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# Effect of Diaphragmatic Lymphatic Contamination on Caudal Mediastinal Node Lymph Flow in Unanesthetized Sheep

**R.H. Demling, M.D., R. Gunther, Ph.D.**

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**Summary**

Our purpose was to determine the effect of diaphragmatic lymphatics on steady state caudal mediastinal node (CMN) or lung lymph flow in the unanesthetized sheep. After standard lung lymph fistula preparation, in which the CMN efferent was cannulated, nine sheep had an additional extensive cautery procedure in the right chest along the esophagus and diaphragm to sever any systemic lymphatics entering the CMN. This was followed by a similar cautery procedure on the left side, one week later. There was no difference in steady state lymph flow, $\dot{Q}_L$, or in the lymph-to-plasma, L/P protein ratio in any paired study when comparing the bilateral cautery procedure with the right side alone, nor was there any difference in these parameters when this data was compared with that from thirty standard lymph fistula preparations. In addition, diaphragmatic lymphatics were cannulated in five sheep. Mean $Q_L$ immediately post-surgery was 1.6 ± 0.4 ml/hr compared to a CMN $\dot{Q}_L$ of 6.1 ± 0.4 ml/hr. By six hours after surgery, diaphragmatic $\dot{Q}_L$ decreased to 0.4 ± 0.2 while CMN flow remained relatively steady. We conclude that residual diaphragmatic lymphatics, if any, entering the caudal mediastinal lymph node, not removed by the standard preparation do not appear to affect steady state CMN lymph flow or L/P ratio, in the normal unanesthetized sheep, especially if measurements are obtained at least 6 hours after surgery.

The chronic lung lymph fistula preparation as described by *Staub* has been used extensively by many investigators for the study of pulmonary edema and acute respiratory failure of trauma and sepsis (1–3). Lymph draining from the caudal mediastinal lymph node, CMN, efferent duct has been felt to originate primarily in the lung (4). Lymph flow and lymph protein content have been used to monitor pulmonary transvascular fluid filtration rate and protein permeability (5, 6). However, a small amount of non-pulmonary lymph has always been felt to be present from diaphragmatic and abdominal lymphatics (4, 7). The majority of this systemic lymph contamination has been reported by *Staub* et al. (4) to be eliminated by transecting the caudal portion of the node at the level of the inferior pulmonary ligament. Recently, *Gabel* et al. (8) reported that there is significant contamination of CMN lymph in the anesthetized sheep amounting to 25–60 percent of total CMN flow, from diaphragmatic lymphatics crossing the esophagus, in both the right and left chest cavities, which are not removed by the standard node division.

Our purpose was to further define the significance of these diaphragmatic lymphatics on steady state CMN flow in the unanesthetized sheep and to determine whether further surgical procedures are necessary to eliminate any existing contamination.

## *Methods*

Chronic lung lymph fistulae were prepared in nine adult female sheep 50–60 kg as described by *Staub* (4). After the sheep were anesthetized with halothane and placed on a volume ventilator (Bird), a right thoracotomy through the sixth intercostal space was performed, and the efferent lymph duct of the caudal mediastinal lymph node, CMN, was cannulated. A smaller right thoracotomy was then performed through the ninth intercostal space and the tail of the

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CMN node was transected at the level of the inferior pulmonary ligament along with surrounding fat and fibrous tissue. This was done to minimize any systemic contamination, in particular from diaphragmatic lymphatics entering the caudal portion of the node. One modification has been made since the original description by *Staub* (4), in that the node transection and efferent cannulation are now done in one operation rather than two. An additional maneuver was then performed to further remove any diaphragmatic lymphatics reported by *Gabel* et al. (8) to enter the node along the esophagus cephalad to the point of transection at the inferior pulmonary ligament. This consisted of cauterizing the mediastinal pleural and the superficial fibers of the esophagus along the right border of the CMN node for a distance of 2–3 inches cephalad to node transection as reported by *Ross* (7) (Fig. 1).

Incisions were closed and the chest cavity was evacuated of air. A Swan-Ganz catheter (Edwards, Santa Ana) was then placed through a neck incision. Animals then recovered from anesthesia. Lymph flow, lymph and plasma protein content and vascular pressures were then monitored intermittently over the next 7 days. Steady state values were defined as those obtained with the animal unanesthetized and standing when all parameters were completely stable. This usually occurs 2–3 days after surgery.

