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RENAL LYMPHATIC FUNCTION FOLLOWING VENOUS PRESSURE ELEVATION

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ABSTRACT

The renal lymphatic system plays an important role in removing excess fluid from the kidneys. Unfortunately, the factors influencing lymphatic flow are difficult to measure. We used a simple model to represent renal lymphatics as a single pressure source (P_L) pushing lymph through a single resistance (R_L). In anesthetized dogs, we cannulated renal lymphatics and measured lymph flow rate (Q_L) as we varied pressure (P_O) at the outflow end of the lymphatics. There was no significant change in Q_L as we increased P_O from -5 to 0 cm H_2O. In other words, there was a plateau in the Q_L vs. P_O relationship. At higher P_O's, Q_L decreased linearly with increases in P_O. From this linear relationship, we calculated R_L as $-\Delta P_O/\Delta Q_L$ and we took P_L as the P_O at which Q_L = 0 µl/min. At baseline, R_L = 0.34 ± 0.14 (SD) cm H_2O•min/µl and P_L = 8.2 ± 4.4 cm H_2O. When we increased renal venous pressure (P_V) from baseline (3.5 ± 3.0 cm H_2O), the plateau in the Q_L vs. P_O relationship extended to higher P_O's, R_L decreased, and P_L increased. Renal interstitial fluid volume and interstitial pressure increased following elevation of P_V. The extension of the Q_L vs. P_O plateau with increasing P_V suggests that renal interstitial pressure may partially collapse intrarenal collecting lymphatics which may compromise lymph flow.

One function of the renal lymphatic vessels is to remove fluid and extravasated plasma protein from the renal interstitium (1-4). Increases in renal lymph flow may be important in maintaining renal function in disease (1-4). Despite the importance of the renal lymphatics, there have been few studies of the physiologic determinants of renal lymph flow. This may be due to the difficulty in measuring the basic factors that affect lymphatic flow. These factors include renal interstitial fluid pressure, the pressure in the initial lymphatics, and the intrinsic contractility of the lymph vessels within the kidney (5-7). However, a model can be used to analyze lymphatic function that does not depend on the direct measurement of any of these factors (8). With this model, the lymphatics of a tissue are represented by an electrical circuit analog consisting of a single pressure source pushing lymph through a single resistance. The values for pressure and resistance are determined from the lymph flow vs. outflow pressure relationship of cannulated lymphatic vessels.

In previous studies, we have used this circuit model to analyze lymphatics from many tissues including lung, liver, and intestine (9-11). In this study, we used the model to investigate renal lymphatics in anesthetized dogs. As in other organs, our results show that increases in renal lymph flow, following augmentation of renal venous

