Origin and Composition of Hepatic Lymph Proteins in the Dog

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Pappenheimer (11) has shown that the walls of capillary vessels behaved as semi-permeable membranes. Applying this concept to the transcapillary transfer of macromolecules, *Grotte* (4) and *Mayerson* et al. (8) measured the permeability of the dog's capillaries by means of the plasma *versus* lymph concentration ratios of dextran molecules of graded molecular size, after intravenous injection of the substance. Both workers concluded that capillary walls contained two kinds of pores of different size. The molecular sieve effect would be largely accounted for by a set of small pores having a radius of about 40 Å according to *Grotte* (4) or 110 Å according to *Mayerson* et al. (8). The data also required the assumption of the existence of larger pores, of undefined diameter, which would allow free passage to particles of any molecular size up to a mol. weight of 300 000.

It was also shown that each type of tissue had its own characteristic capillary permeability, that of the liver being greater than that of the small bowel, and the latter in turn exceeding the capillary permeability of the limbs or cervical region. Such differences would arise both from regional variations in the distribution density of the pores and from shifts in the ratio of large *versus* small pores, the pore sizes remaining constant.

The findings described above were all obtained by using dextran molecules, but in theory they could equally well have been derived from a study of the plasma *versus* lymph concentration ratio of endogenous plasma proteins. This presentation will be concerned with an attempt in that direction, using as a model the plasma and hepatic lymph of the dog.

Materials and Methods

1. *Surgery*

In twelve mongrel dogs a laparotomy was performed and the hepatoduodenal ligament was exposed by reclining the bowel to the left.

A lymph vessel, efferent from a hepatic lymph node, was dissected free and cannulated by means of 0.8 mm bore polyethylene tubing. Lymphatic channels draining from the intestinal wall into the hepatic nodes were made visible by injecting a small amount of Patent Blue® dye into the bowel wall. By ligating all channels dyed by this procedure it was possible to reduce contamination of hepatic lymph with intestinal lymph to the desired minimum.

Lymph was collected over a period of about one hour and, at mid-time, a sample of venous blood was secured for comparison.

2. *Immunochemical quantitation of proteins*

The method of single radial immunodiffusion (7) was used for the determination of albumin, orosomucoid (acid α_1-glycoprotein), transferrin, α_2-macroglobulin, IgG_{2ab} (the counterpart to human immunoglobulin IgG) and immunoglobulin IgM. The appropriate specific antisera have been described elsewhere (14). Reference curves were established by means of a standard dog serum.

Results

As shown in table 1, the concentrations of the different proteins in serum and lymph were of the same order of magnitude, with however the lymph values always lying below the serum values to an extent depending on the type of protein.

Table 1 Concentration of Proteins (mg/100 ml) in Serum and Hepatic Lymph.

Dog No.	Albumin		Orosomucoid		Transferrin		α_2-Macro-globulin		IgG_{2ab}		IgM	
	Serum	Lymph	Serum	Lymph	Serum	Lymph	Serum	Lymph	Serum	Lymph	Serum	Lymph
1	2697	2697	28.8	28.8	125	144	71	15	770	825	91.0	82.6
2	3161	2465	21.6	12.1	264	192	236	15	1595	407	82.6	32.2
3	4669	2929	96.3	90.0	413	295	226	114	836	616	75.6	36.4
4	3422	3219	63.0	56.2	240	226	215	69	594	231	56.0	29.4
5	4205	3683	70.2	66.2	314	209	157	116	1111	814	130.0	104.0
6	3944	3944	–	–	300	252	185	99	935	704	95.2	36.4
7	2871	2175	–	–	545	262	41	30	968	693	63.0	14.0
8	3944	2958	47.7	36.0	413	206	327	103	1452	726	51.8	16.8
9	3306	2378	40.5	23.4	245	163	100	32	1166	792	105.0	67.2
10	3567	3016	46.8	51.3	240	214	236	189	1375	847	85.4	43.4
11	3248	2465	–	–	281	252	763	393	1045	330	44.8	7.0
12	2842	2726	112.5	100.3	300	240	221	101	440	462	126.0	40.6
Mean	3489	2887	58.6	51.6	306	221	231	106	1023	620	83.8	42.5

Table 2 Relationship between the Concentrations of the Different Proteins in Hepatic Lymph and in Serum.

