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Long Term Physiologic Study of Thoracic Duct Lymph and Lymphocytes in Rat and Man

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Summary

Techniques for study of thoracic duct lymph and lymphocyte circulations in rats and in man are presented. Radiographic observations, measurements of lymph flow, lymphocyte output, blood lymphocyte level, intra-thoracic pressures and radioactive determinations in the lymph and blood of ^{51}Cr labelled lymphocytes, indicate that the techniques are suitable for long-term study of thoracic duct circulations under physiologic conditions.

The thoracic duct is the common and major pathway for the circulations of lymph (1) and immunologically competent lymphocytes (2, 3), and sampling of lymph and lymphocytes is done by means of a thoracic duct fistula. The classical technique of thoracic duct fistula of *Bollman* in the rat (4) and of *Bierman* in man (5) interrupts the thoracic

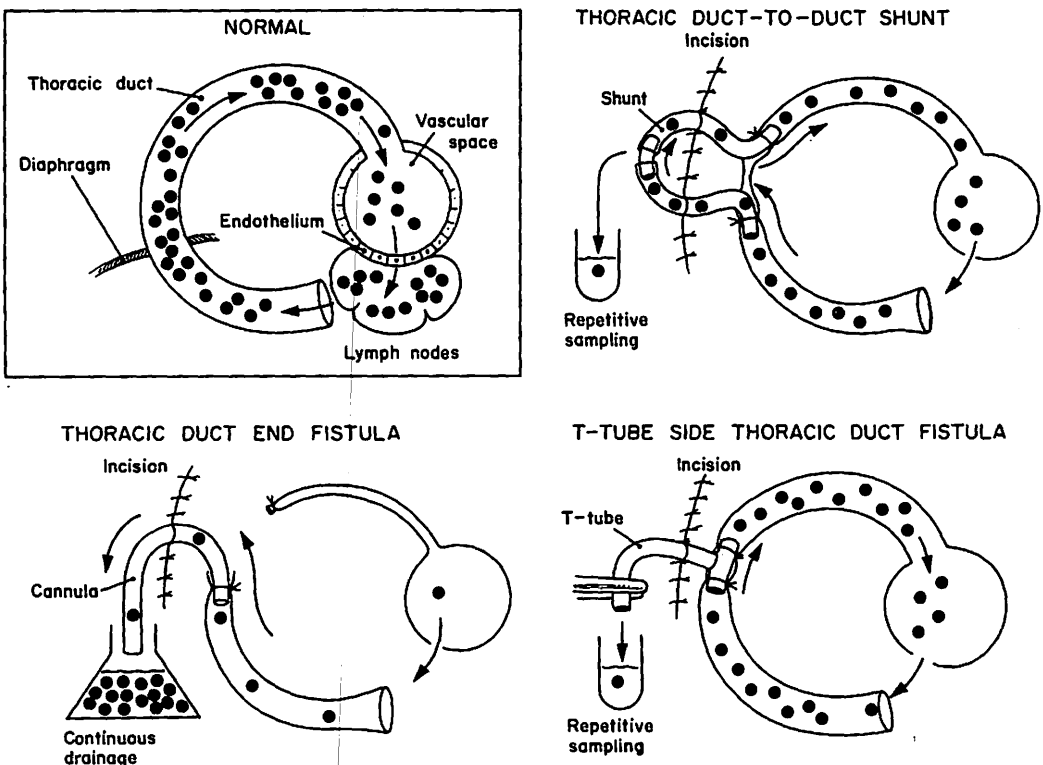

Fig. 1: Schematic representation of the recirculation of small lymphocytes indicated by the black dots (upper left hand corner), and of the principles of three techniques of thoracic duct lymph and lymphocyte sampling.

