Physiologic Sufficiency of Regenerated Lung Lymphatics

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The physiologic sufficiency of regenerated lung lymphatics after surgical transection of the lung hilum was studied experimentally. Dogs were prepared by surgical interruption of all left lung hilar tissues and structures except the skeletonized pulmonary artery and the pulmonary veins; continuity of the bronchus was restored by anastomosis. Anatomic reconnection of lung lymphatics to mediastinal lymph vessels was determined by injecting a sky blue dye marker into peribronchial tissues distal to the bronchial anastomosis at different intervals after surgical preparation. From a series of 50 experimental animals it was demonstrated that the surgical procedure interrupted lymphatic drainage and that anatomic reconnection with mediastinal lymphatics developed 7–28 days after preparation. Physiologic sufficiency of regenerated lymphatics was studied in 60 animals by rapid intravascular volume expansion as a test of lymph flow capacity. By gravimetric determination of lung water and histologic examination of lung specimens this study revealed a significant incapacity to maintain lung water homeostasis 3 days after preparation with return toward normal lymph flow capacity 35 days after preparation. This study indicates that lung lymphatic drainage is re-established 7–28 days after surgical interruption and becomes relatively sufficient after 35 days.

EXTRAVASCULAR LUNG WATER accumulation, hypoventilation and increased pulmonary vascular resistance have all been observed early after experimental lung reimplantation.[4,8,17,21] These findings are most prominent three to five days after reimplantation and spontaneously resolve over a period of several weeks thereafter. Veith and Koerner[19] characterized the morphologic, roentgenographic and functional changes as the lung "reimplantation response" resulting from surgical trauma, ischemia, denervation and lymphatic interruption. Resolution of this self limited, functional disturbance appears to coincide with regeneration of the interrupted lung lymphatic vessels and reconnection with the mediastinal lymphatics.[6] Our experiments were conducted to more clearly define the contribution of lung lymphatic interruption to the functional reimplantation disturbance observed by limiting the alteration of lung hilar tissues to the nonvascular structures. In addition we investigated the physiologic sufficiency of the regenerated lung lymphatics.

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Materials and Methods

Experimental Animal Preparation

Mongrel dogs of both sexes weighing from 9 to 24 kg were used. Sodium nembutal, 30 mg/kilogram of body weight, was administered intravenously supplemented as necessary to provide anesthesia. Room air ventilation was provided through a cuffed endotracheal tube connected to a Harvard respirator adjusted to an appropriate rate and tidal volume. The left thorax was entered through a strategically placed lateral intercostal incision using aseptic precautions. All tissues at the exposed left lung hilum were surgically divided except the skeletonized pulmonary artery and pulmonary veins. This included the left bronchus, pulmonary ligament and posterior parietal pericardium behind the left atrium. Continuity of the bronchus was restored by suture anastomosis invaginating the proximal stump into the distal bronchus as described by Veith and Richards.[20] Chest wall integrity was restored anatomically and definitive studies were done at selected intervals thereafter.

Anatomic Studies

Lung lymphatic regeneration and reconnection to mediastinal lymph vessels were studied in 50 experimental animals. They were divided into study groups as follows: controls, surgically unprepared (10 dogs), surgically prepared animals studied immediately (five dogs), 7, 14, 21 (ten dogs each) and 28 (five dogs) days after preparation. For the definitive study a 2 ml solution containing sky

