Studies on the Plasma Proteins in the Interstitial Fluid of Muscle

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It has been shown that the total extravascular mass of preformed plasma proteins is approximately equal to the mass of plasma proteins within the vascular system (*1–6*). It has also been shown that these proteins in the extravascular pool are in dynamic equilibrium with those in the intravascular compartment (*7*). In studies of their distribution in human tissues, it was demonstrated by qualitative methods that various plasma proteins could be found in connective tissue and, to a lesser extent, in the cells of many organs (*8*). The present investigation (*9*) was undertaken in an attempt to determine the actual concentration of the plasma protein present in the interstitial fluid of muscle.

Experimental procedure. Rabbits weighing about 2 kg were employed in this study. A given plasma protein was injected into the marginal ear vein, and 48 hr later, when this protein had equilibrated between the extravascular and intravascular compartments as shown by its exponential rate of fall (*4*), a second protein was injected into the marginal vein of the opposite ear. Fifteen minutes after injection of the second protein, the animal was exsanguinated from the heart, and a muscle sample was immediately taken from the anterior aspect of each thigh. In one instance, No. 817, three samples were taken and treated separately, one from each anterior thigh and a third from the posterolateral aspect of the right thigh.

The muscle samples were weighed rapidly and homogenized in cold saline in a Waring Blendor for a period of 1 min. A third protein was added to the muscle homogenates and mixed thoroughly. After storage at 2°C for 24 hr, the homogenates were centrifuged at 5000 *g* for 1 hr; the resulting supernates were centrifuged at 5000 *g* for 1 hr and then at 24,000 *g* for another hour. The resulting muscle extracts were analyzed for the three proteins employed, and the serums were analyzed for the two proteins that had been injected into the rabbit.

Calculations. Let A represent the protein given intravenously and allowed to equilibrate between the extravascular and intravascular compartments for 48 hr, let B represent the protein given 15 min before exsanguination, and let C denote the protein added to the muscle homogenate; A_s and B_s are the concentrations of A and B in serum; A_m, B_m, and C_m are the concentrations of the three proteins in the muscle extract; and A_o, B_o, and C_o are the total amounts of these proteins in the muscle sample. If V_m is the volume of distribution of proteins A, B, and C in the muscle homogenate, since C_o is known, then:

$$V_m = C_o/C_m \quad (1)$$

and

$$A_o = V_m A_m,\ B_o = V_m B_m. \quad (2)$$

If S_v is the apparent volume of serum left in the muscle sample,

$$S_v = B_o/B_s. \quad (3)$$

If A_i and A_e represent the intravascular and extravascular amounts of A, respectively,

$$A_i = S_v A_s, \quad (4)$$

$$A_e = A_o - A_i. \quad (5)$$

Table 1. Proteins used.

Rabbit	Protein A*			Protein B†			Protein C‡	
	Protein	Amount (mg)	Volume (ml)	Protein	Amount (mg)	Volume (ml)	Protein	Amount (mg)
845	Human serum albumin	2500	10.0	Radioiodinated rabbit gamma globulin	93.2	4.0	Bovine gamma globulin	119
846	Human serum albumin	2500	10.0	Radioiodinated rabbit gamma globulin	93.2	4.0	Bovine gamma globulin	119
847	Radioiodinated rabbit gamma globulin	233	10.0	Human serum albumin	1250	5.0	Bovine gamma globulin	119
849	Bovine gamma globulin	1000	10.0	Human serum albumin	1250	5.0	Radioiodinated rabbit gamma globulin	46.6
852	Bovine gamma globulin	1000	10.0	Human serum albumin	1250	5.0	Radioiodinated rabbit gamma globulin	46.6
817	Human serum albumin	1250	5.0	Bovine serum albumin	300	2.0	Bovine gamma globulin	11.0

* Injected intravenously 48 hr before injection of protein *B*.
† Animals sacrificed 15 min after intravenous injection of protein *B*.
‡ Added to muscle homogenates.

Table 2. Concentration of homologous and heterologous plasma proteins in the interstitial fluid of rabbit muscle.

Rabbit	Muscle wet wt. (g)	Serum concentrations		Volume of distribution of protein C* (ml)	Total found in muscle sample		Amount of serum in muscle sample† (ml)	Amount A in muscle in excess of contribution by serum‡ (mg)	Interstitial concentration of A§ (mg/ml)	Serum conc. A / interstitial conc. A
		Prot. A (mg/ml)	Prot. B (mg/ml)		Prot. A (mg)	Prot. B (mg)				
845	26.3	6.29	0.608	73.4	10.86	0.276	0.454	8.00	2.25	2.80
846	36.8	7.30	.696	79.3	15.22	.178	.256	13.46	2.75	2.67
847	27.0	0.312	10.39	95.2	0.454	3.90	.375	0.337	0.0924	3.38
849	21.3	2.74	10.90	90.0	3.15	2.16	.193	2.62	.911	2.99
852	32.7	2.38	8.70	80.0	5.76	4.24	.487	4.60	1.04	2.28
817a	18.4	3.44	2.54	93.6	3.18	0.467	.184	2.55	1.03	3.34
817b	17.9	3.44	2.54	65.0	3.25	.660	.260	2.36	0.976	3.52
817c	22.0	3.44	2.54	93.6	3.09	.373	.147	2.58	.868	3.97

* Eq. 1. † Eq. 3. ‡ Eq. 2. § Assuming an interstitial fluid of 13.5 percent; Eq. 6.

Finally,

$$A_t = \frac{A_e}{FW} \times 100, \qquad (6)$$

where A_t is the concentration of A in the interstitial fluid, F is the percentage of free interstitial fluid in muscle, and W is the wet weight of the muscle sample.