One week after this procedure, each animal was again anesthetized and a left thoracotomy through the ninth intercostal space was performed. All visible diaphragmatic lymphatics in the region of the CMN were cauterized as was the mediastinal pleura and superficial muscle fibers of the esophagus along the left border of the CMN (Fig. 2). Animals were allowed to recover and parameters measured as previously described. Values for the nine animals after these procedures were then compared with the last 30 chronic lung/lymph fistulae prepared by the standard procedure during the same time period. This does not include the additional cauterization, but consists of everything except the cautery maneuver along the border of the CMN. In five of the sheep a large diaphragmatic lymphatic near the distal portion of the node was cannulated using a small silastic catheter and parameters measured as previously described.

Statistical analysis: Paired data was analyzed using the paired "t" test with $p < 0.05$ considered as a significant difference. Unpaired data was compared using the unpaired "t" test.

*Results*

Numerous diaphragmatic lymphatics could be seen entering the distal portion of the caudal

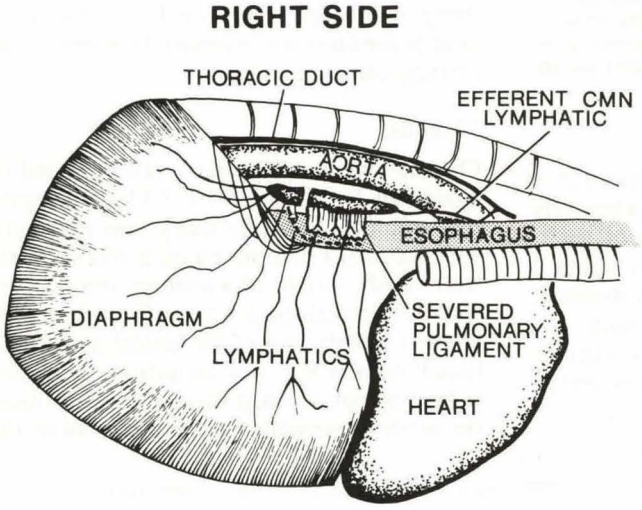


**Fig. 1** A schematic representation of the lymphatic anatomy of the right chest cavity is shown. The lung has been removed. The sausage-shaped caudal mediastinal lymph node CMN has been transected at the right inferior pulmonary ligament removing most of the diaphragmatic lymphatics entering the CMN. The dashed line along the esophagus and border of the CMN represents the line of additional cauterization performed to eliminate any remaining diaphragmatic lymphatics entering the node. The pulmonary ligament is not transected during the cannulation procedure but only for purposes of clarity in the diagram so that the lung could be removed and the lymphatics visualized

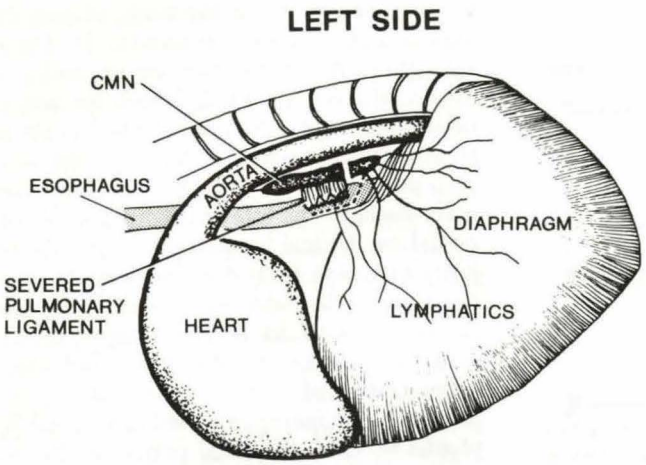


**Fig. 2** A schematic representation of the lymphatic anatomy of the left chest cavity is shown. The sausage-shaped caudal mediastinal lymph node, CMN, has been transected at the inferior pulmonary ligament removing the majority of diaphragmatic lymphatics entering the CMN. The dashed line along the esophagus and the CMN represents the line of cauterization performed to eliminate any remaining diaphragmatic lymphatics entering the node. The pulmonary ligament is not transected during the cannulation procedure but only for purposes of clarity in the diagram

mediastinal lymph node caudal to the inferior pulmonary ligament (Figs. 1, 2). These lymphatics became quite prominent after the node and surrounding fatty tissue were transected. An occasional small lymphatic was seen entering the CMN cephalad to the node division on both the left and right sides. These were easily severed with a cautery.