Protein	Molecular Weight	Number of dogs	$\frac{L}{S}$ Ratio[1]	
			m[2]	σm[3]
Orosomucoid	44 000	9	0,874	0,061
Albumin	69 000	12	0,833	0,035
Transferrin	90 000	12	0,773	0,055
IgG_{2ab}	160 000	12	0,650	0,074
α_2-Macroglobulin	820 000	12	0,506	0,056
IgM	1 000 000	12	0,471	0,049

[1] L: Concentration in hepatic lymph; S: Concentration in serum.
[2] m: Mean of results.
[3] σm: Standard error.

Average lymph: serum concentration ratios, together with their standard deviations, were computed separately for the different proteins and the different animals. These data are presented in table 2.

If it is accepted that the molecular weights of canine and human proteins are the same – a reasonable assumption – then it appears that the lymph/serum concentration ratio bears an evident relationship to the inverse of the molecular weight.

Discussion

The similarity of the protein spectra of hepatic lymph and serum suggests an interrelation between the two media. However, before attempting to analyse this relationship in terms of hepatic lymph/serum concentration ratios for the different test substances, it must be established that all hepatic lymph protein is indeed derived from the blood.

1. *Origin of hepatic lymph proteins.*

Proteins in hepatic lymph need not necessarily be derived from hepatic blood. They might, wholly or in part, originate from the liver cells which actually are their main sites of production in the body. In the rabbit, *Woolley* and *Courtice* (15) have determined the specific activities of albumin and gammaglobulins in serum and hepatic lymph, after intravenous injection of radioactive tracer molecules, and have concluded that hepatic lymph proteins were mainly of plasma origin. Proteins synthesized in the canine liver however, such as albumin and fibrinogen, may directly reach the blood without passing through lymphatic channels (13).

Additional information was gathered from the following experiment which we have described in detail elsewhere (2). Canine plasma, whose proteins where biosynthetically labelled with tritium in a donor dog, was injected intravenously to a recipient dog after withdrawal of an equivalent volume of plasma. A period of three days was allowed to elapse for the different compartments to reach an equilibrium, whereupon samples of hepatic lymph and plasma were collected as described under Methods. Albumin and

Table 3 Comparison of the Specific Activities of Albumin and IgG_{2ab} in Serum and Hepatic Lymph.

Protein	Medium	Quantity of protein mg	Radioactivity[1] d.p.m.	Specific Activity d.p.m./mg
Albumin	Serum	0,5597	2277	*4068*
		1,6791	6905	*4112*
	Lymph	1,6704	6727	*4027*
		2,2272	9163	*4114*
IgG_{2ab}	Serum	0,156	5683	*36429*
		0,104	3864	*37153*
	Lymph	0,126	4561	*36198*
		0,084	3091	*36797*

[1] The determinationes were made on two different volumes of each medium.

IgG_{2ab}-immunoglobulin were determined immunochemically and their specific radioactivities were measured on specific immunoprecipitates. As shown in table 3, both proteins showed identical specific activities (dpm/mg) in the two media, strikingly confirming the conclusion of *Woolley* and *Courtice* (15) and *Smallwood* et al. (13) that hepatic lymph proteins originate mainly from the blood.

2. *Transfer of proteins from blood into hepatic lymph*

Once the plasma origin of the main proteins in hepatic lymph is established, it remains to explain why their relative abundance differs in the two media. The evident relation between the lymph/serum concentration ratio and the molecular weight of any individual protein strongly hints that molecular sieve effects rather than selective reabsorption processes are involved.

Instead of using the mere lymph/serum concentration ratio, it is profitable to refer this ratio, for each individual protein, to the corresponding ratio for albumin. Normalising the data in this manner has the advantage of cancelling uncontrollable causes of variation such as dilution or concentration, as pointed out by *Mayerson* et al. (8) who introduced the use of such parameters, called "relative permeability coefficients" (RPC).

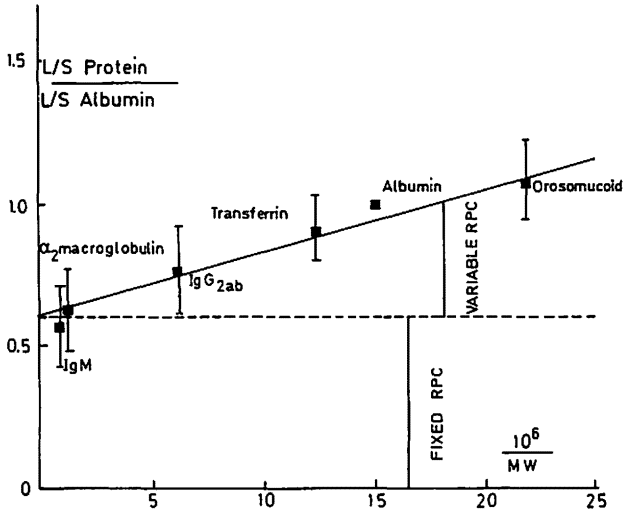

Fig. 1 Relationship between the "Relative Permeability Coefficient" (L/S protein versus L/S albumin ratio) of the proteins and their molecular weight with illustration of the fixed or variable relative permeability coefficients.