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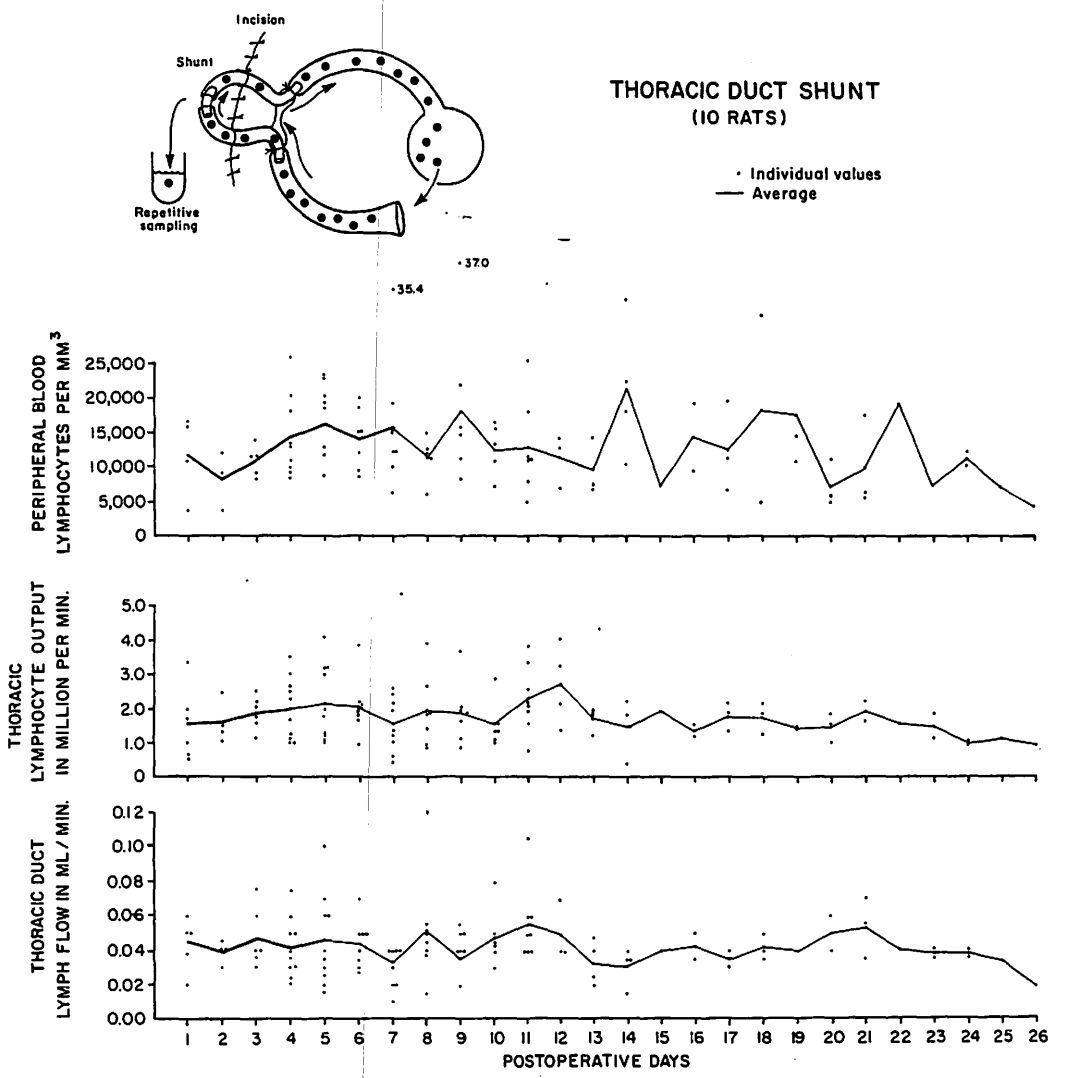

Fig. 2: The means of 26 average daily values are:
peripheral blood lymphocyte, 12,200/mm^3 (range 8,200 to 15,600); thoracic duct lymphocyte output, 1,700,000/min (range 1,540,000 to 2,190,000); thoracic duct lymph flow, 0.042 ml/min (range 0.033 to 0.052).

duct at the site of cannulation and creates a permanent external end-fistula. This method has two serious disadvantages: (a) diversion to the outside of the entire flow of lymph which results in loss of fluid, proteins and lymphocytes, the very objects under study. Techniques for reinfusion of the drained lymph have been described (6, 7) but they are too complex for general use; (b) blockage of the duct, which precludes study of the thoracic duct circulations under physiologic conditions. Because of these disadvantages, the technique of end-cannulation applies only to acute short-term experiments and it has found little acceptance for clinical use.

