Lymph flow pattern in the intact thoracic duct in sheep

Masataka Onizuka*, Torun Flatebø and Gunnar Nicolaysen

Department of Physiology, Institute of Basic Medical Sciences, University of Oslo, PO Box 1103, Blindern, N0317 Oslo, Norway

1. To study the lymph flow dynamics in the intact thoracic duct, we applied an ultrasound transit-time flow probe in seven anaesthetized and four unanaesthetized adult sheep (~60 kg). In unanaesthetized non-fasting animals we found that lymph flow in the thoracic duct was always regular pulsatile (pulsation frequency, $5{\cdot}2 \pm 0{\cdot}8$ min^{-1}) with no relation to heart or respiratory activity. At baseline the peak level of the thoracic duct pulse flow was 11·6–20·7 ml min^{-1} with a nadir of 0–3·6 ml min^{-1}. Mean lymph flow was $5{\cdot}4 \pm 3{\cdot}1$ ml min^{-1}. The flow pattern of lymph in the thoracic duct was essentially the same in the anaesthetized animals.

2. In both the anaesthetized and unanaesthetized animals, the lymph flow response to a stepwise increase in the outflow venous pressure showed interindividual variation. Some were sensitive to any increase in outflow venous pressure, but others were resistant in that lymph flow did not decrease until outflow venous pressure was increased to higher levels. This resistance was also observed in the high lymph flow condition produced by fluid infusion in the anaesthetized animal and mechanical constriction of the caudal vena cava in the unanaesthetized animals. Pulsation frequency of the thoracic duct flow initially increased and then decreased with a stepwise increase in the outflow venous pressure. This initial increase might be a compensatory response to maintain lymph flow against elevated outflow venous pressure.

3. To test the effect of long-term outflow venous pressure elevation in unanaesthetized sheep, outflow venous pressure was increased by inflation of a cuff around the cranial vena cava for 1, 5 or 25 h. The cuff was inflated to a level where lymph flow was reduced. Lymph flow remained low or decreased further during the entire cuff-inflation period. We calculated the lymph debt caused by the outflow venous pressure elevation and the amount 'repaid' when venous pressure returned to normal. Lymph debt for 25 h was 6400 ml but only 200 ml was repaid. Since we observed no visible oedema formation in the lower body of the sheep, the non-colloidal components of the lymph must have been reabsorbed into the bloodstream, most likely in the lymph nodes.

Of long-standing interest has been the extent to which increased systemic venous pressure restricts thoracic duct drainage into the central veins. Such restriction could be a pathological factor in oedema formation in right-sided heart failure. Wégria *et al.* (1963) inserted a bubble flowmeter into the thoracic duct in anaesthetized dogs and observed in acute experiments that lymph flow decreased with increased pressure in the innominate vein. Szabó & Magyar (1967) inserted a tube into the thoracic duct in normal dogs and found that lymph flow decreased with increasing outflow pressure though insignificantly until the pressure exceeded about 6 cmH_2O. In dogs with pericarditis lymph flow was higher at baseline and decreased with any increase in outflow pressure.

There are conflicting data on how other lymph vessels respond to increased outflow pressure. Laine, Allen, Katz, Gabel & Drake (1987) observed in anaesthetized dogs that lymph flow from the heart, liver, small intestine, kidney and skeletal muscles decreased linearly with increasing outflow pressure. In unanaesthetized sheep the same research group (Drake, Giesler, Laine, Gabel & Hansen, 1985) found that in some animals the flow from cannulated lung lymph vessels decreased with any increase in outflow pressure while in others lymph flow did not decrease until the outflow pressure exceeded 5–15 cmH_2O. The authors attributed this latter observation to a Starling resistor effect on the portion of the lymph vessel that was exposed to pleural pressure and not to a compensatory increase in

* To whom correspondence should be addressed at the Department of Surgery, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Ibaraki 305, Japan.

lymphatic pumping. The same group (Drake & Gabel, 1991) later reported that in unanaesthetized sheep cannulated intestinal lymphatics maintained their flow until the outflow pressure exceeded about 15 cmH_2O and this was attributed to increased pumping activity of the lymphatics. McGeown, McHale, Roddie & Thornbury (1987) using anaesthetized sheep found that the flow from cannulated popliteal lymph vessels was more sensitive to increased outflow pressure than hindlimb lymph vessels in that the former showed a continuous albeit non-linear decline while the latter maintained the flow even at an outflow pressure of 30 cmH_2O. In all these studies the lymphatics had been cannulated, and it cannot be excluded that this interfered with the normal pumping function in these thin-walled vessels. Along this line, Hall, Morris & Woolley (1965) reported that the pressure upstream to a clamp on a lymphatic could rise to as much as 55 mmHg. This theoretically means lymph could flow into systemic circulation against such a high outflow venous pressure. The main aim of the present study was to try to establish how the lymph flow in the intact thoracic duct is affected by increased outflow pressure. To this end we applied an ultrasound transit time probe to the duct and increased cranial vena cava pressure stepwise in both anaesthetized and unanaesthetized sheep. Furthermore, we tested the effect of outflow venous pressure elevation maintained for up to 25 h on thoracic duct lymph flow.

Little is known about the role of respiratory movements in the transport of lymph along the thoracic duct. Browse, Rutt, Sizeland & Taylor (1974) using anaesthetized dogs introduced radio-opaque droplets into a peripheral lymphatic and radiographically recorded their movements. They found that cephalad motion took place only as long as there were respiratory movements. In two unanaesthetized dogs they observed a steady fast flow with frequent but small fluctuations. Schad, Flowaczny, Brechtelsbauer & Birkenfeld (1978) on the other hand concluded that a cessation of respiratory movements (bilateral pneumothorax) reduced flow from the cannulated thoracic duct of anaesthetized dogs by only about 35%. A second aim of our study was to study the relation between the flow in the intact thoracic duct and respiration in unanaesthetized animals.

METHODS

The experimental protocols were approved by the local Animal Experimentation Committee.

Lymph flow measurement

Lymph flow in the thoracic duct was measured with an ultrasound transit time flow probe (model 2SB or 3SB, Transonic Systems, Ithaca, NY, USA), connected to a flowmeter (model T206X, Transonic Systems). Although the transit-time ultrasound system is well validated as an accurate method capable of measuring blood flow (Hartman, Olszanski, Hullinger & Brunden, 1994), Depner, Krivitski & MacGibbon (1995) observed a reduction in ultrasound velocity in the saline diluted blood. In order to establish whether differences in lymph protein concentration influence the signal from the ultrasound probe we made calibration curves using latex tubing or excised thoracic duct (one experiment). With flows covering the range 0–5 ml min^{-1} we recorded the flow signal subsequently with plasma, plasma diluted 1 : 1 with saline and saline as fluid in the tubing or duct. With all three fluids the flow signal (y) was linearly correlated to true flow rate (x) and the regression equations were $y = 0{\cdot}75x + 0{\cdot}12$, $y = 0{\cdot}76x + 0{\cdot}06$ and $y = 0{\cdot}80x + 0{\cdot}01$, respectively, and thus not different. Since it is necessary to obtain a real flow volume from the calibration line, we calibrated the probe *in situ* in each animal. At the end of each experiment we inserted a Silastic catheter (no. 602–265, Dow Corning, Midland, MI, USA) into the thoracic duct downstream to the probe, and performed *in situ* calibration by timed collection of lymph. We checked the zero offset values of the probes by blocking lymph. We produced different flow by changing the height of the inserted catheter tip, created different outflow pressure and recorded both probe signal and collected lymph weight. Using the obtained calibration line for each experiment we calculated the real lymph flow rate from computer recordings. Lymph flow was expressed in millilitres per minute in record representations, and as microlitres per minute per kilogram in summary data and the data analysis.

