Vascular-extravascular exchange of $I^{31}$ plasma proteins in the rat

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Rates at which plasma proteins penetrate from the vascular system into extravascular spaces in tissues was measured with $I^{31}$-labeled rabbit gamma globulin, rat gamma and beta globulins, and rat albumin. After vascular mixing was complete, as measured with $Cr^{51}$-labeled erythrocytes, initial rates of increase in the ratio of tissue radioactivity to plasma radioactivity were used to calculate vascular-extravascular transfer rates; these are reported as grams blood plasma equivalents which penetrate the vascular-extravascular barrier in 1 gm tissue each hour. Transfer rates ranged from 0.06-0.76 for visceral organs to 0.0005-0.005 for tissues such as muscle, fat and skin; the transfer rates were roughly proportional to the vascularity of the tissues. Ratios of tissue to plasma radioactivity reached at equilibrium were used to calculate the amounts of plasma proteins present extravascularly. These protein masses ranged from 0.02 gm plasma equivalents per gm tissue for muscle to 0.13 for skin. Significant differences between the various plasma proteins were found only in the liver, spleen and stomach.

METHODS AND PROCEDURES

Rat red blood cells were labeled with $Cr^{51}$ according to the procedure of Cooper and Owen (3) at the level of 10-20 $\mu g$ Cr/ml red blood cells. The $Cr^{51}$ activity not associated with the red blood cells was removed by centrifuging the labeled blood, removing the supernatant and washing the $Cr^{51}$-labeled red blood cells with twice their volume of rat plasma. The red blood cells were used for injection within 24 hours.

Rabbit gamma globulin was fractionated from normal rabbit serum by the method of Jager and Nickerson (4). The gamma globulin was then dialyzed against 2 liters of borate buffer at pH 8.0 as prepared by Pressman and Eisen (5). Protein determinations were routinely carried out by the method of Lowry et al. (6) and were in the range of 10 mg protein/ml. Rat gamma and beta globulin fraction was isolated from rat plasma by the same technique.

The rat and rabbit globulins were labeled with $I^{31}$ by a method similar to that described by Talmage et al.
(7) where iodide is oxidized to iodine before it is added to the protein. The carrier-iodine-free Oak Ridge 131 solution containing NaI04 as reducing agent was prepared as follows: to 1–3 ml of a 1 to 5 dilution of the 131 solution containing approximately 1 mc I131 were added 1–4 drops 0.01 M KI, approximately 3 drops 0.178 N HCl until pH 1.0 was reached, and 2 drops 0.071 N NaNO2. After a 2-minute waiting period in which a slight iodine color developed, 2 ml gamma globulin solution was added at room temperature and rapidly mixed by inversion several times. The iodinated protein was dialyzed twice against 2 liters 0.85% saline at 4°C for at least 5 hours each time. The dialyzed 131 protein was then centrifuged for 10 minutes at 10,000 g to remove any precipitable protein. The percentage of 131 incorporated into protein ranged from 3.3 to 34.5% which labeled the globulin with 0.17–5.5 iodine atoms/protein molecule on the average (assume mole wt. = 160,000). One-half milliliter normal rabbit or rat serum was added as protective protein. By precipitation with 20% trichloracetic acid (TCA) plus 1% NaHSO3 it was found that 97.5–98.5% of the 131 was protein bound. The iodinated material was either used within 1 day or was frozen and stored for subsequent use within a 2-week period. The association of 131 radioactivity with a particular plasma protein was routinely confirmed by paper electrophoretic analysis of both the 131 plasma protein and the plasma obtained from rats which had received the 131 plasma protein.

Rat albumin was fractionated from rat serum by starch electrophoresis as described by Miller et al. (8). The albumin was dialyzed against borate buffer as previously described, and the protein concentration was 3.6 mg/ml. The albumin was iodinated as before except that mixing of the I131 and protein solutions was accomplished by a "jet" procedure comparable to that used by McFarlane (9). Nonprotein I131 was reduced to less than 3% of the total radioactivity by dripping the iodinated protein solution through an amberlite IR-4B (OH) anion exchange resin similar to that used by Talmage et al. (10). The percentages of 131 incorporated into protein were 16.7 and 22.7 for two different iodinations, and the number of iodine atoms per albumin molecule were 0.15 and 0.21, respectively (assume mole wt. = 69,000).