To eliminate these drawbacks, two new techniques of thoracic duct cannulation have been developed, the principles of which are depicted in Fig. 1.

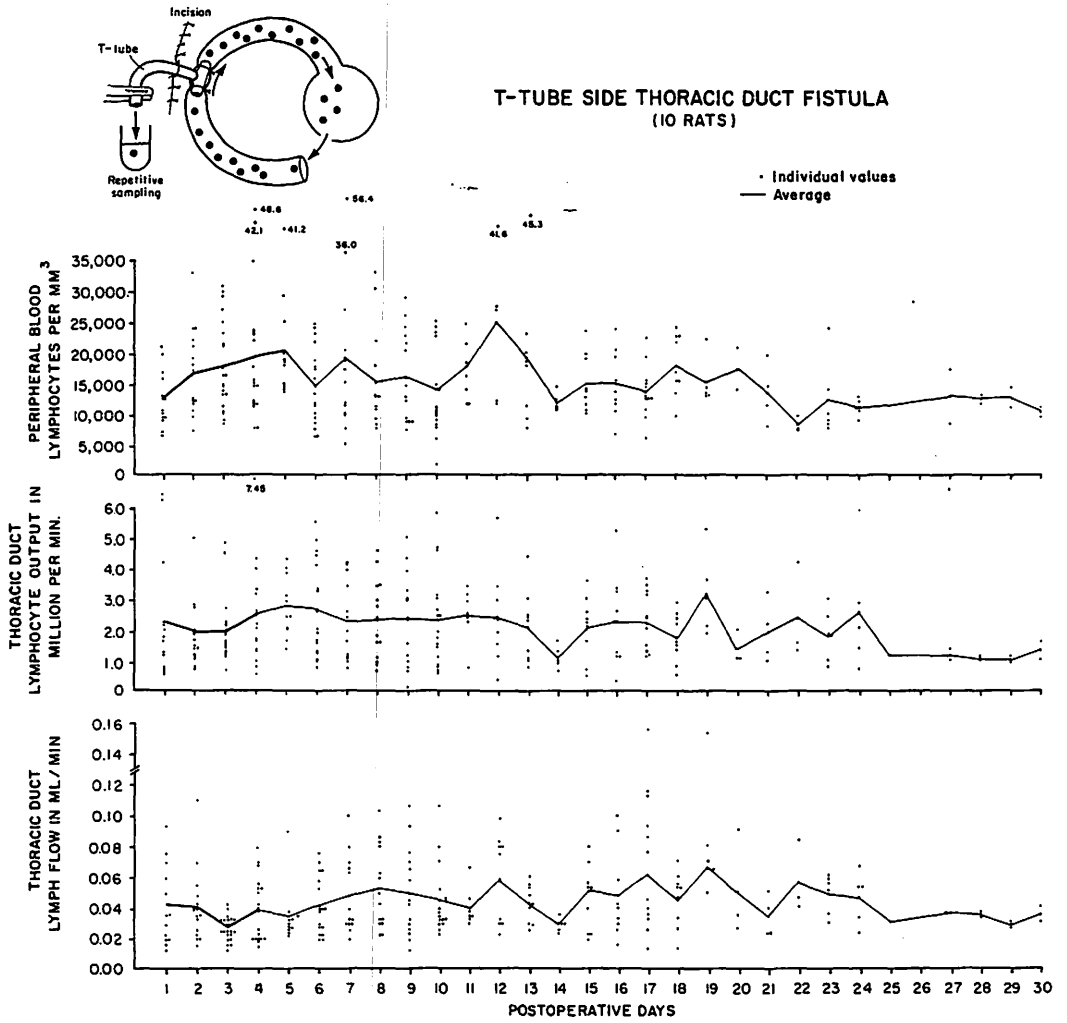

Fig. 3: The means of 30 average daily values are:
peripheral blood lymphocyte, 15,000/mm^3 (range 12,600 to 20,300); thoracic duct lymphocyte output, 2,030,000/min (range 1,980,000 to 2,800,000); thoracic duct lymph flow, 0.044 ml/min (range 0.027 to 0.053).

