Study of the Relationship Between Plasma Volume and Transcapillary Protein Exchange Using I\textsuperscript{131}-Labeled Albumin and I\textsuperscript{125}-Labeled Globulin

By W. Gordon Walker, M.D., Richard S. Ross, M.D., and John D. S. Hammond, M.B., M.D.

A considerable quantity of extravascular albumin and globulin is in equilibrium with plasma albumin and globulin.\textsuperscript{1-4} Tracer studies in man using plasma proteins labeled either in vivo or in vitro have shown concentration-time curves of plasma radioactivity that fall rapidly and at a changing rate during the first 3 to 4 days and at a constant rate thereafter.\textsuperscript{5-8} Disappearance of the labeled material from the plasma during the early phase has been interpreted as representing equilibrium with the extravascular mass of the protein under study. Analysis of edema fluid has confirmed that this equilibrium occurs.\textsuperscript{9-11}

In the present study, transcapillary protein exchange was estimated by measuring the rate of equilibrium of labeled plasma proteins with their respective extravascular protein pools. The relations between the transcapillary exchange rate so measured and such variables as the plasma protein concentrations, total circulating protein mass and plasma volume have been appraised by testing for correlation among these variables.\textsuperscript{12-14} Since it was not feasible to study these interrelationships in man by altering deliberately one or more of these variables in a single individual, correlations among these variables have been studied in the group of individuals. It appears from these data that capillary permeability to protein varies directly with plasma volume and with total vascular albumin (or globulin) mass. At low values for total vascular albumin, transcapillary exchange of this protein appears to cease.\textsuperscript{12-15} In several subjects an effort has been made to eliminate some of the uncertainties of the method through the use of 2 labeled proteins injected simultaneously, permitting comparison of the behavior of 2 proteins in the same subject under identical conditions. For this purpose I\textsuperscript{125} (half-life 60 ± 0.5 d.)\textsuperscript{15-17} has been used in conjunction with I\textsuperscript{131} (half-life 8 d.) to label gamma globulin and albumin respectively.

Methods

Iodinated Proteins

I\textsuperscript{131}-labeled albumin with an initial specific activity of about 75 μCi per mg. of albumin was obtained from Abbott Laboratories (Risa, Abbott). It was always used within approximately 2 weeks following shipment. Twenty-four hour dialysis yielded no more than 2 to 3 per cent unbound or dialyzable iodine. Countercurrent electrophoretic separation of the albumin fraction of sera from 2 patients following injection of the above material revealed that the radioactivity migrated with the albumin fraction.\textsuperscript{8} As has been pointed out previously,\textsuperscript{9} this separation is crude and the albumin fraction contains variable amounts of alpha-1 and alpha-2 globulins, and hence would not pick up the "tailing" demonstrated by paper electrophoresis. Armstrong\textsuperscript{8} has subjected samples of Abbott’s I\textsuperscript{131} albumin, as currently prepared, to paper electrophoresis and reports that with this preparation there is no more "tailing" than is found with S\textsuperscript{35}-labeled albumins in vivo.

I\textsuperscript{125} has a half-life of 60 days and emits a 27 kev x-ray and a 35.4 kev gamma ray.\textsuperscript{10,17} The I\textsuperscript{125} used in the present studies was prepared in the cyclotron at the Oak Ridge National Laboratory. Significant quantities (10-12 per cent) of I\textsuperscript{129} were also produced by this method. However, this isotope has a half-life of 13 days and analyses of the protein data were not begun until approximately 100 days had elapsed after produc-
tion of the material, allowing the interfering radioactivity from I$_{125}$ to decay to a point where it constituted less than 0.05 per cent of the total measured radioactivity.

Processing of the cyclotron target and separation of the iodine was carried out by the chemical division of the Oak Ridge National Laboratory and the processed carrier free I$_{125}$ was forwarded to Abbott Laboratories, where subsequent handling was identical to that of I$_{131}$ except that the material was used to label human gamma globulins. Dialysis and electrophoretic separation gave results similar to those described above for I$_{131}$ labeled albumins.

Because of the reports of low concentration of dialyzable radioiodide in the Abbott preparations and our own findings, the iodinated protein was not dialyzed routinely before use. Urine radioactivity in our subjects during the first few days was no higher than that reported by others who have used only dialyzed material.