As shown in fig. 1, the RPC of the different proteins are linearly related to the reciprocal of the molecular weight (MW), but the line does not cross the origin. This seems to indicate that there are two forms of transfer. On the one hand, the constant term of the equation:

$$RPC = 0{,}606 + \frac{0{,}0216 \times 10^6}{MW} \qquad (1)$$

corresponds to a process of bulk transfer without any modification of the composition of the protein mixture involved. This parameter of *bulk transfer* may be termed "fixed relative permeability coefficient" (FRPC). On the other hand, the variable term refers

to a process by which proteins are transferred at a rate inversely related to their molecular weight. This term, which reflects a *selective form of transport,* will be called the "variable relative permeability coefficient" (VRPC).

The VRPC may be used to derive an estimate of the pore size of the membrane responsible for the molecular sieve effect, on condition however that all pores be uniform diameter.

The passage of solutes, by diffusion and/or filtration, across a semipermeable membrane depends on the dimensions of the molecules as well as on those of the pores. Very small particles pass with the same rate as does the solvent, but the rate of passage becomes the more restricted the closer the particle size approaches the size of the pores. This restriction can be expressed, in terms of filtration, as a restricted pore area (Ar), compared to the total area of the pores (Ap) available for the unrestricted passage of the solvent (12). Alternatively, in terms of diffusion, one might envisage a reduction of the diffusion coefficient (Dr) compared to the free diffusion coefficient (Do) in water. At the molecular scale these two concepts cease to be distinguishable and $Ar/Ap = Dr/Do$.

The concentration ratio of a substance, Cb/Ca, passing from compartment «a» into compartment «b» through a semipermeable membrane, may be described by *Pappenheimer's* (11) equation if the characteristics of the membrane and the molecular dimensions of the substance are known:

$$\frac{Cb}{Ca} = \frac{\left(\frac{Dr}{Qf\,\Delta x}\right) Ap + \frac{Ar}{Ap}}{\left(\frac{Dr}{Qf\,\Delta x}\right) Ap + 1} \qquad (2)$$

where Qf is the rate of filtration of the solvent, Δx the thickness of the membrane, and Ap and Ar respectively the total pore area available to the solvent and to the solute. The ratio of Ar to Ap can be derived from the ratio between the effective diffusion radius of the solute, α, and the radius of the pore, r, according to the equation of *Landis* and *Pappenheimer* (6):

$$\frac{Ar}{Ap} = \left[2\left(1-\frac{\alpha}{r}\right)^2 - \left(1-\frac{\alpha}{r}\right)^4\right]\left[1-2{,}104\left(\frac{\alpha}{r}\right) + 2{,}09\left(\frac{\alpha}{r}\right)^3 - 0{,}95\left(\frac{\alpha}{r}\right)^5\right] \qquad (3)$$

The effective diffusion radius, α, is in turn obtained by the *Stokes* and *Einstein* equation:

$$\alpha = \frac{RT}{6\,\Pi\eta\,Do\,N} \qquad (4)$$

where R is the gas constant, T the temperature, η the viscosity of water, Do the diffusion coefficient of the solute in water and N the number of Avogadro.

Alternatively, when Cb/Ca and Do are known for a given substance, equation (2) provides a means to arrive at an estimate of the pore size, r, provided Qf and Δx be available. In our experiments the latter condition is of course not met. It will be noted, however, that the values of Ap, Δx and Qf are the same for all the test substances employed in a given experiment, so that one may write:

$$\frac{Ap}{Qf\,\Delta x} = K \qquad (5)$$

Now, as discussed above, it is necessary to employ the VRPC ratio instead of the mere Cb/Ca concentration ratio if normalisation of the values is to be achieved. Thereby equation (2) becomes:

$$VRPC = \frac{\dfrac{K\left(Do\dfrac{Ar}{Ap}\right)_i + \left(\dfrac{Ar}{Ap}\right)_i}{K\left(Do\dfrac{Ar}{Ap}\right)_i + 1}}{\dfrac{K\left(Do\dfrac{Ar}{Ap}\right)_a + \left(\dfrac{Ar}{Ap}\right)_a}{K\left(Do\dfrac{Ar}{Ap}\right)_a + 1}} \qquad (6)$$

where data concerning the test substances are indicated by i and those concerning albumin by a.