The first method, called "thoracic duct to duct shunt", creates, with a silicone catheter, a shunt between the caudad and cephalad ends of the duct. The mid-portion of the shunt is located outside the body and permits access to the entire lymph flow for observation and sampling. This technique has been applied first in rats (8) using the cisterna chyli in the abdomen as the site for cannulation, then in man by *Liljeqvist* (9), cannulating the cervical thoracic duct.

The second technique, called "thoracic duct side-fistula", consists in cannulating the thoracic duct through one of its wall only, thereby leaving the duct intact and patent. In rats, a short T-tube is placed within the cisterna chyli (10). In man, a straight catheter is positioned in the cervical portion of the thoracic duct (11). Sampling is performed by simple gravity drainage 30 cm below the level of the fistula. Despite patency of the thoracic duct cephalad to the point of cathe-

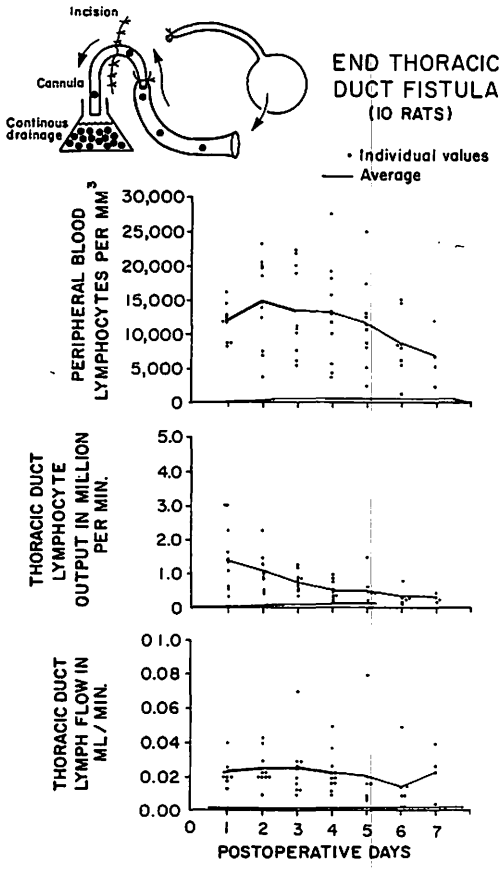

Fig. 4: On day 1, the average values are: peripheral blood lymphocytes, 11,700/mm^3 (range 8,300 to 16,200); thoracic duct lamphocyte output, 1,370,000/min (range 360,000 to 3,010,000); thoracic duct lymph flow, 0.023 ml/min (range 0.013 to 0.04). On day 7, the average values are: peripheral blood lymphocytes, 6,700/mm^3 (range 2,400 to 11,900); thoracic duct lymphocytes output, 260,000/min (range 150,000 to 410,000); thoracic duct lymph flow, 0.024 ml/min (range 0,005 to 0.04).

ter exit during sampling, there is preferential diversion of the entire lymph flow to the outside.

Both methods allow study of thoracic duct circulation under stable and physiologic conditions for several weeks and permit easy and repetitive sampling without loss of lymph or lymphocytes.

Animal studies were conducted in Sprague-Dawley rats. The operation was performed under ether anesthesia and the animals housed in specially designed restraining cages (12). The parameters studied were: blood lymphocyte concentration, thoracic duct lymphocyte output, thoracic duct lymph flow, duration of fistula function and weight loss during the experiment.

Thoracic duct-to-duct shunts were constructed in 10 rats. The results are presented in Fig. 2. The average duration of shunt function was 14.7 days (range 8 to 26 days). The average weight loss during this period was 44 grams per animal, or 9.3 % of the preoperative weight. Thoracic duct side-fistulae were created in 10 other rats and the results are indicated in Fig. 3. The fistulae functioned for an average of 17.8 days (range 8 to 30 days) and the animals lost an average of 81 grams, or 16 % of their preoperative weight. During the entire experiment, all animals in both groups remained in good condition. A *Bollman*'s end-fistula was established in 10 control rats. Average duration of drainage was 6.4 days (range 5 to 8 days). Three animals died and drainage stopped in three others. All were in very poor condition at the time of last sampling. The average weight loss per animal was 101 grams, or 25 % of the preoperative weight. The measured parameters decreased between day 1 and day 7 (Fig. 4).

