Intrarenal Distribution of Nutrient Blood Flow Determined with Krypton$^{85}$ in the Unanesthetized Dog

By Geoffrey D. Thorburn, M.B., Hugh H. Kopald, M.D., J. Alan Herd, M.D., Milton Hollenberg, M.D., Charles C. C. O'Morchoe, M.D., and A. Clifford Barger, M.D.

The development of the countercurrent hypothesis as an explanation for the osmotic gradient from cortex to papilla of the kidney$^1$ has again stimulated investigation of the intrarenal distribution of blood flow. Theoretical considerations have emphasized the necessity of a relatively low medullary blood flow for the maintenance of this gradient.$^2$ However, present methods of measuring renal blood flow in the intact animal do not estimate the distribution of flow to the medulla. Trueba and co-workers$^3$ demonstrated by renal angiography that medullary flow was slower than that of the cortex; these observations have been confirmed by Kramer et al.$^4$ who found that the medullary transit time of Evans blue dye was much longer than that of the cortex.

In the present investigation, a method has been developed for determining distribution of renal blood flow in the unanesthetized dog by monitoring the disappearance of Kr$^{85}$ from the kidney after injection of the isotope into the chronically catheterized renal artery; no blood samples are needed for the measurement. Kr$^{85}$, an inexpensive and readily available isotope, was used by Brun and co-workers$^5$ to measure renal blood flow by the modified ICety technique; they were, at that time, unaware of the gamma emissions from Kr$^{85}$. Although Kr$^{85}$ is primarily a beta emitter, 0.6% of its disintegrations are gamma rays which can be detected externally. Since urine samples are also unnecessary, the method can be used in oliguric or anuric states. The validity and accuracy of this method for measurement of blood flow in the myocardium has recently been demonstrated in this laboratory$^8$; in this tissue, with uniform distribution of blood flow, a single exponential disappearance curve was obtained. The decay curves in the kidney are more complex, and can be described by a series of exponentials, each associated with blood flow through localized regions of the kidney. These regions, which have been identified in acute experiments by the autoradiographic technique to be described below, are (1) cortex, (2) outer medulla and inner cortex, (3) inner medulla, and (4) perirenal and hilar fat.

**Methods**

Polyvinyl catheters (0.030 inches and 0.015 inches) were introduced for chronic use into the renal artery of four anesthetized male dogs by a method previously described$^6$; in three dogs only the left renal artery was catheterized, while both renal arteries were intubated in the fourth animal. The animals were allowed at least one week recovery before the flow measurements were started. During this period the animals were trained to lie quietly for several hours on a padded table (fig. 1A). Before each experiment the animals were fasted for 16 to 20 hours; although they had free access to water, the animals maintained themselves in a relatively dehydrated state, with low basal urine flows. No water was allowed during the two to four hours of blood flow measurements. Preliminary experiments indicate that a negligible amount of krypton is carried into the pelvic urine under these conditions. Two hundred to four hundred microcuries of Kr$^{85}$ dissolved in 0.2 ml to 0.5 ml saline (0.85%) were injected rapidly through a double-barreled adapter into the catheter (fig. 1B) followed immediately.
by 1 ml of saline. A scintillation probe, with a two-inch sodium iodide crystal three inches from the end of a cylindrical collimator, was located over the kidney of the recumbent animal. The output from the probe was led into a linear ratiometer and the decay curve transcribed on a linear recorder.

The decrease in radioactivity as a function of time was plotted on semilogarithmic graph paper. For analysis of the data (fig. 2) a straight line was drawn by inspection through the terminal portion of the curve and extrapolated back to zero time (Component IV). The counts/min of the extrapolated line were then subtracted graphically from the original curve to obtain the third exponential (Component III). The second and first exponentials similarly were drawn in sequential fashion (Components II and I).

