/4 of the parietal pleura facing the lung. The results are reported as means ± SE. Statistical difference between groups was assessed by unpaired t-test.

RESULTS

**Liquid volume.** The volume of liquid in the left pleural space (where no injection was made) was similar in both kinds of experiments of both series, that with labeled albumin and that with labeled dextran 70 (Tables 1 and 2). This volume of liquid was also similar to that previously determined in ad hoc experiments (4, 22), taking into account the small difference in body size by normalizing to body weight $^{2/3}$ (which yields 0.51 ml for the present rabbits). Therefore, the volume of liquid in the right pleural space at the beginning of the experiment should be ~0.81 ml, 0.51 ml being the volume under physiological conditions and 0.3 ml being the injected volume. Because of the variance in the volume of pleural liquid among rabbits and of the error involved in its measurement, to get more reliable data it is better to pool together the values of liquid volume in the right pleural space of both series of experiments in a given condition, i.e., control or with nocodazole, so that each group consists of 16 experiments. By so doing, the volume of the right pleural liquid was smaller ($P < 0.01$) at the end of control experiments, 0.66 ± 0.04 ml, than at the end of experiments with nocodazole, 0.81 ± 0.04 ml. Therefore, transcytosis seems to remove 0.15 ml of liquid from the pleural space in 3 h, i.e., 0.05 ml/h or 26 µl·h$^{-1}$·kg$^{-2/3}$. Moreover, the data suggest that in control experiments approximately half of the liquid injected was removed, whereas in the experiments with nocodazole the net removal of liquid was approximately nil.

**Labeled albumin.** The quantity of labeled albumin in the right pleural liquid was smaller ($P < 0.01$) at the end of control experiments, 318 ± 27 µg, than at the end of experiments with nocodazole, 419 ± 17 µg (Table 1). The quantities of labeled albumin left in the right pleural space should be a little greater than those in the right pleural liquid because of the labeled albumin adsorbed to the walls, which is not measured. This, however, does not represent a problem, because the quantity of labeled albumin adsorbed should be similar in both kinds of experiments and because what matters for our aims is the difference in quantity of labeled albumin left in the liquid between the two kinds of experiments. The difference in quantity of labeled albumin left in the right pleural liquid between nocodazole and control experiments, 101 µg, should provide the quantity of labeled albumin removed by transcytosis in 3 h.

The quantity of labeled albumin in plasma was greater ($P < 0.01$) at the end of control experiments (64.3 ± 6.9 µg) than at the end of experiments with nocodazole (32.5 ± 5.6 µg, Table 1). The finding that the quantity of labeled albumin in plasma is greater in control than in nocodazole experiments fits with the

Table 1. Labeled albumin in pleural liquid and in plasma 3 h after injection of 0.3 ml of Ringer-albumin with 750 µg of labeled albumin in the right pleural space

<table>
<thead>
<tr>
<th>Body Weight, n</th>
<th>Pleural Liquid Volume, µl</th>
<th>Pleural Liquid Concentration, µg/ml</th>
<th>Pleural Liquid Quantity</th>
<th>Plasma</th>
<th>Ventilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right, Left</td>
<td>Right, Left</td>
<td>Right, Left</td>
<td>Right, Left</td>
<td>Volume, ml</td>
<td>Concentration, µg/ml</td>
</tr>
<tr>
<td>Control 8</td>
<td>2.60 ± 0.07, 0.66 ± 0.05</td>
<td>500 ± 44, 11.4 ± 2.5</td>
<td>318 ± 28, 42.5 ± 3.7</td>
<td>103 ± 3</td>
<td>0.62 ± 0.06, 64.3 ± 1.9</td>
</tr>
<tr>
<td>Nocodazole 8</td>
<td>2.74 ± 0.06, 0.79 ± 0.03</td>
<td>549 ± 34, 6.8 ± 2.2</td>
<td>419 ± 17, 55.9 ± 2.2</td>
<td>109 ± 2</td>
<td>0.30 ± 0.05, 32.5 ± 3.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rabbits. *Volume collected at the end of the experiment plus volume adherent to the wall (see METHODS; Ref. 22). †Assumed to be 4% of body weight (6, 10, 15). §$P < 0.01$.

