

Plasma Proteins in the Extravascular Space

While I have frequently seen disappearance plots for parenteral proteins with at least two if not three distinct phases, the importance of the first phase, frequently referred to as equilibration between intravascular space and extravascular space, had eluded me until my recent work on *Biotechnology of Plasma Proteins*(1).

Body water is divided between intracellular and extracellular fluid. The great bulk of body is in intracellular fluid with approximately 30% in extracellular fluid; extracellular fluid is divided between extravascular fluid and intravascular fluid (2). Extravascular fluid can be further segmented into interstitial fluid and transcellular fluid. Transcellular fluid includes fluids such as cerebrospinal fluid, synovial fluid, and ocular fluid (aqueous humor of the eye). The is fluid and solute exchange between the various compartments of the extravascular fluid but the exchange is variable depending on the compartment (see below). Lymph is derived from interstitial fluid and returned to the circulatory system. Thus plasma proteins, excluding extravascular synthesis, would be derived from the intravascular space into the interstitial fluid by a extravasation (3) and then via lymph returning to the circulation. Analytes in lymph may be degraded and at a lower concentration than in the parent interstitial fluid; it should be noted that I could not find data to either substantiate or negate this assumption. It may be easier to obtain reasonable samples of peripheral lymph than interstitial fluid (4) although this writer has no experience with either fluid.

The ratio of IgG in cerebrospinal fluid to plasma is 0.003 while it is 0.80 for urea demonstrating the relative impermeability of the cerebrospinal barrier (5). For comparison, the ratio of IgG concentration between interstitial fluid and plasma is 0.52 while albumin is 0.62 (6). The exchange of fluid between intravascular fluid and the extravascular space is dependent on the physiological state of the individual (7,8). There is also local variation in distribution (9,10). Bar and coworkers (9) observed that insulin stimulated movement of insulin-like growth factor binding protein-1 from the vascular space to tissue in isolated beating rat heart while there was no effect on insulin-like growth factor binding protein-2 but a decrease in endothelial cell IGF binding protein. Juweld and coworkers (10) observed that while the concentration of albumin is higher than that of IgG in normal tissue, the ration approaches unity in inflamed tissue. Reed and Rubin (11) suggest that the edema response in inflammation is of functional significance in promoting the diffusion of plasma protein into the inflamed tissue. The transcapillary escape rate (transport from intravascular space to extravascular space) of albumin, IgG, and IgM increased in angiotensin-II-induced hypertension; the relative increase was much higher for IgG and IgM than for albumin (12). Transport from the vascular space depends on endothelial permeability; transport of plasma proteins can occur either by transcellular or paracellular processes (13,14). As can be surmised from the material cited above as well as material on individual proteins to be presence in later chapters, a substantial portion of a given plasma protein can be found in the interstitial space as the volume of extravascular fluid is 2 to 3 times the size of the plasma volume (15,16). Consider, for example, the interstitial fluid of skin which contains a substantial amount of albumin with considerable exchange with plasma (17). Binding of drugs to albumin is suggested to improve distribution to

tissues (18). In this study (18), it was found that diffusion of a drug to deeper tissues after topical application is facilitated by binding to albumin.

Albumin in human plasma (human serum albumin) constitutes approximately 40% of total body albumin with the remainder in the various extravascular space (19). Hughes described albumin as an interstitial protein rather than a plasma protein in 1954 (20). Rothschild and coworkers (21) used ^{131}I -labeled human serum albumin to determine tissue distribution of albumin in several individual with terminal diseases. These investigators observed that four to five days were required for complete tissue equilibration. The major amount of radiolabel was found in plasma (40%) with 18% in skin and 15% in muscle; there was a minor amount in heart, lungs, kidney, and spleen. The presence of a large amount of interstitial fluid in skin is thought to be responsible for albumin in skin (22). Increased leakage into the extravascular space is considered to be responsible for lower HSA concentrations in acute phase reactions and other clinical situations (23-25). The term transcapillary escape rate (26) is used to describe the process.

