Measurement and Radioautographic Localization of Albumin in Rat Tissues After Intravenous Administration

By Newton B. Everett, Ph.D. and Barbara Simmons, B.S.

Radioautography and conventional counter methods have been used to determine the relative amount and localization of $^{131}$I albumin in a number of tissues at 3, 30, and 60 min. after intravenous injection. Certain tissues showed essentially no change in albumin content with time, others contained extravascular albumin which increased with time, and the liver and adrenal evidenced a decreasing level of albumin with time. The transcapillary passage of albumin and its accumulation in extravascular compartments are correlated with tissue structure.

It has been well established by numerous investigators that albumin and similar-sized molecules, when introduced into the vascular system, undergo exchange with comparable molecules in fluids of the tissue spaces. It is for this reason that in blood volume measurements utilizing $^{131}$I albumin accuracy depends on minimizing the mixing time. During the course of recent studies in which $^{131}$I albumin was used in determining the blood and plasma volumes of rat organs, we found that 3 min. was the most satisfactory mixing time. Within this time mixing was complete and no great amount of tagged material had left the vascular beds. It was observed, however, that at 15 to 30 min., considerable amounts of albumin had accumulated in many organs, whereas in others there was no such accumulation and in certain ones there even appeared to have been a decrease in albumin content from the 3 min. level. In view of these distinct differences among organs in relation to their change in $^{131}$I albumin content with time after injection, the present study was undertaken in order to determine the amount and localization of $^{131}$I albumin in a number of tissues at various times following intravenous injection.

METHODS

The rats used were males of the Sprague-Dawley strain weighing from 180 to 220 Gm. The methods used for the quantitative determinations of organ and tissue plasma volumes were the same as previously described. The injection dose for each rat in this series was 0.2 ml. of serum albumin containing approximately 10 $\mu$g. of albumin and 2 $\mu$g. of I$^{131}$. Twenty rats were frozen in liquid nitrogen after a mixing time of 3 min., 8 after 30 min. and 8 after 60 min. The calculations of tissue radioactivity were made as described previously. The $^{131}$I albumin space in the combined vascular and extravascular compartments of each organ was expressed as plasma in microliters per gram of tissue.

Nine additional rats of approximately 180 Gm. were used for the radioautographic localization studies. Each of these was given 3.5 $\mu$c. of $^{131}$I serum albumin per gram weight, or approximately 630 $\mu$c. of I$^{131}$ per rat. This amount of radioactivity was contained in 0.6 ml., the approximate volume of each injection dose. These rats were frozen in liquid nitrogen in groups of three at 3, 30, and 60 min. respectively after injection. The tissues (adrenal, testis, lung, skeletal muscle, liver, kidney, brain, small intestine, skin and submaxillary gland) were removed in the frozen state and fixed for 24 hours in Bouin's solution. These were processed by the conventional paraffin method, sectioned, and stained with hematoxylin and eosin. The sections were mounted so that each slide would contain a set of tissues from a 3, a 30, and a 60 minute post-injection rat. This was to insure so far as possible the comparable processing of the tissues from different rats and to provide for easier and more reliable comparisons of the radioautographs. Integrated radioautographs were made, after dipping the slides in 0.1 per cent celloidin, by coating with melted (40 C.) NTB-3 emulsion (Eastman Kodak). The sets of sections were kept at 5 C. during the exposure periods of 1, 3, 4 and 6 days.

In order to determine whether labeled material was released into the bile within the time limits of the experiments two rats were prepared with biliary fistulas. Each was given 2 $\mu$c. of I$^{131}$ albumin and the bile was assayed for radioactivity at 15 min. intervals during the collection period.
TABLE 1.—Plasma Volumes of Tissues as Measured with $^{131}$I Albumin at Intervals after Intravenous Administration