**Measurement of Radioactivity**

In the earliest experiments using only I$_{131}$-labeled albumin, radioactivity was measured with an end-window G-M tube. Samples were prepared by drying the plasma and urine (1.0 ml aliquots of each) in nickel planchets. Appropriate corrections were employed for self-absorption due to dried proteins and other solids. The coefficient of variation (C.V.) for a single sample was ± 4 per cent.

For experiments with 2 isotopes and all subsequent experiments, a sodium iodide crystal well scintillation counter was used. For iodine-131 this had an efficiency of 43 per cent. For I$_{125}$ the efficiency was 19 per cent, and showed some variation but by keeping all counting conditions as nearly constant as possible the C.V. for a total of 10,000 counts was 2.5 per cent.

In experiments involving simultaneous use of 2 labeled proteins, radioactivities due to the constituent isotopes were separated by counting each sample on 2 occasions at an interval of 2 to 3 weeks. The fraction of the total measured radioactivity due to I$_{125}$ or to I$_{131}$ respectively was obtained by solution of a pair of simultaneous linear equations. The interval between counting was sufficiently long to allow I$_{131}$ to decay to 30 per cent of its original value or less. The number of counts recorded at each counting was sufficiently large to reduce the coefficient of variation to 0.7 per cent. For a mixture with a ratio of unity, this should have given a coefficient of variation of 1 per cent for the constituent radioactivities.

**Measurement of Protein**

Total serum nitrogen was measured by the Kjeldahl method and converted to protein by multiplication by 6.25 after correction for non-protein nitrogen (NPN). Constituents of the serum proteins were measured by free electrophoresis using the Tiselius instrument using a barbiturate buffer with pH at 8.6. The proportion of the various constituents in the serum was obtained from the area under the appropriate portion of the schlieren diagram. The accuracy of this method has been reported by others. In the first 10 subjects, electrophoretic patterns were not usually done more than once, but total serum nitrogen was measured repeatedly and the coefficient of variation of these reported measurements during the course of an experiment was never more than 4.5 per cent. In subsequent experiments, each serum sample was subjected to electrophoresis using the paper strip technic described by Mackay. Proportions of the constituents of the plasma proteins were measured by the scanner and automatic integrator. Total protein was determined on each sample. The coefficient of variation of replicate measurements of albumin by this technic was 2.5 per cent.

**Experimental Procedure and Subjects**

Studies were carried out on patients from the wards of the Johns Hopkins Hospital. The patients studied had a variety of illnesses and were chosen as a control group for projected studies on patients with heart failure. Availability, absence of heart failure and duration of hospitalization were the primary factors influencing selection of patients. Thus, these were not normal subjects, but represented a variety of illnesses, as shown in table 1. None had any signs or symptoms of congestive heart failure.

Forty to 50 µg of I$_{125}$-labeled globulin and/or 12 to 20 µg of I$_{131}$-labeled albumin were injected intravenously and blood samples were collected 30 minutes after injection, then at hourly intervals for the next 4 hours and finally with progressively decreasing frequency over the next 14 to 18 days. A total of 24 to 28 blood samples were usually collected during the entire observation period. In most cases, complete urine collections were obtained during the first few days of the experiment in order to make certain that there was no rapid initial loss of radioactivity in the urine, which would have suggested the presence of excessive unbound iodine. Patients were kept in bed until extravascular equilibration had occurred to avoid changes in protein concentration associated.
### Table 1