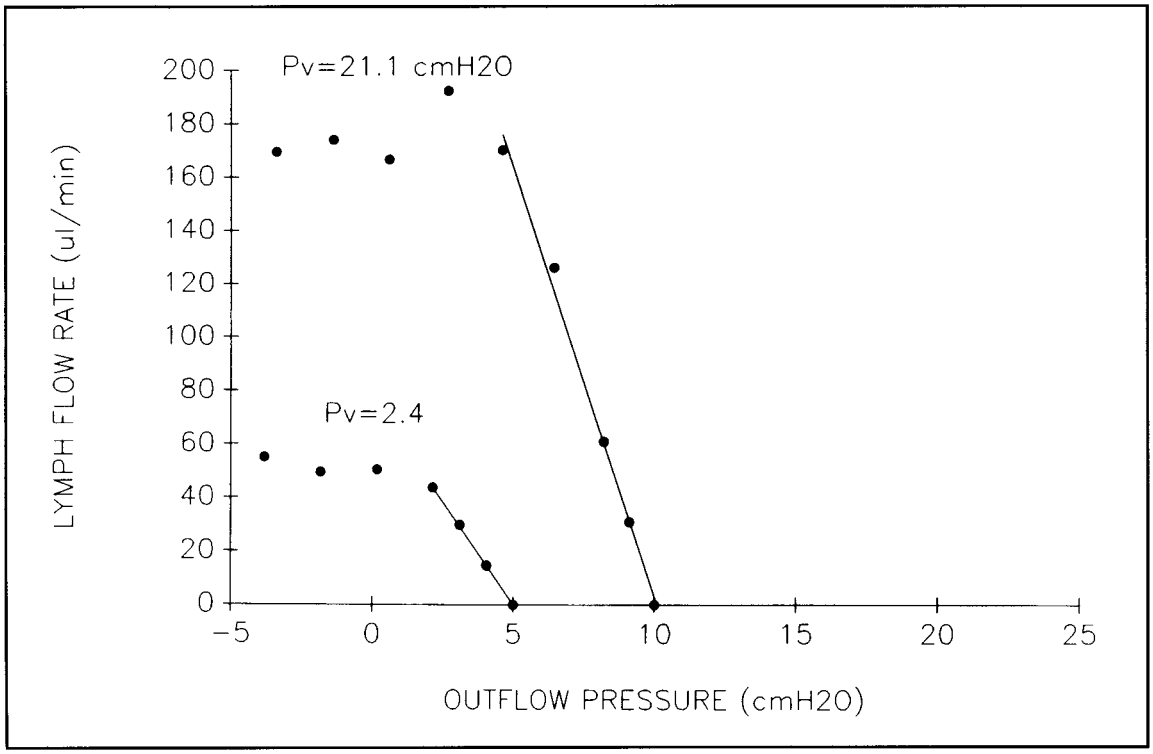

Fig. 1A. Measured lymph flow rate from a cannulated renal lymphatic plotted as a function of lymphatic outflow pressure for a single dog. P_V = renal venous pressure.

pressure, are attributable to decreases in the effective resistance of the renal lymphatics and increases in lymph driving pressure. Furthermore, increases in pressure at the outflow end of renal lymphatics may significantly decrease renal lymph flow.

MATERIALS AND METHODS

All procedures were approved by the University of Texas Animal Welfare Committee and were consistent with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals." Eleven mixed-breed dogs weighing between 20 and 30 kg were anesthetized with thiopental sodium (25 mg/kg) and intubated. Anesthesia was maintained with 1-2% halothane in room air administered using a Harvard ventilator set at a minute volume necessary to produce $PaCO_2$ of 40 mmHg.

The left femoral artery and left femoral vein were cannulated with fluid-filled catheters directed into the aorta and the inferior vena cava, respectively. Aortic and inferior vena caval pressures were measured with Statham P23ID transducers and recorded on a Grass polygraph. The right femoral vein was cannulated with a Fogarty balloon-tipped catheter. This was advanced into the inferior vena cava with the balloon positioned between the hepatic and renal veins. The position of the balloon was confirmed by palpation after the abdomen had been opened.

In five dogs, a left-sided paracostal incision was made and the left kidney was exposed. The prenodal collecting lymph vessel at the hilus of the kidney was identified and cannulated with a 22-gauge catheter (Medicut, Sherwood Medical Industries, St. Louis, MO) in the upstream direction. The