Anaesthetized sheep

Surgery. Seven adult sheep of similar size (40 ± 4 kg, merino, Norwegian strain) were fasted for 12 h before general anaesthesia was administered. Five minutes after intramuscular injection of 0·5 ml xylazin 2% (Rompun vet., Bayer, Leverkusen, Germany), a catheter was introduced into the right external jugular vein by a direct puncture, through which sodium pentobarbitone (20 mg kg^{-1}) was injected. By this catheter we measured the outflow venous pressure of the thoracic duct (pressure transducer Model 840, Sensonor, Horten, Norway). After endotracheal intubation, the sheep were maintained on 0·5–1·0% halothane and 50–55% oxygen in N_2O using a positive-pressure ventilator (G. F. Palmer, London, UK) with a tidal volume of 450–500 ml, a respiratory rate of 15–20 min^{-1} and an end expiratory pressure of 5 cmH_2O. Arterial blood pressure was monitored from a catheter in the right carotid artery (pressure transducer P23Db, Statham, Hatorey, PR, USA). Airway pressure (pressure transducer Model 270, Hewlett Packard, San Diego, CA, USA), arterial blood gas (ABL2, Radiometer, Copenhagen, Denmark), the inspiratory oxygen concentration (M&C Instruments, Bleiswijk, The Netherlands) and expiratory CO_2 concentration (Medical Gas Analyser LB-2 model 240M, Beckman, Schiller Park, IL, USA) were monitored.

The right external jugular vein was exposed to allow the introduction of a balloon catheter (Foley catheter 8 Ch/Fr, Bard, West Sussex, UK or large occlusion balloon catheter OBW/27/8/2/100, Meditech, Boston, MA, USA) and a large bore catheter for the rapid infusion of Ringer lactate solution. The tip of the balloon catheter was placed at the point just caudal to the thoracic duct outlet into the jugular vein. During surgery a drip infusion of Ringer lactate solution, composition (mmol l^{-1}): Na^+, 130; K^+, 4; Ca^{2+}, 1·5; Cl^-, 109; lactate, 28 (200 ml h^{-1}) was maintained.

After intravenous injection of 0·5 ml 0·5% alcuroniumchloride (Alloferin, Roche, Basel, Switzerland), we performed a right thoracotomy in the fifth intercostal space. Through a small incision of the parietal pleura we exposed the thoracic duct about 1 cm cranial to the median mediastinal lymph node and positioned the ultrasound transit time flow probe on the duct. This procedure was done with meticulous care to remove completely the fat tissue around the thoracic duct in order to improve the acoustic conductivity. We fixed the probe by suturing the cable to the parietal pleura at

several points, maintaining the proper alignment of the probe with respect to the thoracic duct (Tanaka & Robotham, 1992), and at the same time we confirmed that the probe did not cause any kinks on the duct.

Protocols. Arterial, venous and airway pressures as well as lymph flow were continuously recorded on a polygraph (model 440406, Gould, Cleveland, OH, USA). The zero level of all pressures was adjusted to the level of the cranial vena cava at its entrance to the heart. Lymph flow was also displayed on a computer screen and the flow signal was digitally recorded every 1 s on a computer disk. The lymph flow data were analysed by a computer program to give the mean flow, peak flow, nadir flow, integrated volume flow and the slope of the mean flow during a selected period.

After baseline measurement, the cranial vena cava was partially obstructed by inflating the balloon in order to increase the venous pressure in the cranial vena cava. The venous pressure was increased in steps of 4–6 cmH_2O (each step maintained for 3 min) until a pressure of 40–45 cmH_2O was reached after which the balloon was deflated completely. When lymph flow returned to the baseline level, body temperature Ringer lactate solution (2500 ml) was infused over a period of 20–30 min in order to increase the baseline lymph flow. After new baseline measurements the same stepwise increase of outflow venous pressure was performed.

Unanaesthetized sheep experiments

Surgery. In all preparations aseptic surgical techniques were used ($n = 4$; body weight: range, 54–78 kg; mean, 63 ± 11 kg). All catheters were impregnated with heparin to retard clotting (TDMC Processing, Polysciences, Warrington, PA, USA). Arterial blood pressure was monitored using a catheter (Intramedic PE50, Clay Adams, Parsippany, NJ, USA) inserted into an ear artery. After setting the flow probe around the thoracic duct at the same position as in the anaesthetized animals, the incised parietal pleura was closed and the cable of the probe was fixed to the parietal pleura at multiple points by suturing. An inflatable latex cuff vascular occluder (model OC20, In Vivo Metric, Healdsburg, CA, USA) was placed around the caudal vena cava. By inflation of the cuff we expect an increase in peripheral venous pressure of the lower body including all abdominal organs and an increase of lymph production in that part of the body. A catheter (PE-90, Clay Adams) was introduced into the caudal vena cava with its open end 15 cm distal to the vascular occluder for pressure measurement. The third intercostal space was also opened to place an inflatable latex cuff vascular occluder (model OC14, In Vivo Metric) around the cranial vena cava. By an inflation of the cuff, outflow pressure of the thoracic duct was increased. At the same time venous pressure in the upper body increased. At the level of the entrance of the cranial vena cava into the heart, a string suture was made in the chest skin to mark the zero level for vascular pressure measurements. A latex balloon with an extension tube was placed in the pleural cavity to monitor respiratory movements. All catheters, tubes and the probe cable were passed to the back of the animal through subcutaneous tunnels and all lines were fixed at their exits. The surgical wound was closed layer to layer after the insertion of a thoracic drainage tube. All vascular catheters were filled with heparin (5000 i.u. ml^{-1}) (Nycomed Pharma, Oslo, Norway).

On the day of surgery and postoperatively for 3 days, we administered 2 g of dihydrostreptomycin and 1·6 g of benzylpenicillin (Streptocillin vet., Boehringer Ingelheim, Ingelheim am Rhein, Germany) subcutaneously to prevent infection. For analgesia we gave an intramuscular injection of 1 g metamizolnatrium (Novalgin vet., Hoechst Veterinar GmbH, Munich, Germany) after extubation of the endotracheal tube and once a day for the next 2 days. We gave the sheep at least 1 week to recover from the operation before starting the experiments. We kept four instrumented sheep together for 3 months in a wide clean open space. For the measurement of the variables, the sheep were moved to a mobile cage, in which they stood quietly throughout each experiment. The baseline acoustic signal from the flow probes when first established did not change over the 3 months.

Protocols. We performed five different experimental protocols on each of the unanaesthetized sheep with at least 1 full day interval between. In each protocol the lymph flows of two sheep were measured simultaneously, one subjected to experimentation, the other subjected only to continuous baseline recording.

Protocol A. After baseline measurement, the cranial vena cava was constricted by inflating the vascular occluder cuff in order to increase the upstream pressure. This pressure was increased in steps of 8–12 cmH_2O (each step held for 3 min) to 45 cmH_2O, then the cuff was deflated.

Protocol B. After baseline measurement the vascular occluder cuff around the caudal vena cava was inflated in order to increase venous pressure of the caudal body, thereby increasing lymph production in that territory. After a new baseline measurement protocol A was carried out.

Protocol C. After baseline measurement for 1 h, the cranial vena cava was constricted to increase the outflow venous pressure to a predetermined level. The pressure was maintained at that level for 1 h. After deflating the cuff, lymph flow was recorded for another hour.

Protocol D. After baseline measurement for 1 h, the vascular occluder around the caudal vena cava was inflated to increase lymph flow. After recording the new high baseline for 1 h, the cranial vena cava was constricted to increase the outflow venous pressure to a predetermined value and this pressure was maintained for 5 h. Then the cuff around the cranial vena cava was deflated. After measurement for another hour the cuff around the caudal vena cava was deflated. Final baseline lymph flow was recorded for 1 h.

Protocol E. After baseline measurement for 1 h, the cranial vena cava was constricted by the cuff inflation in order to increase the outflow venous pressure to a certain level. After recording the parameters for 1 h the sheep were moved back into the open area for 22 h under high outflow venous pressure conditions. The next day the sheep were moved into the cage and lymph flow was recorded for 2 h still with constricted cranial vena cava. The cuff was deflated and the lymph flow was recorded for the next 3 h.

