Capillary Permeability to Macromolecules

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Small infusions of dextran fractions having average molecular weights ranging from 10,600 to 412,000 yield plasma to lymph concentration ratios which are directly proportional to molecular weight. The concentration gradient for a specific molecular weight, however, decreases as the volume of infusion is increased. This volume effect, explained in terms of stretching of capillary pores, consequently provides less resistance to the passage of macromolecules through the capillary wall. The significance of these results in terms of the conventional pore theory of capillary permeability is discussed.

During the past several years we have been interested in determining the permeability characteristics of capillaries to colloid molecules. Inherent in our approach to the problem has been the study of the thoracic duct lymph. Earlier observations indicated that thoracic duct lymph protein is wholly derived from plasma protein and that the thoracic duct returns to the bloodstream about two-thirds of the protein that leaks out of capillaries. Measurement of the concentrations of colloid molecules in thoracic duct lymph as well as in plasma have thus provided direct, quantitative data on the permeability of the capillaries to these molecules. The use of colloid molecules is particularly adapted to the study of capillary permeability because the molecules closely approximate the size of pores as usually conceived in the pore theory.

The present report concerns results of experiments in which we have used eight well-characterized fractions of dextran of different molecular sizes, prepared by The Commercial Solvents Corporation and made available to us through the courtesy of the National Research Council. The characterization of these fractions is given in table 1.

METHODS AND PROCEDURES

Dogs were anesthetized with Nembutal (30 mg./Kg. of body weight). Carotid blood pressure was measured with a mercury manometer; blood sampling was done from the femoral artery through an indwelling Courmand needle. Infusions were given into the femoral vein on the contralateral side. The thoracic lymph duct was dissected free and cannulated with appropriate size polyethylene tubing and lymph was collected continuously in heparinized test tubes.

Two types of experiments were performed. In the first type, dextran fractions of varying molecular weights were mixed and the mixture infused. Plasma, lymph, and urine samples, collected at intervals, were fractionated with methyl alcohol and their dextran concentrations and viscosities measured as outlined in the Appendix. From this data, the molecular weight distribution in each sample was calculated. In the second type of experiment, 5 ml./Kg. of body weight of fractions 1 to 8, respectively, were injected intravenously into nephrectomized dogs as a 6 per cent solution in 0.9 per cent saline. Samples of plasma and lymph were obtained at intervals and their dextran concentrations measured as in the first type of experiment. In addition, plasma volumes were measured with radioactive iodinated albumin before and two and four hours after the dextran injections.
RESULTS

Infusion of a Mixture of Dextran Fractions

Two experiments of this type were performed. In the first experiment, 500 ml. of a 14.5 per cent dextran solution in 0.9 per cent saline were infused into a 22 Kg. male mongrel dog. The solution contained the following: 2 Gm. Commercial Solvents Corporation fraction WR 1416 of average molecular weight 158,000; 22 Gm. C.S.C. fraction R 242-2-C-1, average molecular weight of 125,000 and 12 Gm. of each of N.R.C. fractions 1, 2, 3 and 4 (table 1). The infusion lasted 128 minutes. Samples of plasma were collected at frequent intervals for 7 hours after the end of the infusion, while lymph and urine were collected continuously.

Each sample of plasma, lymph, and urine was successively fractionated with methyl alcohol as described in the Appendix to provide fractions having their average molecular weights within the following ranges:

- Fraction 1—100,000-140,000
- Fraction 2—85,000-100,000
- Fraction 3—75,000-85,000
- Fraction 4—50,000-75,000
- Fraction 5—30,000-50,000
- Fraction 6—less than 30,000

The data obtained in this experiment proved to be less definitive than we had anticipated since all of the dextran fractions with average molecular weights of less than 100,000 came to the same concentration in plasma and thoracic duct lymph and a gradient was evident only for the largest fraction with an average molecular weight range of between 100,000 and 140,000. In the latter case, the concentration in the plasma was about 1.3 times the concentration in thoracic duct lymph. The failure of concentration gradients to occur except above 100,000 suggested the desirability of further fractionating this high molecular weight material. The data was consistent in showing that molecules larger than about 40,000 do not appear in the urine.