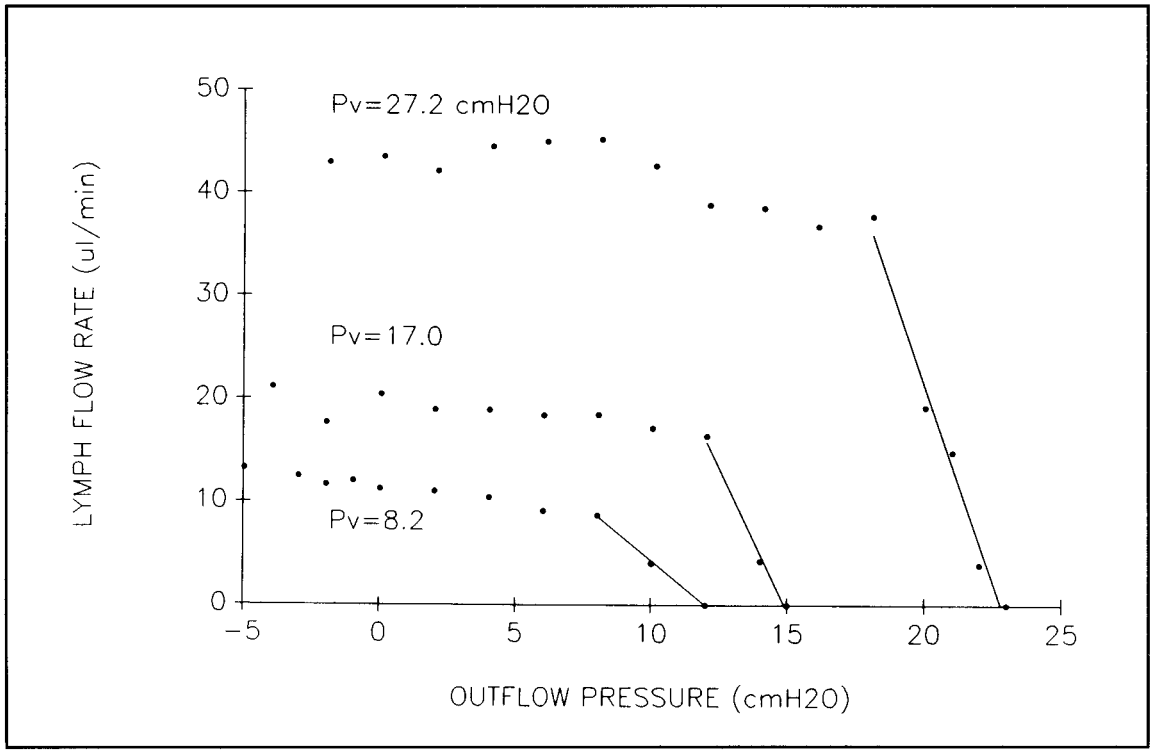

Fig. 1B. Measured lymph flow rate from a cannulated renal lymphatic plotted as a function of lymphatic outflow pressure at different renal venous pressures (P_V) for a single dog. Note the extension of the plateau with increasing P_V.

catheter was fixed in position with a ligature and cyanoacrylate. Following catheterization, dogs were given heparin intravenously at a dose of 100 U/kg body weight. Saline-filled tubing (interior diameter = 1.2 mm) was connected to the catheter. The outflow end of the tubing was connected to a pipette inserted into a clamp attached to a vertical calibrated pole. By raising and lowering the pipette with reference to the site of lymphatic cannulation, we could alter the outflow pressure to the lymph vessel (P_O). The flow rate from the cannulated lymphatic (Q_L) was measured by timing the flow of lymph past calibrated marks on the pipette. We first measured Q_L with the pipette below the site of cannulation. We then raised the height of the pipette 1-2 cm and remeasured Q_L. This procedure was repeated until the pipette was high enough to stop lymph flow (Q_L = 0 µl/min). At each pipette height, the pressure at the site of lymphatic cannulation was calculated as:

P_O = pipette height + Q_L x cannula resistance (1).

Cannula resistance was determined as previously described (11). Q_L was plotted as a function of P_O and a regression line was determined. The effective lymphatic resistance (R_L) was calculated from the regression line as $-\Delta P_O/\Delta Q_L$. The effective driving pressure (P_L) producing lymph flow was taken as the P_O at which Q_L = 0 µl/min. This technique for computing R_L and P_L has been used extensively with lymph vessels in other tissues and has been described in detail by Drake et al (8).

In each experiment the Q_L vs. P_O relationship, obtained by changing pipette height, was first measured at baseline vena caval pressure. The Fogarty balloon was then inflated to raise the pressure within the inferior vena cava and therefore, the renal