Calculation of lymph debt and repayment. We calculated the apparent lymph debt caused by outflow venous pressure elevation lasting 10 min, 1, 5 and 25 h, and the amount that was 'repaid' when venous pressure returned to normal. Lymph debt was calculated simply by taking the baseline flow minus the flow during the venous constriction multiplied by duration of constriction. Lymph repayment was calculated using the flow in the high flow period following the cuff release and in the final baseline period. The duration of high flow was defined as time until the slope of mean lymph flow became less than $-1{\cdot}0 \times 10^{-3}$ ml min^{-1} s^{-1} in the computer recordings.

At the end of the study we anaesthetized the animals and performed *in situ* calibration as described above, then killed the animals by exsanguination. We dissected the thoracic duct completely to the neck from the chest, and confirmed that we had measured the lymph flow of the single main thoracic duct (Lascelles & Morris, 1961).

Data analysis

All data are presented as means ± S.D. unless otherwise indicated. Student's paired t test was applied for comparisons. Correlation was examined by least squares analysis. A value of $P < 0.05$ (two-tailed) was accepted as statistically significant.

RESULTS

In situ calibration

The ultrasound flow probe was calibrated *in situ* at the end of each experiment. Figure 1 shows the high correlation ($r^2 = 0.96$) between the flow probe values and the values from the timed collection. The signal from the probe increases linearly with flow. Although the zero offset values in different animals differed slightly (range, 3·0–5·4), the value was stable in each animal throughout the experiment. In two experiments in which we used the 3SB probe we obtained equally satisfactory data with *in situ* calibration. All values reported for lymph have been corrected according to the actual calibration curve.

Flow pattern of thoracic duct lymph flow

Figure 2*A* shows an actual recording obtained from an anaesthetized mechanically ventilated animal. Lymph flow in the thoracic duct was pulsatile with no relation to arterial blood pulsation or airway pressure fluctuations. We observed exactly the same flow pattern of thoracic duct lymph flow when we stopped the respirator for a moment. In anaesthetized sheep at the baseline the peak level of the thoracic duct flow ranged from 4·8 to 9·9 ml min^{-1} with a nadir of 0·1–1·5 ml min^{-1}. Pulsation frequency was 5·2 ± 1·8 min^{-1}. Mean lymph flow was 2·7 ± 1·9 ml min^{-1}.

In unanaesthetized non-fasting animals (Fig. 2*B*) lymph flow patterns in the thoracic duct were essentially the same as those in the anaesthetized animals. The lymph pulsation showed no relation to outflow venous pulsation or pleural pressure fluctuations (spontaneous respiration). We observed exactly the same flow pattern for approximately 5 min after the animal was killed. At the baseline the peak level of the thoracic duct's flow was 11·6–20·7 ml min^{-1} with a nadir of 0–3·6 ml min^{-1}. Pulsation frequency was 5·2 ± 0·8 min^{-1}. Mean lymph flow was 5·4 ± 3·1 ml min^{-1}.

Response to increased outflow venous pressure

Figure 3 shows a tracing of thoracic duct lymph flow of one anaesthetized animal during stepwise increase in outflow venous pressure. Mean lymph flow was not affected by the 1st or 2nd step inflation of the balloon in the cranial vena cava. However, there was a stepwise decrease with the succeeding steps of balloon inflation. Pulsation frequency increased stepwise, but decreased at the final balloon inflation. In this procedure the venous outflow pressure was increased stepwise from 8 (baseline) to 35 cmH_2O (the highest pressure). After deflating the balloon a large spike flow was observed, but flow returned to baseline within 1 min.

Figure 4*A* summarizes the thoracic duct lymph flow response to stepwise increases in outflow venous pressure in anaesthetized animals. There was interindividual variability in the baseline lymph flow (range, 14–93 μl min^{-1} kg^{-1}; mean, 69 ± 47 μl min^{-1} kg^{-1}). Response to the stepwise increase in outflow venous pressure also varied. Three animals showed decreases in lymph flow with any increase in outflow venous pressure. Four animals showed no

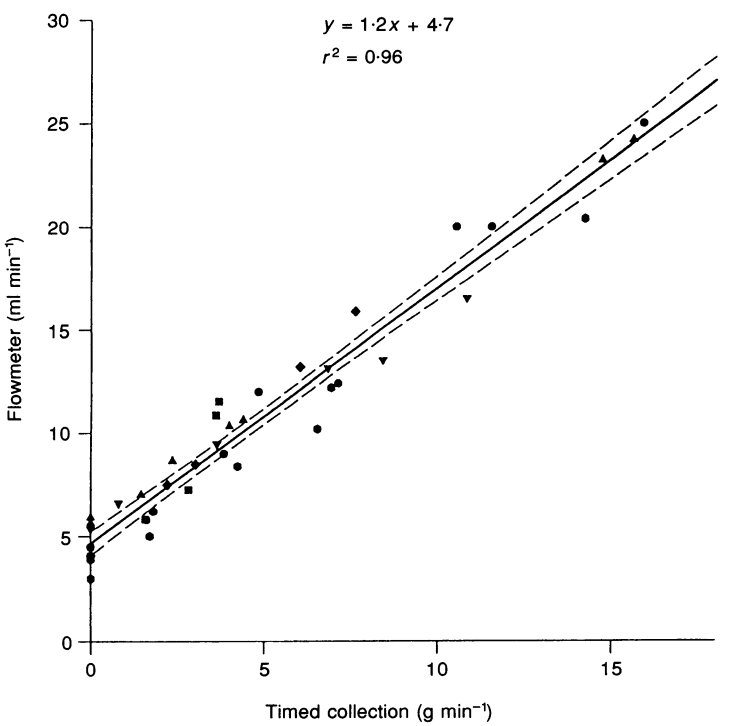


Figure 1. *In situ* calibration of the ultrasound transit time flow probe

The ultrasound transit time flow probe was calibrated *in situ* at the end of the experiments. The regression line with a 95% confidence interval obtained from 7 anaesthetized animals (one symbol per animal) is shown. The data show that the signal from the probe varies linearly with flow and with little scatter.