Since the possibility existed that the relatively large infusion into the normovolemic dog was accompanied by increased leakage of dextran and plasma protein from the plasma, the second experiment was performed on a 29 Kg. greyhound which was bled 1,500 ml. This bled volume was replaced with an equal volume of six per cent dextran solution in isotonic saline. Since the first experiment showed that there was a gradient between plasma and lymph only for molecules over 100,000 M.W., it seemed desirable to add additional high molecular weight dextran to the mixture. This was made up to contain 1 part of each of N.R.C. fractions 1, 2, 3 and 4; 2 parts of N.R.C. fraction 5; and 2 parts of a C.S.C. fraction, RD1-2(1-C-1) with an average molecular weight of 208,000. The infusion took 30 minutes and sampling was continued for about six hours after the end of the infusion.

Concentrations of dextran for each plasma and lymph sample in the ranges from 150,000 to approximately 250,000, 100-150,000, 75-100,000, 50-75,000, and less than 50,000 were determined. Again the only range with an apparent plasma to lymph concentration gradient is for dextran of high molecular weight (over 150,000). The significance of this finding will be discussed later.

Injection of Discrete Fractions of Dextran

Five ml./Kg. of body weight of each of the eight N.R.C. fractions were injected intravenously as a six per cent solution in 0.9 per cent saline into 22 anesthetized, nephrectomized dogs; N.R.C. fractions 1-6 were injected into 3 dogs each, and N.R.C. fractions 7 and 8 into 2 dogs each.

The per cent of injected dextran retained in the plasma at two and four hours after the dextran injection, corrected for blood and

![Graph of percent of injected dextran retained](http://circres.ahajournals.org/)

Fig. 1. Per cent of injected dextran retained (ordinate) in the circulation at 2 and 4 hours (abscissa) after injection of N.R.C. fractions 1 to 8 into 16 anesthetized mongrel dogs.


lymph sampling, is shown in figure 1. The total circulating dextran was obtained by multiplying the \(^{131}\)I labeled albumin plasma volumes at 2 and 4 hours by the plasma dextran concentrations. The dose was corrected for sampling. The percentage retained seems to fall into four distinct groups: fraction 1 is retained least, fraction 2 is retained somewhat better but not as well as fractions 3 to 6. Fractions 7 and 8 are retained best. These differences signify that the rate of dextran disappearance is directly related to molecular weight.

Significant amounts of fractions 1 and 2 apparently disappear from the plasma within the first 15 minutes, the earliest samples taken after the injection, since the volumes of distribution calculated from the 15 minute values show 40 per cent and 20 per cent larger volumes, respectively, than those calculated by the iodinated albumin technic as determined just before the dextran injection and corrected by adding the injection volumes (fig. 2). Volume of distribution of fractions 3 to 8 average about 5 per cent higher than the iodinated albumin plasma volumes. This difference is most likely due to a small amount of dextran left adsorbed to the protein in the plasma separation procedure (see Appendix).

Figure 3 is a plot of the plasma and lymph concentrations for N.R.C. fractions 1 to 8 for 12 typical experiments. Dextran is found in the lymph after injection of all molecular weight fractions, even fraction 8 which has an average molecular weight of 412,000. The plasma and lymph dextran concentrations are the same only for fraction 1. The plasma dextran concentration is higher than the lymph dextran concentration for all of the other fractions. The plasma/lymph dextran concentration gradients for the eight N.R.C. fractions are shown in figure 4. The gradients are directly related to molecular weight, fraction 1 with the smallest molecular weight showing no
gradient and the highest molecular weight fraction (N.R.C. 8) having a gradient of approximately 3.8.