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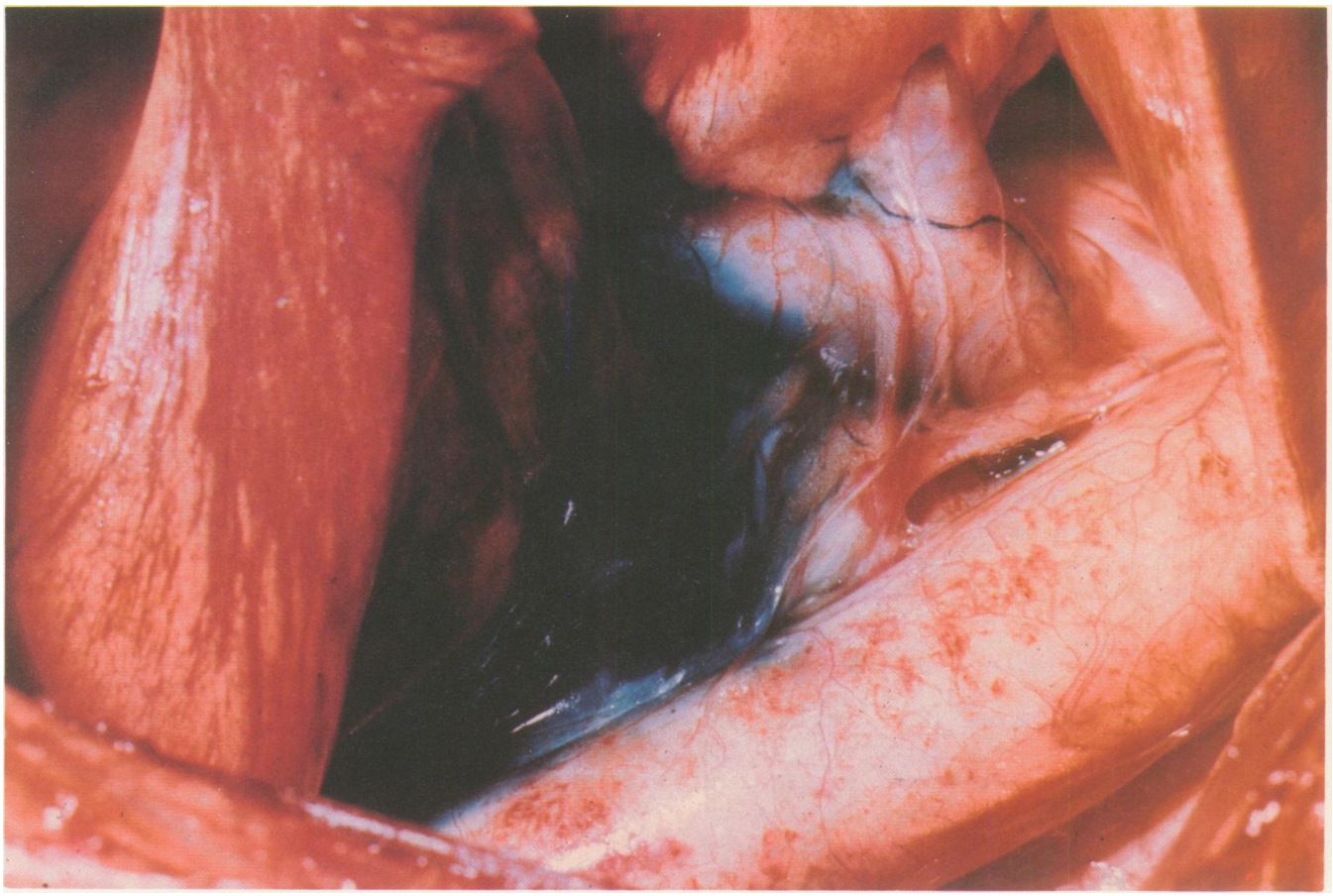

FIG. 1. Intraoperative photograph of a control animal after peribronchial injection of the sky blue dye solution. A dye filled lymph vessel is indicated coursing over the pulmonary artery into the mediastinum.

blue dye* was injected into the peribronchial tissues distal to the bronchial anastomosis. The animal was observed for three hours and visual evidence of blue dye filling the proximal mediastinal lymph vessels was recorded (Fig. 1). The animals were killed and the mediastinal lymph nodes were recovered, sectioned and the presence or absence of dye staining was noted. Also a representative lung sample was secured for gravimetric determination of lung water.

Functional Studies

Physiologic sufficiency of the regenerating lung lymphatics was investigated in 65 animals. They were divided into 13 groups of five subjects each and definitively studied as controls—noninfused, controls—dextran infused and experimental 3, 5, 7, 9, 11, 13, 15, 21, 28, 35 and 56 days postpreparation—dextran infused. Anesthesia was induced as before. An endotracheal tube was placed to secure the airway but the experimental animal breathed spontaneously without ventilatory assistance during the procedure. The right jugular vein was cannulated with a 16 gauge, 20 cm polyethelene catheter advanced to the superior vena cava. The right femoral vein was cannulated for rate controlled infusion using a calibrated pump. The adjacent right femoral artery was cannulated for terminal exsanguination. Low molecular weight dextran† was infused at a rate of 4 ml/kg of dog per minute until the central venous pressure rose 25 cm of water pressure above the initially recorded baseline. The rate of infusion was then adjusted to sustain the central venous pressure within ± 2 cm of the established elevated level for an additional ten minutes. The volume of dextran infused varied for individual experimental animals and ranged from 27 to 114 ml/kg. Animals were killed by exsanguination through

* Fifteen milligrams "pontamine" direct sky blue dye, Dupont, Wilmington, Delaware per ml 0.9% sodium chloride mixed three parts by volume with one part by volume hyalouronidase 150 units/ml.