Table 2. Labeled dextran 70 in pleural liquid and in plasma 3 h after injection of 0.3 ml of Ringer-albumin with 600 µg of labeled dextran 70 in the right pleural space

<table>
<thead>
<tr>
<th>Body Weight, n</th>
<th>Pleural Liquid Volume, µl</th>
<th>Pleural Liquid Concentration, µg/ml</th>
<th>Pleural Liquid Quantity</th>
<th>Plasma</th>
<th>Ventilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right, Left</td>
<td>Right, Left</td>
<td>Right, Left</td>
<td>Right, Left</td>
<td>Volume, ml</td>
<td>Concentration, µg/ml</td>
</tr>
<tr>
<td>Control 8</td>
<td>2.60 ± 0.05, 0.67 ± 0.01</td>
<td>430 ± 33, 6.2 ± 2.3</td>
<td>283 ± 10, 47.2 ± 1.6</td>
<td>107 ± 2</td>
<td>0.21 ± 0.02, 22.3 ± 1.8</td>
</tr>
<tr>
<td>Nocodazole 8</td>
<td>2.61 ± 0.04, 0.83 µg/ml</td>
<td>468 ± 38, 6.4 ± 2.3</td>
<td>381 ± 20, 63.5 ± 1.6</td>
<td>104 ± 2</td>
<td>0.14 ± 0.02, 14.4 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rabbits. *Volume collected at the end of the experiment plus volume adherent to the wall (see METHODS; Ref. 22). †Assumed to be 4% of the body weight (6, 10, 15). §$P < 0.01$; §$P < 0.02$. 

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occurrence of transcytosis because, when transcytosis is operating, more labeled albumin is removed from the pleural space, and therefore more labeled albumin reaches the blood.

**Labeled dextran.** The quantity of labeled dextran 70 in the right pleural liquid was smaller (P < 0.01) at the end of control experiments, 283 ± 10 μg, than at the end of experiments with nocodazole, 381 ± 21 μg (Table 2). The same consideration made above on the labeled albumin adsorbed to the walls of the space also applies to the labeled dextran 70 adsorbed to the walls (which might be relatively greater in quantity, because dextran is not normally present in the pleural space). The difference in quantity of labeled dextran left in the right pleural liquid between nocodazole and control experiments, 98 μg, should provide the quantity of labeled dextran removed by transcytosis in 3 h.

The quantity of labeled dextran in plasma was greater (P < 0.01) at the end of control experiments (22.3 ± 1.8 μg) than at the end of experiments with nocodazole (14.4 ± 1.5 μg, Table 2). The finding that the quantity of labeled dextran in plasma is greater in control than in nocodazole experiments fits with the occurrence of transcytosis for the reason mentioned above for labeled albumin.

**Ventilation.** Pulmonary ventilation, as well as that normalized to body weight, was similar in both kinds of experiments of a given series (Tables 1 and 2).

**DISCUSSION**

The finding that the quantity of labeled albumin or labeled dextran removed from the right pleural liquid is smaller in the experiments with nocodazole than in control ones suggests the occurrence of transcytosis from lumen to interstitium in the pleural mesothelium in vivo, in line with our in vitro evidence on the parietal pericardium (8). Actually, the quantity of labeled macromolecules removed by transcytosis should be somewhat underestimated because of the following reason. The concentration of labeled macromolecules in the right pleural liquid decreases markedly during the experiment because the liquid entering the pleural space during the experiment is essentially free of labeled macromolecules, whereas these leave the space by lymphatic drainage through the stomata of the parietal pleura (2, 5, 19, 21, 23, 25), by convection owing to the Starling forces through the visceral pleura (1–3), by diffusion (7), and by transcytosis (8). In the experiments with nocodazole, the concentration of labeled macromolecules should decrease more slowly than in control experiments because one mechanism removing macromolecules has been blocked. If the concentration of macromolecules in the experiments with nocodazole is a little higher than in control experiments, the quantity of labeled macromolecules leaving the right pleural space with the other mechanisms should be a little greater in the experiments with nocodazole than in control ones.