Mean data for the nine animals and for the standard procedure group of thirty animals, is shown on Fig. 3. There was no change in steady state lymph flow, lymph/plasma protein ratio, or vascular pressures in the nine animals between the right and left diaphragm cautery procedure. This would indicate that residual diaphragmatic lymphatics if any, entering the CMN node on the left side do not contribute significantly to steady state CMN lymph flow or protein content. Also, the addition of the right sided diaphragm cautery procedures did not appear to change the mean lymph flow or protein data obtained from previous animals after just the distal node transection.

Mean data for the five diaphragmatic lymphatic cannulation are shown in Fig. 4. Four of five lymph fistulas remained patent for 48 hours. One fistula ceased to be patent at about 24 hours because of the low flow. Lymph flow was relatively high immediately post-surgery, with flows of 15–40 percent of

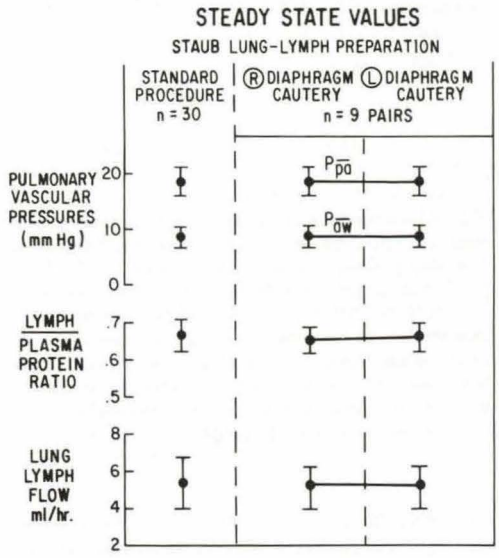


**Fig. 3** Comparison of steady state values after the right cautery procedure and after the left cautery procedure is shown for nine sheep. Data is presented as mean ± S.D. There were no differences indicating that diaphragmatic lymphatics entering the node on the left side not removed with node transection do not contribute significantly to steady state lymph flow. Comparison of the standard node transection procedure with the additional cautery procedure in non-paired animals also revealed no differences indicating additional procedures, beyond adequate node transection does not appear necessary to remove diaphragmatic lymphatic contamination

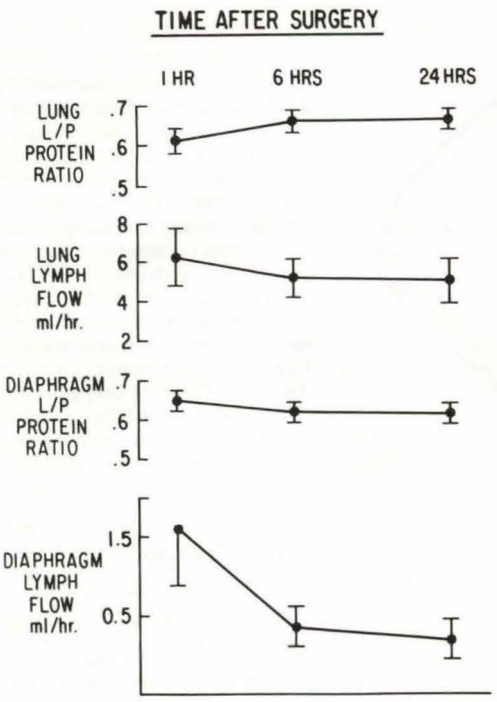


**Fig. 4** Comparison of changes in lung and diaphragmatic lymph flow and protein content is shown for five sheep. Data is presented as mean ± S.D. One hour post-surgery, diaphragmatic lymph flow was 15–20% of that in the lung and protein content was higher than, or equal to, that in lung. By six hours diaphragmatic lymph flow had decreased to less than 10% of that from the lung, while diaphragmatic lymph protein content decreased to a level below that in lung lymph

that seen in the lung. Diaphragmatic lymph protein was nearly equal to that seen in lung in this immediate period. Diaphragm lymph flow decreased rapidly over the next 6 hours then remained stable for the next 24–48 hours until the fistulas ceased functioning due to the low flow rate. Lymph protein content also decreased in all five animals with the value always being lower than that seen in the paired lung lymph sample.