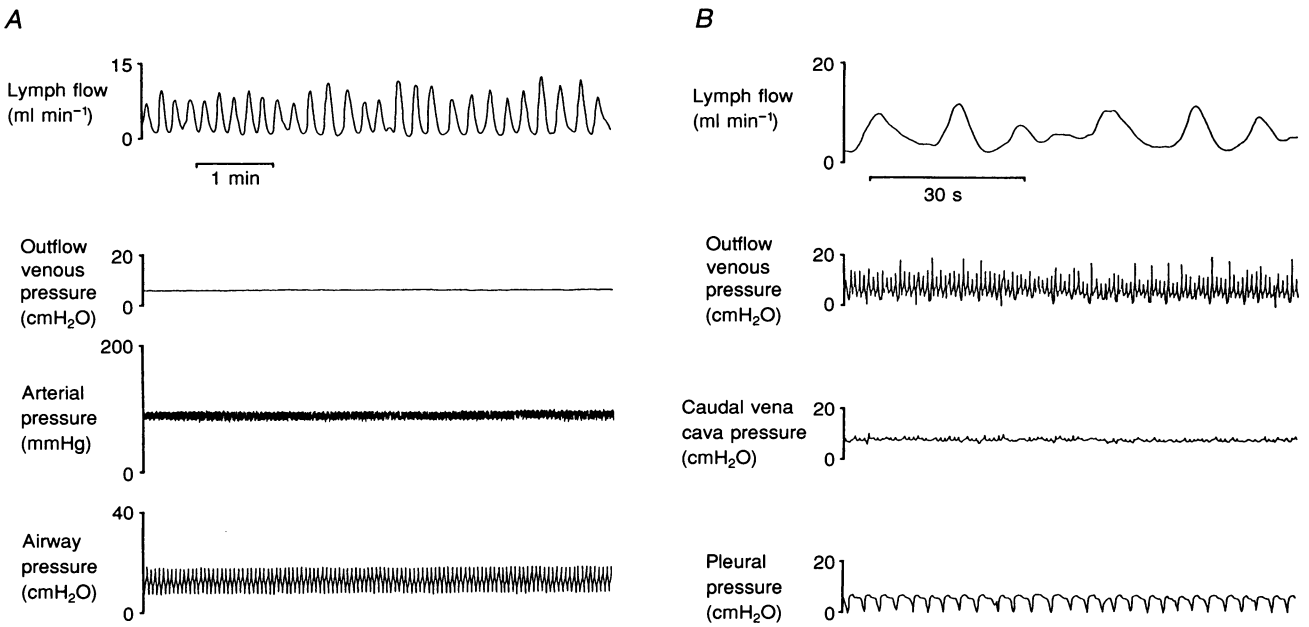


Figure 2. Flow probe recording in anaesthetized and in unanaesthetized animals

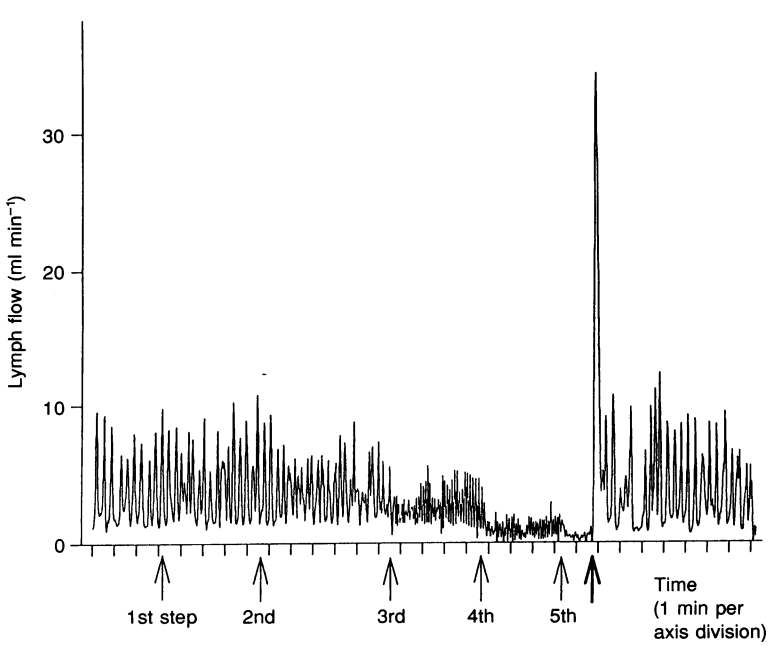
A, in anaesthetized animals lymph flow in the thoracic duct was pulsatile with no relation to arterial blood pulsation or change of airway pressure. *B*, in unanaesthetized animals pulsatile lymph flow was essentially the same as that of anaesthetized animals. The lymph pulsation had no relation to outflow venous pulsation or change of pleural pressure (spontaneous respiration). Note that the time scale is different between *A* and *B*.

decrease in lymph flow until outflow venous pressure increased beyond a certain level. Infusion of Ringer lactate solution (2500 ml) raised outflow venous pressure from 9 ± 2 to 16 ± 3 cmH_2O. Baseline lymph flow increased from 69 ± 47 to 287 ± 136 $\mu l\ min^{-1}\ kg^{-1}$ ($n = 7$, $P < 0{\cdot}01$). Figure 4*B* shows the individual data of lymph flow responding to a stepwise increase in outflow venous pressure after fluid infusion. In this high lymph flow condition three animals were resistant to at least the initial step of increase in outflow venous pressure.

Figure 4*C* shows the individual data in unanaesthetized sheep ($n = 4$), displaying normal baseline conditions (lower 4 lines) and high baseline conditions (upper 4 lines). A high baseline condition was produced by increasing the caudal vena caval pressure from 7 ± 3 to 17 ± 2 cmH_2O by the inflation of the cuff around the caudal vena cava. Baseline lymph flow increased from 83 ± 33 (lower 4) to 181 ± 59 $\mu l\ min^{-1}\ kg^{-1}$ (upper 4; $P < 0{\cdot}01$). By this procedure the cranial vena caval pressure did not change significantly (from 6 ± 3 to 5 ± 3 cmH_2O). In three animals the lymph

Figure 3. Typical tracing of thoracic duct lymph flow during stepwise increase in outflow venous pressure

A computer recording of lymph flow changes by stepwise inflation of the balloon in the cranial vena cava (thin arrows). Mean lymph flow of the thoracic duct decreased non-linearly stepwise (ml min^{-1}: baseline, 3·6; 1st step, 4·0; 2nd step, 3·7; 3rd step, 2·8; 4th step, 1·0; 5th step, 0·4) and pulsation frequency increased stepwise but finally decreased (min^{-1}: baseline, 3·0; 1st step, 3·7; 2nd step, 5·2; 3rd step, 9·3; 4th step, 9·9; 5th step, 3·5). In this procedure the venous outflow pressure was increased stepwise from 8 to 11·5, 18, 23·5, 28·5 and 35 cmH_2O. After deflating the balloon (thick arrow), a large spike flow (34·6 ml min^{-1}) was observed, but the flow returned to the baseline level within 1 min.



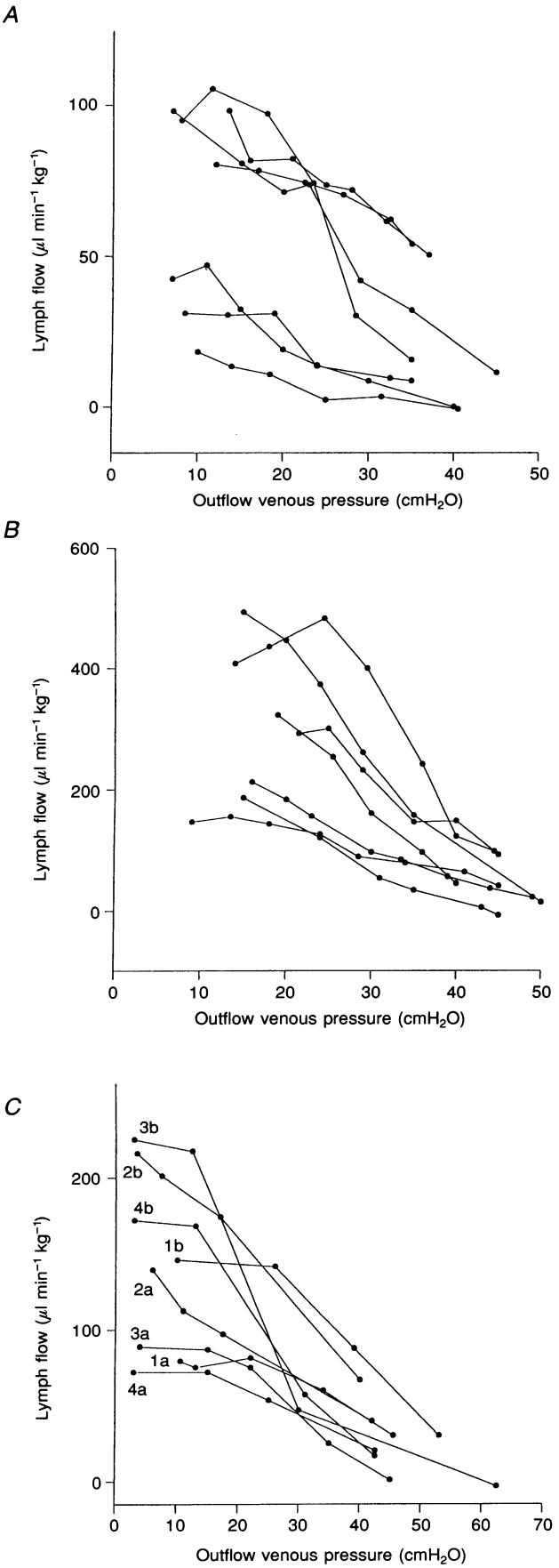


Figure 4. Individual data of mean lymph flow responding to stepwise increase in outflow venous pressure

A, in anaesthetized animals ($n = 7$) lymph flow response to stepwise increase in outflow venous pressure showed a non-linear decrease in lymph flow. *B*, after infusion of Ringer lactate solution (2500 ml), outflow venous pressure increased at baseline. The effect of stepwise increase in outflow venous pressure was similar to that in normal baseline flow conditions. *C*, in unanaesthetized animals ($n = 4$) the lymph flow response to outflow venous pressure also shows a non-linear pattern with a certain threshold with one exception (sheep no. 2). The lower 4 individual lines (1a–4a) are experiments at normal baseline flow. The 4 upper individual lines (1b–4b) are at a high baseline flow condition produced by inflation of the cuff around the caudal vena cava in the same animals.