Because pulmonary ventilation is similar in the two kinds of experiments of a given series, one can rule out the possibility that our results are affected by a different degree of lymphatic drainage from the pleural space caused by a different degree of ventilation. This control has been done because an increase in lymphatic drainage from large hydrothoraxes has been shown to occur when ventilation is increased (10). Moreover, the finding that the quantity of labeled albumin or labeled dextran 70 in plasma is smaller in the experiments with nocodazole than in control ones fits with the occurrence of transcytosis in pleural mesothelium in vivo. One could argue that nocodazole by disrupting the microtubules could modify the shape of the mesothelial (and endothelial) cells, and this, in turn, might reduce the size of the stomata of the parietal pleura, and, hence, reduce the lymphatic drainage through them. On the other hand, the electron micrographs by Hastings et al. (17) show that the shape of the alveolar and endothelial cells 2 h after instillation of nocodazole in rabbit alveoli was not altered. Moreover, the light micrographs by the same authors show that the shape of rabbit alveoli 2 h after instillation of nocodazole was not changed. The alveolar epithelium lacks a strong support like the connective tissue layers of the pleura: hence, after disruption of the microtubules, it should undergo a change in shape more easily than the mesothelium. Therefore, the finding that the shape of the alveoli is not altered by nocodazole makes it unlikely that the disruption of microtubules alters the shape of the mesothelial cells in such a way as to reduce the size of the stomata of the parietal pleura.

The vesicular transport of albumin in the parietal pericardium of rabbits in vitro is only fluid phase, although this transcytosis seems triggered by albumin concentration (8). The situation might be different in vivo, where one cannot rule out the occurrence of receptors for albumin in the vesicular membrane because one cannot perform experiments with very low concentrations of unlabeled albumin, which are required to detect the competition for receptors between labeled and unlabeled albumin. In case the vesicular transport of albumin from the pleural space in vivo is only fluid phase, it may be computed from the vesicular liquid flow (0.05 ml/h, see RESULTS) times the concentration of albumin in the pleural liquid under physiological conditions (~10 mg/ml; Ref. 22) times the lumen-vesicle partition coefficient for albumin (~0.78). This coefficient is given by \((1 - a/r)^3\) (11), where \(a\) is the hydrodynamic radius of the solute (3.55 nm for albumin; Ref. 7) and \(r\) is the radius of the vesicle (~45 nm; Refs. 14, 26). This computation yields a value of 390 μg/h, or 203 μg·h⁻¹·kg⁻²/₃. On the other hand, one may attempt to estimate the vesicular transport of albumin from the pleural space in vivo in a different way that does not require the assumption of a mere fluid-phase transport. This computation is based on the vesicular transport of labeled albumin (see RESULTS) and the ratio between the concentration of unlabeled albumin in the pleural liquid under physiological conditions (~10 mg/ml; Ref. 22) and the mean concentration of labeled albumin in the right pleural liquid during the experiment. To estimate the latter, the time course of the...
concentration of labeled albumin in the right pleural liquid (Calb) during the experiment has to be approximately drawn. To this end, in a separate group of seven rabbits we measured 1 h after injection of the control bolus the same parameters previously measured after 3 h. These data are reported in Table 3. Because of the mechanisms involved, the decay of concentration of labeled albumin should be a curve with upward concavity. The approximate time course of Calb during the experiment was obtained by interpolation through the initial value (see RESULTS), the value after 1 h (Table 3), and the value after 3 h (Table 1). It is shown in Fig. 1 (○), along with the time course of the quantity of labeled albumin in the pleural liquid during the experiment (●). Mean Calb during the experiment was obtained by averaging the readings taken on the corresponding line every 12 min. Its value, 692 μg/ml, is 14.5 times smaller than the concentration of albumin in the pleural liquid under physiological conditions (10 mg/ml). Therefore, the vesicular transport of albumin from the pleural space under physiological conditions should be given by the vesicular transport of labeled albumin during the experiment (101 μg) times 14.5 divided by 3 = ~488 μg/h, or ~254 μg·h⁻¹·kg⁻²/³. This value is 25% greater than that computed from the vesicular liquid flow. This difference should be even greater because the rate of vesicular transport of labeled albumin (as it has been measured) is somewhat underestimated (see above). This difference suggests that the vesicular transport of labeled albumin from the pleural space in vivo is not only fluid phase, but the data on which it is based are not precise enough to afford a conclusion. For the rest of this DISCUSSION, the vesicular transport of albumin in vivo will be taken as the mean between the values obtained by the two procedures, i.e., 439 g/h or 228 μg·h⁻¹·kg⁻²/³.