Female rats of the University of Rochester stock derived from the Wistar strain were used in all experiments. They weighed between 160 and 230 gm and were provided with 6 (10–6) n KI drinking water commencing at least 36 hours before experiments in order to reduce the uptake of inorganic I131 by the thyroid.2 Except for a few experiments with Cr51 red blood cells where the rats were injected under CO2-02 anesthesia, all rats were injected intravenously under ether anesthesia via the saphenous vein with measured volumes ranging from 0.300 to 0.500 ml. The injection material consisted of Cr51 red blood cells (10–20 μc) or I131 plasma protein (about 2 μc). The mass of the protein injected was approximately 1 mg of labeled protein plus 1–15 mg of unlabeled protective protein. The mass of labeled red blood cells injected was approximately equal to that found in 0.2 ml rat blood. After the rats were weighed, they were sacrificed by decapitation from 30 seconds to 7 days after injection, and a sample of circulating blood was obtained by letting the blood drain into a beaker containing about 1 mg (0.1 ml) heparin/5 ml blood.

All radioactivities were determined by measuring gamma emission with a well-type scintillation counter. The standard error of the lowest counts never exceeded ±6%, and the resolving time loss of the highest counts never exceeded 1%. After the tube was counted, it was weighed on an analytical balance, and net weight of sample was obtained by difference. Whole body radioactivity was determined by pushing the live rat to the bottom of an 800-ml beaker placed on a brass platform 10 cm above the scintillation crystal.

In order to determine the amount of radioactivity injected and to account for physical decay of radioisotopes, standards of the injection material were prepared.

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2 It was reported in UR-524 (11) that when I131 plasma protein is injected into rats only 0.5–1.0% of the injected I131 is retained by the thyroid and that the I131 label is not reutilized to any significant extent by components in the plasma or tissues over a 7-day period.

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Fig. 1. Blood content of tissues from exsanguinated rats as determined with I131 rabbit gamma globulin and Cr51 rat blood cells. The mass of blood in the vascular compartment of the spleen could not be distinguished from that in the extravascular compartment. Data were obtained from about 15 rats.
I\(^{131}\) standards were made alkaline with NaOH in order to keep any I\(^{131}\) not attached to protein in the reduced nonvaporizable iodide form, and to prevent adsorption losses of I\(^{131}\) protein to volumetric glassware as discussed by Reeve and Franks (12).

Nonprotein I\(^{131}\) was estimated periodically in tissue and plasma samples by determining the radioactivity not precipitated by a solution of 20\% TCA plus 1\% NaHSO\(_4\). Tissue samples were analyzed by homogenizing 0.5-gm portions in a glass homogenizer containing 3 ml TCA solution. Skin samples were pretreated by freezing them in liquid nitrogen as described in UR-524 (11).

I\(^{131}\) plasma proteins were usually screened in vivo before they were used, according to the method described by McFarlane (13). This was necessary because 7-25\% of the unscreened I\(^{131}\) plasma protein was rapidly removed from circulation by the liver, the I\(^{131}\) activity was subsequently eliminated from the liver and excreted as inorganic I\(^{131}\) (11). About 100 \(\mu\)C of I\(^{131}\) protein were injected into each of four to six rats. After the I\(^{131}\) protein had circulated for several hours, the rats were sacrificed, and the plasma was collected and used for subsequent injections.

**RESULTS**

Rate of vascular mixing and the mass of red blood cells and plasma in the vascular compartment of tissues. The rate of mixing of Cr\(^{51}\)-labeled red blood cells with unlabeled erythrocytes and the mass of red blood cells in the tissues were investigated in about 25 rats sacrificed at various intervals after receiving Cr\(^{51}\) red blood cells. Plots of the ratio of Cr\(^{51}\) radioactivity per unit weight tissue to Cr\(^{51}\) radioactivity per unit weight red blood cells versus time after injection reached constant values within 30 seconds after injection in all the tissues sampled except lymph nodes, skin, and spleen. In the skin and lymph nodes the constant equilibrium values were not reached until about 10 minutes after injection, and in the spleen the equilibrium ratio was not reached until about 1 hour after injection. These results indicate that for most tissues the mixing of injected red cells with native unlabeled blood erythrocytes was very largely complete within 30 seconds. After mixing was complete, the ratio of Cr\(^{51}\) activity per gram tissue to Cr\(^{51}\) activity per gram red blood cells gave the mass of red blood cells contained in 1 gm tissue. The Cr\(^{51}\) activity per gram red blood cells was determined from the Cr\(^{51}\) activity per gram blood divided by the blood hematocrit. The masses of red blood cells contained in the tissues are plotted in figure 1.