<table>
<thead>
<tr>
<th>Number of rats</th>
<th>Three minutes</th>
<th>Thirty minutes</th>
<th>Sixty minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µl./Gm.</td>
<td>µl./Gm.</td>
<td>µl./Gm.</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>182</td>
<td>168</td>
<td>155</td>
</tr>
<tr>
<td>Bone</td>
<td>36.2</td>
<td>43.8</td>
<td>42.7</td>
</tr>
<tr>
<td>Cerebral hemorrhage</td>
<td>20.9</td>
<td>20.4</td>
<td>19.8</td>
</tr>
<tr>
<td>Thalamus and midbrain</td>
<td>21.4</td>
<td>21.8</td>
<td>25.3</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>25.6</td>
<td>26.6</td>
<td>27.5</td>
</tr>
<tr>
<td>Pons and medulla</td>
<td>15.4</td>
<td>13.7</td>
<td>14.4</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>183</td>
<td>195</td>
<td>208</td>
</tr>
<tr>
<td>Hypophysis</td>
<td>58.2</td>
<td>87.5</td>
<td>107</td>
</tr>
<tr>
<td>Kidney</td>
<td>105</td>
<td>106</td>
<td>104</td>
</tr>
<tr>
<td>Liver</td>
<td>185</td>
<td>157</td>
<td>148</td>
</tr>
<tr>
<td>Lung</td>
<td>336</td>
<td>335</td>
<td>357</td>
</tr>
<tr>
<td>Seminal vesicles</td>
<td>14.2</td>
<td>16.0</td>
<td>21.4</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>17.8</td>
<td>20.7</td>
<td>25.3</td>
</tr>
<tr>
<td>Skin</td>
<td>12.4</td>
<td>24.5</td>
<td>32.0</td>
</tr>
<tr>
<td>Small intestine</td>
<td>27.5</td>
<td>53.8</td>
<td>72.6</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>117.7</td>
<td>14.9</td>
<td>15.9</td>
</tr>
<tr>
<td>Spleen</td>
<td>81.4</td>
<td>86.4</td>
<td>82.5</td>
</tr>
<tr>
<td>Submaxillary gland</td>
<td>55.4</td>
<td>60.3</td>
<td>72.6</td>
</tr>
<tr>
<td>Testis</td>
<td>11.6</td>
<td>31.0</td>
<td>48.2</td>
</tr>
</tbody>
</table>

* Standard error of the mean as per cent of mean, or coefficient of variation.

Results

Quantitative Data. The quantitative values for the $^{131}$I albumin content of tissues, expressed as microliters of plasma per gram, are given in table 1. The values for the tissues at 3 min. are most likely to represent only intravascular $^{131}$I albumin and are thus used as baseline values for calculating the percentage change in the $^{131}$I albumin content of tissues for the 30 and for the 60 min. groups. It is observed that for the total rat the calculated value of plasma volume has increased 25 per cent at 30 min. and 33 per cent at 60 min. This is a reflection of the loss of labeled material from the blood. The individual tissues and organs fall into 3 groups in relation to their change of $^{131}$I albumin content with time. The largest group shows an increase. Of these the testis displays the greatest gain: 167 per cent at 30 min. and 316 per cent at 1 hour. Others in this group, listed in decreasing order of per cent increase at 1 hour, include small intestine, skin, hypophysis, seminal vesicle, skeletal muscle, submaxillary gland and bone. A second group of tissues showed no significant change in their labeled albumin content at 30 or at 60 min. These include most divisions of the central nervous system, spleen, lung and kidney. The third category of organs, adrenal and liver, had significant decreases of albumin content at 30 and at 60 min. The decrease was 7.7 and 15 per cent respectively for the adrenal and liver at 30 min., and 14.8 and 20 per cent respectively at 1 hour.

The changes in the calculated plasma volumes of various organs with time are shown graphically in figure 1. Here the changes are plotted as per cent of the 3 min. or control values.

The bile assays from the biliary fistula experiments revealed that only a trace of $^{131}$I, 0.08 per cent of the injected dose, was excreted via this route in 1 hour. After 2 hours 0.2 per cent had appeared in the bile, 50 per cent of which was trichloroacetic acid-precipitable.

At 1 hour after injection 0.039 per cent of the administered $^{131}$I had appeared in the...
urine and at 2 1/2 hours the percentage thus recovered was 0.6 per cent. Less than 1 per cent of the I_131 in urine was trichloroacetic acid-percipient.

Radioautographs. The radioautographs support the quantitative data and, more significantly, show the tissue parts in which the I_131 albumin is contained at the selected time intervals following injection. After a mixing time of 3 min. the radioautographs of representative tissues in group 1 (those in which the I_131 albumin increased with time) show marked reduction of silver overlying the vessels but only a trace appearing in the extravascular areolar tissue (testis, fig. 2; small intestine, fig. 4; skeletal muscle, fig. 6; and skin, fig. 8). It is apparent, however, that in some of these (skin fig. 8), more radiomaterial has accumulated in the tissue spaces than in others sampled at this same time interval.

One hour after injection an increase in extravascular tagged albumin is evidenced in these tissues. This is most pronounced in testis (fig. 3) and small intestine (fig. 5). The intense blackening in the testis overlies the intertubular ground substance. In the small intestine, the exact localization of the tagged albumin is difficult to determine, though it appears to be associated primarily with the tunica propria. Only a little reduced silver overlies the lining epithelium or lumen (fig. 5).