The proteins used in this study for each rabbit are listed in Table 1. It is to be noted that (i) the proteins were used in a variety of combinations in an attempt to eliminate possible errors resulting from a peculiarity in the properties of one or more of the proteins used, such as selective adsorption to tissue elements; (ii) both radioactivity and immunochemical measurements were employed in combination, and (iii) both heterologous and homologous plasma proteins were used in various combinations. The immunochemical (*10*) and radioiodine (*11*) procedures have been described in detail elsewhere. All native proteins studied were estimated immunochemically.

Results. The results are shown in Table 2. It will be noted that the average residual serum in the muscle samples was about 1.5 percent of the muscle wet weight, and the average residual whole blood, therefore, was about 2.5 percent. On the basis of chloride analyses, Harrison *et al.* (*12*) found that the volume of extracellular fluid in muscle obtained from exsanguinated rabbits similar to those studied in this report was 16 percent of the wet tissue weight. Correcting for the average residual whole blood, the average interstitial fluid volume of the muscle samples in Table 2 was about 13.5 percent of the wet weight of the sample (*13*). For the proteins studied, an average ratio of 3 : 1 was found between the concentration of a plasma protein in serum and its concentration in interstitial fluid. The values obtained for the amount of a given plasma protein present extravascularly after 48 hr equilibration in the rabbit compare reasonably well, as can be seen in Table 3, with those obtained from calculations based on the premise that, in the steady state, roughly as much plasma protein is present outside the blood vessels as is present within the blood vessels.

The ratios of serum protein to interstitial plasma protein, given in Table 2, agree with those given by Weech *et al.* (*14*) for the ratios of serum protein to lymph protein and would appear to substantiate the opinion that the lymph protein concentrations are representative of the protein concentrations of the interstitial fluid (*15*). The presence of such relatively large quantities of plasma protein in the fluid outside the capillaries does *not* mitigate against Starling's hypothesis of the capillary (*16*) but instead simply serves to modify quantitatively the balance of forces influencing the transfer of water and solutes across the capillary wall.

Table 3. Comparison of amount of interstitial plasma protein found with that expected.

Rabbit	Amount plasma protein in interstitial fluid	
	Expected* (mg)	Found (mg)
845	8.3	8.00
846	13.4	13.46
847	0.42	0.337
849	2.9	2.62
852	3.9	4.60
817a	3.2	2.55
817b	3.1	2.36
817c	3.8	2.58

* Plasma volume of muscle sample assumed to be 5 percent. On the assumption that $A_i = A_e$, the expected amount = 5 percent wet weight of sample × serum concentration.

References and Notes

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Mass Separation of Reticuloendothelial and Parenchymal Cells of Rat's Liver

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Recent studies (*1, 2*) have emphasized the importance of the hepatic reticuloendothelial (RE) system in the disposition of exogenously derived cholesterol. Consequently it appeared necessary to study these cells more closely than has been done.

A few years ago, Rous and Beard (*3*) were able to collect some RE cells by perfusing the liver of an animal injected with iron. The iron-containing RE cells present in the perfusate were isolated by means of an electromagnet. More recently, Anderson (*4*) described a method by which the total liver could be reduced to a suspension of its constituent cells. We believed these two different procedures, if combined, might offer a method for the separation and collection of relatively pure, viable hepatic parenchymal and RE cells in quantities sufficient so that the functions of each could be studied independently of the other. The method (*5*) described here achieved this separation.

The livers of rats that had been injected intravenously with 1 ml of a suspension of 15 percent pulverized carbonyl iron and 5 percent starch in saline on 3 successive days were perfused forward through the aorta and backward via the superior vena cava with isotonic calcium-sequestering fluid (1 part 0.11*M* versene, *p*H 7.4 to 9 parts Locke).

The entire liver was then removed and forced through a tissue press (garlic press) into a sufficient volume of fresh perfusion fluid to make a 3 to 4 percent suspension, stirred gently for about 10 min, and then poured through a 10 XX silk screen. Approximately 90 percent of the screen filtrate consists of intact liver cells.

The cell suspension was poured into conical siliconed centrifuge tubes and centrifuged at 45 *g* for 2 min. An upper, relatively clear supernatant layer (containing broken cells, vascular elements, and some of the non-iron-containing reticulocytes) was drawn off and discarded. The remaining two layers, the relatively cream-colored upper layer containing mostly hepatic parenchymal cells and the lower grey-black sediment containing RE cells preponderantly, were removed separately, resuspended in the perfusion fluid, centrifuged and separated as before, adding each layer to its respective counterpart obtained after the first centrifugation. This centrifuging washing procedure was repeated 3 times in all.

In order to obtain a pure collection of RE cells, the combined lower layers were suspended in 20 vol of a 4-percent solution of starch in isotonic saline containing 1 ml of 0.3 percent digitonin and 2 ml of Kreb's isotonic phosphate buffer per 100 ml. This suspension, although still containing some parenchymal cells, consists mainly of iron-containing RE cells.

The cell suspension was poured into a conical tube held upright in the field of a large eye magnet. The tube was rotated while being raised so that the magnetized cells described a helix as they were brought from the mouth to the apex of the tube. The fluid was then removed and resubmitted to the magnet as before. The procedure was repeated once more. All the sediments of iron-containing cells were combined, resuspended in the starch solution, and again brought to the bottom of the tube by the magnet. This resulted in a collection of RE cells of high purity (Fig. 1). In order to segregate hepatic parenchymal cells, the upper sedimentary layers obtained from the initial centrifugal process were suspended in perfusate and submitted to the magnet. The few iron-containing RE cells were quickly moved to the bottom of this nonviscous medium, and the upper layer on removal contained a morphologically homogeneous suspension of parenchymal cells (Fig. 2).

Fig. 1. Magnetized hepatic RE cells, unstained (×800).