Table 1. Effect on thoracic duct flow of stepwise increase in outflow venous pressure

Before fluid infusion †		After fluid infusion †		Before CauVC constriction ‡		After CauVC constriction ‡	
Outflow pressure (cmH_2O)	Lymph flow ($\mu l\ min^{-1}\ kg^{-1}$)	Outflow pressure (cmH_2O)	Lymph flow ($\mu l\ min^{-1}\ kg^{-1}$)	Outflow pressure (cmH_2O)	Lymph flow ($\mu l\ min^{-1}\ kg^{-1}$)	Outflow pressure (cmH_2O)	Lymph flow ($\mu l\ min^{-1}\ kg^{-1}$)
9 ± 2 (base)	69 ± 47	16 ± 3 (base)	287 ± 136	6 ± 3 (base)	83 ± 33	5 ± 3 (base)	181 ± 59
16 ± 2	63 ± 47	24 ± 1	251 ± 148	14 ± 2	75 ± 23	15 ± 8	173 ± 55
27 ± 2	33 ± 34**	35 ± 2	113 ± 74**	22 ± 3	65 ± 22	29 ± 9	78 ± 58*
39 ± 5	14 ± 22**	44 ± 5	44 ± 39**	44 ± 2	9 ± 18*	50 ± 10	14 ± 31*

Values are means ± S.D. † Anaesthetized sheep; 2·5 l Ringer lactate solution was infused within 20–30 min; stepwise increase of outflow venous pressure was performed by an inflation of a balloon in the cranial vena cava. ‡ Unanaesthetized sheep; caudal vena cava (CauVC) constriction was induced by an inflation of a cuff around the caudal vena cava; stepwise increase of outflow venous pressure was performed by a cuff inflation around the cranial vena cava. * $P < 0·05$; ** $P < 0·01$; significant differences compared with the value at baseline (base).

flow response to stepwise increase in outflow venous pressure showed a non-linear decrease with a threshold level for decreasing flow at approximately 20 cmH_2O. In one animal thoracic duct lymph flow had decreased already with the first step increase in venous pressure.

Table 1 shows a summary of the data on lymph flow according to the increments of outflow venous pressure at four points (baseline, lower middle, higher middle and high venous pressure). Thoracic duct lymph flow did not decrease significantly until the venous pressure exceeded about 20 cmH_2O.

Long-term effect of high outflow venous pressure

Protocol C (Fig. 5*A*). Outflow venous pressure was increased from 6 ± 3 to 20 ± 1 cmH_2O and maintained at that level for 1 h by inflation of the cuff around the cranial vena cava. Thoracic duct lymph flow (mean value during 1 h baseline, 92 ± 10 $\mu l\ min^{-1}\ kg^{-1}$) was decreased by cuff inflation and the low lymph flow condition remained for the whole hour (mean value for 1 h, 73 ± 4 $\mu l\ min^{-1}\ kg^{-1}$). After the cuff was deflated the lymph flow had already returned to baseline in the succeeding 10 min sampling period.

Protocol D (Fig. 5*B*). The caudal vena caval pressure was increased from 8 ± 4 to 16 ± 5 cmH_2O for 7 h by inflation of the cuff around the caudal vena cava. Lymph flow increased from 61 ± 3 to 139 ± 13 $\mu l\ min^{-1}\ kg^{-1}$ (mean value of each 1 h period). Outflow venous pressure was increased from 4 ± 3 to 29 ± 2 cmH_2O for 5 h in the 7 h period by inflation of the cuff around the cranial vena cava. Thoracic duct lymph flow decreased and the low flow condition prevailed over the next 5 h (mean value for 5 h, 83 ± 10 $\mu l\ min^{-1}\ kg^{-1}$). Following the deflation of the cranial vena cava, cuff lymph flow increased (mean value for 1 h, 142 ± 26 $\mu l\ min^{-1}\ kg^{-1}$). After the cuff deflation around the caudal vena cava, the lymph flow returned to the initial baseline level (mean value for 1 h, 73 ± 19 $\mu l\ min^{-1}\ kg^{-1}$).

Protocol E (Fig. 5*C*). Outflow venous pressure was increased from 3 ± 2 to 37 ± 6 cmH_2O for 25 h by inflation of the cuff around the cranial vena cava. Thoracic duct lymph flow (mean value for 1 h baseline, 90 ± 4 $\mu l\ min^{-1}\ kg^{-1}$) decreased to 55 ± 4 $\mu l\ min^{-1}\ kg^{-1}$ (mean value for 1 h in the 0–1 h interval). The animals then moved freely and ate *ad libitum* for 22 h before the next recording period. The outflow venous pressure was all the time kept at the same high level. The lymph flow showed no sign of recovery but rather decreased further to 25 ± 2 $\mu l\ min^{-1}\ kg^{-1}$ (mean value for 2 h in the 23–25 h interval). After deflating the cuff around the cranial vena cava the lymph flow increased to the level of 50 ± 10 $\mu l\ min^{-1}\ kg^{-1}$ (mean value for 3 h after the 25 h interval). Lymph flow did not return to the starting point values. This might be because lymph production was possibly different in the −2 to 0 h interval than in the period after the 25 h interval.

Lymph debt

Table 2 gives a summary of data on lymph debt (LD) and lymph repayment (LR) calculated from computer recordings. The durations of depressed flow induced by increased outflow venous pressure were as follows. Protocol A, 8·8 ± 2·8 min; B, 7·4 ± 1·5 min; C, 1 h; D, 5 h; and E, 25 h. In protocol E lymph flow during the unmeasured period (22 h) was estimated from the mean value of the flow in the first hour with high venous pressure and that of the final 2 h of the high venous pressure. The percentages of recovery lymph to lymph debt (LR/LD × 100) were as follows: protocol A, 76 ± 23; B, 81 ± 22; C, 68 ± 33; D, 24 ± 19; and E, 3·1 ± 0·4. The percentages in protocols D and E were significantly lower than in A or B ($P < 0·05$).

