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M. Ohkuma, Electron Microscopic Laboratory, Department of Dermatology, Tokyo Medical and Dental University, Yushima, Bunkyo, Tokyo/Japan

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Influence of Lymph Flow Rate on Concentrations of Proteins and Dextran in Dog Leg Lymph

W.L. Joyner*, R.D. Carter, E.M. Renkin

With the Technical Assistance of

Mr. E.S. Mishriky and Miss M. Chumar

Department of Physiology and Pharmacology, Duke University Medical Center, Durham, North Carolina 27710

*Department of Physiology and Biophysics, University of Nebraska Medical Center, Omaha, Nebraska, 68125

Summary

Control measurements of lymph:plasma concentration ratios (R) for total protein, albumin, globulin and Dextran-110 over a more than 60-fold range of spontaneous lymph flows (L) in legs of 72 dogs show an inverse relation of R and L which is in qualitative agreement with the prediction of *Drinker's* theory of lymph formation and some of its more recent elaborations. At low to moderate lymph flow rates, the relation conforms to the simplified relation R = PS/(PS + L) where PS is a permeability-surface area product. PS for plasma globulins (mainly gamma, effective radius 55 Å) is about half that for serum albumin (35.5 Å) and PS for Dextran-110 (71 Å) is about one-sixth. The decrease of PS with increasing molecular size characterizes the sieving properties of the blood-lymph barrier. There is considerable variation of PS values among preparations, even under supposedly normal conditions. Legs with high lymph flows tend to have high permeabilities and show diminished sieving.

According to *Drinker's* theory of lymph formation, the concentration of proteins and other large molecules in lymph, relative to plasma, ought to be inversely related to lymph flow rate, provided capillary permeability to these substances remains constant (1). Experimental support for this contention is limited, however. *White* et al. (2) reported that total protein concentration of dog leg lymph decreased as lymph flow increased when unanesthetized dogs with cannulated leg lymphatics were allowed to walk

about, or when venous congestion was applied. *Sugarman* et al. (3) found an inverse relation between spontaneous lymph flow and protein concentration in dog renal lymph, but *Le Brie and Mayerson* (4) found no consistent pattern in this preparation. *Sass,* cited by *Yoffey and Courtice* (5) (see their Table 4.13, p. 265) found that lymph: plasma concentration ratios (R) for total protein, albumin and globulin decreased with increasing lymph flow (L) for the gravid sheep's uterus as pregnancy advanced. *Garlick and Renkin* (6) also reported an inverse relation between L and R for serum albumin in lymph from dog paws. With the exception of the last series, there is no assurance that changes in capillary permeability were not contributing factors to the changes in lymph flow observed, and indeed, other sets of data for various organs do not show well defined relations between lymph flow and protein concentrations (5, 7).

As part of a series of experiments on agents which alter capillary permeability, we have made a large number of control observations on flow and composition of lymph from paws of anesthetized dogs. Spontaneous flow rates varied over a more than 60-fold range. Though there is much variation between experiments, our results confirm an inverse relation between lymph flow and steady-state lymph:plasma concentration ratios for total protein, albumin, globulin and exogenous Dextran-110.

Methods

Seventy-two dogs of both sexes (wt. 10-15 kg) anesthetized with sodium pentobarbital (30 mg/kg, supplemented as required), had lymphatics of both lower legs cannulated with PE #10 tubing distal to the popliteal node. The paws were flexed at the ankle 100 times per minute to promote flow of lymph from the cannulae. Lymph was collected in tared heparinized vials at 30-minute intervals and flow determined from the weight increase. Samples of arterial blood were taken in heparinized syringes at the midpoint of each lymph collection. No heparin was given to the animals. In 59 animals, Dextran-110 (Pharmacia-R) was infused intravenously to maintain plasma concentrations between 3 and 5 mg/ml.