| Patient | Diagnosis* | Race, sex | Age | Weight (Kg.) | Plasma protein albumin mg./ml. | Plasma volume ml./Kg. | Vascular albumin Gm./Kg. | Extravascular albumin Gm./Kg. | Total exchangeable albumin Gm./Kg. | Extravascular albumin fraction | $\lambda_1$ | $\lambda_2$ | $k_p$ (HR-1) |
|---------|------------|-----------|-----|--------------|-----------------------------|---------------------|--------------------------|-------------------------------|--------------------------------|----------------|-----------|-------------|
| PT      | HASCVD, CVA| CM        | 53  | 47.5         | 32.0                        | 31.2                | 0.99                     | 1.43                          | 2.42                           | .59             | .043      | .0028       |
| PT      | AS, CVA    | CM        | 54  | 47.5         | 32.0                        | 45.0                | 1.43                     | 1.75                          | 2.18                           | .54             | .050      | .0024       |
| LM      | CVA, DM, HCVD | CF      | 56  | 39.5         | 27.0                        | 40.0                | 1.54                     | 4.00                          | 5.50                           | .72             | .048      | .0028       |
| RT      | ASCVD, DM  | CF        | 58  | 62.0         | 25.0                        | 22.4                | 0.56                     | 1.54                          | 2.10                           | .73             | .039      | .0043       |
| TM      | CVA, (L), HASCVD | CM     | 54  | 52.5         | 28.0                        | 53.2                | 1.33                     | 2.27                          | 3.60                           | .63             | .033      | .0022       |
| JE      | Neurosyphilis | WM    | 65  | 63.6         | 29.0                        | 32.0                | 0.91                     | 1.21                          | 2.12                           | .57             | .069      | .0030       |
| HMcl,l  | Myocarditis | CF     | 36  | 60.1         | —                           | 30.0                | —                       | —                             | —                              | .66             | .030      | .0028       |
| HC      | Epilepsy   | CM        | 42  | 49.0         | 31.0                        | 63.0                | 1.96                     | 3.34                          | 5.30                           | .63             | .075      | .0026       |
| WB      | CVA (R), HASCVD | CM    | 66  | 70.0         | 31.0                        | 32.1                | 0.99                     | 2.52                          | 3.51                           | .72             | .046      | .0035       |
| NH      | CVA (L), HCVD | CF    | 65  | 53.5         | 23.0                        | 33.9                | 0.79                     | 1.26                          | 2.02                           | .61             | .038      | .0043       |
| SD      | HCVD, Subarach. Hemorrhage | CF    | 73  | 66.8         | 21.0                        | 22.0                | 0.46                     | 0.26                          | 0.72                           | .36             | .062      | .0052       |
| GP      | CVA        | WM        | 73  | 48.5         | 21.1                        | 39.6                | 0.52                     | 0.67                          | 1.19                           | .56             | .056      | .0057       |
| CL      | Morphone Addict. | WM   | 65  | 54.0         | 17.7                        | 56.7                | 0.98                     | 1.96                          | 2.94                           | .66             | .073      | .0031       |
| IP      | CVA        | CF        | 75.5 | 50.0         | 15.0                        | 24.3                | 0.56                     | 0.60                          | 1.06                           | .66             | .047      | .0041       |
| TD      | Thrombophlebitis | CF    | 35  | 64.4         | 28.6                        | 33.0                | 0.81                     | 1.34                          | 2.15                           | .63             | .052      | .0025       |
| JH      | Schizophrenia, Scurvy | WM   | 46  | 57.6         | 23.3                        | 58.4                | 1.36                     | 2.25                          | 3.61                           | .62             | .068      | .0022       |
| JM      | Convalescent Pneumonia | WM    | 70  | 42.0         | 25.9                        | 44.2                | 1.14                     | 1.91                          | 3.05                           | .63             | .064      | .0035       |

**Mean**

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**Key to abbreviations:** HASCVD=hypertensive and arteriosclerotic cardiovascular disease; CVA=cerebral vascular accident; AS=arteriosclerosis; HCVD=hypertensive cardiovascular disease; DM=diabetes mellitus; $k_p$=fraction of plasma protein exchanged with extravascular protein per unit time.

*Data incomplete.

Comparable values for globulin.
with water shifts during postural changes. A representative plasma concentration curve of radioactivity for a 15-day period is shown in figure 1. Plasma volume was taken as the initial distribution volume of the labeled proteins. In the simultaneous albumin and globulin experiments a duplicate measure of this volume was afforded. The mean difference in these duplicate measurements was 8 percent with no consistent discrepancy between the 2.

Model Used for Analysis of Disappearance Curves

Rates of transcapillary exchange and degradation of the plasma proteins were estimated from data obtained from the concentration-time plots of plasma radioactivity by use of methods similar to those used previously by other investigators in the study of rate phenomena. Mathematical treatment of tracer data has been presented in detail by Sheppard and Householder. The model presented below follows the treatments outlined by Sheppard and Householder and others with certain simplifying assumptions. The limitations which the assumptions place upon the method will be discussed later.