The stability of the thoracic duct lymph flow, lymphocyte output, and blood lymphocyte level, when studied with a duct-to-duct shunt or a side-fistula, indicate that the thoracic duct circulations apparently remain normal under these experimental conditions. The new techniques are suitable for prolonged physiologic studies and therefore they are superior to the *Bollman*'s end-fistula.

Thoracic duct pressure was recorded 4 days after establishement of a T-tube side-fistula in three rats. One representative tracing is shown in Fig. 5. Respiratory fluctuations range between 0.5 und 1 mm of Hg. Sudden elevation of pressure of short duration and up to 2 mm of Hg. occurred every 8 to 10 seconds. This probably refects rhythmic contractions in the lymphatic bed which

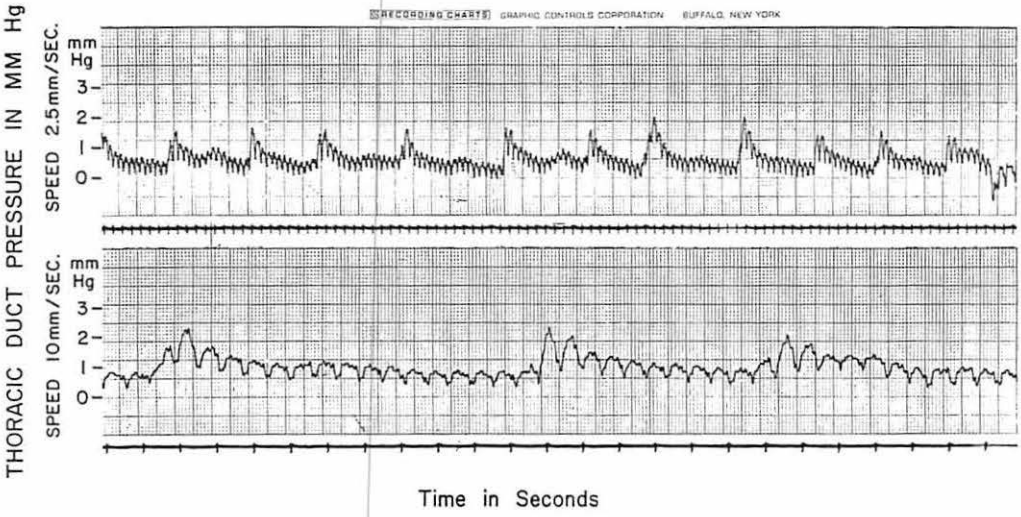

Fig. 5: Thoracic duct pressure in rat recorded via a side-fistula (see text).