† Ten per cent GENTRAN® 40 (Dextran 40) and 0.9% sodium chloride, Travenol Laboratories, Deerfield, Illinois.

TABLE 1. *Anatomic Study of Lung Lymphatic Regeneration*

Time Post-preparation (days)	No. of Animals	Sky Blue Dye Migration		Lung Water Per Cent	
		Yes	No	Right	Left
Control*	10	10	0	79.4	78.8
Immediate†	5	0	5	78.8	78.6
7	10	3	7	78.9	80.7
14	10	8	2	78.3	78.8
21	10	8	2	78.5	78.9
28	5	5	0	78.4	78.8

* Control—not surgically altered.
† Immediate—surgically prepared control.

the femoral artery cannula and samples of shed blood were selectively taken to determine water content. Both lungs were immediately removed, gravity drained and weighed. Samples were taken for preparation of tissue sections and for gravimetric determination of total lung water content.

Lung and Blood Water Determination

The gravity drained lungs were weighed immediately and samples were placed in a drying oven set at 110 C. Total lung water was calculated from the difference in wet and dry weights and an index of experimental change was expressed by the ratio of the right (unaltered) lung water to the left (surgically altered) lung water. Similarly the aliquots of arterial blood were weighed, oven dried to a constant dry weight and the per cent water in the whole blood was calculated.

Histologic Interpretation

Sections were taken from both the right and left lungs and were stained with Hematoxylin and Eosin for examination by light microscopy. The specimens were histologically searched for the presence of perivascular water cuffing, perivascular and peribronchial lymphatic vessels, pleuritis and pleural lymphatic vessels. Perivascular water cuffing and pleuritis were subjectively graded from 0 to +4. A microscopic finding in the left lung was considered positive only if the presence or degree of change exceeded that of the right control lung. Accumulation of perivascular water in the absence of a distinct, identifiable, intact lymphatic vessel was not interpreted as definitive evidence of the presence of dilated lymphatics for the purposes of this study.

Results

Sky blue dye migrated from the site of peribronchial deposition to the mediastinal lymphatics in all of the control animals studied (Table 1). Dye did not migrate in any animal studied immediately after surgical preparation. Dye migration through regenerated lymphatics to mediastinal vessels was observed in three of ten animals day seven, eight of ten animals days 14 and 21 and five of five animals studied 28 days after surgical preparation. Left lung water content was not altered in the group of animals studied immediately after surgical preparation which supports the observation that the procedure did not result in interstitial water accumulation independent of lymphatic obstruction evident seven days later.

The physiologic or functional sufficiency of the regenerated reconnected lung lymphatics was studied in the second series of experiments using an intravascular fluid challenge to discriminate between the right (unaltered) and the left (surgically altered) lungs. The average lung water content of non-infused exsanguinated control animals was 77.7% as compared with an average whole blood water content of 81.6%. Dextran infusion increased the average whole blood content to 86.8%, however, lung water