Consider a model such as that schematically represented in figure 2, consisting of 3 regions in series. One region, by synthesis and catabolism of proteins, maintains the plasma protein pool. Between the plasma protein pool and an extravascular-extracellular protein pool there is a steady state of protein exchange. It is assumed that: (a) the steady state exists unchanged throughout the period of the observations; (b) the behavior and metabolism of the labeled protein is in no way different from the native protein of the individual; (c) metabolic degradation occurs at some site that is in direct equilibrium with the plasma (e.g., liver); (d) no reutilization of the radioiodine occurs following metabolic degradation; (e) mixing within the plasma and within the extracellular pool is rapid and complete; and (f) transeapillary protein exchange can be represented by a single rate. This is clearly an oversimplification and the limitation of this assumption will be discussed further subsequently.

The derivation to be employed is similar to previous presentations.

Let: 

\[ P = \text{total quantity of protein under consideration in the vascular bed.} \]
\[ E = \text{total quantity of that protein that is extravascular.} \]
\[ k_{pe} = \text{fraction of plasma protein exchanged with extravascular protein per unit time.} \]
\[ k_{ep} = \text{fraction of extravascular protein exchanged per unit time with vascular protein.} \]
\[ k_{pm} = \text{fraction of vascular protein metabolized per unit time.} \]
\[ \lambda_1, \lambda_2 = \text{exponential constants appearing in solution (and experimental data).} \]

If now a small quantity \( P^* \) of a protein species is labeled and introduced into the plasma at time \( t = 0 \) and rapid mixing occurs, then the rate of change of the labeled protein is given by:

\[ \frac{dP^*}{dt} = -k_p P^* + k_{ep} E^* \]  
\[ \frac{dE^*}{dt} = k_{pe} P^* - k_{ep} E^* \]  
where \( P^* \) is the quantity of labeled protein in vascular volume at time \( t \) and \( E^* \) is the quantity of labeled protein in extravascular protein pool at the same time.

Converting to concentrations and solving equations (1) yields the second order equation:

\[ \frac{dC_p^*(t)}{dt} + (k_p + k_{ep}) \frac{dC_p^*(t)}{dt} + (k_p k_{ep} - k_{pe} k_{ep}) C_p^*(t) = 0 \]  
which has for its solution:

\[ C_p^*(t) = C_1 e^{-\lambda_1 x_1 t} + C_2 e^{-\lambda_2 x_2 t} \]  
where \( C_1 \) and \( C_2 \) are constants of integration evaluated for the condition \( C_p^*(0) = C_1 + C_2 \) and \( C_p^*(0) = \lambda_1 C_1 + \lambda_2 C_2 \); and \( \lambda_1, \lambda_2 \) are exponential constants determined from the auxiliary equation:

\[ \lambda_1 + (k_p + k_{ep}) \lambda_1 + (k_p k_{ep} - k_{pe} k_{ep}) = 0 \]  
Thus, equation (3) is a description of the plasma concentration of radioactivity as a function of time. By evaluating the constants from the experimental data as described in figure 2, the fractional exchange rates can be determined by the following relations obtained from (4):

\[ k_p = -\lambda_1 C_1 - \lambda_2 C_2 \]  
\[ k_{ep} = -(k_p + \lambda_1 + \lambda_2) \]  
\[ k_{pm} = k_p - k_{pe} \]

While these relations permit calculations of the rates, it should be reemphasized that they are based on the assumptions listed above and, hence, failure of the system to meet these assumptions will result in false estimates of the rates.

Results

Table 1 contains data obtained from 17 subjects chosen from the hospital population to represent a "control" group as described above. Complete studies were obtained on all except one subject. In the figures to follow, each point represents one subject.

Diffusion rather than filtration must be
invoked to explain transcapillary exchange of substances of small molecular weight. If this is the mechanism for transcapillary protein exchange, then it is expected that the quantity of protein exchanged per unit time will vary linearly with its concentration in the plasma, providing that the permeability characteristics of the capillaries are constant, or nearly so.

