Lymphology 11 (1978) 143–146

Protein Concentration in Lymph

G. Nicolaysen

Institute of Physiology University of Oslo, Norway

Summary

A brief review is given on the problem of protein concentration in lymph versus protein concentration in interstitial fluid. The possibility of a concentrating ability of the lymphatics is discussed in the light of recent investigations. It is concluded that the final answer to the problem is not known, but that substantial evidence indicate that the protein concentration in lymph and in the interstitial fluid from which the lymph originate is similar.

In recent years much effort has been devoted to establish whether or not the protein concentration and composition of lymph sampled from central lymphatics are the same as those of interstitial fluid. The main reason for the interest in this question is that it is difficult to sample interstitial fluid directly. Thus the colloid osmotic pressure of interstitial fluid, which is of great importance whenever transvascular fluid exchange is considered, has had to be derived indirectly. In many cases lymph has been used as a substitute.

Several workers have suggested that the lymphatics do have an ability to concentrate the lymph proteins (see f. ex. 1,2), resulting in higher protein concentration in lymph than in interstitial fluid. Such a concentrating mechanism could theoretically exist at the lymph capillary membrane or along the lymphatics.

In the following a brief review of some of the later reports concerning this question will be given. It will not deal with possible mechanisms through which any augmentation in lymph protein concentration theoretically could be explained.

In 1975 *Nicolaysen, Nicolaysen* and *Staub* (3) performed a quantitative radioautographic study in which they compared albumin concentration in different sized lymph vessels in the mouse lung. High specific activity ^{125}I-BSA (bovine serum albumin) was injected i.v. and 4 hrs was allowed for mixing of the tracer. Subsequently the lungs were fixed by rapid freezing. Sections were prepared for radioautography and grain density determined over lymphatics. Fig. 1 shows the results from one such lung and Fig. 2 the composite results from three lungs. The authors concluded that no significant change in albumin concentration occurred during the passage of the lymph along the span of detectable (by light microscopy) intrapulmonary lymphatics. The study did not allow comparison of lymph and interstitial fluid. The authors thus could neither prove nor disprove a concentrating effect at the lymph capillary membrane.

At the same time *Taylor* and *Gibson* (4) published a study in which they had compared fluid sampled from implanted capsules with fluid sampled from small lymphatics draining the region of the implanted capsules. In fluid from capsules implanted for less than 4 weeks

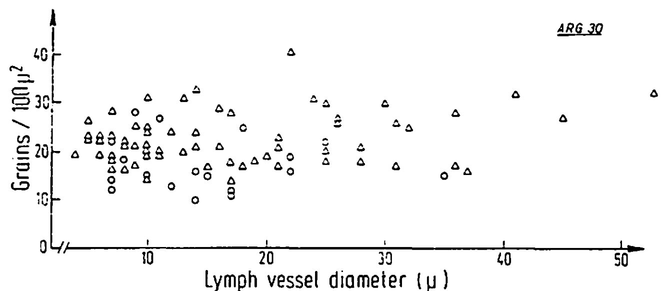

Fig. 1 Grain density in individual lymphatics versus lymph vessel diameters in a rapidly frozen mouse lung. ○: radioautographs exposed 25 days. △ : radioautographs exposed for 24 days. (From Nicolaysen, Nicolaysen and Staub (1975). By permission)

0024–7766/78 1600–0143 $ 04.00 © 1978 Georg Thieme Publishers

Permission granted for single print for individual use.
Reproduction not permitted without permission of Journal LYMPHOLOGY.