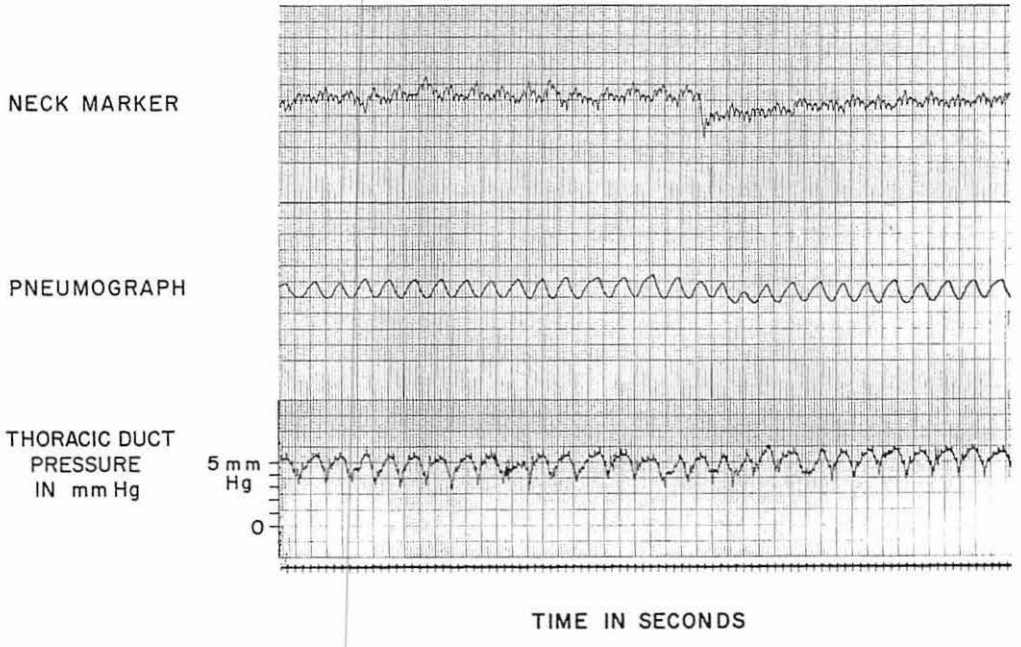

Fig. 6: Respiratory variations of the thoracic duct pressure during quiet breathing (patient J.A.). The downward deflection of the pneumograph indicates inspiration. The baseline pressure fluctuates between + 3.5 mm and + 5.5 mm of Hg.

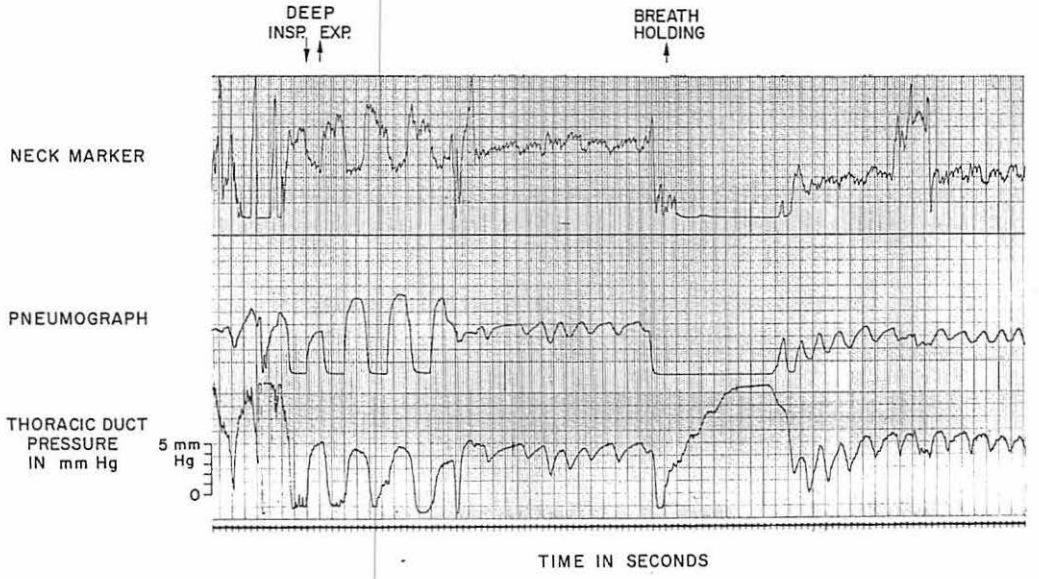

Fig. 7: Respiratory variations of the thoracic duct pressure during deep breathing and breath holding (patient J.A.). During deep inspiratory effort the pressure falls as low as – 1 mm of Hg. During breath holding after deep inspiration, the pressure rises up to 10 mm of Hg.

are believed to be important for the propulsion of lymph. Similar findings have been reported in the lumbar lymph duct of the sheep (13).