Figure 3 is a scattergram showing the relation between serum albumin concentration and the "mean transcapillary exchange rate" for each subject (k_pe times plasma albumin concentration gives a value for this rate in grams exchanged per 100 ml. of plasma per hour). The scatter is great and affords no significant support for a linear relation. Hence, if diffusion is to be retained as the mechanism of transcapillary albumin exchange, it is necessary to postulate considerable variation in capillary permeability to albumin. The permeability constant of a membrane with regard to any given solute is defined as the quantity of solute passing across the membrane per unit surface area of membrane, per unit thickness of membrane, per unit concentration difference across the membrane per unit time. In the intact individual, it is impossible to obtain information about size and thickness of the capillary membrane and consequently a precise measure of the permeability of the capillary membrane to protein is not possible under these circumstances. However, k_pe is a rate constant for transcapillary albumin and in a given individual the value of this constant is determined by the variables above. In trying to evaluate the factors involved in the regulation of k_pe, this rate constant was tested for correlation with serum albumin concentration and plasma volume. These results are shown in figure 4, left and right. There is evidently no correlation between plasma albumin concentration and k_pe in the subjects studied; however, a close correlation exists between the plasma volume and k_pe (r = .75, p < .001). Indeed, partial correlations between the 3 variables, plasma albumin concentration, plasma volume and k_pe, reveal that the correlation between plasma volume and k_pe is the only one of significance and it is not altered appreciably by eliminating any possible correlation between plasma albumin concentration and k_pe. Thus, the larger the plasma volume, the greater k_pe for albumin, and conversely, with small plasma volumes the permeability to albumin becomes quite low.

The Starling hypothesis assigns to the plasma proteins (primarily albumin) the principal role in maintenance of the volume of plasma. Since the resultant of the opposing hydrostatic and osmotic forces on either side of the capillary membrane determines the equilibrium distribution of water across this membrane, the total circulating vascular protein mass is the principal determinant of the plasma volume. One factor regulating the size of this vascular mass is the leakage rate of the protein across the capillaries. Figure 5 is a graph of vascular albumin mass (albumin concentration × plasma volume + body weight) against albumin exchange rate (k_pe × vascular albumin mass). The dashed lines and heavy dots show the relation expected between plasma volume and k_pe shown in figure 4 right (vascular albumin mass calculated for plasma volumes between 10 and 70 ml./Kg. at 10 ml. intervals assuming no change from the main albumin concentration of 2.6 found in these studies; corresponding values for k_pe...
for these plasma volumes obtained from the relation given in figure 4 (right). The open circles represent the data obtained from the subjects studied. The agreement is good and shows that as the vascular albumin mass diminishes the quantity of albumin exchanged approaches zero. Thus, at values below 0.3 Gm. albumin/Kg. body weight, the albumin exchange becomes quite small. From this and observations on animals, it is to be expected that the quantity of albumin in the extravascular pool should diminish quite rapidly as the quantity of vascular albumin approaches 0.3 Gm. per Kg. body weight. Figure 6 confirms this relation (the line of best fit for regression of Y on X has an intercept at y = 0.35 when X = 0). It is also evident that there is considerably greater variation in the quantity of extravascular albumin than in the vascular albumin.

If this phenomenon really represents a change in the permeability characteristics of the capillaries, then these relationships should hold for other plasma proteins. As described under Methods above, information on this point has been obtained using I131-labeled albumin and I125-labeled globulin in experiments designed to obtain simultaneous measures of transepidermal exchange rate of albumin and globulin. A summary of the globulin data is included in table 1.* Because of the difficulty in obtaining I125, it has only been possible to obtain data in 11 individuals; however, in general, the behavior of the globulin appears to be qualitatively the same as the albumin. Figures 7 and 8 are plots of vascular mass versus exchange rate, and vascular versus extravascular globulin (r = +76, p < .01). Globulin exchange rate is plotted against vascular globulin mass in figure 7, while in figure 8 the extravascular globulin mass is plotted against the vascular mass. These plots are comparable to figures 5 and 6, in which the same relationships were shown for albumin. The analysis used in figure 5 for albumin could not be applied to the small number of globulin observations. However, comparison of figures 5 and 7 reveals similar behavior for the 2 proteins. The relationship between the vascular and extravascular globulin masses is shown in figure 8 and is seen to be similar to that for the albumin masses as in figure 6.

