Lymphology 12 (1979) 208–216

Fibrinogen Clearance from the Pulmonary Interstitium

E.C. Meyer, R. Ottaviano

Departments of Surgery and Pathology, Fitzgerald Mercy Division,
Mercy Catholic Medical Center, Darby, Pennsylvania 19023

Summary

Previous experiments demonstrated that ^{125}I-human fibrinogen (125-RIF) instilled into the distal airway of the right lower lobe in intact dogs passed through the microairway into the interstitium. This report analyzed the subsequent interstitial clearance of this fibrinogen by analyzing plasma and lymph samples for ^{125}I activity. This activity was separated into clottable, and trichloroacetic acid (TCA) insoluble and soluble components. Clottable and TCA-insoluble 125-RIF in the same ratio appeared in lymph from the right lymphatic duct (RD) and thoracic duct (TD). From standard mixing equations we calculate that 1.4 times as much lymph from the right lower lobe flows into the TD as into the RD. With one exception, clottable 125-RIF appeared in plasma only in the absence of an RD; this suggested the presence of accessory lymphatico-venous communications rather than back flux of 125-RIF at the capillary wall. By injecting ^{131}I-human fibrinogen intravenously, we observed a small reduction in clottability (maximum of 10%) in lymph both from the RD and TD. Equilibration in RD lymph was only 1/5 as fast as albumin, and from the measured equilibration rate constant, and the assumption that interstitial fibrinogen clearance is entirely convective, we estimate that the pulmonary interstitial distribution volume for fibrinogen is 70% that for albumin. Non dialyzable 125-RIF products generated in the distal airway adhered to intracapillary, circulating erythrocytes. These products retained fibrinogen antigenicity, and appeared to be further degraded in the vascular compartment.

Introduction

It has been established that tracer albumin instilled into alveoli of intact dogs, passes through the microairway into the interstitium (8, 13, 15). Using this technique, we were able to separate subsequent interstitial clearance of the albumin into transcapillary (permeative) and lymphatic (convective) components (15). The permeative component averaged approximately six times the convective component. Recently, using tracer fibrinogen solutions of varying concentrations as the alveolar instillate, we demonstrated that fibrinogen is degraded within the distal airway by non linear kinetics. The degradation resulted principally in the production of small tracer molecules which were soluble in trichloroacetic acid and became evenly distributed in body extracellular water (16). In this experiment, however, small quantities of clottable tracer fibrinogen did pass through the microairway by first order (concentration dependent) kinetics, and appeared in lymph collected from the right lymphatic duct. The present report is an analysis of the clearance routes of interstitial fibrinogen, and indicates not only the complexity of lung lymph drainage pathways, but also a clearance mechanism by erythrocytes.

Methods

Although the experiment has been described in detail previously (16), it is summarized briefly. Nine dogs of either sex were used in the study. All were anesthetized with sodium pentobarbital (25 mgm/kgm [IV]) initially, and 25 to 50 mgm IV subsequently every 2 hours. In the first 7 dogs we explored the right and left neck for the right lymphatic duct (RD) (12) and thoracic duct (TD). We identified and cannulated the RD in 4 of the 7 dogs, and the TD in 6 of the 7 dogs. We placed cuffed tubes into the tracheas and sampling catheters into both femoral veins and a femoral artery. Under fluorscopic control, a radioopaque catheter with a 2 mm diameter was placed into the wedged position in the lateral (costal) aspect of the right lower lobe. Through the catheter we instilled approximately 1 ml of citrated, isoosmolar radioio-