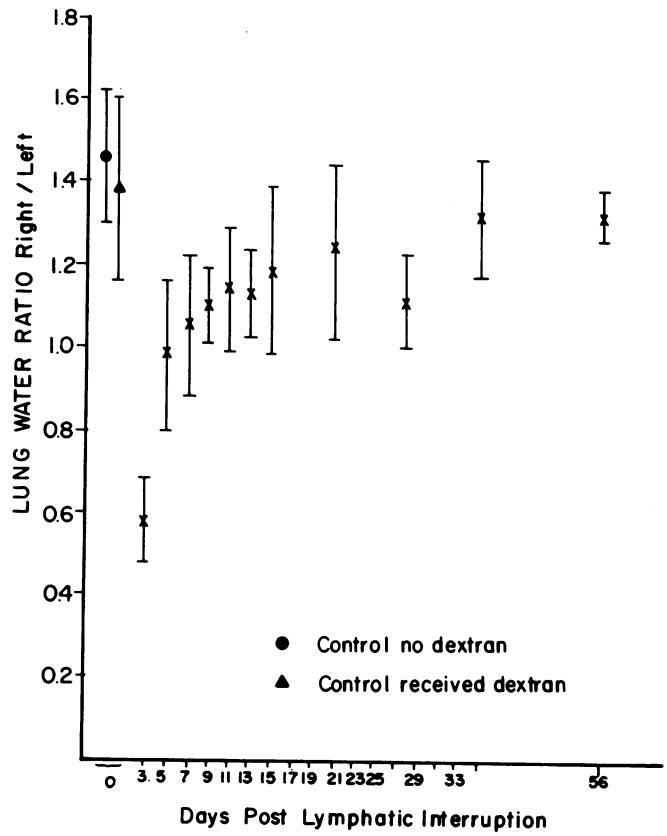

FIG. 2. The ratio of right to left lung water content over time postsurgical preparation of dextran infused experimental animals reveals a significant ($p < 0.001$) increase in left lung water at three days compared with controls. Prompt early improvement in lung water clearance is noted; however return to normal lymph flow capacity is delayed beyond 28 days (control vs. 56 days no significant difference, 3 days vs. 56 days ($p < 0.001$).

