Diverse Distribution of Red Cells and Albumin in the Dog Kidney

By Lawrence S. Lilienfield, M.D., Ph.D., John C. Rose, M.D., and Niels A. Lassen, M.D.

Cr\(^{51}\) labeled red cells and I\(^{131}\) human serum albumin were injected intravenously into 8 mongrel dogs. After 1 hour kidneys were removed and frozen rapidly with dry ice. Sections of outer cortex, inner cortex, outer medulla, inner medulla, outer papilla and inner papilla were removed and analyzed for Cr\(^{51}\) and I\(^{131}\) radioactivity. Tissue radioactivity was compared with that of arterial blood and concentrations of labeled red cells and albumin were calculated. No significant difference was found in relative red cell and albumin content between outer and inner cortex. The magnitude of the I\(^{131}\) albumin content in the cortex is interpreted to indicate a sizeable extracellular exchangeable albumin pool. The renal papillae were extraordinarily deficient in red cells, but contained per 100 Gm., albumin equivalent to that of 39 ml. of plasma.

STUDIES of mammalian kidneys by analysis of homogenated tissue have demonstrated considerably more plasma albumin relative to red cell content than would be expected from analysis of blood.\(^1\)\(^-\)\(^4\) This observation has been part of the basis of a series of reports by Pappenheimer and Kinter who recently formulated the cell separation theory of regulation of renal blood flow.\(^2\)\(^-\)\(^6\)

The present study is concerned with the distribution of red cells and plasma albumin in various anatomic regions of the kidney. These observations will be discussed with special regard to the use of albumin as an indicator of renal plasma volume.

METHODS

Eight normally hydrated mongrel dogs weighing approximately 10 Kg. were anesthetized with sodium pentobarbital, 25 mg./Kg. One mc. of I\(^{131}\) labeled human serum albumin and 3 mc. of Cr\(^{51}\) labeled dog red cells were then injected intravenously. Forty-five minutes later the abdomen was opened, and a loose tie placed around the pedicle of each kidney. The kidneys themselves were not handled during the procedure. Approximately 60 min. after the injection of the radioactive material, an arterial blood sample was taken and both kidney pedicles were simultaneously ligated. The kidneys were removed and frozen rapidly in dry ice.

A thin transverse slice from the frozen kidneys was cut with a band saw. Sections from different regions of the slices were removed, weighed, and assayed for radioactivity in a well-type scintillation counter. The remaining bulk of the kidney was homogenized with equal parts by weight of saline in a Waring Blendor. Aliquots of the kidney homogenate and arterial blood samples were also analyzed for radioactivity.

Determination of the amount of the two isotopes separately, by differential decay, was made by recounting after a 3 week interval. The concentration of labeled red cells in the tissues was expressed as the ratio of the amount of Cr\(^{51}\) in 100 Gm. of tissue to the amount of Cr\(^{51}\) in 1 ml. of red blood cells. Similarly, the concentration of labeled albumin was expressed as the ratio of the I\(^{131}\) activity in 100 Gm. of tissue to that of 1 ml. of blood plasma.

The tissue sections were mechanically divided as follows: 1, outer cortex: the outer 2 mm. of the cortex (capsule was stripped off the frozen kidney); 2, inner cortex: the layer between outer cortex and the arcuate vessels; 3, outer medulla: the outer half of the medulla; 4, inner medulla: the inner half of the medulla which was considered to end at the base of the pale protruding papilla; 5, outer papilla: upper half of the papilla; 6, inner papilla: lower half of the papilla. Care was taken to avoid the inclusion of any large vessels in these tissue blocks.

At the end of the experiment urine was collected from the bladder and analyzed for radioactivity.
Urine radioactivity varied from zero to less than 2 per cent of the blood radioactivity. Nonprotein-bound $^{131}$I activity in tissue homogenates was less than 2 per cent of the total $^{131}$I activity in the tissues, as determined by trichloroacetic acid precipitation. Thus it is assumed that section analyses actually represent tissue red cell and albumin concentrations.

Femoral arterial blood pressure was monitored throughout the procedure, employing a strain gage transducer and direct-writing oscillograph. In all studies accepted, pressure remained between 125 to 150 mm. Hg.

**RESULTS**

**Red Cell Concentration.** One hundred Gm. of outer cortex contained the $^{51}$Cr radioactivity of 5.5 ml. red cells, whereas 100 Gm. of inner cortex contained the activity of 6.9 ml. red cells. Similarly, 100 Gm. of outer medulla, inner medulla, outer papilla and inner papilla had radioactivity corresponding to 10.4, 6.3, 3.6 and 3.5 ml. red cells, respectively (table 1, fig. 1). The over-all $^{51}$Cr activity per 100 Gm. of total kidney tissue corresponded to that of 7.2 ml. red cells.