The studies on the pharmacokinetics of antithrombin have, however, demonstrated complex behavior seen with many other protein therapeutics. Two-compartment (27) and three-compartment (28) models have been proposed for the pharmacokinetics of **antithrombin**. A common observation in antithrombin pharmacokinetics studies is rapid equilibration with the extravascular space (29). Collen and coworkers (27) estimated that 45% of antithrombin remained in the intravascular pool while Carlson and coworkers (28) estimated that 40% of the antithrombin was in the intravascular pool. Carlson and coworkers proposed a three-compartment model where 10% of the antithrombin was bound to the vascular wall in a non-circulating fraction. Both of these groups used radiolabeled proteins and demonstrated that the rapid initial loss of infused material was not due to denatured protein. Carlson and coworkers had performed earlier studies in a rabbit model system (30) where the issue of protein denaturation secondary to the iodination process was addressed by a "first pass" of radiolabeled material through a rabbit and plasma containing radiolabeled antithrombin was taken to a second rabbit. Antithrombin is active as a protease inhibitor in the extravascular space with matriptase (31). Latent antithrombin, a conformer, has antiangiogenic activity (32) implying activity in the interstitial space. The expression of antithrombin in benign prostate epithelium has been reported by Cao and coworkers (33) suggesting an extravascular source for the synthesis of antithrombin.

Makino and Reed (34) showed that there was a rapid distribution of **α_1 -antitrypsin** into the extravascular space after administration of partially purified α_1 -antitrypsin to 3 patients homozygous for α_1 -antitrypsin deficiency. Jones and coworkers (35) studied the clearance of native and disialylated α_1 -antitrypsin using radiolabeled protein. Approximately half of the α_1 -antitrypsin distributed into the extravascular space. The disialylated proteins were cleared in minutes associated with a rapid uptake of radiolabel into liver.

While a substantial portions of antithrombin and α_1 -antitrypsin are in the extravascular space, an even greater proportion of heparin cofactor II is found in the extravascular space (36,37). Hatton and coworkers (36) proposed a three-compartment model for heparin cofactor II with 20% distribution in the intravascular space and 60-70% in the extravascular space. It is then not surprising that heparin cofactor II is not thought to have an important factor in the

regulation of solution blood coagulation but likely functions at the vascular wall or in the extravascular space (38). The tissue distribution data supports this concept as well as the stimulation of the heparin cofactor II reactions by dermatan sulfate (39) and smooth muscle cells (40).

Plasminogen activator inhibitor-1 is thought to control the activity of tissue plasminogen activator in the vascular space and urokinase-type plasminogen activator on cell surface and in the extravascular space (41). **Plasminogen activator inhibitor-2** is thought to function primarily inside the cell (42) or in the extravascular space as an inhibitor of urokinase-type plasminogen activator (43) and is missing definition of function (44).

The demonstration of **protein Z and protein Z inhibitor** outside of the vascular space (45,46) permits the suggestion of the importance of extravascular function for these proteins(47).

Plasminogen likely has substantial activity in the extravascular space. There is little quantitative information on the concentration of plasminogen in the extravascular space but it is documented that there a number of extrahepatic sites of synthesis (48) and that plasminogen is present in the extravascular space available to generate angiostatin (49). There is one study with radiolabeled plasminogen in rabbits showed that there was more plasminogen in the extravascular space (46% for plasminogen I, 64% for plasminogen II) than in the intravascular space (41% for plasminogen I, 23% for plasminogen II). Plasminogen I and plasminogen II are glycoforms of plasminogen (50). It is not unreasonable assume, as with other plasma proteins of similar size, that at least half of the available plasminogen is in the extravascular space.

The presence of other coagulation proteins in the extravascular space as well as the process of extravasation of plasma protein and production of derivative products such as thrombin and fibrin is complex. Jacob and coworkers (51) observe that there is physiological heterogeneity in the vascular barrier in the coronary system where arterial and capillary segments are relatively impermeable while the venous system permits facile passage of plasma proteins into the interstitial space. A consideration of the older work in transcapillary transport was presented by Aschheim in 1977 (52) and there are several more recent studies which considerable value (11,53). Borge and coworkers (53) evaluated changes in vascular permeability in inflammation and reviewed the various approaches to the collection of interstitial fluid while Reed and Rubin (11) discussed the role interstitial fluid pressure and the extracellular matrix in capillary permeability. Borge and coworkers (53) also mention that pharmacologically active low-molecular weight compounds such as docetaxel and PGE₁ increase the extravasation of albumin; it is not unreasonable that the transcapillary transport of other proteins would also be affected (54).