DISCUSSION

Most previous studies on lymph transport in the thoracic duct have been carried out with invasive techniques in the sense that the ducts were cannulated and the animals were anaesthetized (see Introduction). A study by Sakai *et al.* (1985) is the only exception to this in that the duct was not cannulated though also in their study the animals were anaesthetized. Sakai *et al.* (1985) applied cross-thermocouples to the thoracic duct and could thus obtain relative lymph flow values. Also studies on lymph propulsion in other lymph vessels have required cannulation. The possibility exists that cannulation and in many cases anaesthesia affects the ability of the lymph vessels to propel lymph. Ultrasound transit time probes afford a minimally invasive technique that can be used in both acute and chronic experiments in unanaesthetized animals. With such probes we obtained a linear relationship between flow values obtained with the probes and with the timed collection of lymph, with little scatter around the regression line. This was true also for probes that had been implanted for many weeks. We found that in all animals during the first days

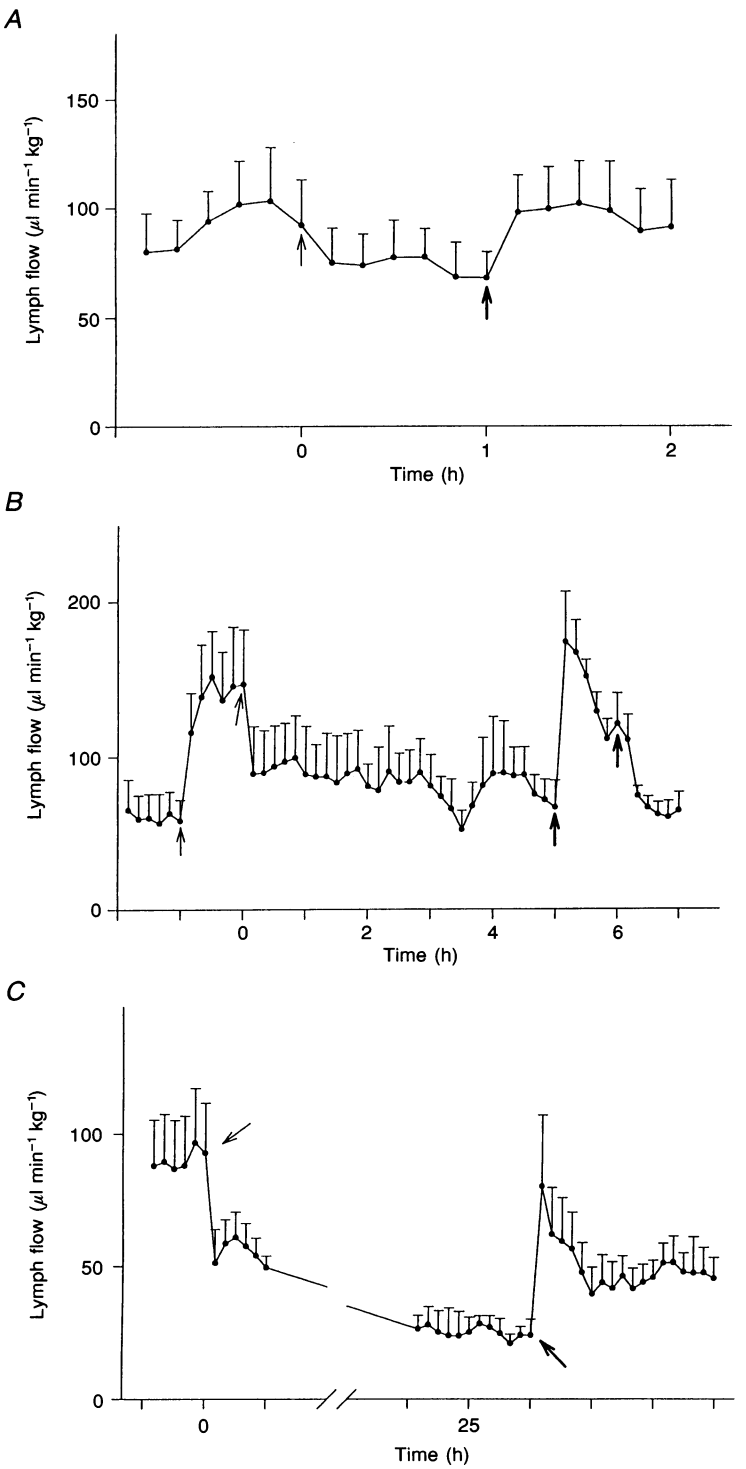


Figure 5. Thoracic duct flow during long-term outflow venous pressure elevation

A, the cuff around the cranial vena cava was inflated (thin arrow) and kept inflated to increase pressure by 20 ± 1 cmH_2O for 1 h. Thoracic duct lymph flow remained low until the cuff was deflated (thick arrow). *B*, the cuff around the caudal vena cava was inflated to increase lymph flow (left thin arrow), then the cuff around the cranial vena cava was inflated (right thin arrow) for 5 h. Thoracic duct lymph flow remained reduced to the same level during the whole period but returned to the baseline level after deflation of the cuff (right thick arrow). *C*, the cuff around the cranial vena cava was inflated (thin arrow) and kept inflated for 25 h. Thoracic duct lymph flow did not recover during this period. After deflating the cuff (thick arrow) the lymph flow increased. Each dot represents the mean value obtained from 4 animals (each mean flow for 10 min). Error bars are ± 1 S.E.M.

Table 2. Summary of lymph debt in unanaesthetized sheep

	Protocol				
	A	B	C	D	E
Lymph debt (LD) (ml)	18 ± 14	38 ± 16	76 ± 67	1042 ± 718	6430 ± 981
Lymph repayment (LR) (ml)	11 ± 6	29 ± 9	48 ± 51	163 ± 36	205 ± 57

LD = (baseline flow − low flow by constriction) × duration of constriction; LR = (high flow after cuff release − final baseline flow) × duration of high flow. Duration of high flow was defined until the time when the slope of the mean lymph flow became less than $-1{\cdot}0 \times 10^{-3}$ ml min^{-1} s^{-1} in the computer recording. Values are means ± S.D.

after implantation no flow signal could be obtained even though adequate signals were obtained immediately after surgery. After these first days we obtained good signals for the remaining period of observation. It is most likely that air bubbles were formed postoperatively and were temporarily trapped in the sound path.

Values for the lymph flow in the thoracic duct in mammals have been reported for more than a hundred years; thus Lesser (1871) reported a flow of 2 ml kg^{-1} h^{-1} in the anaesthetized dog. Colin (1873) found a similar value (3 ml kg^{-1} h^{-1}) in sheep. In our experiments the thoracic duct lymph flow in undisturbed non-fasting unanaesthetized sheep was 5·4 ± 3·1 ml min^{-1}, a value which appears to be several times higher than the recently reported value of 0·8 ± 0·4 ml min^{-1} also in unanaesthetized adult sheep but with a chronically implanted cannula in the duct (Drake, Anwar, Kee & Gabel, 1993), though not so different from the value reported by Colin (1873).

Intrinsic and extrinsic factors in propulsion of lymph in the thoracic duct

In guinea-pig mesenteric lymphatics Heller (1869) showed that rhythmic contractions were completely independent of respiration, heart rate and gut movement. Active contractions have been demonstrated in larger lymphatics including the thoracic duct (Werner, 1965; Staub, 1974; Reddy & Staub, 1981). In this study we confirmed the active spontaneous contractions of the thoracic duct by measuring real-time volume flow. Since the thoracic duct is very distensible we believe that the flow pulses we observed could not have originated in abdominal lymphatics transmitted to the thoracic duct. In our study the frequency of the pulse wave was regular at the resting condition (range, 3–7 min^{-1} in anaesthetized and 4–6 min^{-1} in unanaesthetized sheep). Campbell & Heath (1973) reported pulsation frequencies of the thoracic duct in sheep from pressure measurements that ranged from 2 to 20 min^{-1}. The different results might suggest that the pressure waves in the thoracic duct were not always parallel to the flow waves. With the present method we observed a sine-like shape of the flow wave.

Florey (1927) and later Smith (1949) proposed that the contractions of lymphatics were stimulated by increased distending pressure in the lymphatics. In our study the contraction frequency of the thoracic duct increased after intravenous fluid infusion. This might be the result of distension of the thoracic duct due to increased lymph production and flow. It could also be due to the increase in outflow pressure induced by the infusion. Furthermore, if the increasing outflow venous pressure directly increases the transmural pressure of the lymphatics (Drake *et al.* 1993), the same mechanism could explain the increase in contraction frequency induced by the elevation of outflow venous pressure in our study (Fig. 3). Although Waldeck (1965) demonstrated that the contraction frequency continued to increase with increasing transmural pressure in isolated liver lymph vessels from rats, we found at the highest outflow venous pressure that the contraction frequency of the duct decreased as judged from flow pulsation, suggesting that complete pumping failure occurred in this extreme high pressure condition. In a lower pressure range lymph flow decreased stepwise while the contraction frequency increased with stepwise increases in venous pressure (Fig. 3). Ohhashi, Azuma & Sakaguchi (1980) suggested that both a rise and a rapid fall in transmural pressure may initiate contraction in isolated bovine mesenteric lymphangions. In our study, after deflation of the cuff around the cranial vena cava a large spike flow occurred. The spike flow might be produced by increased contractility due to high pressure in the highly distended thoracic duct. These intrinsic activities of thoracic duct lymph flow were not affected by halothane–N_2O anaesthesia in our study.