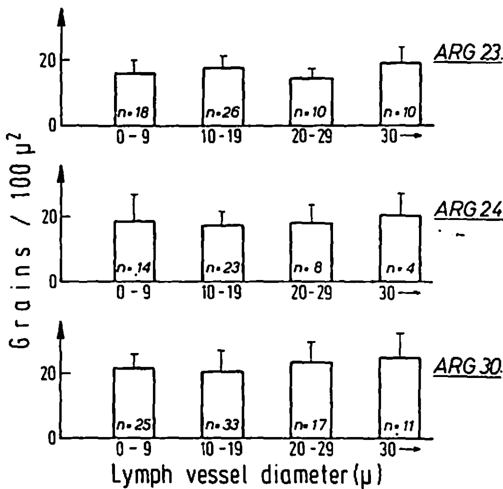

Fig. 2 Radioautographic grain density in lymph versus lymph vessel diameter in each of the three rapidly frozen lungs. Lymph vessels grouped according to diameter. The columns give the mean number of radioautographical grains per 100 μm^2 lymph vessel cross-sectional area with the bars giving 1 SD. The number (n) of lymph vessels in each group is indicated. (From Nicolaysen, Nicolaysen and Staub (1975). By permission)

the protein concentration was significantly higher than in the regional lymph. When fluid was sampled from capsules implanted more than 4 weeks previously the protein concentration in this fluid was not significantly different from that of lymph. *Taylor* and *Gibson* concluded that inflammation at least in part caused the high protein concentration in capsular fluid during the first 4 weeks of implantation.

Casley-Smith and *Sims* (5) attacked the problem using the radioautographic approach. They counted in electron-microscopic radioautograph grains caused by ^{125}I-BSA over lymph vessels at the bases of the villi and in lymph vessels in the serosa/muscle of the intestine in the mouse. They also determined grain density over the interstitial tissue. According to their tabulated data the grain density over lymphatics at the bases of the villi was more than twice as large as that in the serosa/muscle region and also more than twice that of interstitial tissue. *Casley-Smith* and *Sims* interpreted their data to confirm their hypothesis (6) that the initial lymphatics concentrate the proteins but that a redilution takes place in the collecting lymphatics. Comparisons of lymph and interstitial fluid protein concentration by radioautography do however, require special assumptions concerning the interstitium. The basis for these assumptions is in my opinion not very convincing. Another point which make their work difficult to assess is that they compared protein concentration in lymphatics in the villi bases with that in lymphatics in the serosa/muscle. A problem here must be the inhomogenity of the vascular beds involved. The lymph in the lymphatics in the serosa/muscularis region must represent lymph both from the mucosa and from the serosa/muscle region itself. Although a concentrating-rediluting ability of the lymphatics cannot be denied these experimental results do not establish a firm evidence for such an ability.

Using micropipettes *Hargens* and *Zweifach* (7) sampled lymph from pre- and postnodal lymphatics in the mesentery and in the omentum. They found protein concentration values increasing from 1.5 to 4.1 g% going from initial to post-nodal lymph. *Hargens* and *Zweifach* are very cautious in their conclusion, saying that "although our measurements suggest that protein is concentrated as it flows from the periphery to the thoracic duct, the definite measurement remains to be determined". One should note that the data of Hargens and Zweifach go in the opposite direction of what should be expected from Casley-Smith's theory that a redilution takes place in the collecting lymphatics.

Very recently *Brace, Taylor* and *Guyton* (8) have performed experiments from which they to some extent, reach another conclusion than in the 1975 study by *Taylor* and *Gibson* (4). In the newest work they collected lymph from fore- and hind limb, the cervical duct and thoracic duct in dogs. They found that during the first 2 hrs of anesthesia a continuous increase in protein concentration in lymph from all regions except the thoracic duct took place. This occurred under condition of constant plasma protein concentration and also, in several instances, at constant lymph flow. A

Permission granted for single print for individual use.
Reproduction not permitted without permission of Journal LYMPHOLOGY.

gentle massage of the tissue resulted in an abrupt decrease in lymph protein concentration. One must agree with the authors that these observations are not so easily explained other than through a variable concentrating of the proteins in the lymph. The finding of no change in thoracic duct lymph protein is puzzling since the protein concentration in lymph from the hindlimb increased from less than 2 g/dl to somewhat more than 3 g/dl during the first 2 hours of anesthesia. One would expect this more local effect to cause measurable changes in composition of the lymph in the thoracic duct.