Total protein concentration of lymph and plasma were measured with the Folin reagent according to the method of *Lowry* et al. (8). Albumin was determined by the same method after precipitation of globulins with saturated sodium sulfate, globulins by difference. In many experiments, electrophoresis on polyacrylamide gel was also used to measure concentrations of individual plasma proteins (9); the results agree closely with chemical separation of albumin and globulins and only chemical data were used in compiling the graphs presented here. Dextran was determined by the anthrone method of *Roe* (10). The preparation of Dextran used in these experiments is a relatively narrow fraction of weight-average molecular weight 110,000. Its effective molecular radius is about 71 Å, compared to 55 Å for gamma globulin and 35.5 Å for serum albumin (6).

Results

The experimental data are presented in Figure 1. On separate ordinate scales, against a common abscissa of lymph flow rate (L), are plotted lymph-plasma concentration ratios (R) for Dextran-110 (R_D), serum albumin (R_A), globulin (R_G) and total protein (R_T), as well as albumin-globulin ratio (R_A/R_G). Lymph flow is scaled both in ml/hr and ml/sec, the latter being the standard of reference for permeability calculations.

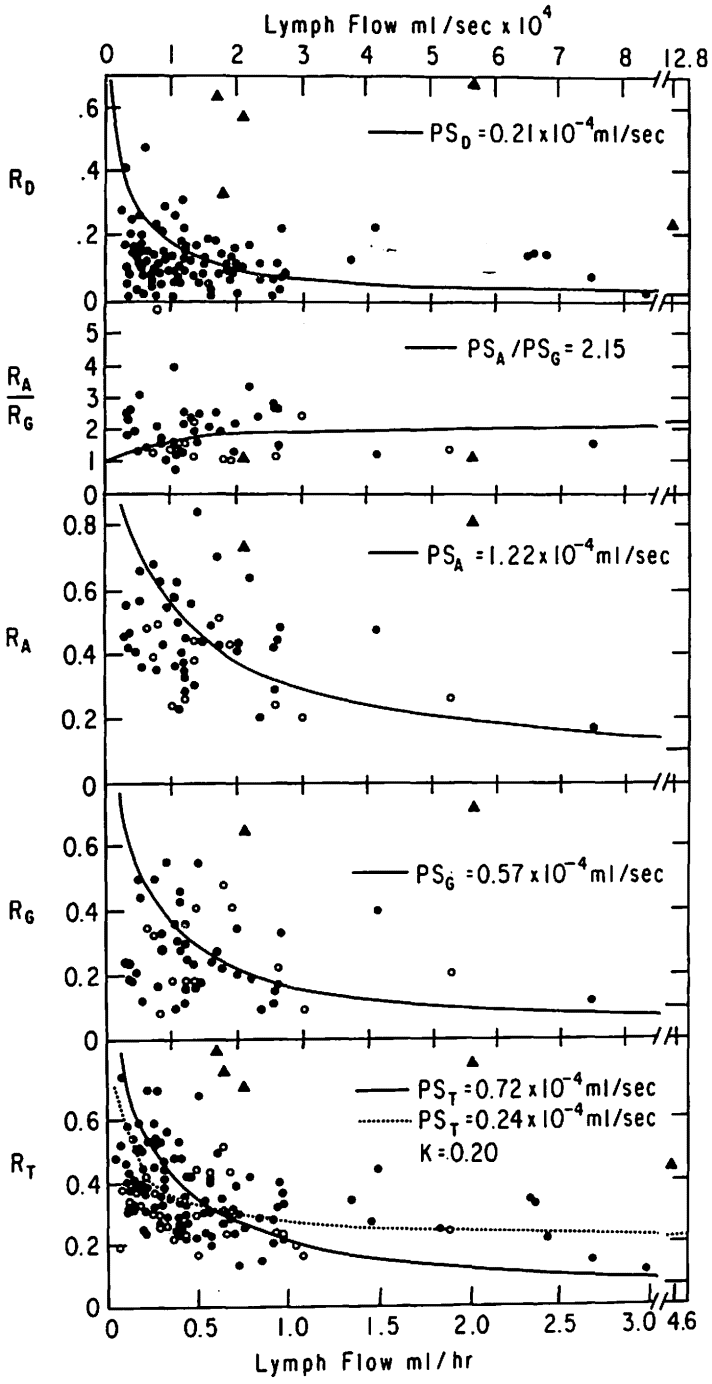

Fig. 1. Lymph:plasma concentration ratios for Dextran-110 (R_D), plasma albumin (R_A), plasma globulin (R_G) and total protein (R_T) as a function of lymph flow (L) in control collections from 72 dogs under pentobarbital anesthesia. Lymph flows are indicated both in ml/hr (practical units) and in ml/sec (for calculation of permeabilities). The ratio R_A/R_G is equal to the quotient of A:G ratios of lymph and plasma. Experimental points: ○ 13 dogs receiving no dextran, ● 59 dogs receiving Dextran, main population, ▲ 3 dogs receiving Dextran presumed to have abnormally high permeability (see text). The solid curves represent the best theoretical fit to the data (excluding ▲'s) by Equation (2) in text, the dashed curve (for total protein only) is a closer fit obtained with Equation (3). The parameters used are indicated on each graph.

The filled circles are data from 59 dogs in which Dextran-110 was infused; the open circles are from 13 animals without dextran. Lymph flows and plasma protein R's do not differ by very much for the two groups. The mean lymph flow for all these preparations was 0.54 ml/hr, with a range of 0.05 to 4.6 ml/hr. The upper two-thirds of this range corresponds to the flow rates reported by *White* et al. (2) for cannulated leg lymphatics of dogs walking about or running. In dogs at rest, with massage or passive limb motion, they obtained flows in the lowest third of our range, where most of our points lie.