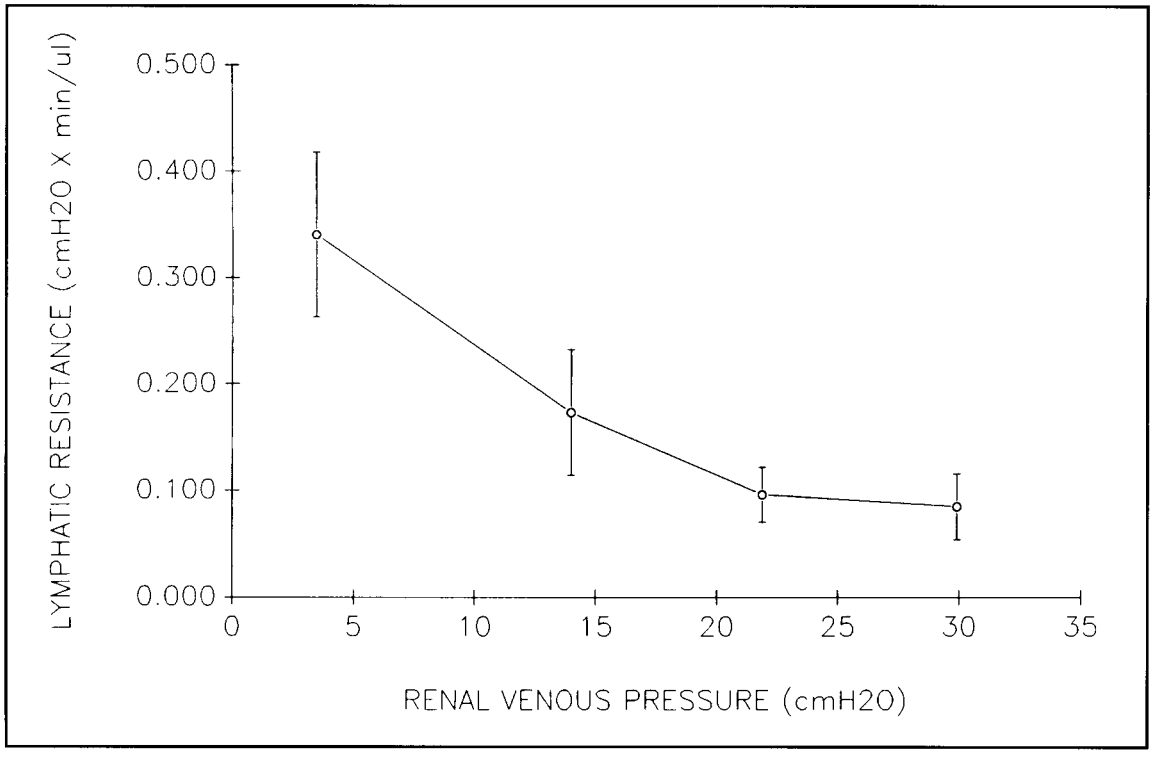

Fig. 2. Renal lymphatic resistance plotted as a function of renal venous pressure. Values are mean ± SE.

veins. Because the renal veins drain directly into the inferior vena cava, we recorded the inferior vena caval pressure as renal venous pressure (P_V). Elevated P_V was maintained with a constant pressure device (12). After a new steady-state Q_L had occurred (approximately 30-45 minutes), the Q_L vs. P_O relationship was redetermined. This process was repeated until we had recorded the Q_L vs. P_O relationship for several P_V's. P_L and R_L were calculated at each P_V.

In two additional groups of dogs, each containing three animals prepared with a midline laparotomy, two new parameters were measured. In the first group, the cisterna chyli was exposed and cannulated with a 22-gauge catheter. The catheter was connected to fluid-filled tubing and a pressure transducer (Statham P23ID), and the pressure in the cisterna chyli was recorded. In the second group, an index of interstitial pressure within the kidney (P_{int}) was obtained by injecting Ringer's solution (0.1 ml) into a subcapsular pocket. A 22-gauge needle, connected with saline-filled tubing to a Statham P23ID pressure transducer, was inserted into the subcapsular pocket. Following an initial spike, P_{int} fell to a steady value in approximately ten minutes. Pressure within the pocket was then recorded as P_V was elevated. In these animals, the volume of interstitial edema fluid was determined using a unitless blood-free wet-to-dry weight ratio technique previously described (13). A right nephrectomy was performed at the beginning of the experiment and each animal was used as its own control for quantitation of renal edema. The left kidney was removed at the conclusion of the P_V elevation protocol for interstitial volume determination.