The general form of the equation describing such a series of exponential curves is:

\[ A = A_e^{k_1t} + A_{i_1}e^{k_2t} + A_{i_2}e^{k_3t} + A_{i_3}e^{k_4t} \]  

(1)

Where \( A \) = total radioactivity (counts/min at time \( t \)), \( k_1, k_2, k_3, \) and \( k_4 \) = the slopes of the four lines, and \( A_{i_1}, A_{i_2}, A_{i_3}, A_{i_4} \) = the number of counts present initially in Components I, II, III, and IV as determined by the intercept of each line with the ordinate. The percentage of the total counts distributed initially to each compartment was obtained by dividing \( A_i \) by \( A \) at time zero and multiplying by 100.

From the general form of the Fick equation,

\[ \frac{dQ}{dt} = F(C_a - C_v) \]  

(2)

Where \( \frac{dQ}{dt} \) = moles removed from the tissue per unit time, \( F \) = arterial inflow = venous outflow in ml/min, \( C_a \) and \( C_v \) = concentration in arterial and venous blood in moles/ml. Since re-circulation of krypton is extremely small, it can be assumed that \( C_v = 0 \) immediately after injection is completed. Thus

\[ \frac{dQ}{dt} = - F Q_a \]  

(3)

If the assumption is made that equilibration of krypton between blood and tissue is extremely rapid, the rate of desaturation will depend upon the nutrient or capillary blood flow, and

\[ C_v = \frac{C_i}{\lambda} \]  

(4)

Where \( C_i \) = concentration of the gas in the tissue, and \( \lambda \) the partition coefficient for the inert gas between tissue and blood, and since

\[ C_i = \frac{Q}{V} \]  

(5)

Where \( Q \) = number of moles in the tissue, \( V \) = volume of the tissue in ml.

Then

\[ C_v = \frac{Q}{V \lambda} \]  

(6)

By substituting for \( C_v \) in equation 3

\[ \frac{dQ}{dt} = - \frac{FQ}{V \lambda} \]  

(7)

*For renal parenchyma \( \lambda \approx 1.0 \).
Typical $\text{Kr}^{84}$ disappearance curve (heavy black line) following injection of the isotope into the renal artery. Graphic representation of the resultant exponentials is shown by thinner lines (see text). The accompanying table presents pertinent data and derived values obtained from such a curve.

Rearranging this for integration

$$\frac{dQ}{Q} = -\frac{F}{\lambda \nu} \, dt \quad (8)$$

and integrating we obtain

$$Q = Q_0 e^{-\frac{F}{\lambda \nu} t} \quad (9)$$

Where $Q =$ number of moles in the tissue at time $t$, $Q_0 =$ initial number of moles at time $t = 0$. Since the activity (counts/min) is proportional to the number of moles, equation 9 may be written

$$A = A_0 e^{-\frac{F}{\lambda \nu} t} \quad (10)$$

Thus, $k$ (slope of line) $= \frac{F}{\lambda \nu}$, or $\frac{F}{\nu} = k \lambda$.

To convert the nutrient flow into ml/100 g of tissue/min, $k \lambda$ is multiplied by 100, and divided by the specific gravity ($\rho$) of the tissue. Thus,

$$F (\text{ml}/100 \, \text{g/min}) = \frac{k \times \lambda \times 100}{\rho} \quad (11)$$

and

$$k = \frac{\log a_t - \log a_s}{t_2 - t_1} = 0.693 \frac{t_2}{t_1} \quad (12)$$

Where $a_t =$ counts/min at time $t_1$, $a_s =$ counts/min at time $t_2$.

**FIGURE 2**

**FIGURE 3**

Autoradiograph of renal tissue equilibrated with saline solution containing $\text{Kr}^{84}$, showing uniform distribution of radioactivity in the kidney parenchyma, and the greater isotope concentration in the hilar and perirenal fat.