The surface area of the parietal pleura in our 2.65-kg rabbits should be ~115 cm² (see METHODS). Therefore, if transcytosis occurred only through the parietal pleura, the vesicular transport of albumin from the pleural space would be ~3.8 μg·h⁻¹·cm⁻² and the vesicular liquid flow ~0.4 μl·h⁻¹·cm⁻². Though the morphological features of mesothelial cells seem similar on the parietal and visceral side, except for a greater density of microvilli on the visceral side (27), no direct evidence of transcytosis has been yet provided for the visceral mesothelium. The surface area of the visceral pleura in our rabbits should be ~132 cm² (see METHODS). Therefore, the overall surface area of the parietal and visceral pleura in our rabbits should be ~247 cm². If the rate of transcytosis in the visceral pleura were similar to that in the parietal one, albumin removal from the pleural space by transcytosis would be ~1.8 μg·h⁻¹·cm⁻² and the vesicular liquid flow ~0.2 μl·h⁻¹·cm⁻². In vitro albumin transcytosis through specimens of parietal pericardium of rabbits, with an albumin concentration in the solution similar to that occurring under physiological conditions (10 mg/ml), was 5 × 10⁻⁴ μmol·h⁻¹·cm⁻² or 36 μg·h⁻¹·cm⁻² (8). Therefore, the rate of albumin transcytosis found in the parietal pericardium in vitro is one order of magnitude greater than that found in the pleural space in vivo in the present research. Part of this difference was expected because morphological studies showed a greater concentration of vesicles in the cytoplasm of the pericardial mesothelium (18, 19) than in that of the pleural mesothelium (26, 27). Moreover, the rate of albumin transcytosis measured in the present research should be somewhat underestimated for the reason indicated above. This underestimation, however, should not be such as to fill the gap.

The lymphatic drainage from the pleural space of dogs has been determined by Miniati et al. (20), who made a 0.5-ml intrapleural injection of Ringer solution with ¹³¹I-labeled albumin and a simultaneous intravenous injection of ¹²⁵I-labeled albumin. Plasma activity of both tracers was followed for 24 h: the ¹³¹I-labeled

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### Table 3. Labeled albumin in pleural liquid and in plasma 1 h after injection of 0.3 ml of Ringer-albumin with 750 μg of labeled albumin in right pleural space

<table>
<thead>
<tr>
<th>Body Weight, kg</th>
<th>Pleural Liquid Volume, ml</th>
<th>Pleural Liquid Concentration, μg/ml</th>
<th>Pleural Liquid Quantity, μg</th>
<th>Plasma Volume, ml</th>
<th>Plasma Concentration, μg/ml</th>
<th>Plasma Quantity, μg</th>
<th>Ventilation ml⁻¹·min⁻¹·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>% Injected</td>
<td>Left</td>
</tr>
<tr>
<td>7</td>
<td>2.72</td>
<td>0.75</td>
<td>0.55</td>
<td>741</td>
<td>6.5</td>
<td>532</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>± 0.06</td>
<td>± 0.08</td>
<td>± 0.07</td>
<td>± 61</td>
<td>± 2.7</td>
<td>± 31</td>
<td>± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rabbits *Volume collected at the end of the experiment plus volume adherent to the wall (see METHODS; Ref. 22). †Assumed to be 4% of the body weight (6, 10, 15).