The mass of plasma in the vascular compartment of tissues was determined by distinguishing the I\(^{131}\) activity in the vascular compartment from the I\(^{131}\) activity in the extravascular compartment of tissues obtained from rats which were sacrificed at various intervals after receiving I\(^{131}\) plasma proteins. The ratio of I\(^{131}\) activity per gram tissue to I\(^{131}\) activity per gram plasma was extrapolated by the method of least squares described by Ostle (14) to the time when vascular mixing is complete. Since the I\(^{131}\) protein accumulates slowly in the extravascular compartment of tissues (see fig. 3) compared to the rate of accumulation in the vascular compartment of the tissues, the extrapolation was made to zero time. Only in the spleen where the I\(^{131}\) protein activity ratio increased more rapidly than the Cr\(^{51}\) activity ratio, was it impossible to distinguish the I\(^{131}\) protein activity in the vascular compartment from the I\(^{131}\) protein activity in the extravascular compartment. Values for gram plasma per gram tissue are plotted in figure 1 as determined in about 15 rats which were injected with I\(^{131}\) rabbit gamma

**FIG. 2.** Extravascular I\(^{131}\) tissue activity per I\(^{131}\) plasma activity versus time after intravenous injection of I\(^{131}\) rabbit gamma globulin. The extravascular radioactivity ratio was determined by subtracting from the total radioactivity ratio the vascular radioactivity ratio as plotted in fig. 1. Points represent individual animals.
globulin. Only the value determined with in vivo screened \textsuperscript{113}I rabbit gamma globulin was plotted for the liver since the unscreened \textsuperscript{113}I rabbit gamma globulin gave a significantly higher value. Values were also determined for all of the tissues with in vivo screened \textsuperscript{113}I rat gamma and beta globulins and with in vivo screened \textsuperscript{131}I rat albumin, but no values significantly different from those plotted in figure 1 were found.

Rate of extravascular accumulation of intravenously injected \textsuperscript{131}I plasma proteins. It is assumed that increases in the ratio of tissue radioactivity to plasma radioactivity in rats sacrificed subsequent to vascular mixing represents migration of \textsuperscript{131}I plasma protein into the extravascular compartment. A plot of extravascular cpm/gm tissue divided by cpm/gm plasma is shown in figure 2 for kidney and skin taken from rats which received \textsuperscript{131}I rabbit gamma globulin; the values for kidney were determined with in vivo screened protein. The extravascular radioactivity ratio was determined by subtracting from the total radioactivity ratio for a particular tissue the vascular radioactivity ratio (fig. 1) for the same tissue. Figure 2 shows that the \textsuperscript{131}I rabbit gamma globulin penetrates to the extravascular compartment of the kidney quite rapidly and that vascular-extravascular equilibrium is reached within a few hours. In the skin, on the other hand, the vascular-extravascular exchange is much slower, and equilibrium is not reached until 3-4 days after injection. The accumulation of \textsuperscript{131}I rabbit gamma globulin in the extravascular compartments of most of the tissues in the rat is shown in figure 3. The plot of the liver includes only values obtained from 38 rats which received in vivo screened protein. Screened and unscreened values obtained from 100 rats were pooled for spleen, lung, heart, kidney and skin since no differences were detected between the screened and unscreened \textsuperscript{131}I rabbit gamma globulin. Only unscreened values obtained from 62 rats were available for the other tissues.

Similar results were obtained from experiments in which 58 rats received in vivo screened \textsuperscript{131}I rat gamma and beta globulins and in which 53 rats received in vivo screened \textsuperscript{131}I rat albumin. The initial slopes of the plots of radioactivity in unit weight tissue divided by radioactivity in unit weight plasma versus time after injection are indicated in figure 4. The initial slopes with 95\% confidence limits on the mean were computed by the method of least squares as described by Ostle (14). Only 6-28 points during the initial portion of the slope which appeared to be somewhat linear were used. For example, in the case of kidney the points prior to 5 minutes after injection were used, and for the skin points prior to 2 hours after injection were used; these plots are shown in figure 2. Slopes for the other tissues were determined in the same way. In figure 5 the initial slopes for the whole tissue arc plotted. These slopes and confidence limits were determined by multiplying the slopes and confidence limits for unit weight tissue, shown in figure 4, by the fraction of the body weight comprising the tissue as reported by Caster et al. (15).