**Albumin Concentration.** One hundred Gm. of outer cortex contained the $^{181}$I radioactivity of 22.8 ml. of plasma, and the inner cortex the activity of 24.0 ml. of plasma. Similarly, 100 Gm. of outer medulla, inner medulla, outer papilla and inner papilla had albumin radioactivity corresponding to 30.7, 34.6, 37.0

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### Table 1.—Concentrations of $^{51}$Cr Labeled Red Cells in the Dog Kidney

<table>
<thead>
<tr>
<th>Kidney no.</th>
<th>Outer cortex (ml/100 Gm.)</th>
<th>Inner cortex (ml/100 Gm.)</th>
<th>Outer medulla (ml/100 Gm.)</th>
<th>Inner medulla (ml/100 Gm.)</th>
<th>Outer papilla (ml/100 Gm.)</th>
<th>Inner papilla (ml/100 Gm.)</th>
<th>Whole kidney (ml/100 Gm.)</th>
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</thead>
<tbody>
<tr>
<td>1017 A1</td>
<td>6.7</td>
<td>10.3</td>
<td>11.2</td>
<td>10.4</td>
<td>37.3*</td>
<td>4.3</td>
<td>7.0</td>
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<tr>
<td>1017 A2</td>
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<td>12.2</td>
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<tr>
<td>1017 Cl</td>
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<tr>
<td>1017 C2</td>
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<tr>
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<td>&lt;0.1</td>
<td>0.4</td>
<td>4.5</td>
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Average: 3.5 6.9 10.4 6.3 3.6 3.5 7.2
S.E. ±0.5 ±0.7 ±1.4 ±0.8 ±1.1 ±1.0 ±0.8

*Not included in average. Probably included a large vein in section.
†Not included in average. Values statistically too far outside of group range. See contralateral kidney no. 1022 A2.
and 38.5 ml. plasma, respectively (table 2, fig. 2). The over-all I\(^{131}\) activity per 100 Gm. of total kidney tissue corresponded to that of 25.6 ml. of plasma.

**Tissue “Hematocrit.”** If it is assumed that at the end of 60 min. all the labeled albumin and red cells are intravascular, and neither concentrated nor diluted with respect to arterial blood, then the concentrations may directly be translated into intravascular volumes of distribution. The sum of the red cell and albumin volumes would give the total vascular volume of each tissue section, and the tissue hematocrits could be calculated (table 3). The cortex would contain about 29 ml. of blood per 100 Gm. with a hematocrit ratio about one half that of the arterial blood. The papilla on the other hand would contain about 42 ml. of blood, with a hematocrit about one fifth that of the arterial blood. The total vascular volume of the kidney would be 33 ml./100 Gm., and the average intrarenal hematocrit would be approximately one half that of the arterial blood.

**DISCUSSION**

Pappenheimer and Kinter have suggested that plasma skimming occurs in the interlobular arteries. However, in that case the outer cortex would have a higher ratio of red cell concentration to albumin concentration than would the inner cortex. In the present investigation, no significant difference was found in red-cell content/100 Gm. of tissue between outer and inner cortex (table 2). Both regions contained a similar and disproportionately large amount of albumin when compared to arterial blood (table 2). Unless redistribution of red cells occurs in the cortex between the time of ligation of the renal vessels and the freezing of the kidney, these observations suggest that there is no significant cell separation from plasma in the interlobular arteries.

The red-cell concentration of the outer medulla was higher than it was in any other area of the kidney. This is to be expected, since it represents the most vascular anatomic zone.

The greatest variability in red-cell concentration was shown in the papilla (table 1). In several instances the red-cell concentration was less than 1.0 ml./100 Gm. The pale appearance of the papilla is well known, as is its propensity for infarction and necrosis. Its red cell content may be under some control (the nature of which is still unknown) since, in another group of traumatized dogs.

### Table 2.—Concentration of I\(^{131}\) Albumin in the Dog Kidney

<table>
<thead>
<tr>
<th>Kidney no.</th>
<th>Outer cortex (ml./100 Gm.)</th>
<th>Inner cortex (ml./100 Gm.)</th>
<th>Outer medulla (ml./100 Gm.)</th>
<th>Inner medulla (ml./100 Gm.)</th>
<th>Outer papilla (ml./100 Gm.)</th>
<th>Inner papilla (ml./100 Gm.)</th>
<th>Whole kidney (ml./100 Gm.)</th>
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<td><strong>Average</strong></td>
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<td><strong>30.7</strong></td>
<td><strong>34.6</strong></td>
<td><strong>37.0</strong></td>
<td><strong>38.5</strong></td>
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<td><strong>S.E.</strong></td>
<td>±1.1</td>
<td>±1.2</td>
<td>±1.1</td>
<td>±3.2</td>
<td>±3.1</td>
<td>±2.5</td>
<td>±0.8</td>
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</table>
in shock, the papillae were found to be quite red and had a much greater red-cell concentration than was in this series.

Estimates of renal vascularity by latex injections have been made by Weaver, McCarver and Swann. These authors conclude that the kidney contains approximately 14 ml. of blood/100 Gm. This is in much closer agreement with direct histologic evidence than the 33 ml./100 Gm. obtained in the present study from summing the red cell and albumin "volumes of distribution" (table 3). If the intrarenal plasma volume is truly 3 times that of the red-cell volume, the transit time of injected albumin would have to be 3 times longer than that of the red cells. This has been shown to be not the case. The discrepancy presumably is due mainly to the assumptions made in calculating the plasma volume from the albumin content.