All data are expressed as mean ± SD in

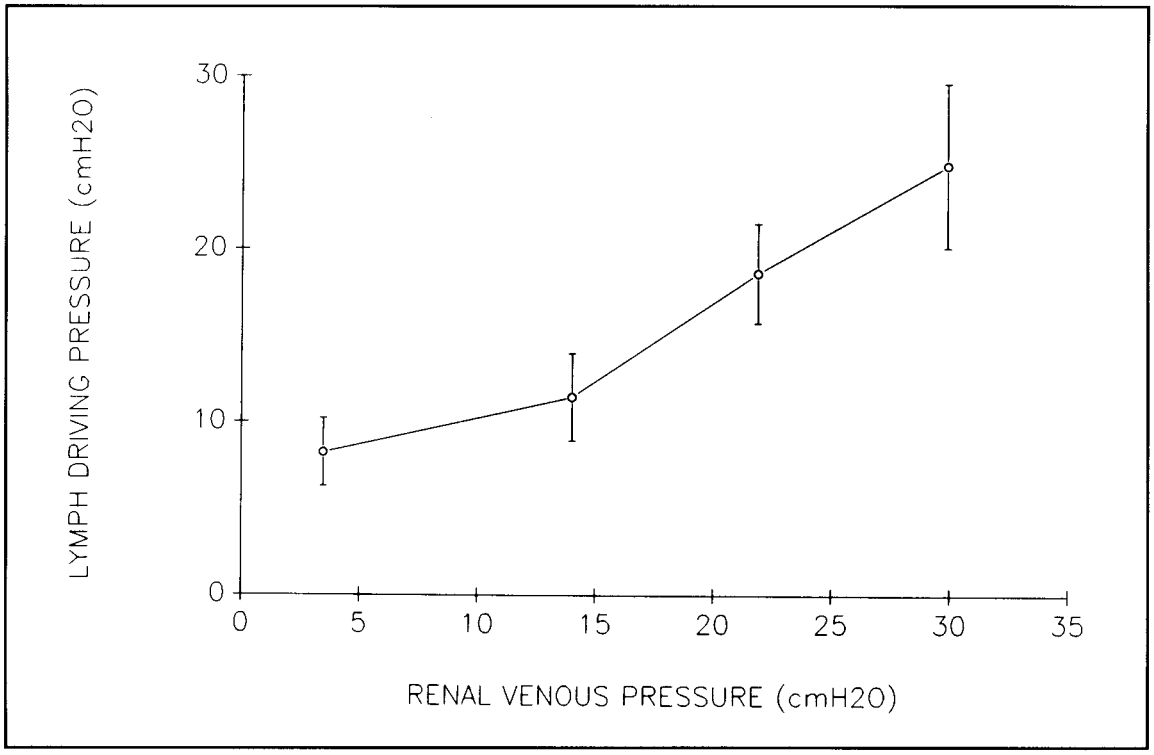

Fig. 3. Renal lymphatic driving pressure plotted as a function of renal venous pressure. Values are mean ± SE.

the text and mean ± SE in the figures. We used the method of least squares to determine regression lines (14). Two-way analysis of variance was used to test statistical significance between animals and between different P_V's (15). The wet-to-dry weight ratios were compared with a Student's *t*-test. A p value <0.05 was considered significant.

RESULTS

Data from a total of 11 dogs are presented. At baseline P_V (P_V = 3.5 ± 3.0 cm H_2O), we found little change in Q_L as we increased P_O from -5 to 0 cm H_2O *(Fig. 1A)*. In three dogs, this plateau in the Q_L vs. P_O relationship extended to higher P_O's as we increased P_V *(Fig. 1B)*. In two other dogs, the plateau did not appear to shift substantially with augmented P_V *(Fig. 1A)*.

Q_L decreased linearly with increases in P_O above the plateau region. At baseline P_V, we calculated R_L = 0.34 ± 0.14 cm H_2O•min/µl and P_L = 8.2 ± 4.4 cm H_2O from the linear Q_L vs. P_O relationships. When we increased P_V, the Q_L vs. P_O relationship became steeper and shifted to the right *(Figs. 1A and 1B)*. Accordingly, the calculated R_L's decreased *(Fig. 2)* and the P_L's increased *(Fig. 3)*. These changes in R_L and P_L with elevations in P_V were significant, but we found no significant between-animal differences in the data. As found in other studies (9,16), the absolute value for Q_L varied considerably between animals.