0024–7766/69 1500–0218 $ 02.00 © Georg Thieme Publishers

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

dinated ^{125}I-human fibrinogen (125-RIF)* in carrier human fibrinogen containing two drops of stock Evans Blue Dye. We varied the quantity of carrier fibrinogen so that fibrinogen concentration in the instillate ranged from 0.75 mgm/ml to 4 mgm/ml in individual animals. Simultaneously in all 7 dogs, we injected approximately 20 μCi ^{131}I-human fibrinogen (131-RIF)* intravenously. The purpose for this was to analyze the degradation of radioiodinated fibrinogen within the vascular compartment, and within the interstitium during plasma to lymph transit. In the remaining 2 dogs we instilled 125-RIF (3 mgm/ml and 4 mgm/ml concentration) into alveoli but no 131-RIF iv. Otherwise, we prepared the animals in the same manner as the other 7, but used their lung tissue for autoradiography and immunocytochemical marking instead of homogenization for tracer recovery. The osmolalities of 131-RIF and 125-RIF solutions were determined by freezing point depression, and all were dialyzed against cold phosphate-buffered saline (PBS) using Visking cellulose. Usually the dialyses were carried out over a 24 hour period during which the bath was changed three times. At the time of instillation and injection more than 95% radioiodine activity was clottable, and less than 3% was soluble in 30% trichloroacetic acid (TCA). These "ions" were subtracted from total quantities instilled or injected in order to remove them from further calculation. All drained lymph, and 3 ml blood samples, were collected each 20 min throughout the experiment. The samples were collected in ice-cooled plastic tubes containing 0.03 ml 38% sodium citrate and 0.07 ml 5 M aminocaproic acid (EACA) per 3 ml sample. Blood samples were centrifuged immediately, and the plasma was decanted, frozen, and stored at -40 °C. No clotting occurred in any lymph sample, and each was stored at -40 °C immediately after the collection period. Plasma and lymph samples were analyzed within 4 days of the experiment by the following method. One ml of each sample was analyzed for ^{125}I and ^{131}I activity in a Picker well counter and scaler. In the case of RD lymph, less than 1 ml was usually available. To an additional 1 ml of each sample, 0.2 ml clotting solution was added. The clotting solution contained 22 mgm EACA, 1.2 units thrombin, and 1.2 mgm calcium chloride. The clots were wound on glass rods, washed three times with PBS, and analyzed in the well counter for both isotopes. This yielded the clottable quantity of RIF in the sample. The remainder of the sample was diluted 1:1 with TCA. The volume of the resulting supernate was decanted, the volume measured, and analyzed in the well counter. This yielded the quantity of TCA-soluble RIF products in the sample. The quantity of TCA-insoluble RIF products was calculated by subtracting the sum of clottable and TCA-soluble quantities from that in original sample. In 4 of the 7 dogs receiving both 125-RIF and 131-RIF, we measured the quantities of ^{125}I and ^{131}I adherent to red cells by collecting the cells after decanting the plasma, washing them three times with PBS, and analyzing them in the well counter. Fibrinogen concentrations in plasma and lymph samples were determined using a Lancer Fibrinogen Analyzer. All dogs were killed by pentobarbital overdose at 311 ± 8 (SE) min. In the 2 dogs not receiving 131-RIF we identified the blue zone of instillate in the right lower lobe after opening the chests by a sternal splitting incision. A 5 mm thick slice of lung containing instillate was processed for both autoradiography and immunocytochemical labelling of the human fibrinogen. For autoradiography, a portion of the slice was fixed in cold, cacodylate-buffered 2.5% glutaraldehyde for 2 hours, diced into 1 mm cubes, and fixed for an additional 10 hrs. The tissue cubes were washed in several changes of cacodylate buffer, post-fixed in osmium tetroxide, and pre-stained with uranyl acetate and phosphotungstic acid. After embedding in Epon 812, the blocks were sectioned with an LKB ultratome and diamond knife. Sections of silver interference color were placed on glass slides coated with celloidin-amyl acetate. The sections were coated with a monolayer (gold-

* Kindly supplied by Dr. *Jose Martinez*, Cardeza Foundation, Thomas Jefferson University, Philadelphia, Pa.

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

silver interference color) of Kodak NTE emulsion. As prepared, the emulsion and sections were in direct contact. The slides were stored at 4 °C for 10–12 weeks. After developing in Dektol for 2 min and fixing for 1 min, the celloidinamyl acetate film was stripped from the slides, and the sections mounted on copper grids. From section and emulsion thicknesses, halide crystal diameter, and the size of developed silver grains, we estimated resolution for ^{125}I by the equations of *Bachman* and *Salpeter* (2). By these equations, resolution is defined as the diameter of the 50% probability circle around each silver grain. This indicates the 50% probability that the radioactive source in the tissue originated within the circle area. With our preparation, we estimate that the 50% probability circle had a diameter of approximately 150 nm. In view of the long fixation and wash times, we assumed that no ^{125}I-activity in the tissues was produced by ions.