The discrepancy in the plasma/lymph dextran concentration gradients in the two types of experiments presented is due, we believe, to differences in the experimental approach. As previously indicated, the concentration ratios in the first type of experiment exceeded 1.0 only for a molecular weight of above 100,000. On the other hand, the concentration ratios in the second type of experiment were always above 1.0 except for the fraction of average molecular weight of 10,600 (fig. 4). The concentration ratios for the fractions above 100,000 in the first type of experiment correspond to those for a molecular weight of 35,000 in the second type of experiment. In the first type of experiment, there was a large loss of infused volume within a half-hour after the infusion. This loss is related to the relatively large amounts infused and is not significant when smaller infusions are given. It is always accompanied by an increase in lymph flow and a decrease in the plasma/lymph concentration gradient for macromolecules. This is well illustrated by the results of a series of additional experiments in which 40 ml./Kg. of body weight of 5 per cent albumin were infused into anesthetized dogs (fig. 5). In these experiments 80 to 100 per cent of the infusion

* The albumin was generously furnished by the American National Red Cross through the courtesy of Dr. Sam T. Gibson.
fusion volumes had left the circulation four hours after the infusion had ended. The ratio of plasma/lymph protein concentrations before infusion in the experiment illustrated was 2.2. Following the infusion, the ratio approached unity. The reduction in the plasma/lymph concentration ratio is due mainly to a rise in lymph protein concentration which occurs at this time even though the lymph flow increases. This indicates that the increased transudation occurring after a 5 per cent albumin infusion of this size is a high protein transudate. If increased capillary pressure increases filtration or water activity with no change in effective pore size of the capillary, the resulting filtrate should contain a lower concentration of large molecules than when the pressure and consequent transudation are low. Since we obtained the opposite effect, namely, that the resulting filtrate (lymph) contains a greater concentration of large molecules when the transudation rate is high, we conclude that an increase in capillary pressure, as caused by plasma volume expansion, causes the capillaries to stretch and their pores to enlarge with a resultant increased leakage of large molecules. Cassen and Kistler also postulate elastic capillaries with labile pore size as a result of their work on acute pulmonary edema in mice and rats. They observe pulmonary edema fluid to have approximately the same per cent of solids as blood plasma, following blast injury.

The relationship of infusion volume to plasma/lymph concentration gradients is further illustrated by data from other experiments, given in table 2. Here we infused volumes of 10 and 40 ml./Kg. of body weight of clinical and of high molecular weight dextran into anesthetized dogs. The clinical dextran had an average molecular weight between 60,000 and 80,000 but was undoubtedly raised by loss, within four hours, of 35 to 50 per cent of the infused dextran as a result of excretion of small molecules by the kidney. The results indicate that the relatively small infusion volumes of between 5 and 10 ml./Kg. of body weight again result in high plasma/lymph concentration ratios as was the case with similar small infusions in the earlier experiments (fig. 4). Increase of infusion volumes to 40 ml./Kg. of body weight, on the other hand, always results in significantly lower ratios. There thus appears to be an inverse relationship between the volume infused and the plasma/lymph concentration gradients.

Our concept that effective pore size is vari-
able and can be increased by increasing the plasma volume is dramatically supported by the results of experiments shown in figure 6. After a plateau in the level of lymph dextran had been reached, following the injection of 5 ml./Kg. of body weight of N.R.C. fractions 3 and 7, respectively, 40 ml./Kg. of body weight of 5 per cent albumin solution were infused and the observations continued for several hours after the end of the infusion. Lymph flow increased immediately after the start of the infusions and the plasma/lymph dextran concentrations dropped from 1.9 to 1 for fraction 3 and from 3.85 to 1.8 for fraction 7. The initial gradient for fraction 7 is so high that the gradient is reduced after the infusion, not only by a fall in plasma dextran due to dilution, but also by a rise in lymph dextran concentration.

It should be emphasized that in these experiments, we are concerned with changes in permeability and not in capillary fragility. Increased capillary fragility is easily detected by the appearance of red blood cells in the lymph and occurs infrequently under a variety of non-specific conditions. The changes described in these experiments were not associated with the appearance of red blood cells in the lymph and thus are not the result of increased capillary fragility.