Additional information regarding the relationship between albumin and globulin transcitapillary exchange may be obtained by comparing the simultaneously measured rates directly. As indicated by equations 5a–d, the data from each individual yield 2 fractional rates for the transcortexillary exchange, one being the fraction of the plasma protein moving into the extravascular spaces (kpe) and the other fraction moving from extravascular to vascular space (kvp) per unit time. Both of these fractional rates have been converted to

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*Table 2, which contains details of the globulin data has been deposited as Document number 6312 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D.C. A copy may be obtained by citing the Document number and by remitting $1.25 for photoplants, or $1.25 for 35 mm. microfilm. Advance payment is required. Make checks or money orders payable to: Chief, Photoduplication Service, Library of Congress.
A comparison of the albumin exchange rate, Gm./100 ml./hour, with the serum albumin concentration in 16 control subjects. The correlation is low and of doubtful significance. At normal concentrations the exchange rate appears to vary independently of the albumin concentration.

Gm./Kg./hour and the data from these simultaneous studies are shown in figure 9. The correlation coefficient for the group is .78 (p < .01). Thus, the relation between albumin transcapillary exchange and simultaneously measured globulin exchange appears to be a linear one over the ranges found (regression equation: albumin exchange rate = 1.20 globulin exchange rate + .013) and the globulin data appear to support the inferences concerning capillary permeability to protein drawn from the albumin data. It is interesting that after correcting the differences in concentration of albumin and globulin, the ratio of the exchange rates (i.e., the ratio of the fractional exchange rates, k_{pe} albumin/k_{pe} globulin) of albumin to globulin is 1.6, a value very close to the ratio of their diffusion coefficients (D_{alb.}/D_{glob.} = 1.61 at 20 C. in aqueous solution). This relation is to be expected if the exchange process is primarily one of diffusion.

Discussion

These results reveal a much more highly significant correlation between the plasma volume and the transcapillary exchange rate of the proteins studied than between this rate and serum protein concentration. The correlation between this rate and the vascular albumin mass (Gm. albumin/Kg. body weight) is also greater than the rate-concentration correlation. These observations, plus the relationship observed between the quantities of protein in the vascular and extravascular regions and the other data presented above, are consistent with the interpretation that the capillary permeability to protein is greatest with large quantities of protein and large plasma volumes and diminishes as these 2 quantities diminish. Indeed, extrapolation suggests that below a value of 0.3 Gm. albumin/Kg. capillary permeability to protein is insignificant. Such fluctuating permeability would serve to maintain plasma volume within relatively narrow limits despite wide fluctuation.
The relation between the albumin exchange rate (Gm./Kg./hour) and the total circulating vascular albumin (Gm./Kg.). The dashed line and closed circles are calculated from the relation shown in figure 4 right (plasma volume and $k_{av}$) for plasma volumes between 10 and 70 ml./Kg. body weight assuming a constant plasma albumin concentration. (Coordinates for closed circles were obtained from the relations: Y in Gm./Kg. body weight = plasma volume ml./Kg. body weight $\times$ average plasma albumin concentration; X in Gm./Kg. body weight = Y in Gm. vascular albumin per Kg. expected from figure 4 right.) Open circles are actual values for these parameters in the group of subjects studied. It can be seen that at lower values for vascular albumin the albumin exchange approaches zero, while at higher values the exchange increases much more rapidly than does the vascular albumin.

Quantities in total exchangeable protein. There appear to be 2 possible explanations for this variation in permeability with variation in plasma volume. The first is that the individuals with the largest plasma volumes have a greater number of capillaries per unit body weight, providing a greater surface for exchange; the second is that there is an actual change in permeability of the capillary (i.e., a change in the fraction of the capillary area available for diffusion). The results of these studies do not provide information that permits a choice between these 2 possibilities.

It must be emphasized that the relations described above are obtained from studies on a number of subjects and it does not necessarily follow that these relations hold in a given individual when one or more of the quantities measured above is varied. Evidence from other sources to be considered below suggests that these observations also hold for the individual.

Other criticisms are to be raised concerning this type of approach to the study of capillary permeability, and these are concerned with the assumptions made in order to analyze these plasma disappearance curves of labeled protein. The first of these is the assumption of integrity of the labeled protein. Evidence has been offered to support the suggestion that iodination of the protein may alter it sufficiently to change its rate of destruction. However, since the present data suggest that the transcapillary exchange appears to be a function of the physical characteristics of the molecule (size, diffusion coefficient), this criticism has less force here than in studies concerned primarily with degradation rates.