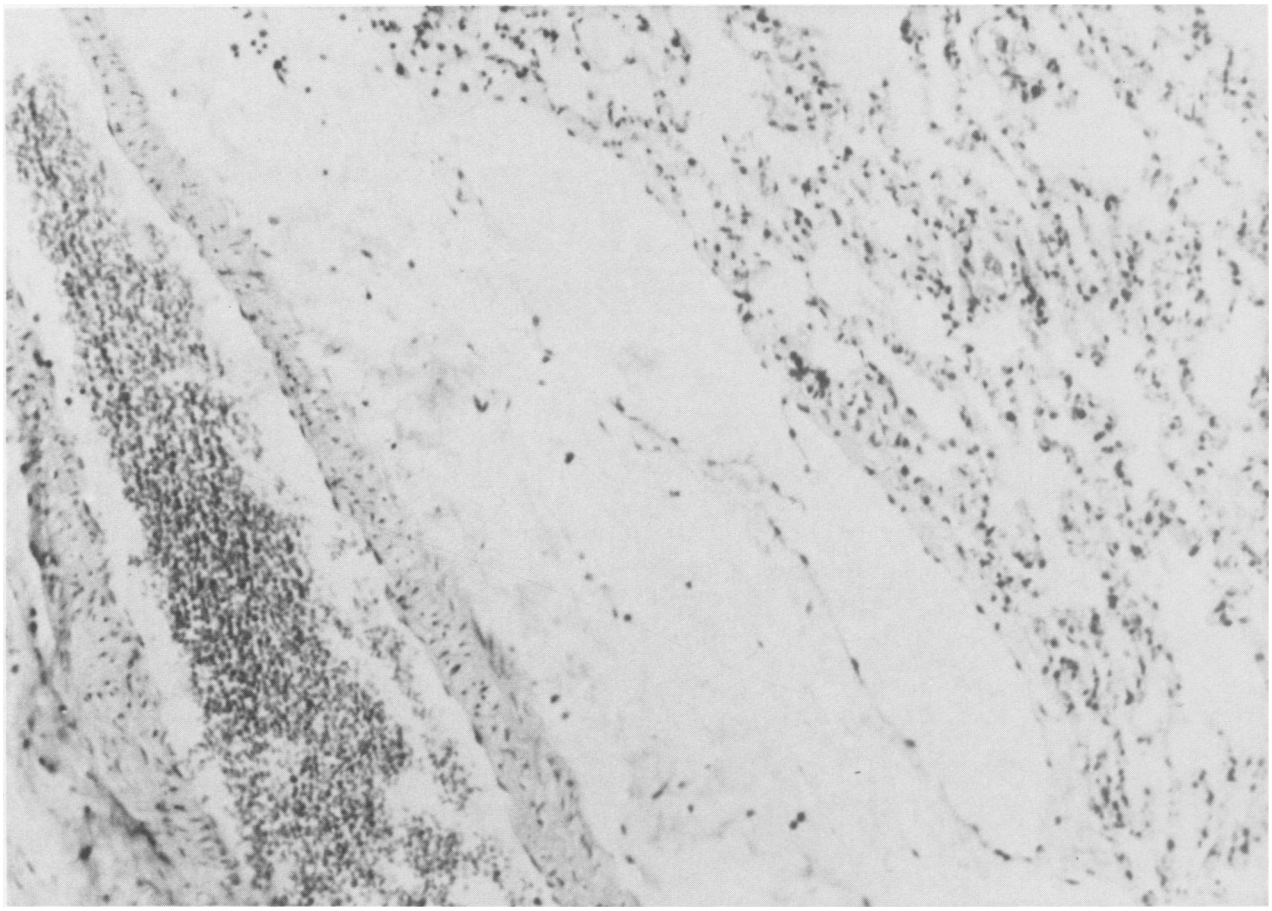

FIG. 3. Photomicrograph (×125 magnification) of a left lung recovered three days after surgical preparation of a dextran infused experimental animal. Moderately extensive perivascular fluid cuffing is apparent along with a distended lymph vessel complete with an intact valve.

content was only 81.0% in the group of exsanguinated, infused controls. In addition the average right lung water content for all dextran infused, surgically prepared experimental animals (55 dogs) was 79.6%. These data support the probability that the observed differences in interstitial lung water accumulation between the right and left lungs of surgically prepared, dextran infused, exsanguinated animals was independent of acutely increased blood water content. Maximal left lung water accumulation occurred in the group of animals studied three days after surgical preparation (Fig. 2). Left lung water clearance improved promptly but did not approach a near normal capacity until 35 days postpreparation. Microscopic examination of the sections prepared from lung specimens recovered from this series of experimental animals revealed the presence of perivascular fluid cuffing and perivascular lymphatics early after surgical preparation (Fig. 3). These changes resolved partially by 13 days. In contrast pleuritis and pleural lymphatic dilation became prominent after three days and persisted with residual findings in the surgically altered left lung as late as 56 days postpreparation (Fig. 4 and Table 2).

Discussion

The lungs are supplied with a rich network of lymphatics that are distributed in the visceral pleura and around the pulmonary blood vessels and

TABLE 2. *Positive Microscopic Findings, Left Lung, Series 2 Animals*

Time Post-preparation (days)	Perivascular Cuffing	Parenchymal Lymphatics	Pleuritis	Pleural Lymphatics
3	5	5	0	1
5	5	3	5	4
7	4	3	5	5
9	3	1	3	3
11	3	3	5	5
13	1	0	2	4
15	1	1	5	5
21	1	0	5	5
28	2	2	4	5
35	1	1	5	4
56	2	1	3	4