**DISCUSSION**

Anatomically, the capillary has been described as a mosaic of endothelial cells held together by a cement substance whose occasional gaps provide openings or pores for exchange of substances between the plasma and interstitial fluid. Pores are not necessarily of uniform size and most probably are randomly distributed between two extremes of sizes, much as the macromolecules used in this study. Information as to the distribution of pore sizes in various capillary beds is furnished by an analysis of the extravascular volume of distribution of dextran of different average molecular weights as shown in figure 7. This
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Fig. 7. Extravascular volume of distribution in ml/Kg (ordinate) of dextrans NRC-1-8 in (abscissa) in anesthetized, nephrectomized dogs four hours after injection of the dextran. Each point, different dog. See text for details.

The smallest molecular weight fraction, NRC-1, has the largest volume of distribution which is approximately the same as the volume of extravascular, extracellular fluid determined by the inulin method.7 The larger molecular weight fractions (NRC-3 to 8) show small extravascular volumes of distribution of approximately 30 ml/Kg of body weight or approximately 0.6 of the plasma volume at four hours after the infusions. These values are probably lower than the true extravascular volume of distribution of these macromolecules, since a specific dextran fraction of higher molecular weight probably is not as uniformly distributed throughout the entire interstitial space as the smaller molecular weight fraction, NRC-1. The thoracic duct concentration of these macromolecules may therefore not be representative of the average concentration of these molecules in the extravascular space. However, the constancy in size of the extravascular distribution volume for dextran of molecular weights between 51,000 through 412,000 can be interpreted as indicating that if a space contains dextran of a molecular weight of 51,000, it also contains dextran of a molecular weight of 412,000. In other words, the constant extravascular volumes of distribution suggest that all of the capillaries which leak dextran of molecular weight of 51,000 or more under the conditions of these experiments have the same size pores or the same proportion of large to small pores. This does not deny, however, that the population of pores is not greater in one capillary bed than in another. It merely means that the distribution of pore sizes is uniform.

The higher volumes of distribution for the smaller molecular weight fractions (NRC-1 and 2) leave open the question as to whether some capillary beds leak dextran of molecular weight less than 51,000 and no dextran of higher molecular weight.

Our method of calculation of dextran extravascular distribution volume expresses the ratio of the amount of dextran which has left the plasma to the thoracic duct lymph concentration. The calculated value thus depends not only on the capillaries that have contributed to thoracic duct lymph but on all capillaries that leaked dextran from the circulation. In the anesthetized dog, Cain and associates8 indicate that the liver contributed one quarter to one-half of the lymph in the thoracic duct. The intestine presumably contributes the bulk of the remainder. Our analysis thus indicates that the distribution of pore sizes in these major organs, at least, is similar.

Pores assume particular importance in the exchange of substances which, because of size or chemical composition, cannot readily diffuse through the endothelial cell. The size of these "non-cell penetrators" relative to the size of the pores is the critical factor in determining their rate of penetration through the capillary wall. This concept of "restricted diffusion" as developed by Pappenheimer3 accounts for the findings on "molecular sieving" presented in figure 4. Pappenheimer uses the term "molecular sieving" when referring to the ratio of concentrations in the filtrate to that in the filtrand (the reciprocal of the
plasma/lymph concentration ratio). He further points out that the degree of molecular sieving of any given solute would depend upon the ratio of its restricted diffusion coefficient to the filtration rate. Working with collodion membranes, he demonstrates the existence of an inverse relationship between molecular sieving (filtrate/filtrand) and filtration rate. This, however, is contrary to our present findings of an increasing ratio of concentrations in filtrate to filtrand with increasing filtration rate (figs. 5 and 6, table 2). The sound physical basis of Pappenheimer's findings with the collodion membrane suggests that the analogy made between the collodion membrane and the biologic membrane in the study of molecular sieving is not a good one. The analogy is at fault in assuming a constant restricted diffusion coefficient, expressing the relationship between pores of constant size relative to constant molecular size. In the relatively rigid collodion system, this is true. On the other hand, the capillary is a relatively elastic system and changes in size of the capillary would conceivably alter the restricted diffusion coefficient as a result of a change in pore size.