For immunocytochemical labelling of the instilled human fibrinogen we fixed thin (1 x 5 mm) lung sections in 2.5% cacodylate-buffered glutaraldehyde for 15–30 min at room temperature. After fixation, the strips were washed in cacodylate buffer and embedded in 7% agar. Using a Smith and Farquhar TC-2 tissue sectioner, we cut the strips into sections 40 μm thick, and incubated them in serial dilutions (1:10 to 1:100) of ferritin conjugated IgG fraction rabbit anti-human fibrinogen (Cappel Laboratories, Inc.) for 24 hours, and washed them in several changes of PBS for an additional 24 hours. Then the tissue sections were post-fixed in osmium tetroxide, dehydrated, and embedded in Epon 812. The tissue capsules were analyzed in the well counter for ^{125}I activity. Those with the greatest activity were selected for subsequent sectioning. Sections of gold interference color were mounted on copper grids, and post-stained with bismuth subnitrate for improved definition of the ferritin label (1). Control studies included (a) antigen-antibody blocking by absorbing the ferritin-conjugated antibody with human fibrinogen, and (b) treatment of control lung with the ferritin conjugate. All sections were viewed with a Zeiss EM 9 electron microscope.

Results

All animals appeared to be in a steady state with regard to cardiopulmonary function. There was no measurable pulmonary edema from lung homogenate analysis: mean lung water/bloodless dry lung weight was 3.46 ± 1.2 (SE) ml/gm (16). Lymph flows from the RD and TD decreased slowly and linearly with time to 75% baseline levels during 5 hours of observation. Measured fibrinogen concentrations in plasma and lymph were constant (steady state) and were (in mgm/ml): 3.25 ± 0.06 (SE) for plasma, 1.23 ± 0.04 (SE) for TD lymph, and 1.17 ± 0.04 (SE) for RD lymph.

The measured fractions of clottable, TCA-insoluble, and TCA-soluble tracer in plasma, RD lymph, and TD lymph are presented in Table 1. Since the concentration of TCA-soluble products was equal in all three fluids, RD lymph/plasma ^{125}I concentration ratio was 91.7/17.8 = 5.3, and that for RD lymph/TD lymph was 80.7/17.8 = 4.5. It was apparent that lymph from the right lower lobe drained into both RD and TD systems, and that the ratio of clottable 125-RIF in RD/TD lymph (53.9/12.0) was approximately equal to the TCA-insoluble ratio (28.3/7.3). This indicated that clottable and TCA-insoluble 125-RIF molecules passed together in both ducts without binding or absorption of one molecular species as opposed to the other within lymphatics or lymph nodes. An estimate of relative flow into both ductal systems can be calculated from the ratio of clottable and TCA-insoluble flux in each duct, that is: flux (cts/min) = lymph concentration (cts/ml) x lymph flow (ml/min). Therefore RD/TD flux ratio was 4.5 (RD/TD concentration ratio) x (53.9 + 28.3)/(12.0 + 7.3) (RD/TD clottable and TCA-insoluble fractional quantities x $(0.42 \times 10^{-3})/(1.12 \times 10^{-2})$ (RD/TD flow ratio [Table 3]) = 0.7. This indicated that nearly 1.4 times as much lymph from the right lower lobe zone of instillate drained into the TD as into the RD when both ducts were present. On the other hand, when only a TD was present (Table 1) there were two noteworthy observations. First, there was more fractional clottable 125-RIF in TD lymph,

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

Tab. 1 Coagulation Properties of 125-RIF Following Intra-Alveolar Instillation

Dog	Plasma (%)			RD Lymph (%)*			TD Lymph (%)*		
	Clottable	+ TCA ± Insoluble	TCA ± Soluble	Clottable	+ TCA ± Insoluble	TCA ± Soluble	Clottable	+ TCA ± Insoluble	TCA ± Soluble
1	11.0	0	89.0	–	–	–	2.0	9.0	89.0
2	25.6	0	74.4	–	–	–	21.6	5.4	73.0
3	12.8	0	87.2	–	–	–	40.0	0	60.0
4	0	0	100.0	63.6	20.1	16.3	–	–	–
5	8.6	0	91.4	49.0	32.0	19.0	3.4	14.6	82.0
6	0	0	100.0	40.0	36.5	23.5	1.5	5.6	92.9
7	0	0	100.0	63.0	24.6	12.4	3.4	9.3	87.3
Mean	8.3		91.7	53.9	28.3	17.8	12.0	7.3	80.7
± SE	3.6		3.6	5.7	3.7	2.3	6.4	2.0	5.0