Before the slopes in figures 4 and 5 can be expressed in terms of vascular-extravascular transfer rates, certain corrections must be considered. First of all, the slopes are somewhat too high because of the accumulation of nonprotein \textsuperscript{131}I, but from measurements of nonprotein \textsuperscript{131}I by TCA precipitation it was found that this correction would not reduce the slopes by more than 10\%. Secondly, the slopes for accumulation of \textsuperscript{131}I activity in the extravascular compartments of tissues as plotted in figures 4 and 5 were determined as the rate of increase in the ratio of tissue radioactivity to plasma radioactivity. Since the plasma radioactivity decreases while the tissue radioactivity increases, the increase in the radioactivity ratio is naturally a function of both of these processes. The rate desired is that attributed only to radioactivity transfer into the extravascular compartment of the tissue, viz., the increase in the ratio of tissue...
VASCULAR-EXTRAVASCULAR EXCHANGE OF $^{131}I$ PLASMA PROTEINS

Fig. 4. Rates of transfer of $^{131}I$ plasma proteins from the vascular compartment to the extravascular compartment of unit weights of individual tissues. Tissue values in this figure represent initial slopes of plots of cpm/gm divided by cpm/gm plasma; these plots were obtained by sacrificing rats at various intervals after intravenous injection. One gram represents $1\%$ body weight. Lines indicate $95\%$ confidence limits on the estimate of the mean slope. Where no confidence limits are indicated, the limits were too close together to be shown. For each tissue the lower closed symbol for $^{131}I$ rat albumin indicates the value after correcting for decreasing plasma activity. Where no correct value is indicated, the correction was too small to be shown. Corrections are not indicated for the other $^{131}I$ proteins because the relative corrections are the same as for the $^{131}I$ rat albumin.

Fig. 5. Rates of transfer of $^{131}I$ plasma proteins from the vascular compartment to the extravascular compartment of the whole tissue. The values in this figure were obtained by multiplying the values in fig. 4 by the tissue weights. It is assumed that tibia is representative of all bone. For further details see the legend of fig. 4.

radioactivity to plasma radioactivity when the plasma radioactivity remains constant. Theoretically, this rate could be determined directly from a plot of the percentage dose per gram tissue versus time, but it is difficult to determine the absolute slope this way because the plasma radioactivity decreases rapidly and causes this slope to quickly deviate from a straight line. However, the relative slopes which were determined over a time interval $t_i$ were converted easily into absolute slopes by assuming that the initial rate of accumulation of $^{131}I$ protein in a tissue is proportional to the concentration of $^{131}I$ protein in the plasma. Then

$$\frac{dx}{dt} = \rho f(t)$$

Integrating equation 1

$$\int_0^{t_i} dx = \rho \int_0^{t_i} f(t) dt$$

$$\rho = \frac{\int_0^{t_i} dx}{\int_0^{t_i} f(t) dt} = \frac{x_i - o}{\int_0^{t_i} f(t) dt}$$

where $x_i$ is the percentage dose per gram tissue in the extravascular compartment at time $t_i$, and the integral of $f(t) dt$ is the area under a curve of percentage dose per gram plasma plotted from zero time to time $t_i$. From plots of extravascular cpm/gm tissue divided by cpm/gm plasma and percentage dose per gram plasma versus time, the values in equation 2 were determined over the time interval $t_i$. The transfer rates, $\rho$, ranged from about $97\%$ to $71\%$ of the uncorrected slopes as shown for $^{131}I$ rat albumin values in figures 4 and 5. Corrected slopes for $^{131}I$ rabbit gamma globulin and $^{131}I$ rat gamma and beta globulins were not indicated in figures 4 and 5 because the percentage corrections were practically identical to those shown for $^{131}I$ rat albumin.

The corrected slopes as they are plotted in figure 4 now represent the rate at which plasma proteins pene-
trate the vascular-extravascular barriers. These transfer rates are expressed as the number of gram plasma equivalents transferring to extravascular space of 1 gm tissue/hr., where 1 gm plasma equivalent is the amount of the particular plasma protein fraction contained in 1 gm of blood plasma. For very low values which can not be read from figure 4 refer to table 2 presented later. The corrected transfer rates for the whole tissue are plotted in figure 5.

