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Studies on Human Peripheral Lymph

I. Sampling Method

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Summary

A method is described for sampling of peripheral lymph in man. A peripheral lymphatic of the leg has been cannulated in 59 patients with various malignancies, most of whom had lymphoproliferative diseases. The cannulation was successful in 40, most of the failures were from the early period. The cannulation could be maintained for many days. The mean lymph flow was 0.78 ml/hour, the cell content 162/mm^3 with a mean output of 129 x 10^3 cells/hour. Differential counts revealed 70-96 per cent lymphocytes. The method is considered harmless and generally useful for lymphocyte circulation studies.

It is now well established that lymphocytes are migrating and recirculating cells, though this probably applies more to those derived from the thymus (T cells) than to the plasma cell precursors (B cells) (1,2). The main area for recirculation is in the lymph nodes where the blood lymphocytes reenter the lymphoid tissue through the walls of the post-capillary venules (1). However, some lymphocytes also migrate through the blood vessel into *non*-lymphoid tissue (for instance, sub-cutis) and hence into the lymphatics and back to the lymph nodes (3, 4, 5). Experiments in animals have revealed that the peripheral lymph contains few cells (3, 4, 5) compared with the intermediate and central lymph which, after passage through lymph nodes, has been enriched by numerous lymphocytes.

There are a few reports based on studies of the thoracic duct lymph indicating disturbances of lymphocyte recirculation in patients with chronic lymphocytic leukemia (6, 7), and probably normal recirculation in patients with Hodgkin's disease (8). Further observations of this type are required to gain more insight into the patho-physiology of the lymphocyte population in lymphomas. Thoracic duct cannulation is, however, rather a grave intervention for experimental purposes in man, even though it can be performed without serious complications. It must be assumed that disturbances in the kinetics of lymphocyte circulation will be reflected also in the cells of the peripheral lymph. Alterations could for example be caused by defects in the lymphocytes themselves, or a shift in the relative amounts of B and T cells in the blood. A technique has therefore been developed for prolonged collection of peripheral lymph in man as has previously been done in sheep (4, 5). The present paper describes the method and summarizes the results obtained in the first 59 cannulations.

Method

All cannulations have been done on one of the subcutaneous lymphatics in the lower half of the leg, usually at a point about 15 cm above the ankle joint. The procedure has changed somewhat during the period of development. For the last 20 cannulations it has been as follows:

Cannulation is performed with a 20 - 25 cm polyethylene tube No. 60 (outer diameter 1.22 mm). The tube is drawn out at one end to a thin tip, and then siliconized, followed by sterilisation in 2% cetyl pyridinium chloride, Pyrisept®, for 24 hours. The tube is then flushed with sterile 0.9 per cent saline, dried and stored. Lymph is collected in 15 ml sterile glass vials containing 1 ml 0.9 percent saline with 20 IU of heparin. These have perforated rubber corks through which the catheter is inserted. To insure cold sampling the vials are placed in a 4 x 8 x 10 cm isopore box together with an ice-filled vial. The box has an opening in the front through which the vials can be removed (Fig. 1).

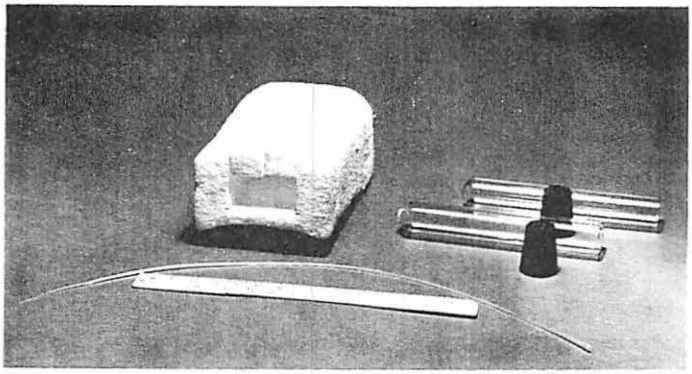

Fig. 1. Equipment for cannulation: isopore box with glass vials and rubber corks (one pierced for tubing). Polyethylene tube drawn out to a slender tip.