* RD = right lymphatic duct TD = thoracic duct
\+ Clottable = % of tracer activity clotted by thrombin and ca^{++}
± % of tracer insoluble and soluble in 30% trichloroacetic acid

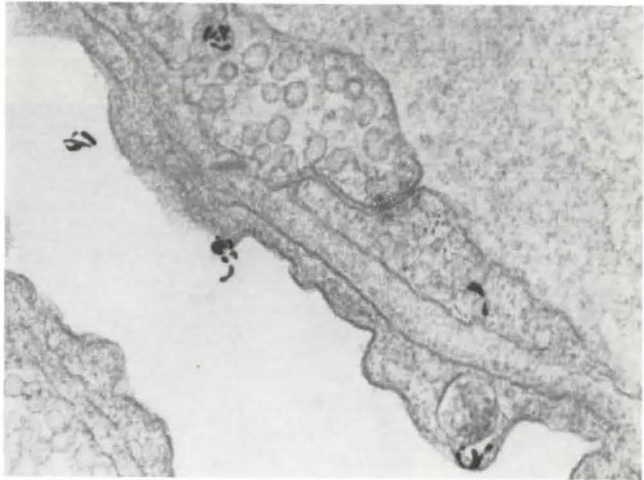

Fig. 1 High power electron microscopic autoradiograph of the thin portion of the air-blood barrier in dog lung following intraalveolar instillation of ^{125}I-human fibrinogen. Developed silver grains appear to penetrate intact alveolar epithelium, and pass through capillary endothelial vesicles toward the plasma-filled capillary lumen to the right. There is no apparent damage to epithelium or endothelium (original magnification 52,000X)

and second, there was clottable 125-RIF in plasma. The second observation suggested the presence of accessory lymphatico-venous junctions, since clottable 125-RIF appeared in plasma of only 1 dog with an RD (# 5 in Table 1).

If accessory junctions existed, and if both clottable and TCA-insoluble 125-RIF products flowed together in lymph, why were no TCA-insoluble products identified in plasma? Autoradiographs indicated the passage of non-ionic tracer (washed tissue) across intact epithelium and endothelium (fig. 1) and then selective binding to erythrocytes (fig. 2). These tracer products retained fibrinogen antigenic sites since they were labelled with immunoferritin (fig. 3). Moreover, in the course of time, they were released from erythrocytes (fig. 4), presumably as TCA-soluble products since no TCA-insoluble products were found in plasma. Since no selective adherence of intravenously injected 131-RIF products was found on washed erythrocytes, it was apparent that processing of fibrinogen within the distal airway was necessary for the development of molecules with an affinity for erythrocytes. In addition, erythrocytes apparently contain an enzyme system which further degrades

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

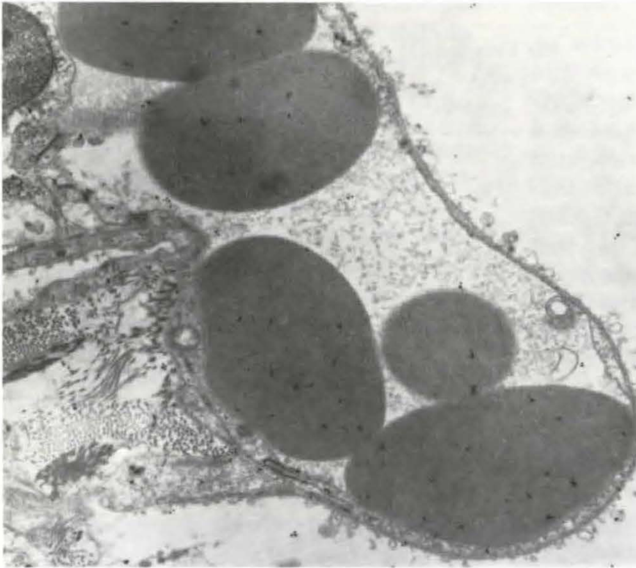

Fig. 2 Lower power electron microscopic autoradiograph of dog alveolar capillary following intra-alveolar instillation of ^{125}I-human fibrinogen. Developed silver grains lie over erythrocytes. There is damage to both epithelium and endothelium. Approximately half the microairway was damaged following instillation, but developed silver grains lay over erythrocytes in all sections (original magnification 9,000X)