Renal P_{int} varied directly with changes in P_V. The blood-free wet-to-dry weight ratio increased significantly from 4.20 ± 0.26 at baseline to 4.90 ± 0.20 following two hours of P_V elevation. We could not detect a change in P_{int} associated with this edema formation as determined by the P_{int} vs. time relationship. A

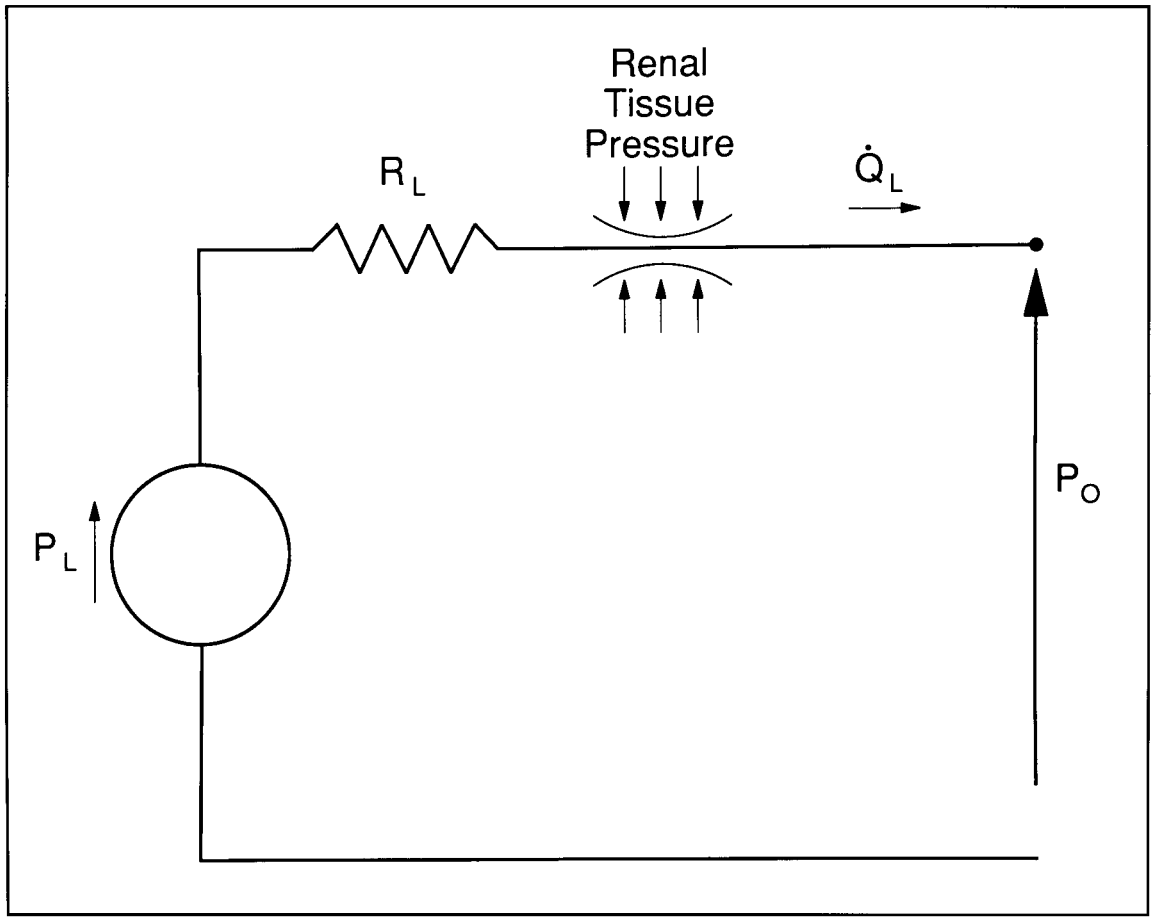

Fig. 4. Equivalent circuit diagram of a cannulated renal lymph vessel. P_L = effective lymph driving pressure; R_L = effective resistance of lymphatic vessels; Q_L = lymph flow rate; P_O = pressure at the site of lymph cannulation (calculated as $P_O = Q_L$ x cannula resistance + height of the pipette above the cannulation site).

line with a constant slope was found indicating that increases in P_{int} were directly attributable to venous engorgement secondary to increased P_V. Control cisterna chyli pressure was 3.2 ± 1.5 cm H_2O.