A second assumption, much more difficult to justify, is the assumption that transcapillary exchange can be represented by one rate (this assumption is inherent in the model described above). This is an obvious oversimplification, since evidence is available in the literature indicating that there are several different rates. In a more general treatment, Sheppard and Householder have shown that in a system such as that considered here,
Correlation between the total vascular gamma globulin and the transcapillary globulin exchange rate. Comparison with Figure 5 reveals that the behavior of albumin and globulin is very similar, \( r = +.76, p < .01 \). If there is a large number of rates normally distributed about a single mean, instead of one transcapillary rate, the error introduced in measuring this mean rate by assuming that it is a single rate is quite small. However, if the distribution is not normal, or if the rates are distributed about 2 or more means, then the error introduced may be appreciable. The closeness with which the data can be represented by a function of the form of equation (3) (i.e., the sum of 2 exponentials) is one measure of the validity of this assumption. Although in some subjects considerable scatter was evident in the concentration of radioactivity in the early samples, in only 2 cases did it appear that the data could be better represented by the sum of 3 exponentials. This observation is not consistent with the experience reported by Berson, his report emphasized the necessity for postulating at least 2 rates to describe transcapillary exchange. The difference between his data and those reported here may have arisen as a result of the difference between the duration of observation in the 2 groups. His observations usually extended over a period of 30 or more days and it was impossible to follow the present group for more than 2 weeks. Because of this, it is possible that an intermediate rate (with half-life 4 to 6 days) may have been overlooked. If this is a true discrepancy, then the estimates of the transcapillary exchange rate will be in error by a variable amount, depending upon the magnitude of this second phase. Our average figure for transcapillary albumin exchange rate \( k_{ae} \) of 0.026 per hour agrees reasonably well with the value of 0.031 per hour reported by Schoenberger and others using a similar method of estimation of the transcapillary exchange rate from the plasma disappearance curve of I\( ^{131} \) labeled albumin. The 5 curves obtained by these authors containing 3 exponential terms gave values for the total exchange rate (transcapillary plus metabolic) that did not differ greatly from values obtained from the curves containing only 2 exponential terms. The values for plasma volume and transcapillary exchange rate obtained by Lewallen and others on their patients studied in the euthyroid state fall within one standard deviation of the regression line when compared with our data presented in Figure 4 right. Another consequence of overlooking an intermediate rate in our data would be that we underestimate the fraction of protein that is extravascular. The average value for the extravascular fraction of the above data is \( .61 \pm .03 \) (SEM), which compares very favorably with Berson’s value of \( .60 \pm .01 \) (SEM). Although we cannot be certain that we have not overlooked a very fast component of transcapillary albumin exchange, this should not invalidate the relation between plasma volume and transcapillary exchange rate described in Figure 4 right, since such an error would yield plasma volumes that are too high and exchange rates that are too low. Such errors would tend to obscure the positive correlation between plasma volume and transcapillary exchange rate.

Two other assumptions require justification. The first is that reutilization of the radiiodine does not occur. Evidence tending to justify this has been presented by others. The second is that metabolism of serum albumin is not a property of all body cells, i.e., that
it occurs at some site supplied by only a small segment of the total circulation. Evidence to support this assumption has been obtained by Miller and Bale by perfusion studies of the rat liver and carcass. The curves published by Berson are presumptive evidence that this is also true of man. The assumption that the steady state obtained is likewise an approximation at best since there is evidence that the relation between vascular and extravascular compartments varies throughout the day. However, if there are no net trends, no serious error should arise. While it seems reasonable that this should be so in normal individuals, it is much less likely that these conditions should be met in a variety of diseases associated with disturbances in the vascular and interstitial fluid volumes; however, some index of the validity of the steady state assumption in such situations should be afforded by following body weight, plasma protein concentration and hematocrit. These were stable in the above subjects during the period of study.

There is evidence from several sources suggesting that this relation between total circulating protein, plasma volume and protein permeability holds in individual subjects. Chinard demonstrated a marked increase in urinary albumin clearance following intravenous infusion of serum albumin to nephrotics. Moreover, when expressed as the ratio albumin clearance/creatinine clearance, this increase persists (i.e., the albumin clearance increases out of all proportion to increase in creatinine clearance, with this divergence of the clearances becoming much more pronounced as the serum albumin concentration approaches normal values). It is also interesting that this ratio correlated much more closely with the plasma volume than with the serum albumin concentration. This was interpreted as evidence that the permeability of
the glomerular capillaries to protein was changed following the protein infusions, the direction of change being consistent with the data above. Gitlin's observation that the concentration of albumin in edema fluid of nephrotics was .001 and .002 Gm. per cent on 2 occasions constitutes evidence from still another source. This value for protein concentration is much less than one per cent of the values reported by Stead and Warren for normal individuals.