The experimental data marked by filled triangles are from three dogs with unusually high lymph concentrations of plasma proteins and Dextrans relative to lymph flow. In all of these experiments, Dextran-110 had been infused. These values have been omitted from subsequent calculation of average parameters for the control population.

The solid and dotted lines in Figure 1 show tentative theoretical relations between R and L for comparison with the experimental values. Their significance is discussed below.

Discussion

Even if we disregard the three specially designated experiments (▲ in Figure 1) there is great scatter of individual data points. However, this does not entirely obscure an inverse relation between L and R for total protein, albumin, globulin and Dextran-110. Much of the variability of R is due to individual differences within our population of presumed normal dogs. Comparison of data for R and L in opposite legs of the same dogs shows a highly predominant tendency to smaller R's in the leg with higher L; for example, this is the case in 45 out of 65 animals for total protein. Lymph-plasma ratios for globulin tend to be lower than for albumin, and lymph-plasma ratios for Dextran-110 are usually lower than either, supporting the concept of progressive restriction to blood-lymph transport with increasing molecular size (6, 11). In the three dogs with exceptionally high R values, there is less decline in R with increasing molecular size than in the rest. For this reason, as well as the high R in relation to L, we consider them to represent abnormal states of increased capillary permeability. Some of the points which we have not specially designated may also belong to this category.

Mathematically, the simplest form of inverse relation between R and L is

$$R = m/l \qquad (1),$$

which implies constant protein or Dextran transport rate (m) from plasma to lymph. This equation does not provide a satisfactory conformation to the data; R falls too steeply with increasing L. A considerable improvement in fit is obtained using the simplified equation for steady-state diffusion interchange between plasma and lymph (12, 13):

$$R = \frac{PS}{PS + L} \qquad (2),$$

where PS represents the diffusion capacity (product of permeability and surface area) of the blood-lymph barrier for a macromolecule. The solid curves in Figure 1 were fitted to the experimental data by calculating PS from individual measurements for simultaneous L and R for total protein, albumin, globulin and Dextran-110 (omitting ▲'s), determining the mean values for each substance, and plotting the curves which represent each mean. Equation (2) permits a satisfactory fit to the data for values of L up to about 1.0 ml/hr. At higher flows, for which there are relatively few points,