DISCUSSION

Our results show that, as in other tissues, increases in P_O to renal lymphatics cause Q_L to decrease. However for P_O's lower than a critical level, there was a plateau in the Q_L vs. P_O relationship. Although this plateau occurs in the Q_L vs. P_O relationships of other organs (9,16), it was more prominent in the renal lymphatics of this study *(Figs. 1A and 1B)*. Furthermore in some experiments, the P_O at which Q_L began to decrease, increased when we elevated P_V. In other words, the plateau extended farther to the right *(Fig. 1B)* with augmented P_V. We have not found this phenomenon in lymphatics from any other organs.

We have previously proposed that the plateau of the Q_L vs. P_O relationship is attributable to the "Starling resistor" phenomenon (9). That is, if the pressure within a flaccid lymphatic vessel is less than the

external pressure, the vessel will collapse. Q_L through collapsed lymphatics will not vary with changes in P_O if this pressure is less than the external pressure acting on the lymphatics. For instance in our previous lung experiments (9), that part of the lymphatic vasculature outside the lung parenchyma was exposed to an external pressure equal to atmospheric pressure, and the plateau was evident for $P_O < 0$ cm H_2O. In the experiments of this study, the plateau often extended well above $P_O = 0$ cm H_2O. We believe this results from the relatively high renal tissue pressure surrounding the intrarenal portion of the lymphatics. This tissue pressure collapses the intrarenal portion of the collecting lymphatics so that, for P_O's less than P_{int}, there should be little change in Q_L with alterations in P_O. This is in contrast to the effects of increasing P_{int} on the initial intrarenal lymphatics which are tethered via anchoring filaments to the interstitium. The equivalent circuit model for the kidney is illustrated in *Fig. 4*. At $P_O < P_{int}$, the intrarenal portions of the collecting lymphatics would be collapsed and $Q_L = (P_L - P_{int})/R_L$. However when $P_O > P_{int}$, $Q_L = (P_L - P_O)/R_L$. When we increased P_V in this study, we observed that the kidney became more tense, P_{int} increased, and renal edema developed. Consequently, the intrarenal collecting lymphatics may have remained collapsed at higher P_O's. This could explain the extension of the plateau with increasing P_V's shown in *Fig. 1B*.

The equivalent circuit model, when applied to the kidney, predicts that R_L represents the effective hydraulic resistance and P_L approximates the driving pressure of the renal lymphatics (9,16). The decrease in R_L *(Fig. 2)* and the increase in P_L *(Fig. 3)* associated with increases in P_V are consistent with the R_L and P_L changes in lymph vessels of other tissues (9-11). Because anesthesia inhibits lymphatic contractions, we believe that the increase in P_L may be attributable to an increase in interstitial fluid pressure at the entrance to the lymphatic vessels (16). The decrease in R_L may be associated with lymphatic distention or recruitment of previously collapsed lymphatic vessels (9).

We do not believe that lymph shunting contributed to variations in flow from the cannulated lymphatic. Previously, we showed that lymphatic to lymphatic shunting may not significantly contribute to removal of interstitial fluid (17). Furthermore, when we isolated the lymphatic to be cannulated at the renal hilus, a single collecting prenodal lymphatic vessel resulting from the anastomosis of several smaller lymphatic vessels was frequently found. We selectively cannulated this larger collecting lymphatic. Although the kidney has two lymphatic drainages, hilar and capsular (18), we contend that the measured Q_L accurately represents the lymph flow arising from the renal hilus.