Mass of plasma protein in the extravascular compartment of individual tissues. Values were obtained for the ratios of extravascular cpm/gm tissue divided by cpm/gm plasma after vascular-extravascular equilibration occurred; these values obtained from 8 to 30 rats are plotted in figure 6. In figure 7 the ratios of radioactivity in the whole tissue divided by plasma radioactivity are plotted; these values were obtained by multiplying the ratios for unit weight tissue shown in figure 6 by the fraction of body weight comprising the tissue.

Before the equilibrium ratios for the different I\(^{131}\) plasma proteins are compared and expressed in terms of mass of extravascular plasma protein, certain corrections must be considered. First of all, the I\(^{131}\) activity ratios must be corrected for nonprotein I\(^{131}\). By TCA precipitation of plasma and tissue samples it was found that these corrections reduced the ratios by 4-15%.; these corrections are indicated in figures 6 and 7. Secondly, the final equilibrium values plotted in figure 6 after correction for nonprotein I\(^{131}\), are dependent essentially upon three factors, the rate of I\(^{131}\) protein transfer into the extravascular compartment, the fractional rate of I\(^{131}\) protein return to the vascular compartment, and the rate and location of I\(^{131}\) protein catabolism. From evidence reported by McFarlane (13) and from data presented in UR-524 (11), it appears that plasma proteins are catabolized in compartments rapidly equilibrating with the vascular compartment; this could include the extravascular compartments of visceral organs such as liver, kidney and spleen. Actually, this means that the I\(^{131}\) protein activity in most extravascular compartments will lag behind the decreasing I\(^{131}\) protein activity in the vascular compartment where catabolism is effectively occurring. This contrasts with the situation for homologous plasma protein where the plasma protein concentration in the vascular compartment does not decrease in concentration because protein catabolism is balanced by protein anabolism. Thus, it is necessary to reevaluate the tissue radioactivity ratios for the condition of constant plasma radioactivity. These corrections are actually manifested in accounting for specific activity in the extravascular compartments exceeding the specific activity in the vascular compartment.

In order to estimate the magnitude of these corrections, the following assumptions were made: all extravascular cpm/gm tissue divided by cpm/gm plasma, these plots were obtained by sacrificing rats at various intervals after intravenous injection. Lines indicate 95% confidence limits on the estimate of the mean. Where no confidence limits are indicated, the limits were too close together to be shown. For each tissue the upper open symbol indicates the value corrected for nonprotein I\(^{131}\) activity, and the lowest closed symbol indicates the value corrected for both non-protein I\(^{131}\) activity and decreasing plasma radioactivity. Where no corrected value is indicated, the correction was too small to be shown. The corrected values can be expressed as the number of gram plasma equivalents per gram tissue where 1 gm plasma equivalent is the amount of the particular plasma protein fraction contained in 1 gm of blood plasma.

FIG. 6. Amount of I\(^{131}\) plasma protein in the extravascular compartment of unit weights of individual tissues. The values in this figure represent the constant equilibrium values reached in plots of extravascular cpm/gm tissue divided by cpm/gm plasma, these plots were obtained by sacrificing rats at various intervals after intravenous injection. Lines indicate 95% confidence limits on the estimate of the mean. Where no confidence limits are indicated, the limits were too close together to be shown. For each tissue the upper open symbol indicates the value corrected for nonprotein I\(^{131}\) activity, and the lowest closed symbol indicates the value corrected for both non-protein I\(^{131}\) activity and decreasing plasma radioactivity. Where no corrected value is indicated, the correction was too small to be shown. The corrected values can be expressed as the number of gram plasma equivalents per gram tissue where 1 gm plasma equivalent is the amount of the particular plasma protein fraction contained in 1 gm of blood plasma.

FIG. 7. Amount of I\(^{131}\) plasma protein in the extravascular compartment of the whole tissue. The values in this figure were obtained by multiplying the values in fig. 6 by the tissue weights. It is assumed that tibia is representative of all bone. For further details see the legend of fig. 6.
vascular compartments communicate directly with the vascular compartment; there is a random transfer of I\(^{131}\) proteins from the vascular to extravascular compartments, and this transfer occurs at rates proportional to the concentration of I\(^{131}\) protein in the vascular compartment; the I\(^{131}\) protein entering the extravascular compartment mixes uniformly and instantaneously with the unlabeled plasma proteins in the compartment; a constant fraction of the I\(^{131}\) protein in the extravascular compartment returns to the vascular compartment per unit time; and catabolism of I\(^{131}\) protein occurs in the vascular compartment. Mathematically this can be expressed as