The radioautographs of cerebral cortex, cerebellum, lung and kidney (figs. 10 to 14) are representative of those tissues which had no significant increase in albumin content with time. Except for the kidney, the radioautographs of these at 3 and at 60 min. were very similar and only the 60 min. ones are presented. It is apparent that in both the cerebral cortex (fig. 10) and cerebellum (fig. 11), essentially all of the reduction is confined to blood vessels. It appears, however, that in these a trace of albumin has escaped into the tissue space of the arachnoid layer but has not traversed the pia-glial membrane.

In the lung the intense vascularity and the intimate association of the capillaries with the alveoli prevent a discrete localization of the reduction in relation to the underlying tissue. It is apparent, however, (fig. 12) that the respiratory passages and the center of the alveoli are essentially free of reduced silver. The most intense spots overlie the larger vessels and the moderately reduced areas appear over smaller vessels between adjoining respiratory elements.

The radioautographs of the kidney at 3 and at 60 min. are similar in over-all intensity of reduction, but show certain differences in the distribution of labeled albumin. At 3 min. (fig. 13) distinct reduction is associated with the glomeruli, with blood vessels elsewhere, with the proximal convoluted tubules, and with the tissue between the collecting tubules of the medulla. At 60 min. (fig. 14) the intensity over the glomeruli is comparable to that at 3 min., but the reduction over the convoluted tubules is less. The blackening over the intertubular tissue of the medulla is increased at this time.

The radioautographs of the adrenal gland (figs. 15, 16) and liver (figs. 17, 18) betray widespread evidence of intra and extravascular labeled protein at both time intervals, but silver reduction is more intense over the larg-
Fig. 2. Radioautographs of tissue which were fixed at 3 or at 60 min. after intravenous 111m albumin. All sections were cut at the same thickness and received the same treatment throughout the radioautographic process. 2, 3 and 11 are X 100, 10-16 are X 150, 4-9, 17 and 18 are X 250. G., glomerulus; M., medulla; P., proximal convoluted tubule; V., vein.
er vessels than over the parenchymal cells. It is apparent, however, that the intensity of reaction is less for both liver and adrenal at 1 hour than at 3 min.

**DISCUSSION**

It has been known for many years that most capillaries throughout the body leak protein into the tissue spaces and that part of this protein subsequently appears in the lymph. It is likewise known that the ease with which the protein moves from the capillaries varies from organ to organ, as the capillaries of liver and intestine have generally been shown to be freely permeable to vascular proteins, whereas those in the central nervous system do not permit the passage of protein. Furthermore, Pappenheimer pointed out that capillary walls in different tissues may differ widely in their resistance to permeating plasma components. More recently Caster, Simon and Armstrong reported that the transfer of Evans blue dye into the tissue spaces of the rat begins immediately after injection and continues for many hours at different rates in different tissues.

The results of the present study would indicate, however, that various tissues differ more with respect to the transcapillary passage of albumin and its extravascular accumulation than had been appreciated. It now appears to be possible to make further correlations of tissue structure with the tissue content of labeled protein with time following intravascular administration. That this labeled material localized in tissues represents $^{131}$I albumin only and not degradation products is evidenced by the following: (1) essentially none of the $^{131}$I albumin was metabolized during the time limits of the experiment since only 0.1 per cent of the injected dose appeared in the urine and bile within the first hour, (2) the fixation and subsequent treatment of the tissues would remove any trace of free $^{131}$I. This was verified in control radioautographic studies using $^{131}$I sodium iodide.

It would appear that 3 min. mixing time is adequate for all the tissues studied, but even at this time certain organs show a small but discernable leakage into the tissue spaces (see testis fig. 2 and skin fig. 8). These observations are in line with those of Wasserman and Mayerson, who found that in dogs $^{131}$I albumin mixes uniformly in plasma within 3 to 5 min. and begins to leave the plasma immediately after injection.

The skin and testis, like other organs in the group having an increasing albumin content with time, are characterized by having considerable amounts of loose areolar connective tissue, or "structureless" matrix, as in the intertubular spaces of the testis. It is observed that the extravascular albumin accumulates in these loose areas, as evidenced by the density of the radioautographs. Contrariwise the parenchyma shows no associated silver reduction (fig. 2 and 3). The group of tissues which showed no significant change in albumin content with time (brain, spleen, lung and kidney) all differ from the above group in their relative absence of loose areolar connective tissue and thus have less extracellular space. The radioautographs of brain at 1 hour (fig. 10 and 11) reveal clearly that the reduction is limited to the underlying vessels. Thus the tagged albumin has not crossed the so-called blood-brain barrier. Although not clearly resolvable with $^{131}$I autographs, it would appear that the barrier to albumin is at the outer capillary border of the glial cells, which lies against the extracellular basement membrane comprising part of the capillary wall. The increase in albumin content of thalamus and midbrain with time probably reflects the escape of albumin into the more abundant connective tissue within the choroid plexus, vessels, and infolded meninges which were included with the material subjected to assay.