Since there is no reason to believe albumin is highly concentrated intravascularly in the renal cortex, the most likely explanation is that some of the cortical albumin measured in this study is located extravascularly. Such a hypothesis would explain the discrepancy between the renal "hematocrit" estimated by equilibrium techniques and the value estimated by transit time studies following rapid arterial injection of labeled albumin.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Tm albumin radionuogram of transverse slice of dog kidney removed 1 hour after injection of 1 mc. Tm albumin. Note concentration of albumin in papilla. (Exposure time 3 days on Ansco nonscreen x-ray safety film.)

In such single circulation studies, injected albumin has insufficient time to exchange completely with extravascular albumin.

Electron microscope studies by Pease have shown that the capillaries of the renal cortex are exceedingly thin-walled, and that the endothelial lining is fenestrated, a feature not found in any other organ. Thus it seems likely that these capillaries are specialized to facilitate transmural exchange to a larger extent than most other capillaries. It is possible that these vessels permit protein to exchange relatively freely with the interstitial fluid. The unusual permeability of the renal capillaries has also been demonstrated by Freis, Schnaper, Rose and Lilienfield, who compared transcapillary exchange of inulin in the kidney with that of the forearm.

Swann, Valdivia, Ormsby, and Witt have estimated the minimum amount of interstitial fluid in the dog kidney to be about 13 ml./100 Gm. with an albumin content about two thirds that of arterial or venous blood. If this albumin is freely exchangeable it would contribute 9 ml. (2/3 × 13) to the calculated albumin volume of distribution. Additional
Contributions might be made by any intracellularly located, exchangeable albumin. In addition, the magnitude of the albumin content of the papilla found in the present study (table 2) (equivalent to 38 ml. of plasma/100 Gm. of papilla) is so great as to suggest hyperconcentration with respect to blood plasma. Evidence reported in another communication implies that some of this papillary albumin is located extravascularly, since the rate of accumulation of $^{131}$I albumin (85 per cent in 3 min.) seems faster than that of $^{131}$I gamma globulin (ref. 14 and figs. 3 and 4).

These studies are interpreted to indicate the existence within the kidney of a large pool of exchangeable albumin. If, as seems to be the case, much of this albumin is located extravascularly in the cortex, attempts to measure vascular volumes or kidney tissue hematocrits by equilibrium techniques with labeled albumin will yield vascular volumes too large and hematocrits too low. It probably will be necessary, therefore, to employ larger molecules than those of albumin for the estimation of renal plasma volume.

**SUMMARY**

Cr$^{51}$ labeled dog red cells and $^{131}$I labeled human albumin were injected systemically. After 1 hour, analysis was made of the concentration of the two isotopes in large-vessel blood and in various anatomical regions of the kidney as well as in homogenates of whole kidney.

Whole kidney, outer cortex, inner cortex, outer medulla, inner medulla, outer papilla and inner papilla contained, per 100 Gm. of tissue, an amount of radioactivity corresponding to 7.2, 5.5, 6.9, 10.4, 6.3, 3.6, 3.5 ml. red cells, respectively, and an amount of albumin radioactivity corresponding to 25.6, 22.8, 24.0, 30.7, 34.6, 37.0, 38.5 ml. of plasma, respectively.

The discussion is primarily concerned with
the data from the renal cortex, where blood and interstitial fluid is assumed to be isosmotic with plasma. The data fail to support the cell separation theory of intrarenal circulation. However, the findings are consistent with the existence of a sizeable extravascular pool containing exchangeable albumin.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of Mr. Thomas Doyle, Miss Jean Pietras and Miss Marcheta Alion.

SUMMARIO IN INTERLINGUA

Erythrocytos canin marcate con Cr\textsuperscript{51} e albumina human marcate con I\textsuperscript{131} esseva injicite in le circulation systemic. Un hora plus tarde, le concentration del duo isotopos essava analysate in sanguine de vasos major e in varie regiones anatomic del ren e etiam in homogenatos del ren integre.

Ren integre, cortice exterior, cortice anterior, medulla exterior, medulla interior, papilla exterior, e papilla interior contineva per 100 g un quantitate de radioactivitate que corrispondeva, respectivemente, a 7,2, 5,5, 6,9, 10,4, 6,3, 3,6, e 3,5 ml de erythrocytos e un quantitate de radioactivitate albuminie que corrispondeva, respectivemente, a 25,6, 22,8, 24,0, 30,7, 34,6, 37,0, e 38,5 ml de plasma.

Le presente articulo discute primarimente le datos pertinente al cortice renal in que il as supponite que le sanguine e le fluido interstital es iso-osmotic con le plasma. Tamen, le constatationes es compatibile con le concepto che il existe un considerabile reservoir extravascular de albumina excamiabile.

REFERENCES

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Circ Res. 1958;6:810-815
doi: 10.1161/01.RES.6.6.810

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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