The values for r and K have been determined by means of the least squares method, using the experimental VRPC results obtained for the different proteins. Then, a theoretical VRPC versus α curve has been constructed (fig. 2). The best agreement with the experimental data was obtained for a pore radius of 102 Å K being: 1.556×10^7.

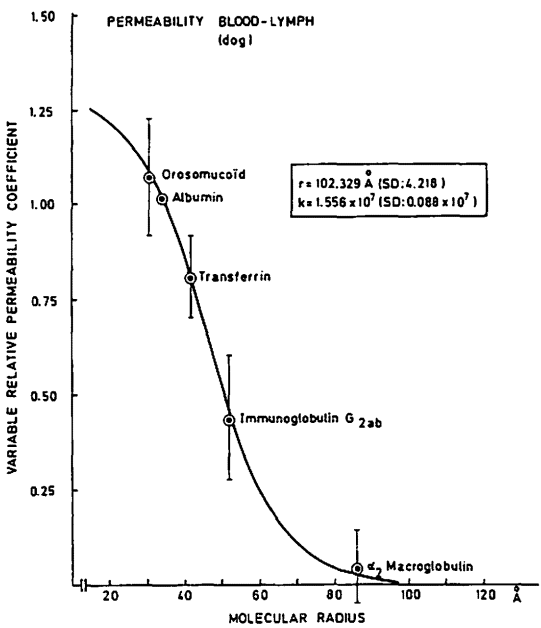

Fig. 2 Pores radius of the semipermeable membrane determined by means of the "variable relative permeability coefficient" and dimension of protein molecules.

3. *Types and sites of passage*

From the preceding data it may be concluded that proteins are transferred from plasma to hepatic lymph by a combination of two processes; one of which consists of an indiscriminate bulk transfer, whereas the other one involves a molecular sieving. Evidently, then, there must exist at least two types of pores in the semipermeable membrane separating hepatic lymph from plasma, one type having a radius of 102 Å and one being of a

size such that no restriction to filtration is encountered. As suggested by *Mayerson* et al. (8), it is however conceivable that the process of bulk transfer does not involve any pore system at all but is achieved by transcellular transfer, process termed *cytopempsis* by *Moore* and *Ruska* (9) or diacytosis by *Jacques* (5).

As to the precise site where the molecular sieving takes place, two kinds of structures may be considered: the vessel walls separating the plasma from interstitial spaces and the barriers between the latter and the lymphatic channels.

The studies of *Grotte* (4) and *Mayerson* et al. (8) on the passage of macromolecules from blood to lymph led them to postulate a similar double system of transfer for all regions studied, which comprised the legs, the cervical area, the small bowel and the liver. Lymphatic vessel walls seem rather unlikely candidates for the qualification as sieving membranes since electron microscopic studies have shown that lymph capillaries are open vessels unable to oppose any resistance to the inflow of proteins (1, 3, 10). It is more reasonable to assume that the barrier responsible for the selective as well as for the bulk transfer of plasma proteins into hepatic lymph resides in the walls of the hepatic blood vessels. Whether the dual capillary and sinusoidal structure of the liver, or the hypothetical boundaries between intra- and perilobular spaces play any role in this respect, appears very doubtful.

Summary

1. The concentrations of albumin, orosomucoid (acid α_1-glycoprotein), transferrin, α_2-macroglobulin, IgG_{2ab} (corresponding to human immunoglobulin IgG) and IgM have been determined in the serum and hepatic lymph of 12 dogs.
2. Protein concentrations were lower in lymph than in serum and the lymph: serum concentration ratio of the different proteins was inversely related to their molecular weight.
3. Evidence is presented that hepatic lymph derives the bulk of its proteins from the plasma.
4. There appear to be two processes involved in the transfer of proteins from plasma to hepatic lymph: on the one hand a bulk transport and on the other hand, a filtration through a semipermeable membrane with pores having a radius of 102 Å.
5. It is probable that this double system of transfer resides in the capillary wall.

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