As shown in *Figs. 1A* and *1B*, Q_L from the cannulated lymphatic vessels varied with P_O. Flow from a cannulated lymphatic vessel should equal flow in uncannulated vessels only when P_O equals the pressure normally opposing lymphatic flow. Renal lymphatics drain into the cisterna chyli. Thus, P_O to uncannulated renal lymphatics should approximately equal the pressure within the cisterna chyli. For each experiment, we estimated uncannulated renal lymph flow from the Q_L vs. P_O relationships with P_O = cisterna chyli pressure that we measured (3.2 ± 1.5 cm H_2O). The resulting Q_L's are plotted vs. P_V in Fig. 5. It illustrates our estimate of the relationship between Q_L in uncannulated renal lymph vessels and P_V.*

*In previous studies, we used calculated R_L's and P_L's to estimate Q_L in uncannulated lymphatic vessels from various tissues (9-11). However, in some experiments of the present study, the Q_L vs. P_O plateau region included P_O equal to cisterna chyli pressure. Because R_L and P_L were calculated from Q_L vs. P_O data at P_O's above the plateau, we could not use P_L and R_L to estimate Q_L that would have occurred in lymphatics with P_O = cisterna chyli pressure.

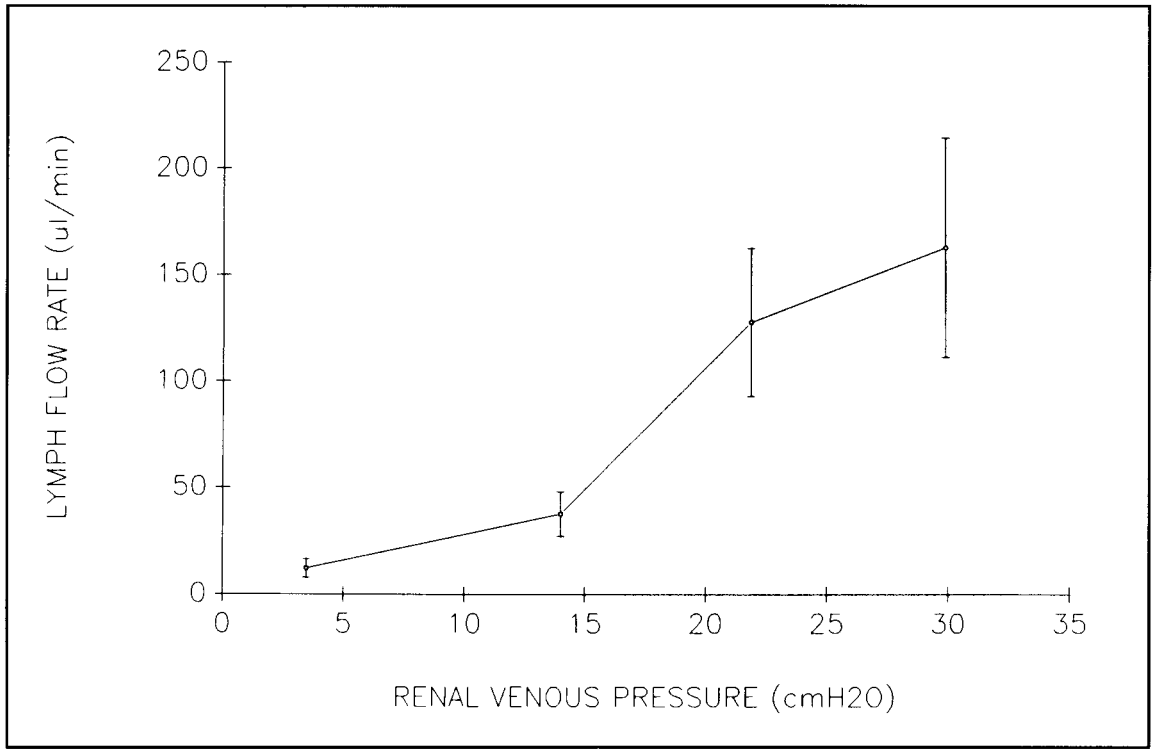

Fig. 5. Estimated lymph flow rate in uncannulated renal lymphatics (P_O = cisterna chyli pressure) as a function of renal venous pressure.

We conclude that for renal lymphatics, as in lymphatics from other tissues, Q_L varies inversely with P_O. Increases in renal Q_L resulting from P_V elevation are attributable to increases in P_L driving lymph from the kidneys and decreases in R_L of the renal lymphatic vessels. In contrast to previous studies, there was an extension of the Q_L vs. P_O plateau associated with high renal tissue pressure. Extension of this plateau suggests that compensatory increases in Q_L may be compromised by increases in renal interstitial pressure because the intrarenal portions of the collecting lymphatics may partially collapse.

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