\[
\frac{dx}{dt} = pf(t) - K_2 x
\]

where \(K_2\) = fraction of I\(^{131}\) protein in extravascular compartment returned to vascular compartment per hour, and the other symbols are defined as before. If the concentration of I\(^{131}\) protein in the plasma remained constant after vascular-extravascular equilibrium, viz. there were no catabolism, \(f(t)\) would be a constant, \(f(t)k\) and \(dx/dt\) would equal zero. Then

\[
f(t)k = K_2 x
\]

and

\[
\frac{x}{f(t)k} = \frac{p}{K_2}
\]

By letting \(y\) equal \(f(t)\) and solving for \(K_2\) in equation 3,

\[
y = K_2 \frac{dy}{f(t)k} - x
\]

where \(x/f(t)\) is the uncorrected radioactivity ratio for different tissues plotted in figure 6. Substituting for \(K_2\) in equation 4

\[
\frac{x}{f(t)k} = \frac{y}{f(t)} - \frac{x}{f(t)k} \frac{1}{p} \frac{dy}{dt}
\]

After vascular-extravascular I\(^{131}\) activity equilibrium is reached, a semilogarithmic plot of the plasma radioactivity versus time is a straight line with slope \(a\). Therefore,

\[
y = K_2 e^{-at}
\]

and differentiating

\[
\frac{dy}{dt} = -K_2 ae^{-at} = -ay.
\]

Dividing the numerator and denominator in equation 5 by \(y\) and substituting \(-a\) for \((dy/dt)/y\),

\[
\frac{x}{f(t)k} = \frac{y}{f(t)} \frac{x}{f(t)k}
\]

The corrected radioactivity ratios, \(x/f(t)\), were estimated by substituting into equation 6 values for \(p\) and \(x/f(t)\) plotted in figures 4 and 6, respectively, and values for \(a\) measured experimentally from the exponential part of the plasma radioactivity curve. These corrections for plasma protein catabolism which are plotted in figures 6 and 7 were less than 4% for all the tissues except the stomach, fat, muscle and skin in which they ranged from 14 to 38% largely because of the slow vascular-extravascular turnover (fig. 3).

The masses of plasma protein in the extravascular compartment of individual tissues are plotted in figure 6 as the corrected radioactivity ratios and are expressed as the number of gram plasma equivalents per gram tissue. The amounts of plasma protein located extravascularly in the whole tissue are plotted in figure 7.

**Distribution of plasma proteins in the extravascular compartment as a whole relative to the vascular compartment as a whole.** The ratio of total extravascular radioactivity to total vascular radioactivity was determined in experiments where the whole body radioactivity as well as the plasma and tissue radioactivities were determined. The percentage of injected radioactivity remaining in the whole rat at various intervals after injection was determined by relating the radioactivity in the rats at time of sacrifice to the radioactivity in the rats immediately after injection. The percentage dose in the vascular compartment was determined by multiplying the percentage dose per mass of blood plasma equal to 1% body weight by the mass of the blood plasma in the rat, also expressed in terms of percentage body weight (3.75 ± 0.1—standard error of mean). The difference between the radioactivity in the whole body and the radioactivity in the vascular compartment gave the radioactivity in the extravascular compartment. The ratios of I\(^{131}\) activity in the vascular compartment to I\(^{131}\) activity in the whole rat

<table>
<thead>
<tr>
<th>Protein Injected</th>
<th>Intravenous</th>
<th>Intravenous</th>
<th>Intravenous</th>
<th>Intravenous</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>I(^{131})</td>
<td>I(^{131})</td>
<td>I(^{131})</td>
<td>I(^{131})</td>
</tr>
<tr>
<td>Rat Albumin</td>
<td>2.69</td>
<td>3.4</td>
<td>4.0</td>
<td>5.6</td>
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<tr>
<td>Rat y and (\beta) Globulin</td>
<td>0.295±0.015</td>
<td>0.342±0.015</td>
<td>0.340±0.016</td>
<td>0.374±0.016</td>
</tr>
<tr>
<td>Rat y Globulin</td>
<td>2.34</td>
<td>1.92</td>
<td>1.94</td>
<td>1.67</td>
</tr>
<tr>
<td>Rabbit y Globulin</td>
<td>2.24</td>
<td>1.84</td>
<td>1.86</td>
<td>1.61</td>
</tr>
</tbody>
</table>

\(p\) = content in vascular compartment; \(L\) = content in extravascular compartment; * \(L/P\) corrected for nonprotein I\(^{131}\); † \(L/P\) corrected for decreasing plasma radioactivity by eq. 7.