In our study flow pulsation in the thoracic duct was completely independent of respiratory movements both in unanaesthetized and anaesthetized sheep. This finding is in apparent contrast to that of Browse *et al.* (1974) and in part to that of Schad *et al.* (1978). Both of these previous studies indicate a prominent or even dominant (Browse *et al.* 1974) role of respiratory movements in anaesthetized dogs. In two unanaesthetized dogs Browse *et al.* (1974) observed a fast

flow with no relation to respiration as judged from radiographic monitoring of lipiodol droplets, though in contrast to our findings the flow was steady. These differences could be due to different techniques or to species differences.

Effect of outflow venous pressure

We observed in three out of four unanaesthetized animals that the cranial vena cava pressure could be increased by at least 10 cmH_2O both at normal and at increased baseline lymph flows without any decrease in lymph flow over the next 3 min. We have no ready explanation as to why one animal showed some decrease (~20%) when cranial vena cava pressure was increased by 10 cmH_2O. Also, in some but not all the anaesthetized animals we observed such a threshold for the effect of cranial vena cava pressure. As shown in Table 1, there generally seems to exist a threshold level in the outflow venous pressure above which thoracic duct flow decreases significantly. In the experiments inserting a catheter in the thoracic duct or in the efferent duct of the caudal mediastinal lymph node, such a threshold has also been observed when raising the outflow level of the catheter (Cole, Witte, Kash, Rodger, Bleisch & Muelheims, 1967; Szabó & Magyar, 1967; Drake *et al.* 1985). In experiments using the interposed cannula in the thoracic duct (Wégria *et al.* 1963), a threshold was, however, not observed in anaesthetized dogs. Both Szabó & Magyar (1967) and Drake *et al.* (1985) found no such threshold when the lymph flow was artificially increased. We believe that an activity to maintain lymph flow against the elevation of outflow pressure exists physiologically in the thoracic duct. The most likely reason for why such a threshold has not been observed is the invasiveness of the methods applied. Our study might be less invasive compared to others, so that such a threshold phenomenon could be observed more frequently. This mechanism might work as a safety factor to minimize oedema formation in pathologically increased lymph flow conditions such as heart failure (Földi & Papp, 1961; Wégria, Entrup, Jue & Hughes, 1967).

Sakai *et al.* (1985) observed that in anaesthetized dogs thoracic duct lymph flow as measured with heated cross-thermocouples continuously increased during saline infusion in spite of a marked rise in central venous pressure to about 40 cmH_2O. At such high pressures all other studies have shown a decrease in lymph flow.

Contraction frequency of the thoracic duct initially increased and then decreased with stepwise increase in outflow venous pressure in our study (Fig. 3). This initial increase might be a compensatory response to maintain lymph flow against elevated outflow venous pressure. When the outflow venous pressure exceeded a threshold level, lymph flow decreased even though the contraction frequency increased. With further increases in the outflow venous pressure, lymph flow decreased significantly together with an apparent decrease in flow pulse frequency. Possibly, if we had monitored electrical activity or measured contractions with higher sensitivity, high frequency activity would be apparent similar to that observed by Hall *et al.* (1965). They observed an increase from 8 to 22 min^{-1} in the frequency of pressure pulses in the lumbar lymph trunk when the outflow of the inserted catheter tip was raised to 68 cm above the head of the humerus. In their study the frequency of pressure pulsation increased while lymph flow stopped.

Long-term effect of increased outflow venous pressure

Hall *et al.* (1965) demonstrated that pressure in the efferent lymphatic of the popliteal lymph node increased to about 55 mmHg when the inserted cannula was clamped. This suggests that lymph can be propelled from the lymphatics even when the outflow pressure is quite high. We therefore expected that even if thoracic duct lymph flow decreased with acute elevations of outflow pressure it could recover over time due to the propulsive activity in intact lymphatics. However, thoracic duct lymph flow did not recover during a 1, 5 or 25 h elevation of outflow venous pressure (Fig. 5), at least not with the outflow pressures we applied. These results obtained with non-invasive techniques are similar to those obtained by Drake *et al.* (1985) and Laine, Allen, Katz, Gabel & Drake (1986). They found that flow from cannulated lung lymphatics did not recover during 3 or 6 h periods of elevated outflow pressure in unanaesthetized sheep. They suggested that the produced lymph accumulated within the lung or associated tissues during the period. We observed no visible oedema formation in the lower body of the sheep. If lymph formation in our protocols were unchanged during long-term outflow venous pressure elevation, there must be some alternative pathway through which lymph was drained into the blood circulation. Probably lymph was reabsorbed in nodes along the lymphatics, since in our study the pressure in the caudal vena cava was much lower than that of the cranial vena cava during the period. The ability of lymph absorption by lymph nodes was confirmed by the observation of higher protein concentrations in efferent than in afferent lymph of popliteal and renal nodes in sheep (Quin & Shannon, 1977). Furthermore, Adair & Guyton (1985) found that increased pressure in the efferent lymphatic increased fluid reabsorption in the node. The same investigators (Adair & Guyton, 1983) reported that a rise in nodal venous pressure increased efferent flow and reduced lymph protein concentrations. We did not monitor the thoracic duct pressure and did not measure nodal venous pressure. Therefore, further investigations are necessary to prove that the alternative pathway for lymph would be the lymph nodes during long-term outflow venous pressure elevation. In the prenodal collectors there is little evidence for the addition or removal of water as recently reviewed by Aukland & Reed (1993).

Our calibration experiments (see Methods) show that an increase in protein concentration in thoracic duct lymph due to reabsorption in lymph nodes of water and small solutes during long-term outflow venous pressure elevation would not affect the signal from the ultrasound probe.

It is unlikely that lymph production was decreased in the period of high pressure in the cranial vena cava, because such a reduction would require a high interstitial pressure in our experimental setting. A high interstitial pressure would only be present with massive interstitial oedema (Aukland & Nicolaysen, 1981).

We calculated the lymph debt in different periods of outflow venous pressure elevation (Table 2). If a volume equal to the 25 h debt had accumulated in the interstitium, severe swelling of the lower body or legs would be present, but this was not the case. However, some lymph must be accumulated in the interstitium or in the lymphatics. Thus after returning to normal outflow venous pressure, lymph flow exceeded the baseline for a while. We calculated the excess lymph amount as payback lymph during the high lymph flow period after the outflow venous pressure was normalized (Table 2). We found that the ratio of the payback to the lymph debt decreased significantly as the duration of outflow venous pressure elevation increased. The data suggest that the payback lymph was drained from a compartment with a maximum volume of about 200 ml; most likely this compartment is distended lymphatics from initial lymphatics to the thoracic duct. Therefore, peripheral lymphatic pressure probably increased along with the distension of the lymphatics during long-term elevation of outflow venous pressure. Drake & Abbott (1992) demonstrated that increased neck vein pressure caused little pressure increase in peripheral lymphatics in unanaesthetized sheep. They kept outflow pressure high for less than 1 h, however, and it might take several hours for the pressure to build up in peripheral lymphatics.

In summary, we studied the lymph flow pattern in the intact thoracic duct by recording real-time absolute volume flow using a non-invasive flow probe. A regular pulsatile flow was independent of cardiac activity and respiratory movement. With stepwise increase of outflow venous pressure, thoracic duct lymph flow was maintained in most animals until the outflow venous pressure was elevated to 20–30 cmH_2O, suggesting that the intrinsic propulsive activity of the thoracic duct can compensate for a considerable rise in outflow pressure. With the elevation of outflow pressure to a level that initially depressed thoracic duct lymph flow, the flow showed no recovery within a 25 h observation period and during this period some of the fluid in the peripherally formed lymph was probably transported into the bloodstream, most likely in the lymph nodes.