The thoracic duct circulation was studied in 2 patients by means of a side-fistula constructed in the neck (11). *Case 1:* S.P. was a 50-year old male admitted for post-orchiectomy treatment of metastatic seminoma to the lungs (11). *Case 2:* J.A. was a 44-year old male in whom Hodgkin's disease involving cervical, mediastinal and aortic lymph nodes, had been diagnosed two and one half years previously. The patient had then received a course of combined chemotherapy (nitrogen mustard) and radiotherapy to the supradiaphragmatic lymph nodes, followed 8 weeks later by irradiation to the nodes below the diaphragm. He was in clinical remission at the time of this study. The fistulae were kept functioning for 5 days (S.P.) and 21 days (J.A.), then electively pulled out without complications.

Thoracic duct lymph flow, lymph cellular composition, lymphocyte output, and blood lymphocyte count were measured daily. The results are presented in Table 1.

Thoracic duct pressure measurements made in the cannula filled with saline and in the supine position are shown in Table II. Recordings of intra-thoracic duct pressure obtained on the 18th post-operative day in patient J.A. during quiet breathing, deep breathing and breath holding are given in Fig. 6 and Fig. 7.

Radiographic studies of the terminal thoracic duct obtained by injection of contrast medium directly into the duct were done in both patients. The appearance of the duct in patient J.A., on the first, 8th and 12st post-operative days is shown in Fig. 8 and compared with a conventional lymphangiogram performed from the foot three years previously. Despite the presence of the catheter in place, lymph flowed normally past the catheter from the thoracic duct into the internal jugular vein.

In rats and in the patients, thoracic duct lymphocytes were labelled with ^{51}Cr according to the technique of *McMillan* (14). The

Table 1 T.D.* Lymph Measurements

	Patient	Days postoperative 1	2	3	4	5	7	9	10	11	15	21
T.D.* Lymph flow (ml/kg/hr)	S.P.	1.5	1.5	1.2	1.2	1.5						
	J.A.	0.8	0.3	0.5	0.5	0.5	0.7	0.9	0.6	0.4	0.3	0.3
T.D. lymphocyte concentration/mm^3	S.P.	10,770	8,600	8,500	9,000	8,100						
	J.A.	1,250	2,175	1,770	1,250	1,000	1,750	1,038	1,100	1,450		1,013
% small lymphocytes in T.D. lymph	S.P.	94	88	94	95	96						
	J.A.			73	86		95	68	87	91	98	80
T.D. RBCX $10^3/mm^3$	S.P.	170	265	275	333	260						
	J.A.			168	65		280	167	241	152	117	69
Peripheral blood absolute lymphocyte count/mm^3	S.P.	3,000	2,300	3,800	3,800							
	J.A.	1,189		2,400	1,625	1,456		1,682	974	2,220		1,450
51 Cr counts/10^6 lymphocytes in T.D. lymph	S.P.		0,19	1.22	1.48	1.24						
	J.A.	4.3	5.0			1.3		3.8		4.3		2.9

* Thoracic duct

Table II T.D.* Pressures

	Patient	Days postoperative 1	2	3	4	5	7	9	10	11	15	21
Quiet breathing	S.P.		+ 5 to + 6		+ 5 to + 6	+ 5 to + 6						
Deep inspiration			0		0	− 1						
Deep expiration			+ 14	+ 12		+ 17						
Quiet breathing	J.A.	+ 8	+ 9	+ 6	+ 9			+ 8	+ 8		+ 9	+ 9
Deep inspiration		+ 3	+ 1	+ 1	0			+ 4	− 2		0	− 1
Deep expiration		+ 11	+ 12	+ 12	+ 10			+ 12	+ 10		+ 12	+ 12