R tends to lie above the theoretical curve. The deviation is, however, much less than with Equation (1). Equation (2) fits the data of *Sass* (cited in Ref. 5), *Sugarman* et al. (3) and of *Garlick and Renkin* (6) without consistent deviation at high lymph flows; therefore, we consider it a reasonable standard of reference for our data, representing the average transport capacity of capillaries in the dog's paw for albumin, globulin and Dextran-110 (the value for total protein is, of course, a weighted average for albumin and globulin). The mean values of PS obtained by this procedure (mean ± S.E.M.): $(1.22 \pm .17) \times 10^{-4}$ ml/sec for serum albumin, $(0.57 \pm .07) \times 10^{-4}$ for globulin, $(0.72 \pm .05) \times 10^{-3}$ for total protein and $(0.21 \pm .03) \times 10^{-4}$ for Dextran-110. The values for serum albumin and for Dextran-110 do not differ significantly from those reported (for another group of dogs) by *Garlick and Renkin* (6). The ratio of permeabilities for albumin:globulin:Dextran-110 is 1:0.47:0.17.

A more nearly complete theoretical relation between R and L takes into account direct ultrafiltration transport of solute as well as diffusion (12, 14, 15). It may be written as follows:

$$R = \frac{PS + KL}{PS + L} \qquad (3).$$

In addition to PS, a second constant K is required, representing the fractional contribution of solute to the lymph by ultrafiltration (ratio of membrane pore area available to solute to that for water). With two constants it is easier to fit the data, but more difficult to assign unique values to PS and K.

In Figure 1, a curve according to Equation (3) has been fitted to the experimental points for total protein, using a process of trial and error (dotted line). With K = 0.20, it yields a PS value one-third as large as that obtained by Equation (2), and appears to fit the experimental points more satisfactorily. PS values for individual R's calculated by Equation (2) tend to increase with increasing L in our experimental population, while those calculated by Equation (3) tend to scatter about a constant value. However, we do not believe it is justified to assume constant parameters over the entire range of lymph flows in our experiments, since these were spontaneous flows and it is possible that PS and/or K may have been elevated in at least some of the high-flow preparations. Our values for the ratio R_A/R_G (Figure 1) provide evidence that this is indeed the case. This ratio is a measure of the ability of the capillary membrane to discriminate between albumin and globulin molecules. At lymph flows above 1 ml/hr, R_A/R_G values are distinctly smaller than the average at lower flows, indicating reduced ability of the capillary to separate molecules on the basis of size. The solid line in the graph is the theoretical ratio of R_A/R_G for the mean PS values determined by Equation (2). For constant values of PS_A and PS_G the ratio of R's at high lymph flows should approach that of the PS's. For this reason, we believe that most of the discrepancy between the experimental data and the theoretical curve according to Equation (2), equivalent to Equation (3) with K = 0, is due to an elevated level of permeability in preparations with high lymph flow rather than values of K as large as 0.20.

Variation in permeability even in the low range of lymph flows does not appear to be entirely random, and is certainly not due to analytical error. There seems to be an upward dispersion of R's from a basal level. Preparations with elevated R and PS for individual macromolecules also tend to show reduced discrimination with respect to molecular size. The points marked ▲ in Figure 1 represent the extreme of this dis-

persion. We do not know if such high values of R and PS represent capillary injury from experimental manipulations, or an upper range of normal variability of capillary permeability. In some particularly sensitive dogs, the Dextran-110 infused may have provoked a mild inflammatory response. Such responses are common in rats but rare in dogs (16). The majority of Dextran-treated dogs (filled circles) gave mean PS values for albumin, globulin and total protein according to Equation (2) which were not statistically distinguishable ($p > .05$) from those calculated for the animals receiving no Dextran (open circles). Comparable variation in PS for serum albumin and Dextrans of different molecular sizes in a population of presumably normal dogs (all receiving Dextran) were reported by *Garlick and Renkin* (6), and similar variability is discernible in the publications of other workers (3, 11). Its physiological significance remains to be discovered.

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W.L. Joyner, Ph.D., R.D. Carter, Ph.D., E.M. Renkin, Ph.D., E.S. Mishriky, B.S. Miss M. Chumar, M.Ed., Department of Physiology and Pharmacology Duke University Medical Center, Durham, North Carolina 27710/U.S.A.*
**Department of Physiology and Biophysics, University of Nebraska Medical Center, Omaha, Nebraska, 68125/U.S.A.*