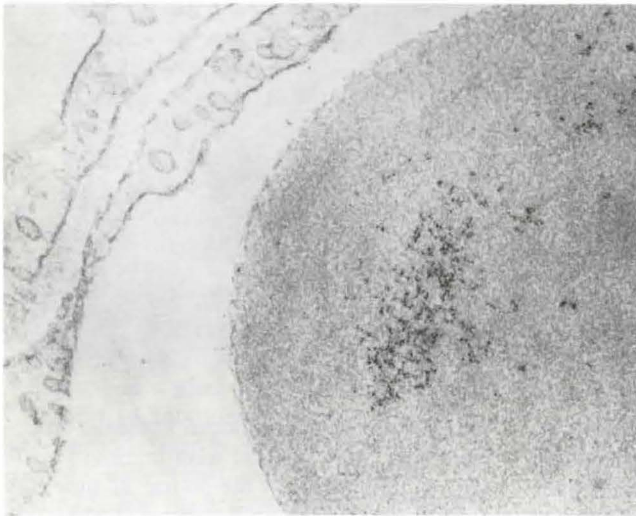

Fig. 3 High power electron micrograph of intracapillary erythrocyte in dog lung following intra-alveolar instillation of ^{125}I-human fibrinogen. The tissue was incubated with ferritin conjugated rabbit-antihuman fibrinogen. Ferritin labelling lies over the erythrocyte (original magnification 52,000X)

these products. Our data did not exclude the possibility that clottable fibrinogen penetrating the microairway became adherent to erythrocytes, but the usual presence of clottable 125-RIF in plasma in the absence of an RD, suggested that there was no back flux of clottable 125-RIF at the capillary wall. Therefore, interstitial clearance probably occured exclusively by convective flow in lymph.

Is fibrinogen altered within the interstitium? The results of 131-RIF analysis in lymph following intravenous injection (Table 2) suggests that some alteration occurs along the plasma to lymph transit route. Only clottable and TCA-insoluble fractional quantities in the three fluids are presented in Table 2 because injected TCA-soluble molecules were subtracted from futher analysis. The data indicate a reduction in clottable 131-RIF in passing from

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

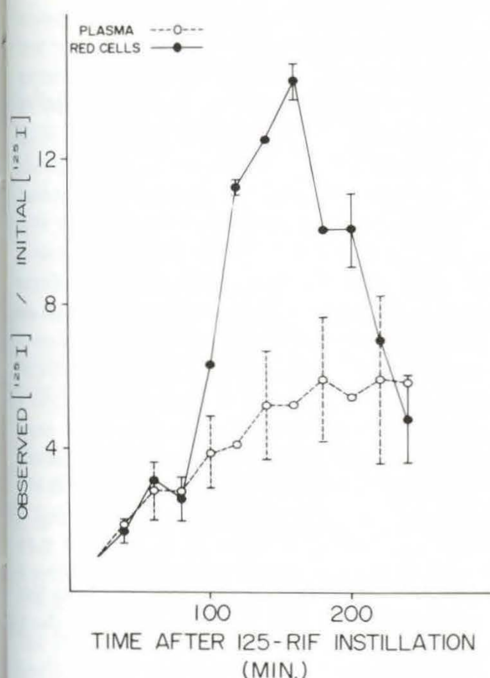

Fig. 4 Relationship between proportional increases in washed red cell ^{125}I activity (counts/ml) and plasma ^{125}I activity (counts/ml) following intra-alveolar instillation of ^{125}I-human fibrinogen (125-RIF) in dogs. The points are means and the brackets ± 1 SE of measurements made on 4 dogs. Note the 14-fold increase in washed red cell activity at 150 min which decreases toward normal at 250 min. Plasma activity was caused exclusively by tracer molecules soluble in 30% trichloroacetic acid. The peak level of activity in red cell samples was only 7% that of plasma