* Thoracic duct

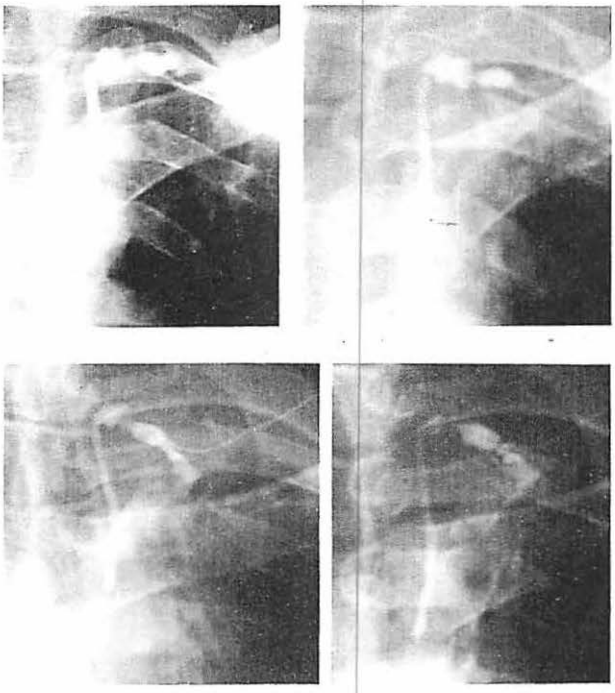

Fig. 8: Radiographic appearance of the terminal thoracic duct (J.A.).
A: during conventional foot lymphangiogram (1968).
B: 1 day after construction of the side-fistula (1971).
C: on the 8th postoperative day.
D: on the 21st postoperative day.
Note that the irregular configuration of the terminal portion of the duct was present 3 years prior to construction of the fistula and did not change thereafter. During the 3-weeks interval (C-D), the duct remained of the same caliber, an observation speaking in favor of an unobstructed thoracic duct circulation despite the presence of the catheter.

labelled cells were reinfused directly into the cephalad thoracic duct (rat) or intravenously (patients). A total number of 80–100 x 10^6 cells (rat), 653.4 x 10^6 cells (S.P.) and 95 x 10^6 cells (S.P.) and 95 x 10^6 cells (J.A.) were injected, representing a net radioactivity of 13.6 x 10^6 counts/min (S.P.) and 0.15 x 10^6 counts/min (J.A.). The radioactivity of lymph and blood samples was expressed as counts/min/10^6 lymphocytes, after correction for RBC tag. The demonstration of a prompt appearance of labelled lymphocytes in the thoracic duct (within 2 hours in patient J.A.) proves the existence of a re-circulation of lymphocytes in rats and in man. This finding and the stable recovery of labelled cells in the lymph over a prolonged time (Table 1) give an example of the usefulness of the side-fistula and indicate a means to study the pool of re-circulating lymphocytes.

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EDITORIAL

In 1976, a brief questionnaire was circulated to the members of the International Society of Lymphology seeking information regarding the attitudes of the members towards the official Journal of the Society, *Lymphology.* Of the *400* questionnaires mailed to the active members, 101 were returned by November, 1976, and form the basis for the following short report.

Approximately 40% read all, or almost all, papers published in *Lymphology;* 39% read all, or almost all, clinical papers; 15% read all, or almost all, laboratory papers; and 6% read usually no papers. Approximately 47% felt that the balance between clinical and laboratory papers was fine; 12% felt there were too many clinical articels, whereas 33% believed there were too many laboratory articles; and 8% did not respond.

Approximately 70% felt the size of the Journal was fine, the remainder feeling the Journal was too small. However, only 47% stated their willingness to support an increase in size by paying more for subscription rates, as compared to 36% who felt contrary-wise, and 17% who did not respond.

Impressions regarding the overall quality of the published articles were as follows (for the clinical and basic science papers, respectively): above average, 17% and 26%; average, 63% and 43%; below average, 10% and 5%; and, can't evaluate, 7% and 16%, with no comments from 3% and 10%.

Fifty-eight percent of the respondes believe that Lymphology should be continued as is; whereas 22% suggested considering merger with another society journal or journals, which might have similar, parallel interests; and 7% recommended discontinuation of publication (which probably would decrease ISL dues). Thirteen percent did not respond to this final question.

Although the above responses are open to numerous interpretations, I have tentatively concluded that: a) most of the responding members find the Journal to be satisfactory, although perhaps they feel more clinical articles could be included; b) that most find the quality of the articles to be average or above average; and c) that there is some indication that an increase in size should be considered, although this means increasing the subscription rates. There appears to be very little interest in discontinuing the publication of the Journal. Interestingly, there appears to be some interest to consider merging the Journal with another scientific publication, although the majority of the members believe that the Journal should continue as is.

R.A. Castellino