Standard errors of means indicated in line 3.
after vascular extravascular equilibration are tabulated in table 1 in the column designated $P'/(L + P)$. These ratios did not change significantly from 3 days to 7 days after injection. Before these values can be compared and evaluated in terms of the distribution ratios of homologous plasma proteins, however, they must be corrected for nonprotein $^{131}$I activity and reevaluated for the situation where the $^{131}$I protein activity in the plasma remains constant with time.

In the first place, the corrections for nonprotein $^{131}$I were of little consequence (11); the corrected values of $L/P$ were lower than the uncorrected values by only about 4%. Second, in order to account for $^{131}$I plasma protein catabolism, equation 6 was utilized. This equation can be modified as follows:

$$L \over P_k = {L \over P} \left(1 + 3.75 \times {1 \over I_0 \times {P \over P}}\right)$$

where

- $P' = \text{transfer rate into extravascular compartment expressed as gm plasma equivalents/hr.}$
- $L/P_k = \text{distribution ratio for the condition of constant }^{131}\text{I protein concentration in the plasma.}$

In order to estimate $P'$, which is the average transfer rate into the extravascular compartment as a whole, it is necessary to consider the tissues responsible for the majority of the extravascular accumulation and to exclude those tissues in which small amounts of extravascular plasma protein are exchanging much more rapidly than the average. Figure 7 shows very clearly that the skin, fat, muscle, small intestine and bone account for over 95% of the extravascular protein. Therefore, $P'$ was estimated by summing the transfer rates for these tissues (fig. 5); this gave a value of 0.012 gm plasma equivalents/hr. for $^{131}$I rat albumin, and other $^{131}$I plasma protein fractions were not significantly different. The corrected distribution ratios of extravascular to vascular plasma protein, $L/P_k$, tabulated in table 1 are practically identical (1.37-1.48) for the different $^{131}$I proteins, and agree very well with the distribution ratios determined by Campbell and associates (16) who accounted for the complications of plasma protein catabolism by a different method.

**DISCUSSION**

The rates at which plasma proteins penetrate from the vascular compartment to the extravascular compartments differ greatly from one tissue to another and are roughly proportional to vascularity. The transfer rates were expressed as the number of gm plasma equivalents which transfer each hour into the extravascular space of 1 gm tissue, where 1 gm plasma equivalent is the amount of a particular protein fraction contained in 1 gm of blood plasma. In the visceral organs which are quite vascular (fig. 1) the transfer rates (fig. 4, table 2) range from 0.06 to 0.76 and are much higher than the transfer rates (0.0005-0.0005) in the muscle, fat and skin which are low in vascularity. It seems reasonable that in tissues with a more extensive vascular network there would be more capillary wall surface through which the plasma proteins could penetrate. Of course, as discussed by Grotte (17), there are probably differences between tissues in the number and size of pores in the capillary walls.

The masses of plasma protein contained in the extravascular compartments also differ from one tissue to another and do not correlate with the vascular-extravascular transfer rates. The mass of a particular plasma protein fraction in the extravascular compartment was expressed as the number of gram plasma equivalents per gram tissue. These masses (table 2, fig. 6) range essentially from zero for brain (a demonstration of the blood-brain barrier) and 0.04 for kidney to 0.193 for Walker 256 carcinoma and 0.105 for skin. Note, however, that over 90% of the total extravascular plasma protein is located in the muscle, fat and skin (fig. 7). The mass of plasma protein contained in the extravascular compartment is probably dependent largely on a combination of rate of transfer into the extravascular compartment and the rate of clearance from the extravascular compartment. In the visceral organs, for example, where the

