

Time of Exchange of ^{131}I -Labeled Albumin between Plasma and Peripheral Lymph in Man

A. Engeset*, M. Aas**, W. Olszewski*** and J. Sokolowski*

Laboratory of Hematology and Lymphology*, Department of Nuclear Medicine**, Norsk Hydro's Institute for Cancer Research, Norwegian Radium Hospital, Montebollo, Oslo, Norway, and the Surgical Research and Transplantation Laboratory***, Medical Research and Transplantation Laboratory***, Medical Research Center, Polish Academy of Sciences, Warsaw, Poland

Summary

The passage from blood to peripheral lymph of ^{131}I -labeled human serum albumin has been studied in 6 male patients (30-70 years) with malignancies without metastases. On the first day the concentration of radio-labeled albumin in the blood was kept almost constant by repeated i.v. injections. Lymph was collected continuously from a cannulated subcutaneous lymph vessel on the leg.

Two hours after the first i.v. injection of radio-labeled albumin the lymph contained significant amounts of radioactivity in all patients. Equilibrium between radioactivity in blood and lymph was reached after 26 hrs. This indicates a long "wash out time" of unlabeled protein in the interstitial tissue from where lymph has been sampled.

Introduction

In previous studies on 24 hour variation of leg lymph composition in man, no correlation was found between lymph flow and protein concentration (3, 6). The highest flow and protein concentration was found in the early morning hours after night rest. During the day a continuous decrease in protein concentration occurred on a stable or slightly decreasing lymph flow. We attributed this lack of correlation to the time required to wash protein-rich lymph formed during the night rest out of the interstitial space and the lymph vessels, causing a lag between lymph formation and lymph collection. In the present report this time lag has been investigated by registration of the first appearance in sampled lymph of i.v. injected labeled albumin and the equilibrium time between plasma and lymph.

Materials and Methods

The study was performed on 6 male patients mean 55 years (range 30-70), all giving their appropriate consent. They were under treatment for carcinomas (ca. bronchiale 3, ca. cutis 1, ca. gingiva 1, malignant melanoma 1). All were in good physical condition and out of bed during the day.

Lymph collection. A superficial leg lymph vessel was cannulated as previously described (2). Lymph was collected continuously into plastic syringes containing 20 IU of heparin in 0.2 ml saline, taped to the leg. The syringes were replaced at the end of each sampling period. The content of each syringe comprising all lymph sampled during the preceding interval, was analysed separately.

Injection of ^{131}I -labeled human albumin. The transcapillary escape rate of albumin in man is about 5%/hr (7). To achieve a relatively stable level of labeled serum albumin in plasma; it was therefore necessary to give repeated injections. The mean total dose was 28.4 μCi . About 80% of this dose was given at hr. 08.00 in the morning while the patient still was in bed. Later 4% of the total dose was injected every second hour with the last injection at hr. 18.00 in the night. Immediately after the first injection in the morning the patient rose and stayed out of bed for the rest of the day. ^{131}I -labeled human serum albumin was obtained from Institute of Atomic Energy, Kjeller, Norway (code I.K. 21S).

Blood and lymph sampling. The radioactivity was injected through a plastic infusion cannula

placed in the left cubital vein. Blood samples were collected from a cannula in the right cubital vein every second hour, immediately before injection of labeled albumin. At the same time the syringes with the lymph samples were replaced. The last samples were taken at hr. 20.00 in the night. During night rest there was no sampling. On day II blood and lymph sampling started at hr. 08.00 in the morning and every second hour until hr. 16.00. No radioactivity was injected on day II. Lymph flow was measured and the protein concentration and radioactivity measured in the plasma and lymph.

Measurement of protein concentration and radioactivity. Protein concentration was determined by the biuret method. The radioactivity was measured in an Intertechnique CG 4000 gamma counter with counting efficiency about 40%. The count rates were corrected for decay back to the time of injection.

Results

The first lymph samples taken 10–15 min after the patients were out of bed in the morning, contained lymph accumulated during the night when there was a low flow of concentrated lymph. Throughout the day there was, as previously found (3, 6), a drop in lymph/plasma protein concentration ratio and a relatively stable lymph flow (Fig. 1).

The mean radioactivity in plasma and the peripheral lymph is given in Fig. 2. The figure shows that the radioactivity per mg pro-

tein in plasma remained at a relatively stable level during the first 16 hrs., around 75 cpm/mg protein. On the second morning, the plasma radioactivity dropped to about 2/3 of that of the first day with a trend towards further drop during the day.

There was no measurable radioactivity in the first lymph samples on day I. The second samples, however, contained significant amount of radioactivity in all patients (Fig. 2). The radioactivity of the lymph increased continuously throughout the first day to about 1/3 of the plasma level late in the evening. The individual variations were small. On day II there were greater variations from patient to patient. In the first morning sample the specific activity in the lymph was still lower than that of the plasma, but the difference was not significant. Later on the mean values of radioactivity were at the same level in plasma and lymph.