plasma to lymph in both ducts. This reduction might be accounted for partially by injected TCA-insoluble molecules passing the microvascular membranes more rapidly than clottable fibrinogen. However, the TCA-insoluble molecules may range in molecular weight from 4000 to more than 300,000 daltons, and since 131-RIF transit into TD lymph was approximately six times the transit into RD lymph (Table 3), the TCA-insoluble molecules would have to have been very large to equilibrate at precisely the same rate as clottable fibrinogen in both ducts. This seemed unlikely. There is an another explanation which appears more reasonable: fibrinogen is degraded by first order kinetics in passing the microvascular membrane. If it is true that approximately (98–86)/96 = 10% of fibrinogen is altered in passing the microvascular membrane, and if this is one mechanism of fibrinogen catabolism, then the catabolic half time should be approximately 10 times the plasma to lymph equilibration half time. This is approximately true (19).

Equilibration of clottable 131-RIF was much faster in TD lymph than RD lymph (Table 3). Assuming that the equilibration with RD lymph was monoexponential (14), the rate constant can be calculated as (7):

$$\lambda = 1n\ (1\text{-}C(t)/C(ss))/t \qquad (1)$$

Tab. 2 Coagulation Properties† of 131-RIF Following Intravascular Injection

Dog	Plasma (%)		RD Lymph (%)*		TD Lymph (%)*	
	Clottable+	TCA-Insoluble±	Clottable+	TCA-Insoluble±	Clottable+	TCA-Insoluble±
1	98	2	–	–	89	11
2	95	5	–	–	79	21
3	96	4	–	–	94	6
4	94	6	81	19	–	–
5	97	3	84	16	86	14
6	97	3	84	16	92	8
7	97	3	94	6	88	12
Mean	96	4	86	14	88	12
± SE	1	1	3	3	2	2

* RD = right lymphatic duct; TD = thoracic duct
\+ % of tracer activity clotted by thrombin and ca^{++}
± % of tracer activity insoluble in 30% trichloroacetic acid
† Excludes products soluble in 30% trichloroacetic acid

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

Tab. 3 Lymph Characteristics

	Thoracic Duct			Right Duct		
Dog	Flow x 10^2 *	R^+	$C(t)/C(ss)^{\pm}$	Flow x 10^3 *	R^+	$C(t)/C(ss)^{\pm}$
1	0.70	0.33	0.56	–	–	–
2	0.78	0.53	0.73	–	–	–
3	1.88	0.33	0.36	–	–	–
4	–	–	–	$1.84^{\dagger}$	$0.33^{\dagger}$	$0.90^{\dagger}$
5	1.35	0.41	0.33	0.43	0.35	0.04
6	1.13	0.35	0.90	0.49	0.31	0.14
7	0.92	0.37	0.84	0.34	0.42	0.11
Mean	1.12	0.38	0.62	0.42	0.36	0.10
± SE	0.18	0.03	0.10	0.04	0.03	0.03

* in ml/kgm body wt/min
\+ steady state lymph/plasma fibrinogen concentration ratio
± The ratio of clottable 131-RIF concentration in lymph at the end of the experiment (C(t)) to that at a steady state (C(ss)). The (Css) was calculated as the product of R x plasma 131-RIF concentration assuming the latter was constant.
† Not included in the calculated mean in view of the absence of a thoracic duct.

where λ = rate constant (min^{-1}) and $C(t)/C(ss)$ = ratio of equilibration at time = t (min). Substituting from Table 3 where t = 311 min experimental time, $\lambda = 4 \times 10^{-4}$ min^{-1}. This is only 1/5 the rate constant for albumin (14).

In general, clearance of proteins from the interstitium can be expressed most simply as (20):

$$\lambda V = PS + L \quad (2)$$

where V = interstitial distribution volume of the protein, PS = permeability-surface area product of the microvascular membrane for the protein (ml/min), and L = lymph flow (ml/min). Since we identified no apparent back flux of fibrinogen at the capillary wall, $(PS)_F << L$, where subscript F refers to fibrinogen. In the case of albumin (15), we found that $(PS)_A \cong 6L$, where subscript A refers to albumin. Therefore, $\lambda_F V_F \cong L$, and $\lambda_A V_A \cong 7L$. Since $\lambda_A/\lambda_F = 5$ then $V_F/V_A \cong 5/7$. This is a fairly big ratio considering the difference in molecular sizes between albumin and fibrinogen, and suggests that the interstitial gel contains fairly large pores.