It is evident from the studies reviewed that the final answer to the question of whether lymphatics (in some or all organs) concentrate protein during normal conditions or not is not known. In the opinion of this author, however, the experimental evidence showing that lymph protein concentration changes take place in the lymphatics, is not very convincing.

References

1 *Rusznyák, I.M. Földi, G. Szabo:* "Lymphatics and lymph Circulation: Physiology and Pathology". 2nd Ed. Oxford Pergamon, 971 pp., 1967
2 *Casley-Smith, J.R.:* The role of the endothelial intercellular junctions in the functioning of the initial lymphatics. Angiologica 9 (1972) 106
3 *Nicolaysen, G., A. Nicolaysen, N.C. Staub:* A quantitative radioautographic comparison of albumin concentration in different sized lymph vessels in normal mouse lungs. Microvasc. Res. 10 (1975) 138
4 *Taylor, A., H. Gibson:* Concentrating ability of lymphatic vessels. Lymphology 8 (1975) 43
5 *Casley-Smith, J.R., M.A. Sims:* Protein concentrations in regions with fenestrated and continous blood capillaries and in initial and collecting lymphatics. Microvasc. Res. 12 (1976) 245
6 *Casley-Smith, J.R.:* Osmotic pressure: A probably important force for the entrance of material into lymphatics. In "Proc. III. Int. Cong. Lymphology, Brussels (J. Gruwez, ed.) Int. Soc. Lymphology (1970) 148
7 *Hargens, A.R., B.W. Zweifach:* Transport between blood and peripheral lymph in intestine. Microvasc. Res. 11 (1976) 89
8 *Brace, R.A., A.E. Taylor, A.C. Guyton:* Time course of lymph protein concentration in the dog. Microvasc. Res. 14 (1977) 243

Gunnar Nicolaysen, Institute of Physiology, University of Oslo, Karl Johansgt. 47 Oslo 1 Norway

Discussion

Lassen: Would you comment on the problem of concentrating ability of lymphatic capillaries?

Nicolaysen: It is difficult to know exactly what happens at the lymph capillary membrane. There have been several theoretical studies done concluding that concentrating can take place. Others, for example by Taylor et al are arriving at the opposite conclusion. My own opinion is that the information we have as today goes against any concentrating ability at the lymph capillary membrane.

Aukland: If you are contending that there is no concentrating ability of lymphatics then you must assume the water permeability of the wall is zero, or that there is no transmural gradient for water flux.

Nicolaysen: This will be different, whether we speak of the lymph capillary or of the larger lymphatics. The lymph capillary is a very thin walled structure with may be even not a continuous endotelium, whereas the larger collecting lymphatics do have a much thicker wall with smooth muscle cells. Of course the wall permeability for water is not zero, but it is probably very small in these larger vessels. So the most important part is the lymph capillary membrane, and what we know is that at least there is a flux from the interstitium into the lymphatic.

Rossing: Concerning your elegant lungs slices technique I would like to ask you whether you have counted your grains in other areas than just in the lymph. Have you counted the number of grains over cells. What I am after, actually, is whether you draw a film of plasma over your slice?

Nicolaysen: There are several sources of artifacts in this technique and one of the reasons that I did not show more results than from three lungs is that we

Permission granted for single print for individual use.
Reproduction not permitted without permission of Journal LYMPHOLOGY.

worked very hard on finding a satisfactory method. We tried different techniques for fixation of the lungs and the only one in which we found no loss of tracer from the tissue during the whole processing was rapid freezing. The grain density over the lumen of airways which would give us a background, was close to zero. So we did not even have to subtract that from our numbers. We did a test on what we call both positive and negative chemography. A positive chemography meant that a section without labelled albumin, could show grains with different density over different structures. There was no such positive chemography. If one partly exposes radiographs to light you get an even grain density all over the tissue. If one then exposes this section one can in some instances see that there is a decade of grain density over some structures but not over the others, that is a negative chemography. We did not see anything of that so we do think that the method gives a fair estimate on the real grain density. A problem here was to get a freeze dried lung block infiltrated with the embedding medium.

Permission granted for single print for individual use.
Reproduction not permitted without permission of Journal LYMPHOLOGY.