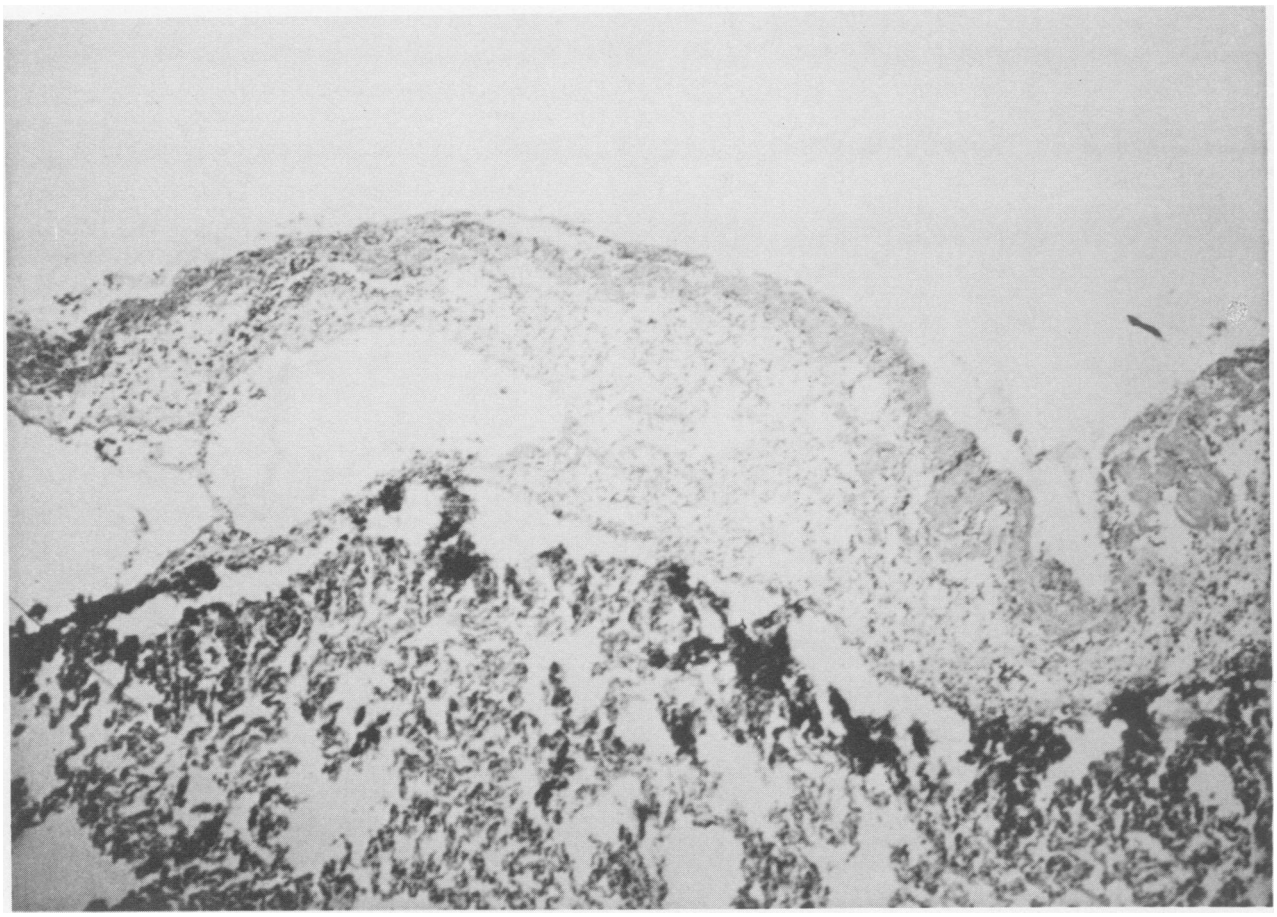

FIG. 4. Photomicrograph (×50 magnification) of a left lung recovered seven days after surgical preparation of a dextran infused experimental animal. The visceral pleura is thickened, edematous and infiltrated with inflammatory cells. A distended pleural lymphatic is shown.

bronchi.[11,15] Lymph flow is directed centrally toward the hilum by strategically placed valves.[18] The lymph vessels coalesce into larger collecting lymphatics at the hilum which empty into the mediastinal drainage system. Lung reimplantation, accomplished by complete excision of the hilum, obstructs lung lymph flow.[4,6,21] However, with the temporary interruption of pulmonary blood flow there is a potential for ischemic injury and for imperfect vascular reconstruction which may contribute to the lung "reimplantation response." Lung ischemia and vascular anastomoses were avoided in our experimental animals as well as those prepared for dye migration studies by Shanik, et al.[16] Our procedure denervated the lung but this reportedly does not alter function.[21] Also bronchial artery blood flow was obliterated in our experimental preparation as a result of surgical division of the main bronchus and restoration by suture anastomosis. This may affect healing of the bronchial anastomosis adversely in some animal species but it was not critical in the canine model prepared for these studies.[9]

The anatomic dye migration studies were done to demonstrate hilar lymphatic vessel interruption and obstruction resulting from the preparatory procedure. Shanik, et al[16] demonstrated obstruction to lymph flow by dye migration studies after bronchial transection in a similar canine model in which they managed to spare the bronchial arterial blood flow. They observed evidence of lymph vessel regeneration 10–21 days after surgical transection. The sky blue dye injection method used in our study to outline lymph vessels and stain lymph nodes was a modification of the successful technic reported previously.[6] Our results confirm the findings of Eraslan, et al[6] who investigated dogs after lung reimplantation as well as the observations of Shanik, et al[16] made in dogs after bronchial transection only. We observed satisfactory interruption of lung lymph vessels with absence of dye migration in animals studied immediately after surgical preparation and noted evidence of lymphatic regeneration and reconnection to mediastinal lymphatics 7–28 days after preparation.