Finally, although the number of dogs was small a single instance of an RD without a TD was found (dog # 4). In Table 3, the large flow rate and rapid equilibration rate of clottable 131-RIF in lymph collected from this duct indicated that it functioned as a thoracic duct.

Discussion

The alveolar and interstitial compartments appear to provide different environments for fibrinogen. In the former, fibrinogen is slowly degraded by non-linear kinetics, and this may be why *Bignon* et al. (3) found no fibrinogen in normal rat alveolar lining liquid by immunoperoxidase staining. Presumably the degradation occurs by macrophages although *Colvin* and *Dvorak* (5) could find no fibrin/fibrinogen receptors on alveolar macrophages as opposed to peritoneal macrophages. We have observed alveolar macrophages engulfing fibrin (11), and macrophages are known to produce plasminogen activator when stimulated (10). Within the interstitium, or at some point in transit from plasma to lymph, fibrinogen appears to be slowly degraded by first order kinetics. However, this conclusion is speculative since it depends upon the applicability of standard plasma clotting solutions to lymph. After microvascular damage, the interstitial compartment is highly fibrinolytic (11).

Autoradiographs indicated that tracer fibrinogen molecules, produced in alveoli, penetrated into erythrocytes, and were not merely

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

adsorbed to their surfaces. This distinction was well within the resolving power of the autoradiographs. There is a close relationship between erythrocytes and fibrinogen. A spectrum of fibrinogen-like proteins have been identified in saline elutes of sucrose-washed bovine erythrocytes (9), and human erythrocyte membranes have been shown to alter N-terminal amino acids of fibrinogen (17). A number of proteolytic enzymes are bound by erythrocyte membranes (4), and erythrocyte hemolysate contains a plasminogen activator (erythrokinase) (21). Our data demonstrated a decrease in washed erythrocyte ^{125}I-activity from 150–200 min, and this proves release of tracer. The fact that no TCA-insoluble 125-RIF products were identified in plasma suggests that the erythrocyte-bound products were released as TCA-soluble molecules. Presumably this involved some enzyme system within the erythrocyte itself.

The fact that erythrocyte bound tracer was labelled with immunoferritin requires some interpretation. Tissue procedures for immunoferritin marking have three requisites: (a) adequate preservation of fine structure, (b) adequate preservation of antigenic sites which are crosslinked by fixatives, and (c) adequate penetration of large ferritin molecules into the cell. The antigenic sites of fibrinogen proved to be very sensitive to fixatives. Fibrinogen in alveolar instillate and epithelial vesicles stained poorly with the immunoferritin whereas that on erythrocytes consistently stained well. We assume this was related to different glutaraldehyde penetration rates. Since no staining of erythrocytes was observed in control preparations, we conclude that the fibrinogen-like molecules on the erythrocytes contained fibrinogen antigenic sites. Therefore, they were either fragment D, fragment E, or the terminal carboxy portion of the A α chain (6). It is important to reiterate that no intravenously injected 131-RIF, either clottable or TCA-insoluble, adhered to erythrocytes. Thus some action by alveolar enzyme systems on fibrinogen is necessary before the degration products develop an affinity for erythrocytes.

Finally, the lymphatic drainage noted in the experiment emphasizes the variability of lung lymph ducts in the dog. Direct lymphaticovenous communications appear to exist primarily in the absence of an RD, and direct communication of lung lymph trunks into the azygos vein has been reported (18). On the other hand, when no TD is present, the RD serves as a functional TD. Lymph production in the right lower lobe apparently enters both the RD and TD when both are present. These observations indicate the caution that must be exercised in interpreting lung lymph flows as recorded by RD flow in dogs. Experiments should state not only whether a TD is present, but the flow relationship between the two ducts.