The quantitative data for the kidney reveal no changes in $^{131}$I content between 3 and 60 min., but it is apparent from the radioautographs (figs. 13 and 14) that there are certain differences in albumin distribution. The reason for the decrease in reduction over the proximal convoluted tubules at 1 hour is not apparent. The decrease in $^{131}$I albumin content of blood has been relatively small by this time and it is known that protein molecules of this size are resorbed by these tubules. It
may be that the initial greater accumulation of radiomaterial over the proximal convoluted tubules is due to a small amount of denatured I\textsuperscript{131} albumin in the injection dose. A precise assignment of the reduction over the kidney medulla cannot be made to underlying tissue elements. Clearly, however, this reduction which is increased at 1 hour is not associated with the collecting tubules. Probably it results from the labeled albumin in the numerous small vessels in relation to the loops of Henle.

Perhaps the most surprising results from this study are that the liver and adrenal had their highest levels of I\textsuperscript{131} albumin at 3 min. and progressively less at 30 and 60 min. after injection. The fact that the 3 min. values for the I\textsuperscript{131} albumin content of liver and adrenal were the highest suggests that intravascular albumin has free entry into the tissue spaces of these organs. The recent electron microscope observations of Fawcett,\textsuperscript{10} Rouiller\textsuperscript{11} and Hampton\textsuperscript{12} would support this proposal for the liver since these investigators have shown that the subendothelial spaces of Disse communicate with the lumen of the sinusoid through relatively large endothelial gaps. It may be that a similar situation exists for the adrenal since Lever\textsuperscript{13} has reported for the rat adrenal distinct spaces between the endothelial lining of the blood sinusoids and the parenchymal cells, which communicate with the interparenchymal cell spaces. That these spaces may communicate through endothelial gaps with the blood sinusoids is suggested by the observed presence of perforations in the endothelium and basement membrane lining the sinusoids of the cow adrenal fixed several minutes after the animal was stunned.\textsuperscript{14} These perforations were not observed, however, after the adrenal had been restored by perfusing for 1 hour or more with oxygenated beef blood.

The decline in I\textsuperscript{131} content of the liver and adrenal after 3 min. may perhaps be due to rapid drainage via the lymphatics. Morris\textsuperscript{15} has shown that in the cat appreciable amounts of I\textsuperscript{131} albumin appears in liver lymph within 30 min. after intravenous administration.

This study suggests two important structural features which relate to the transcapillary passage of protein into extra-cellular tissue compartments and to the accumulation of protein in tissue spaces. The first appears to be the structure of the capillary wall, including the endothelium and basement membrane. It is noteworthy that the organs most permeable to albumin—liver and adrenal—are ones in which perforations have been described in the capillary endothelium and endothelial basement membrane. The other important structural feature is the character and amount of the extracellular tissue. Next to the liver and adrenal, the most permeable tissues are those having an abundance of loosely organized connective tissue, e.g., skin, intestine, and testis. Furthermore, these are organs in which large amounts of extravascular albumin accumulate. The organs which appear least permeable to albumin, and in which there is minimal accumulation of extravascular albumin, are those with proportionately more parenchyma and with a reduced amount of areolar or "loose packing" substance. The brain, which shows the least amount of extravascular albumin, would fall into this category, as recent studies have shown that the central nervous system has relatively little extracellular space.\textsuperscript{8,16} The proposal that the amount and character of the extracellular tissue is important in the permeability of an organ to protein is in accord with recent report of Maynard, Shultz and Pease\textsuperscript{7} that the blood-brain barrier may be an illusion due to the lack of extracellular space.

Although the vascularity of an organ and its blood flow are unquestionably related to its transcapillary passage of protein, it should be pointed out that some of the least vascular organs evidenced the greatest per cent increase of extravascular albumin, e.g., testis and skin.

**Summary**

I\textsuperscript{131} albumin was administered intravenously to rats. After mixing times of 3, 30, and 60 min. the animals were frozen in liquid nitrogen. The organs were removed in the frozen state and assayed for radioactive material which was expressed as activity per unit weight of tissue. Samples of organs from animals of each group were fixed while frozen,
sectioned, and processed for radioautography using the integrated method.

The quantitative measurements and the radioautographic observations were in agreement in revealing that the various tissues differ widely with respect to the transcapillary passage of albumin and its extravascular accumulation. Most organs had an increasing albumin content with time, some showed essentially no change and others had less albumin at the longer time intervals. It is suggested that these observed differences can be explained in part by the structure and amount of connective tissue in organs and by the structure of the capillary wall.

REFERENCES
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doi: 10.1161/01.RES.6.3.307

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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