Before use the rubber cork is threaded onto the polyethylene tube. The thick end of the tube is connected by a needle to a syringe containing 0.9 per cent saline with 20 IU heparin/ml and is filled with this solution.

The vessel is exposed after visualization with Patent Blue-Violet (PBV), isolated for 2 - 3 cm and thoroughly stripped of fat and fibrous tissue, using the same technique as for lymphography (9)..The drawn-out tip of the tube is then cut at the level where it has about the same diameter as the lymph vessel. The lymphatic is punctured with a needle and the polyethylene tube is inserted 5 - 10 mm in a distal direction, fastened with a catgut ligature around the vessel and taped to the skin outside the wound. The syringe is removed and the open end of the tube lowered so that the lymph flow is aided by gravity. When lymph begins to flow, the tube is inserted into the glass vial with the open end under the heparin solution. The isopore box is taped to the leg at a level which insures that the end of the tube is at a lower level than the tip, when the patient is standing as well as when he is lying on his back (Fig. 2). After the wound has been closed, the patient is asked to walk around and do so repeatedly during the days of lymph sampling.

The collection vials are changed at least three times a day, depending on the lymph flow. The ice containing vials are changed about every two hours. The sampling time and lymph volume are recorded on each specimen. The cells are counted in a Bürker counting chamber after 1/1 dilution with 1 percent orcein in 60 percent acetic acid. The cell content of the whole chamber is counted. Differential counts are made on concentrations prepared with a Shandon Cytocentrifuge operated at 750 r.p.m. for 15 minutes. After fixation the preparations are stained with *May Grünewald-Giemsa.*

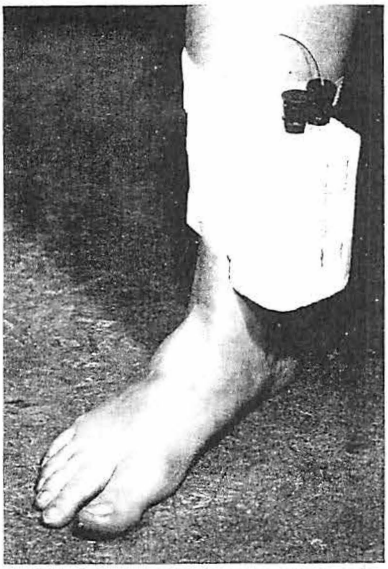

Fig. 2 Cannulated leg with collecting equipment in position

Table 1 Peripheral lymph from the leg in 40 patients with various malignacies including lymphomas. Mean values.

Lymph flow, ml/hr	0.78 ± 0.08
Leucocytes/mm³	162 ± 24.5
Leucocyte output x 10^3/hr	129 ± 28.2

At the end of the sampling period the tube is closed. A roll of gauze is placed over the lymph vessel and pressure is applied with an elastic bandage. The tube is pulled out the next day. Elastic pressure is maintained for two days.

Results

Fifty-nine cannulations have been performed in patients with various malignancies, mostly lymphomas. In the first seven cases a tube of about 1 meter in length was used causing the flow to stop after a few hours due to coagulation. Forty of the remaining 52 cannulations with a shorter tube (20 - 25 cm) have been successful. In the first 15 of these, lymph sampling was maintained for 2 days, but in the last 25 cases it has been continued for 5 or more days. A thread of fibrin was formed in the catheter in most of the patients. The threads usually projected out of the catheter and could be removed once or twice a day.

The lymph is blue for the first 24-48 hours due to PBV, and later the colour changes to yellow. The flow is higher during the day than during the night, probably due to exercise. The mean flow in 40 cases was 0.78 ml/hour, the mean cell number 162/mm³ and the mean output per hour 129 x 10^3 cells (Table 1). Samples from the first hours after cannulation demonstrated regularly fewer cells than at later intervals. Also, the night lymph contained significantly more cells/volume than the day lymph. The cell output/hour was usually also somewhat higher during the night than the day, but the difference was not significant.

Differential counts revealed that 70 - 96 percent of the cells were small lymphocytes. Most of the other cells were macrophages and monocytes with some granulocytes. Most of the samples also contained some erythrocytes. Histological sections of the fibrin threads shows numerous cells; lymphocytes as well as phagocytes and granulocytes.