Consideration was given to the selection of an

appropriate parenteral fluid that would expand the intravascular volume without precipitating pulmonary edema in a normal animal or in the unaltered lung but would reflect compromised lung lymph flow. Low molecular weight dextran was chosen because of its molecular size and because we had data indicating the tolerable limits of intravenous administration in dogs.[13] During infusion animals breathed room air spontaneously without mechanically assisted ventilatory support to simulate conditions of normal lung water homeostasis without altering lung lymph flow by changing airway pressure.

Compromised lung lymph clearance, as produced by lymphatic interruption, results in interstitial water accumulation. The best estimate of regenerated lung lymphatic sufficiency would be a comparative measurement of maximal lymph flow before and after surgical interruption. Since definitive measurements of canine lung lymph flow are impossible, extravascular lung water estimation has been used by many investigators as an approximation of adequate lung lymph drainage. Levine, et al.[12] concluded from their experiments that 50% of total lung water determined gravimetrically was extravascular water. They reported that extravascular lung water averaged 3.5 ml/kg in dogs. Cowan, et al.[4] determined the extravascular water content of the lung at intervals after reimplantation using radioactively chromated red blood cells to estimate and exclude intravascular water. Using their tabulated data the ratio of right lung water to left lung water in control animals would be 1:38, very similar to the data derived from our experiments. Magno and Szidon[14] studied lung water homeostasis in dogs after interruption of lung lymph drainage in the superior mediastinum and at both subclavian jugular lymphovenous junctions. They were only able to demonstrate interstitial lung water accumulation four days after preparation in animals with elevated left atrial pressure. The authors suggested two possible explanations for the failure to observe increased lung water without establishing left atrial pressure elevation; one that lymphatic obstruction was not absolute in all experimental subjects or two that the accumulated interstitial fluid had the water content of whole blood which is similar to the water content of normal lung. Our experimental preparation differed in that lymphatic interruption was complete and unilateral and, in addition the unaltered right lung lymph flow provided additional control observations. In order to simplify the measurement method we used the gravimetric determination of total lung water. The proportionate contribution of intravascular water to total lung water was minimized by exsanguinating the animals at the time of lung recovery and by calculating changes in left lung water as a function of right lung water (Fig. 2). The validity of the methods we used is based on the additional assumption that there was no change in vascular permeability as a result of the surgical procedure. The dextran induced increase in whole blood water content did not produce an increase in lung water in control animals or in the right lungs of experimental animals within the limits defined for rate of administration. Therefore, since our data compliments that reported by others who have studied lung lymph flow it is probable that fluid changes noted in the left lung of the experimentally prepared dogs are attributable to lymphatic obstruction.

The microscopic findings correlated with the presence of increased lung water as determined gravimetrically. Prominent findings three, five and seven days after surgical preparation were the striking perivascular fluid cuffing and the dilated perivascular and peribronchial lymph vessels. Visceral pleural lymphatics appeared in thickened edematous pleura after three days postpreparation. Perivascular fluid cuffing and presence of parenchymal lymphatic vessels were less frequently seen in lung specimens recovered 11 days after preparation; however pleural changes persisted throughout the period of 56 days of observation. Other investigators have reported that ventilation of the reimplanted lung is reduced during the period of increased lung water accumulation and evolving histologic changes.[17] Ventilatory restriction is consistent with the presence of the lymphedematous pleuritis noted in our study. Controversy exists regarding reversal of ventilatory restriction. Strieder, et al.[17] and Brownlee, et al.[2] have reported a return to normal ventilation 6 months and 24 months postlung reimplantation. However, in a very recent study Blumenstock, et al.[1] observed persistent decreased ventilation in both reimplanted and transplanted canine lungs in animals studied serially up to 673 days postpreparation. We are unable to make a definitive observation about the probability of achieving unrestricted ventilation after 56 days from the histologic data available from our study.

We were prompted to initiate this investigation of lung lymphatic regeneration by a suggestion from earlier work indicating that attenuated lymphatic alterations may be detrimental in immunosuppressed canine lung allograft recipients.[10] The present study was designed to eliminate vascular and immunologic contributions to the observed and measured changes in the lung and enhance the probability that the changes could be ascribed to surgical interruption of the lung lymphatics at the hilum. Anatomical

regeneration and reconnection of lung lymphatics with mediastinal lymph vessels in 7–21 days disclosed by this study is within the period reported for regeneration of lymph vessels after transection in other tissues and animals species.[3,5,7] The possibility of a more remote residual ventilatory deficit is suggested by our investigation of lymphatic sufficiency.

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