References

1 *Ainsworth, S.K., M.J. Karnovsky:* An ultrastructural staining method for enhancing the size and electron opacity of ferritin in thin sections. J. Histochem. Cytochem. 20 (1972) 225–229

2 *Bachmann, L., M.M. Salpeter:* Autoradiography with the electron microscope. A quantitative evaluation. Lab. Invest. 14 (1965) 303–315

3 *Bignon, J., M.C. Jaurand, M.C. Pinchon, C. Sapin, J.M. Warnet:* Immunoelectron microscopic and immunochemical demonstrations of serum proteins in the alveolar lining material of the rat lung. Am. Rev. Respirat. Diseases 113 (1976) 109–120

4 *Burkholder, D.E., A.S. Brecher:* Interaction between proteases and bovine erythrocyte membranes. Biochim. Biophys. Acta 282 (1972) 135–145

5 *Colvin, R.B., H.F. Dvorak:* Fibrinogen/fibrin on the surface of macrophages: detection, distribution, binding requirements, and possible role in macrophage adherence phenomena. J. Exp. Med. 142 (1975) 1377–1390

6 *Gaffney, P.J.:* The Biochemistry of fibrinogen and fibrin degradation products. In: *Haemostasis: Biochemistry Physiology, and Pathology* edited by *D. Ogstom* and *B. Bennett.* John Wiley and Sons, New York, 1977, pp. 105–168

7 *Garlick, D.G., E.M. Renkin:* Transport of large molecules from plasma to interstitial fluid and lymph in dogs. Am. J. Physiol. 219 (1970) 1595–1605

8 *Gee, M.H., N.C. Staub:* Role of bulk fluid flow in protein permeability of the dog lung alveolar membrane. J. Appl. Physiol. 42 (1977) 144–149

9 *Goodger, B.V.:* Fibrinogen-like proteins and fibrinolytic proteins in a saline eluate from sucrose washed bovine erythrocytes. Aust. J. Exp. Biol. Med. Sci. 54 (1976) 381–390

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

10 *Gordon, S., J.C. Unkeless, Z.A. Cohn:* Induction of macrophage plasminogen activator by endotoxin stimulation and phagocytosis. J. Exp. Med. 140 (1974) 995–1010
11 *Meyer, E.C.:* Acute and chronic clearance of lung fluids, proteins, and cells. In: *Lung Water and Solute Exchange*, edited by *N.C. Staub*. Marcel Dekker, New York, 1978, pp. 277–321
12 *Meyer, E.C.:* The collection of pulmonary lymph in dogs. J. Surg. Res. 8 (1968) 544–550
13 *Meyer, E.C., E.A.M. Dominguez, K.G. Bensch:* Pulmonary lymphatic and blood absorption of albumin from alveoli. Lab. Invest. 20 (1969) 1–8
14 *Meyer, E.C., R. Ottaviano:* Right lymphatic duct distribution volume in dogs: relationship to pulmonary interstitial volume. Circulation Res. 35 (1974) 197–203
15 *Meyer, E.C., R. Ottaviano, J.J. Higgins:* Albumin clearance from alveoli: tissue permeation vs. airway displacement. J. Appl. Physiol. 43 (1977) 487–497
16 *Meyer, E.C., R. Ottaviano, J.J. Higgins:* Fibrinogen clearance from alveoli. J. Appl. Physiol. (In Press)
17 *Murray, M., D.E. Rearick:* Interaction between red cell membranes and fibrinogen. Thromb. Res. 4 (1974) 261–269
18 *Rabin, E.R., E.C. Meyer:* Cardiopulmonary effects of pulmonary venous hypertension with special reference to pulmonary lymphatic flow. Circulation Res. 8 (1960) 324–335
19 *Reeve, E.B., J.J. Franks:* Fibrinogen synthesis, distribution, and degradation. Sem. Thromb. Hemostasis 1 (1974) 129–183
20 *Renkin, E.M.:* Transport of large molecules across capillary walls. Physiologist 7 (1964) 13–28
21 *Semar, M., L. Skoza, A.J. Johnson:* Partial purification and properties of a plasminogen activator from human erythrocytes. J. Clin. Invest. 48 (1969) 1777–1784

E.C. Meyer, Dept of Surgery and Pathology, Fitzgerald Mercy Division, Mercy Catholic Med. Center, Darby, Pennsylvania 19023, USA

This study was supported in part by National Heart, Lung, and Blood Institute Grant HL-13596

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