There have been several studies on the presence of coagulation factor in lymph and one on synovial fluid. These results are shown in Table 1 below. There are substantial amounts of coagulation proteins in lymph with the studies largely in agreement; there was one exception on the concentration of fibrinogen. The studies reported the presence of sufficient coagulation proteins to support coagulation. The presence of a functional coagulation pathway in synovial fluid may have functional consequences in rheumatoid arthritis (55).

Table 1: Coagulation Factors in Extravascular Fluids

Factor	Thoracic-Duct Lymph (1) ^a	Thoracic-Duct Lymph (2) ^b	Peripheral Afferent Lymph (3) ^d	Limb Lymph (4) ⁱ	Synovial Fluid (5) ^l
Fibrinogen	33%	39%	71.8 ^e	28% ^j	
Prothrombin	63%	65%	61% ^f /30% ^g	26%	21%
Factor V	20%	26%	Nd ^h /21% ^g	8%	<1%
Factor VII	50%	38% ^c	35 ^f /10 ^g	17%	
Factor VIII	30%	-	-	8%	<1%
Factor IX	26%	-	15% ^f /8 ^g		10%
Factor X	60%	52%	25% ^f /29% ^g	37%	
Factor XIII	34%	-	-		
Antithrombin	73%		77% ^g	38%	73%
TFPI ^k				40%	
vWF					<1%
α ₂ -Macroglobulin					

^aData presented as % of normal plasma; data obtained with canine thoracic-duct lymph. It was possible to perform a prothrombin time, thrombin time, and partial thromboplastin time; the values obtained were greater (less activity) with lymph.

^b Human thoracic-duct lymph. Data is presented as % of normal plasma. It was possible to perform a thrombin time and thromboplastin time on the lymph; the as with the studies with the canine lymph (1), the activity of lymph was less than plasma.

^c Determined as factor VII complex ().

^d Human peripheral lymph was obtained by cannulation of lymph vessel in the leg.

^e mg/dL determined as antigen; fibrinogen biological activity is 5-6 times less than antigen.

^f Activity

^g Antigen

^h Factor V activity was not detected (Nd).

ⁱ Rabbit limb lymph

^j All fibrinogen was clottable; no fibrin degradation products were detected.

^k Tissue factor pathway inhibitor.

^l Synovial fluid from patients with osteoarthritis. Thrombin generation was obtained with the addition of tissue factor.

References for Table 1.

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Much of the information on the distribution of plasma protein between intravascular and extravascular space is based on pharmacokinetics following infusion. Two classic papers in this area deserve consideration. The first has to do with pharmacokinetics of fibrinogen in patients with congenital afibrinogenemia (56). Gitlin and Borges (56) concluded that total body fibrinogen mass is divided equally between extravascular and intravascular space. This is consistent with the volume of distribution of plasma proteins is approximately twice the plasma volume such that extravascular protein mass is equal to the intravascular protein mass and that extravascular plasma protein is in dynamic equilibrium with intravascular plasma protein. In situations such as inflammation, capillary permeability may increase with a concomitant increase in total extravascular protein. The second paper was published by Cohen and Freeman in 1960 (57). These investigators evaluated the pharmacokinetics of human γ -globulin isolated from plasma by electrophoresis or chromatography. The γ -globulin fractions were labeled with ^{131}I and infused into human subjects. A rapid initial decrease was observed with the material prepared by electrophoresis representing loss to the extravascular space; this was followed by a second phase associated with equilibration with extravascular space. The material prepared by chromatography did not show the rapid initial decrease followed by equilibration with the extravascular space. These investigators suggest that, unlike the chromatographic material, the fractions obtained by electrophoresis are heterogeneous in turnover rate and distribution into the extravascular space (the electrophoretically purified fractions showed a somewhat higher mass in the extravascular space. The half-life for the electrophoretic material (average 18 days) was somewhat less than that observed for the chromatographic fractions (23 days). The heterogeneity observed with the fractions obtained by electrophoresis might reflect heterogeneity in glycosylation. It was somewhat disappointing to find that, as far as this writer could determine, this heterogeneity and factors influencing distribution between intravascular and extravascular space has not been the subject of subsequent research. There have been subsequent studies on the effect of glycosylation on IgG clearance (58-60) but the potential of distribution into the extravascular space as a factor in

changes in pharmacokinetics is not address (the writer would appreciate being corrected on this statement if incorrect).

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