There was considerable variation from patient to patient concerning the cell number in the lymph, probably related to the disease. The highest mean output was 742 x 10^3 cells/hour in a patient with lymphosarcoma. The lowest output was 11 x 10^3 cells/hour in a patient with stage IV *Hodgkin's* disease. Details about the lymphoma patients will be published. No controls in healthy patients have been studied so far. However, successful cannulation has been performed in 13 patients with malignancies other than lymphomas. Six of these had had no treatment other than small surgical interventions and were all in good condition. Table 2 shows that there is variation in the cell output also in this group. There is no obvious correlation between lymphocyte number in blood and lymph, but the material is small and not homogenous what drainage time and diseases are con-

Table 2 Cell content and flow of peripheral lymph from the leg in 6 patients with malignancies other than lymphomas.

			Lymph					Blood	
				Leucocytes		Lymphocytes		Leucocytes	Lymphocytes xx
Pat. No.	Diagnosis	Cannulation time in hours	ml/hr	per mm^3	%[x]	per mm^3	x 10^3 per hour	x $10^3/mm^3$	x $10^3/mm^3$
15	Seminoma testis	24	1.10	37	73	27	29	4.0	2.7
16	" "	25	0.80	131	81	106	85	6.7	3.9
19	" "	28	0.98	89	90	80	78	5.1	2.9
33	Ca. labii	267	0.72	274	96	265	189	9.5	3.0
36	Ca. cutis	214	0.54	303	93	282	152	7.0	3.5
53	Ca. cutis	91	0.34	241	94	227	77	10.0	5.5
Mean		108	0.75	179	88	165	102	7.1	3.6

x Based on differential counts of 400 - 1000 cells of day lymph.
xx Based on differential counts of 500 - 1000 cells of the early morning sample prior to cannulation.

cerned. Further studies are therefore necessary to answer this question. – The method is very simple and can easily be performed by those experienced in lymphography. The procedure may be considered harmless and no complications have been observed in the 59 cannulations performed.

Discussion

Abundant peripheral lymph can easily be obtained in man by cannulation of a subcutaneous lymphatic on the leg. A relatively pure suspension of lymphocytes can be obtained. It is suggested that studies of this lymph can contribute valuable information about the physiology and pathophysiology of the lymphocyte population. There are some important points concerning the technique. Cannulation at a relatively low level in the leg usually gives a better lymph flow than cannulation at a higher level. The reason for this is probably that the lymphatics may divide into two or more branches higher up. Also, to achieve a good flow it is important to use as short and as wide a tip as possible and to keep the tube short. The fibrin threads formed within the catheter have to be removed to maintain the flow. When removed daily, they do not influence the flow rate. They were found just as often in patients with a high flow as in those with a low flow rate. The threads contained a lot of cells and the cell counts in the lymph must therefore be regarded as minimum values. There is, however, no indication that cell trapping in the fibrin threads can account for the variation in cell number among the various groups of patients. There seem to be fewer cells within the threads in those with a low output than in those with a high output. Also, the cell output was lower during the first hours after cannulation when fibrin threads were rarely found than at later intervals when the threads were formed more regularly. Thus, Table 2 shows that the mean cell number/mm^3 lymph is lower in those cannulated for 24 hours than in those cannulated for a longer period.

Cooling the lymph during sampling may be unnecessary. It is our impression, however, that the cell morphology has been somewhat better maintained in samples where this has been done. The heparin concentration in the vials is of significance. In the first cannulations a concentration of 200 IU heparin/ml saline was used in the vials. These samples contained a lot of pycnotic and dead granulocytes, especially in lymph collected during the night. Pycnotic cells are rarely found in samples where 20 IU heparin/ml is used.

Working independantly, *Bujar* and *Bărbulescu* (10) sampled peripheral lymph in 25 patients with lymphomas. Lymph was obtained by aspiration from the wound immediately after withdrawal of the needle following lymphography. By this method the lymph can be collected only for a short period and the composition of the lymph might be influenced by the injection of PBV, by röntgen contrast media and by long stasis in the exposed lymph vessel before sampling. *Bujar* and *Bărbulescu* give no data on the composition of the lymph. Their method is, however, very simple and it would be of interest to compare data obtained by their method with that obtained by protracted cannulation.

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