*Discussion*

The chronic lung lymph fistula preparation as described by *Staub* has been used by a number of investigators for the study of lung fluid balance and respiratory failure (1–3). The advantages of this preparation are the ability to obtain relatively pure lung lymph for long periods of time. Sheep can be studied in the unanesthetized state several days after the surgical procedure when steady state values have been reached. The efferent lymphatic of the caudal mediastinal lymph node drains the majority of lymph formed in the lung and flow rate reflects the pulmonary transvascular fluid filtration rate (5, 6). However, any systemic lymph also drained by this duct could alter interpretation of results. *Staub* et al. (4) reported that diaphragmatic and abdominal lymphatics do enter the distal portion of the node at the diaphragm and can account for up to 40% of total lymph flow. They reported that the majority of this lymph contamination can be eliminated if the node is severed at the level of the inferior pulmonary ligament. *Gabel* et al. (8) however, recently reported that there were large diaphragmatic lymphatics on both the left and right sides which crossed the esophagus and entered the node beyond the point of node resection, thereby contributing to CMN lymph flow. They reported that flow from these contaminating lymphatics in the anesthetized sheep accounted for from 25–60 percent of CMN flow and lymph protein content was equal to or exceeded that reported in CMN lymph. The high protein content of these contaminating vessels could erroneously elevate protein content in CMN lymph which is considered to be representative of that from the lung, thereby altering results.

We studied the importance of these potential contaminating lymphatics on steady state CMN lymph flow and protein content since a large amount of research on lung edema after trauma and sepsis is based on the validity of this model. We also wanted to determine whether a more extensive procedure was needed to remove this contamination than that outlined by *Staub* et al. (6) and more recently by *Roos* et al. (7). Transection of the pleura and of the outermost muscle fibers of the esophagus along the course of the node by cautery should eliminate most of these reported contaminating lymphatics as they cross the esophagus to

enter the node (Figs. 1, 2). The addition of this procedure on the left side did not alter steady state lymph flow or protein content in nine sheep when compared to values after the same procedure on the right side. This would indicate that residual diaphragmatic lymphatics on the left side not severed with the caudal portion of the node do not contribute to any significant amount of steady state CMN lymph flow in the unanesthetized state. The cautery procedure along the node on the right side also appears not to be necessary as there was no change in lymph flow or protein content when compared to a large group of sheep with only transection of the caudal portion of the node. Although this latter comparison is less sensitive, one would expect to see some change if 25–60 percent of the CMN flow was due in fact to contamination.

The finding that diaphragmatic lymph flow was nearly 5 fold higher immediately post-surgery compared to even 6 hours later when the animal had totally recovered from anesthesia, was most interesting. Lymph protein content was also higher immediately post-surgery. These results may help explain the difference in our results compared to *Gabel* et al. (8), where diaphragm lymphatic flow was measured immediately after cannulation when the sheep was anesthetized. The anesthetized state, exposure to positive pressure, or simply manipulation of the diaphragm during surgery, may accentuate the flow from these vessels. Although we did not measure flow during the anesthetized state, the high flow immediately post-anesthesia would suggest that it may well be elevated. All small diaphragmatic lymphatics were probably not removed by our cautery procedure, but even if present, did not contribute significantly to lymph flow several days post-surgery. These small vessels may, however, be more significant in the immediate post-surgery period because of the apparently higher flow of these vessels during this period. This finding indicates the need for an adequate recovery period prior to the study of the animal in the unanesthetized state, so as to minimize the contribution of any lymphatics contaminating CMN efferent flow. It also appears essential that the node be carefully transected at and not below, the inferior pulmonary ligament, because of the high density of diaphragmatic lymphatics entering the CMN in this area.

We, therefore, conclude that the standard method of removing systemic contamination from CMN lymph appears adequate. Remaining lymphatics, if present, do not appear to contribute to a significant amount of CMN lymph when measured in the normal animal in unanesthetized steady state. Potential contamination can be further minimized by avoiding acute experiments in the immediate post-surgery period. Further studies will be necessary to determine whether systemic insults such as endotoxin will increase flow in these minute contaminant lymphatics vessels, to rates which can become quite significant.

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