Discussion

Labeled albumin injected intravenously will very rapidly be evenly distributed throughout the whole vascular system. Therefore the albumin transported to the interstitial fluid from the blood capillary in the area studied, will have the same specific activity as the plasma albumin at the same time. However, in the lymph sampled about 15 min. after i.v. injection of labeled albumin the count rate was not significantly above zero. The explanation is that this lymph contains practically only protein which has accumulated in the tissue and

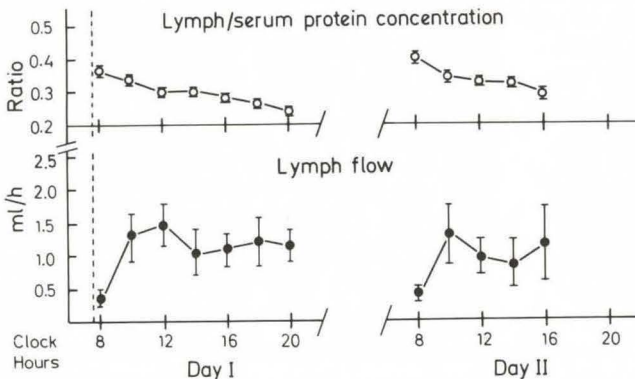


Fig. 1 Lymph flow (●) and ratio between total protein concentration in leg lymph and serum (○). Mean values \pm 1 S.E. from 6 male patients (30–70 years) with malignancies.

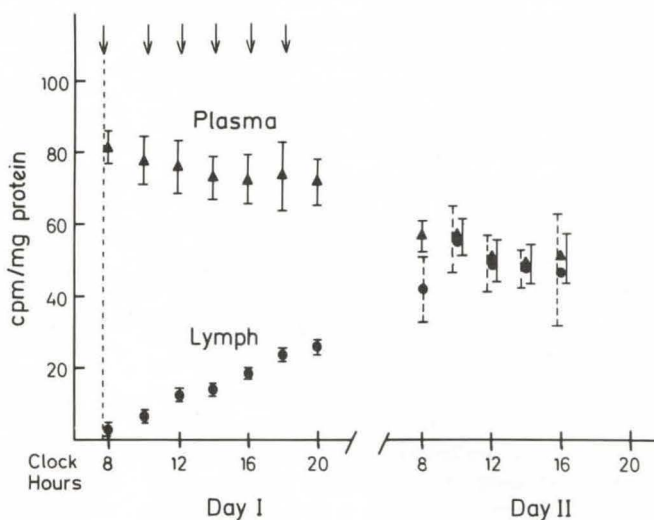


Fig. 2 Radioactivity in plasma and peripheral leg lymph after i.v. injections of ^{131}I -labelled human serum albumin expressed as counts/min. per mg protein. Mean injected dose $28.4 \mu\text{Ci}$. Mean values ± 1 S.E. Arrows indicate time of injections. Same patients as Fig. 1.

initial lymphatics during the preceding night. The very first appearance of labeled albumin in the lymph was not registered, but after two hours significant amounts of radioactivity were present in the lymph samples. This means that it takes less than two hours for the first albumin molecules to cross the capillary membrane, traverse the interstitial fluid space and enter the lymph vessel and collection tube. It cannot be excluded, however, that some of the ^{131}I has passed to the lymph as free iodine. However, the first time of appearance is in good agreement with the findings of Taylor et al. (8) after subcutaneous injection of labeled protein. In the following lymph samples there was a steady but slow increase in radioactivity. This means that there is a slow, gradual disappearance of unlabeled protein in the interstitial tissue with which the labeled protein is mixed.

It has been suggested that the ground substance in the interstitial tissue consists of a colloid-rich, water-poor phase and a water-rich colloid-poor phase (4). Most of the macromolecules forming the interstitial ground substance have a low isoelectric point and accordingly a net negative charge at physiological pH (5). This means that the colloid-rich phase of the ground substance will have very little tendency to bind the negatively charged albumin. Consequently, most of the albumin will stay in the water-rich phase. The necessary time

for reaching equilibrium between the plasma albumin and the lymph albumin in a certain area will be determined by the transcappillary escape rate of serum albumin, the amount of unlabeled protein in the interstitial tissue and lymph in the area studied (9), the distance the molecules have to pass including the length of the tube and the outflow rate of the lymph.

Equilibrium is reached after 26 hours. For the whole body the shortest possible equilibrium time can be calculated: Based on the transcappillary escape rate of between 5 and 6 per cent (7), a back flow to the plasma compartment of about the same order and a tissue fluid lymph protein pool of about the same size as the plasma albumin-pool (1), equilibrium should be reached after about 12 hours. This fits well with the studies performed on dogs by Wasserman et al. (10) where the radioactivity in *central* lymph was studied in anaesthetized dogs after i.v. injection of labeled human serum albumin. They found equilibrium between plasma and thoracic duct lymph after 7–13 hours. This is a significantly shorter equilibrium time than in the present study. This difference might be explained by two factors: First of all the liver contributes 25–50% of the thoracic duct lymph in anaesthetized dogs (11). Because of the discontinuous capillaries in the liver, the lymph from this organ contains nearly as much protein as the circulating plasma (11). This might shorten

the equilibrium time. The disappearance rate of the unlabeled protein with which the labeled protein is mixed is probably also faster in the liver than in peripheral tissue, because of the higher lymph flow.

In conclusion: The first appearance of labeled protein in sampled peripheral lymph in the present experiment shows that the lag due to passage of the protein molecules throughout interstitial tissue, the lymph vessel and collecting tube is less than 2 hours. Equilibrium between plasma and lymph is reached when excess unlabeled protein from the time before the i.v. injection has passed out from the interstitial tissue and lymph vessel. In the present study this took 26 hours. The patients were studied during normal every day activities and night rest. The changing position and changing muscular activity of the extremities will influence capillary filtration and lymph flow and consequently also the wash out time of the interstitial proteins. The time of the first appearance of labeled protein and the equilibrium time between plasma and lymph might be significantly different from the present findings in various steady state situations, for instance when the patients are observed resting in bed for several days.

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A. Engeset, M.D., Lab. of Hematology and Lymphology, Norsk Hydro's Institute for Cancer Research, Norwegian Radium Hospital, Montebello, Oslo, Norway