Weech and others in plasmapheresis experiments made serial simultaneous measurements of lymph and plasma protein and noted "... that the fall in lymph protein occurred at a more rapid rate than the decline in plasma protein." In an attempt at explanation they comment, "... diminished permeability of the capillaries has to us been the most acceptable explanation..." They did not, however, have any data on plasma volume or total circulating protein, and hence it was not possible to relate this to changes in plasma volume. Wasserman, Joseph and Mayerson have published the half-times and vascular-extravascular albumin ratios for dogs before and after infusion of an amount of albumin approximately equal to the circulating plasma albumin. Calculation of exchange rates from their data using equation (4) above reveals that, on the average, infusion of this quantity of albumin resulted in a 3-fold increase of the transcapillary exchange rate of albumin. Evidence of a different sort was obtained by these workers in experiments using infusions of large quantities of dextrans of varying molecular weights. They noted that when the plasma volume was markedly expanded with high molecular weight dextrans the concentration of protein in the plasma and lymph tended to become equal. Thus, the experimental data available both in man and in animals following infusion of albumin and plasmapheresis are all consistent with the interpretation of the data presented in the present report.

**Summary**

A mean transcapillary exchange rate for albumin and gamma globulin has been obtained from plasma disappearance curves of I\(^{131}\)-labeled albumin and I\(^{125}\)-labeled gamma globulin. A much closer correlation exists between transcapillary exchange rate for albumin and the plasma volume than between the rate and serum albumin concentrations. Also, for albumin this rate varies with plasma volume and circulating protein in such a manner as to suggest that the capillary permeability changes with changing plasma volume. The relation between vascular and extravascular albumin is consistent with this interpretation, the extravascular mass of albumin appearing to approach zero as vascular albumin approaches the vicinity of 0.35 Gm./Kg. In experiments in which I\(^{131}\)-labeled albumin and I\(^{125}\)-labeled globulin were employed to obtain simultaneous data on albumin and gamma globulin, a good correlation has been obtained between the simultaneously measured albumin and globulin transcapillary exchange rates. The ratio of the fractional capillary exchange rates of these 2 substances is very nearly the same as the ratio of their diffusion coefficients, a relation to be expected if diffusion is the mechanism of transcapillary exchange.

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**Summario in Interlingua**

Un valor medie pro le intensitate del exambio transcapillar de albumina e globulina gamma eseva obtenite ab curvas de disparition de albumina a I\(^{131}\) e de globulina gamma a I\(^{125}\) ab le plasma. Un multo plus directa correlation existe inter le exambio transcapillar de albumina e le volume del plasma quo inter ille exambio e le concentration de albumina in le zero. In plus, in le caso de albumina le intensitate del exambio varia con le volume del plasma e le proteina del circulacion in un maniera que suggere que le permeabilitate capillar se altera con alterationes del volume de plasma. Le relation inter albumina vascular e albumina extravascular es in congruentia con iste interpretation, in tanto que le massa extravascular de albumina pone aproximare zero quando le
albumin vascular approach un nivello de 0,35 g per kg. In experimentos in que albumina a 1^st e globulina a 1^st essee utilizite pro obten data simultanea pro albumina e globulina gamma, un bon correlation essee obtenite inter le simultaneomente mesurate intensitates del exchange transcapillar de albumina e de globulina. Le proportion del intensitates de exange capillar pro le 2 mentionite substantias es plus o minus identic con le proportion de lor coeffieientes de diffusion. Un tal relation es a expeetar si diffusion es de facto le mecanismo del exange transcapillar.

References

Study of the Relationship Between Plasma Volume and Transcapillary Protein Exchange Using $^{131}$I-Labeled Albumin and $^{125}$I-Labeled Globulin

W. GORDON WALKER, RICHARD S. ROSS and JOHN D. S. HAMMOND

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