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Transfer Rate/Unit Wt. Tissue</th>
<th>Mass of Plasma Protein/100 gm Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>0.012±0.02</td>
<td>0.08±0.009</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.009±0.05</td>
<td>0.024±0.011</td>
</tr>
<tr>
<td>Liver</td>
<td>0.15±0.06</td>
<td>0.052±0.004</td>
</tr>
<tr>
<td>Heart</td>
<td>0.08±0.002</td>
<td>0.054±0.004</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.22±0.04</td>
<td>0.036±0.004</td>
</tr>
<tr>
<td>Ovaries</td>
<td>0.78±0.02</td>
<td>0.144±0.013</td>
</tr>
<tr>
<td>Tibia</td>
<td>0.0013±0.0001</td>
<td>0.015±0.001</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.001±0.007</td>
<td>0.001±0.004</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.0034±0.002</td>
<td>0.001±0.004</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>0.015±0.002</td>
<td>0.001±0.002</td>
</tr>
<tr>
<td>Brain</td>
<td>0.002±0.02</td>
<td>0.0002±0.0008</td>
</tr>
<tr>
<td>Walker tumor</td>
<td>0.027±0.003</td>
<td>0.004±0.003</td>
</tr>
<tr>
<td>Murphy tumor</td>
<td>0.013±0.003</td>
<td>0.004±0.001</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0008±0.0002</td>
<td>0.005±0.005</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0006±0.0002</td>
<td>0.005±0.008</td>
</tr>
<tr>
<td>Skin</td>
<td>0.005±0.0009</td>
<td>0.105±0.008</td>
</tr>
</tbody>
</table>

Standard errors of means indicated. * Extravascular transfer rates are expressed as the number of grams plasma equivalents penetrating the vascular extravascular barrier each hour in 1 gm tissue, where 1 gm plasma equivalent is the amount of gamma globulin contained in 1 gm of blood plasma. † The mass of gamma globulin in the extravascular compartment of tissues is expressed as the number of grams plasma equivalents per gm tissue. These values are the equilibrium ratios of tissue radioactivity to plasma radioactivity (fig. 6) after being corrected for nonprotein $^{131}$I and plasma protein catabolism as discussed in the text.
transfer rate is high and the mass of extravascular plasma protein is low, the rate of clearance of plasma protein from the extravascular compartment must be high. This is consistent with the high rates of hepatic lymph flow, for example, which have been reported in the literature (18). In contrast, the transfer rate in the skin is low, and the mass of extravascular plasma protein is large; therefore, the rate of clearance for skin must be low as was shown by Taylor et al. (19). The rate of clearance is probably related to the nature of the interstitial space and the lymphatic system in each tissue.

The values reported for the masses of plasma protein in the extravascular compartments are generally consistent with the hypothesis that plasma proteins are confined to the extracellular phase of the extravascular system. In UR-524 (11) the concentration of plasma protein in the interstitial water of the tissues was computed by assuming that the plasma proteins are located extracellularly. The concentration of plasma protein in the interstitial water relative to the concentration of plasma protein in vascular water ranged from 0.2 to 0.7 in most tissues. These values for heart, lung, muscle and skin compare very well with similar values derived from actual measurements of plasma protein concentration in interstitial water and lymph from these same tissues as reported in the literature. In the liver, however, these values (0.04 to 0.20) were much lower than values reported for hepatic lymph (0.77 to 0.97).

No over-all differences in transfer rates and masses of extravascular plasma protein were found between the different plasma proteins (table 1), if differences in the rates of plasma protein catabolism were taken into account. In the rat as a whole the uncorrected distribution ratios of mass of plasma protein in the extravascular compartments divided by mass of plasma protein in the vascular compartment were 1.86, 1.84 and 2.24 for rabbit gamma globulin, rat gamma and beta globulins, and rat albumin, respectively. After corrections were introduced to account for plasma protein catabolism, however, the differences disappeared and the distribution ratios were 1.43, 1.43 and 1.48, respectively. Although there were significant differences in transfer rates in a few tissues between the different plasma proteins, there were no over-all differences which would indicate that one protein fraction penetrated the capillary walls more rapidly than another (figs. 4 and 5). As pointed out in UR-524 (11), reports in the literature are contradictory as to whether or not capillary walls are equally permeable to albumin and globulins.

A striking difference between the different plasma proteins was noticed in the spleen and liver, however, where the apparent transfer rate and mass of extravascular plasma protein were significantly less for rat albumin than for rat gamma and beta globulins and for rabbit gamma globulin (figs. 4 and 6). As Miller et al. (8, 20) and other workers have shown, the liver is a very active site in plasma protein metabolism, and these variations between the different plasma proteins in the liver and possibly the spleen (21) are probably associated with the metabolic processes. In fact, in any organ where plasma protein synthesis or degradation is occurring in the extravascular compartment, the value reported in this paper for mass of extravascular plasma protein would be too low.

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REFERENCES