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![Fig. 1. Time course of concentration (○) and quantity (●) of labeled albumin in pleural liquid during the experiment. For further information, see text.](http://jap.physiology.org/)

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albumin curve provided the output function from the pleural space, whereas the $^{125}$I-albumin curve served as input function for the interstitial space (including the serosal cavities). They found a lymphatic drainage from the pleural space of 0.02 ml·h$^{-1}$·kg$^{-1}$. This lymphatic drainage of albumin was considered to occur mostly through the stomata of the parietal pleura. Therefore, taking into account that the concentration of albumin in the pleural liquid of dogs under physiological conditions is 6.1 mg/ml (22), the lymphatic drainage of albumin from the pleural space of their 17.5-kg dogs should be $\sim$122 $\mu$g·h$^{-1}$·kg$^{-1}$, i.e., $\sim$314 $\mu$g·h$^{-1}$·kg$^{-2/3}$. On the other hand, the lymphatic drainage from the pleural space does not necessarily occur mostly through the stomata, because albumin leaving the pleural space outside the stomata is also eventually drained into blood by the lymphatics of the connective tissue of the pleura (3). Miniati et al. pointed out that the initial upward concavity of the time course of plasma recovery of labeled albumin injected into the pleural space (Fig. 9 of their article) is due to a large liquid volume for albumin distribution interposed between pleura and plasma. Because they believed that most of albumin removal from the pleural space occurs through the stomata of the parietal pleura, they concluded that the lymphatic network was adequate to explain the delayed appearance of labeled albumin in plasma. The present finding of albumin transcytosis by the pleural mesothelium suggests that the lymphatics contribute only part of the initial upward concavity of the above-mentioned curve, the rest being due to the interstitial liquid of the pleural connective tissue, where albumin removed by transcytosis (parietal and, perhaps, visceral pleura) and by convection (visceral pleura) is distributed before being drained into blood by lymphatics. The possibility that a substantial part of albumin leaves the pleural space outside the stomata was considered by Broaddus et al. (9) after they found in sheep that the removal rate of hydrothorax computed from the clearance of labeled erythrocytes (which may leave the space only through the stomata) was 89% of that computed from the clearance of labeled albumin. Their hydrothoraxes, however, were very large (10 ml/kg body wt), and under this condition the lymphatic drainage from the pleural space may increase more than 10 times (9). Convection too increases (because of the increase in pleural liquid pressure), but to a smaller extent. No information is available on transcytosis, but its increase should be smaller than that of the lymphatic drainage. Consequently, the above difference of 11% between the overall (direct plus indirect) lymphatic drainage from the pleural space and that through the stomata of the parietal pleura (direct) should be smaller than that occurring under physiological conditions.

Unfortunately, no data are available on the lymphatic drainage of albumin from the pleural space of rabbits. It has been stated that the turnover rate of pleural liquid per unit pleural surface area is $\sim$3 times greater in rabbits than in dogs (23). This statement, however, is based on a comparison between an estimate of liquid filtration through the parietal pleura of rabbits (24) and the lymphatic drainage of liquid from the pleural space of dogs, computed from albumin clearance from this space (20). This comparison, in turn, implies the assumption that the lymphatic drainage of liquid from the pleural space represents most of the liquid outflow from the pleural space, and that it occurs through the stomata of the parietal pleura (23). This is not the case because of transcytosis and because of the liquid outflow caused by Starling forces through the visceral pleura and that coupled to the active absorption of NaCl, which are not negligible under physiological conditions (5). Hence the turnover rate of pleural liquid per unit pleural surface area does not seem to be so much greater in rabbits than in dogs. Therefore, our results suggest that under physiological conditions the vesicular transport of liquid and albumin from the pleural space might contribute a substantial part of the overall removal of liquid and albumin from this space.

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