It thus appears that the current concept of capillary permeability must be modified to include the possibility of changes in pore size. Changes in pore size undoubtedly account for the large loss of protein and macromolecules following infusion of plasma, blood and blood substitutes and may also account for much of the edema formation in pathologic conditions associated with high venous pressure. The degree of pore size lability, under other conditions than those of the present investigation, remain to be determined by future work.

**SUMMARY**

Plasma and lymph concentration gradients for varying molecular weight dextrans and protein are presented and a direct relation of gradient to molecular weight demonstrated. All sizes of dextran fractions including that with an average molecular weight of 412,000, the largest molecule studied, penetrate capillaries and enter thoracic duct lymph.

The pores in the capillary wall stretch when blood volume is significantly increased. Hence the current concept of capillary permeability should be modified to include a labile capillary pore size, subject to change with variations in capillary volume.

All capillary beds which permit leakage of dextran with molecular weight of 51,000 also permit leakage of dextran with molecular weight of 412,000. All major capillary beds through which macromolecules leak have the same pore size distribution although they may differ with regard to total pore population.

**APPENDIX**

**Determination of Dextran Concentration and Molecular Weight Distribution.** In the first type of experiment five ml. of fluid (plasma, lymph or urine) were diluted with an equal volume of saline and then freed from protein by the addition of 3 ml. of 20 per cent trichloracetic acid and centrifugation. The supernatant was decanted into a 250 ml. centrifuge bottle and the precipitate washed with 5 ml. of 5 per cent trichloracetic acid. The wash supernatant was also added to the 250 ml. centrifuge bottle. This procedure gave a recovery of better than 95 per cent of the dextran, present in the sample. One hundred ml. of cold absolute methyl alcohol was added to the combined supernatants and the samples were left in the refrigerator overnight. The next day, the precipitated dextran was centrifuged, the supernatants were decanted and discarded and the dextran precipitates placed in a vacuum desiccator for drying. After several days of drying, when there was no perceptible odor of CH$_3$OH in the bottles, the dextran was dissolved in 10 ml. of distilled water by constant agitation. The samples were then transferred to 40 ml. conical graduated centrifuge tubes and absolute methyl alcohol was added to give a concentration of methyl alcohol of 45 per cent. This precipitated 50 to 60 per cent of the dextran in the plasma and lymph samples consisting mainly of dextran molecules of over 100,000 molecular weight. The tubes were centrifuged and the supernatants were decanted and saved for subsequent methyl alcohol precipitation at higher concentrations. The precipitates were dried and redissolved in saline and analyzed as detailed below.

Viscosity determinations were made on solutions...
of the fractionated dextrans dissolved in 0.15 M saline in ASTM no. 50 Ostwald-Cannon-Fenske type viscometers at 25 ± .02 C. All solutions were filtered through an M-frit sintered glass filter prior to loading into viscometers. Four viscometers were used during the course of this work. Constants for the viscometers were determined from the flow times of distilled water and redistilled iso-butyl alcohol. From the flow times for the standardizing liquids, the constants A and B in the following equation were determined in the solution of the two simultaneous equations from the data on water and iso-butyl alcohol.

$$\eta = A d t - \frac{B d}{t}$$

$\eta$ for water at 25 C. = 0.8987; $d$ for water at 25 C. = 0.9791; $t$ = flow time in seconds; $\eta$ for iso-butyl alcohol at 25 C. = 3.3155; $d$ for iso-butyl alcohol at 25 C. = 0.7970. The second term in the equation involves a correction for kinetic energy loss during discharge. For dextrans solutions having flow times of about 300 seconds, this correction amounted only to about 0.1 per cent.