ADAIR, T. H. & GUYTON, A. C. (1983). Modification of lymph by lymph nodes. II. Effect of increased lymph node venous blood pressure. *American Journal of Physiology* **245**, H616–622.

ADAIR, T. H. & GUYTON, A. C. (1985). Modification of lymph by lymph nodes. III. Effect of increased lymph hydrostatic pressure. *American Journal of Physiology* **249**, H777–782.

AUKLAND, K. & NICOLAYSEN, G. (1981). Interstitial fluid volume: Local regulatory mechanisms. *Physiological Reviews* **61**, 556–643.

AUKLAND, K. & REED, R. K. (1993). Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiological Reviews* **73**, 1–78.

BROWSE, N. L., RUTT, D. R., SIZELAND, D. & TAYLOR, A. (1974). The velocity of lymph flow in the canine thoracic duct. *Journal of Physiology* **237**, 401–413.

CAMPBELL, T. & HEATH, T. (1973). Intrinsic contractility of lymphatics in sheep and in dogs. *Quarterly Journal of Experimental Physiology* **58**, 207–217.

COLE, W. R., WITTE, M. H., KASH, S. L., RODGER, M., BLEISCH, V. R. & MUELHEIMS, G. H. (1967). Thoracic duct-to-pulmonary vein shunt in the treatment of experimental right heart failure. *Circulation* **36**, 539–543.

COLIN, G. (1873). *Traité de Physiologie Comparée des Animaux*, 2nd edn. Balliere, Paris.

DEPNER, T. A., KRIVITSKI, N. M. & MACGIBBON, D. (1995). Hemodialysis access recirculation measured by ultrasound dilution. *American Society for Artificial Internal Organs Journal* **41**, M749–753.

DRAKE, R. E. & ABBOTT, R. D. (1992). Effect of increased neck vein pressure on intestinal lymphatic pressure in awake sheep. *American Journal of Physiology* **262**, R892–894.

DRAKE, R. E., ANWAR, Z., KEE, S. & GABEL, J. C. (1993). Intestinal lymphatic pressure increases during intravenous infusions in awake sheep. *American Journal of Physiology* **265**, R703–705.

DRAKE, R. E. & GABEL, J. C. (1991). Effect of outflow pressure on intestinal lymph flow in unanesthetized sheep. *American Journal of Physiology* **260**, R668–671.

DRAKE, R., GIESLER, M., LAINE, G., GABEL, J. & HANSEN, T. (1985). Effect of outflow pressure on lung lymph flow in unanesthetized sheep. *Journal of Applied Physiology* **58**, 70–76.

FLOREY, H. (1927). Observations on the contractility of lacteals. Part I. *Journal of Physiology* **62**, 267–272.

FÖLDI, M. & PAPP, N. (1961). The role of lymph circulation in congestive heart failure. *Japanese Circulation Journal* **25**, 703–708.

HALL, J. G., MORRIS, B. & WOOLLEY, G. (1965). Intrinsic rhythmic propulsion of lymph in the unanaesthetized sheep. *Journal of Physiology* **180**, 336–349.

HARTMAN, J. C., OLSZANSKI, D. A., HULLINGER, T. G. & BRUNDEN, M. N. (1994). *In vivo* validation of a transit-time ultrasonic volume flow meter. *Journal of Pharmacological and Toxicological Methods* **31**, 153–160.

HELLER, A. (1869). Ueber selbstständige rhythmische Contractionen der Lymphgefässe bei Saugetieren. *Zentralblatt für die medizinischen Wissenschaften* **7**, 545–548.

LAINE, G. A., ALLEN, S. J., KATZ, J., GABEL, J. C. & DRAKE, R. E. (1986). Effect of systemic venous pressure elevation on lymph flow and lung edema formation. *Journal of Applied Physiology* **61**, 1634–1638.

LAINE, G. A., ALLEN, S. J., KATZ, J., GABEL, J. C. & DRAKE, R. E. (1987). Outflow pressure reduces lymph flow rate from various tissues. *Microvascular Research* **33**, 135–142.

LASCELLES, A. K. & MORRIS, B. (1961). Surgical techniques for the collection of lymph from unanesthetized sheep. *Quarterly Journal of Experimental Physiology* **46**, 199–205.

LESSER, K. A. (1871). Eine Methode um grosse Lymphmengen vom lebenden Hunde zu gewinnen. *Berichte über die Verhandlungen der königlich Sächsischen Gesellschaft der Wissenschaften zu Leipzig. Mathematisch-Physische Classe* **23**, 590–616.

MCGEOWN, J. G., MCHALE, N. G., RODDIE, I. C. & THORNBURY, K. (1987). Peripheral lymphatic responses to outflow pressure in anaesthetized sheep. *Journal of Physiology* **383**, 527–536.

Ohhashi, T., Azuma, T. & Sakaguchi, M. (1980). Active and passive mechanical characteristics of bovine mesenteric lymphatics. *American Journal of Physiology* **239**, H88–95.

Quin, J. W. & Shannon, A. D. (1977). The influence of the lymph node on the protein concentration of efferent lymph leaving the node. *Journal of Physiology* **264**, 307–321.

Reddy, N. P. & Staub, N. C. (1981). Intrinsic propulsive activity of thoracic duct perfused in anesthetized dogs. *Microvascular Research* **21**, 183–192.

Sakai, T., Yabuki, S., Chang, K., Kambayashi, T., Nakamura, R., Takeyasu, N., Taira, K., Hirota, A., Machii, K. & Seki, K. (1985). Effect of increased systemic venous pressure on thoracic duct and peripheral lymph flow in dogs. *Lymphology* **18**, 64–67.

Schad, H., Flowaczny, H., Brechtelsbauer, H. & Birkenfeld, G. (1978). The significance of respiration for thoracic duct flow in relation to other driving forces of lymph flow. *Pflügers Archiv* **378**, 121–125.

Smith, R. O. (1949). Lymphatic contractility. A possible intrinsic mechanism of lymphatic vessels for the transport of lymph. *Journal of Experimental Medicine* **90**, 497–509.

Staub, N. C. (1974). Pulmonary edema. *Physiological Reviews* **54**, 678–811.

Szabó, G. & Magyar, Z. (1967). Effect of increased systemic venous pressure on lymph pressure and flow. *American Journal of Physiology* **212**, 1469–1474.

Tanaka, M & Robotham, J. L. (1992). Effects of inspiratory diaphragmatic descent on inferior vena caval venous return. *Journal of Applied Physiology* **72**, 597–607.

Waldeck, F. (1965). Zur Motorik der Lymphgefäße bei der Ratte. II. Die contractilen Eigenschaften der Muskulatur der Leberlymphgefäße. *Pflügers Archiv* **283**, 294–300.

Wégria, R., Entrup, R. W., Jue, J. & Hughes, M. (1967). A new factor in pathogenesis of edema of cardiac origin. *American Journal of Physiology* **213**, 94–101.

Wégria, R., Zekert, H., Walter, K., Entrup, R., De Schryver, C., Kennedy, W. & Paiewonsky, D. (1963). Effect of systemic venous pressure on drainage of lymph from the thoracic duct. *American Journal of Physiology* **204**, 284–288.

Werner, B. (1965). Thoracic duct cannulation in man. I. Surgical technique and a clinical study on 79 patients. *Acta Chirurgica Scandinavica*, **353**, suppl. 5–32.

Acknowledgements

We want to thank Morten Eriksen and Bruce Piercey for their help with the computer work. This study was supported by The Scandinavia-Nippon Sasakawa foundation and The University of Tsukuba Project Research on The Circulatory Biosystem, The Anders Jahres Foundation for the Promotion of Sciences and The Norwegian Research Council on Cardiovascular Diseases.

Author's email address

M. Onizuka: masataka@md.tsukuba.ac.jp

Received 8 July 1996; accepted 12 May 1997.