N.R.C. fractions 1, 2B, 3, 4 and 5, as well as two fractions furnished by Commercial Solvents Company, were used to determine the intrinsic viscosity-molecular weight relationship applicable to dextran in physiological saline. Viscosity determinations were made on each of these fractions at three concentrations in the range 0.1 to 0.5 g/100 ml. The observed viscosities, $\eta$, were divided by the viscosity of the solvent, $\eta_0 = 0.9007$, to give $\eta_r$, the relative viscosity: $\eta_r = \eta/\eta_0$. The relative viscosity less 1 gives the specific viscosity: $\eta^*_r = \eta_r - 1$.

The reduced viscosity is obtained by division of $\eta^*_r$ by the concentration C in Gm./100 ml.: $\eta^*_r/C = \eta_p$. The intrinsic viscosity, defined as

$$\lim_{C \to 0} \frac{\eta^*_r}{C} = [\eta]$$

were determined from the extrapolated value of plots of $\eta^*_r/C$ vs C at C = 0. In all cases the plots were linear. For fraction 1 the reduced viscosity appeared to be independent of concentration. For the other fractions, a slight positive slope was observed which increased with increasing molecular weight.

The intrinsic viscosity-molecular weight data were plotted according to the relation: $[\eta] = kM$. The log-log plot was found to be linear from approximately 25,000 M.W. through the highest molecular weight fraction examined, 208,000. Molecular weights of the fractionated dextrans obtained from the experimental animals were read directly from this log-log plot.

Due to the small size of the samples obtained from the fractionation experiments, only single concentration viscosity determinations could be obtained. Since the solutions of the fractionated materials were quite dilute, usually less than 0.2 Gm./100 ml., these reduced viscosities may be taken as good approximations of the intrinsic viscosities. From the slopes of the $\eta_p/C$ vs. C curves for the standardizing fractions, it appears that the highest molecular weight samples would be most affected by this approximation. For a solution containing 0.25 Gm./100 ml. of a dextran of molecular weight 210,000, the molecular weight as read from the reduced viscosity would be about 10 per cent higher than that obtained from the intrinsic viscosity. The percentage error decreases with decreasing molecular weight.

Dextran concentrations were determined by optical rotation using a 1 decimeter polarimeter tube. Observations were made at about 25 C. using the d line of sodium as the source of illumination. A value of 199 for the specific rotation of dextran from B-512 strain of Leuconostoc mesenteroides was employed as recommended in private communication from Dr. Homer E. Stavely of Commercial Solvents Corporation. The polarimeter used was graduated to .01 degree.

A total of 6 or 7 fractions were obtained from each plasma and lymph sample and 3 from the urine. For the purpose of comparing the plasma and lymph, the fractions of each sample were plotted on rectilinear coordinates as discrete points with the average molecular weight on one axis and one-half of the per cent of the fraction in the total sample added to the total percentage of dextran of higher molecular weight in the sample on the other (fig. 8), similar to

**Fig. 8.** Each frame contains a plot of dextran fractions in a plasma sample. Number of points indicates number of fractions into which dextran in plasma sample was broken down. Average molecular weight, determined from the intrinsic viscosity-molecular weight relationship outlined in the appendix, is plotted on ordinate. Cumulative percentage of dextran precipitated, plus the percentage of dextran in the fraction indicated by the point, is plotted on the abscissa. B 41, B 101, B 151, and B 181 blood samples collected 10, 130, 277 and 365 minutes, respectively, from the end of the dextran infusion. Note that curves shift upward with time, because of preferential loss of small molecules.
the method of Cragg and Switzer. A smooth curve was then drawn through the points. Such a procedure of plotting would presuppose a Gaussian distribution for molecular weight versus concentration for each fraction with the average molecular weight as the midpoint. Figure 8 is a typical plot for four of the plasma samples collected at times ranging from 10 minutes to 6 hours after the end of the infusion. From such plots, the per cent of each sample in a molecular weight range in the first type of experiment was determined.

The plasma and lymph in the experiments in which the N.R.C. fractions were studied individually (second type of experiment) underwent the same procedure outlined above up to and including the dissolution of the dextran in distilled water after total precipitation with methyl alcohol. The dextran concentrations in the plasma and lymph were subsequently determined by polarimetry as outlined above.

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