Circulation Research. Volume XIII. October 1962
AUTORADIOGRAPHS

In order to relate each exponential to a specific anatomical region of the kidney, autoradiographs were made from kidneys removed at various times after intrarenal injection of Kr$^{85}$ in a series of anesthetized animals (pentobarbital sodium 25 mg/kg). The kidney and pedicle were exposed through a lumbar incision, and a catheter inserted into the renal artery. In each experiment a control Kr$^{85}$ "washout" curve was obtained to demonstrate the presence of four exponentials; the numerical value for each exponential was similar to that observed in the unanesthetized dog. After the completion of the control curve a second injection, containing approximately the same amount of radioactivity, was made. At a predetermined time after Kr$^{85}$ administration, the renal pedicle was ligated, the kidney extirpated and frozen immediately in an acetone and dry-ice mixture. The frozen kidney was sliced into one-mm cross-sections which were placed in contact with X-ray film* between ferrotype plates and stored at —50°C for varying periods of time, depending upon the amount of activity remaining in the tissue. The exposure time (one hour to nine days) for each autoradiograph is given in the legends. Finally, each tissue section was photographed for comparison with the corresponding autoradiograph.

As an additional control, kidney sections without radioactivity were placed in contact with film under identical conditions; the films were free of artifact when developed. To demonstrate uniform affinity for krypton throughout the kidney parenchyma, 1-mm slices of renal tissue were equilibrated with a saline solution containing Kr$^{85}$ and autoradiographs were prepared after freezing the sections (fig. 3). Uniform density was observed throughout the kidney parenchyma. However, because of the greater solubility of krypton in fat (partition coefficient for fat/blood = 9), the hilar and perirenal fat contained more activity and appeared more dense. Diffusion of Kr$^{85}$ in the frozen tissue during exposure did not appear to be a significant problem, since reaplication of film to the same section of tissue one to two weeks after the original exposure showed excellent duplication of the margins.

Results

Figure 2 illustrates a typical disappearance curve following the rapid injection of Kr$^{85}$ into the renal artery. The heavy line represents the original decay curve, while the various components resulting from the analysis of the curve are shown as thin lines. Representative calculations from such a curve are given in the table in figure 2.

A. ANATOMICAL LOCALIZATION OF Kr$^{85}$ IN THE KIDNEY BY AUTORADIOGRAPHY

The anatomical distribution of Kr$^{85}$ in the kidney was determined autoradiographically

*Kodak Blue Brand Medical X-ray Film.

Circulation Research, Volume XIII, October 1963
in acute experiments by clamping the vessels and removing the kidney at various time intervals after intrarenal injection of the isotope. When the kidney was removed within five seconds of the administration of Kr$^{85}$, the distribution was limited to the cortex, large blood vessels, and fat (fig. 4). Fifteen seconds after injection (fig. 5), radioactivity was present in the cortex, outer medulla, large veins, and fat; no Kr$^{85}$ was detected in the inner medulla. Two minutes after the injection of the same amount of Kr$^{85}$ (fig. 6), the outer cortex was largely cleared of radioactivity; Kr$^{85}$ in the renal parenchyma was found chiefly in the outer medulla and inner cortex, with no detectable activity in the papilla. Thus, it may be concluded that Component I of the Kr$^{85}$ removal curve represents disappearance of the isotope from the outer cortex.

At six minutes (fig. 7) the inner cortex and outer medulla no longer contained appreciable amounts of Kr$^{85}$; the parenchymal activity was confined to the inner medulla. Therefore,
Component II of the removal curve represents disappearance of $Kr^{85}$ not only from the outer medulla, but also from the inner cortex, the region containing the juxtamedullary glomeruli. In all of the autoradiographs made from kidneys removed two to six minutes after injection of $Kr^{85}$, the juxtamedullary region of the cortex and the outer medulla cleared at the same uniform rate, behaving as a single unit. Upon reflection, this observation might have been predicted from previous knowledge that the efferent vessels of the juxtamedullary glomeruli are the sole supply of blood for the medulla.

Figures 7 and 8 illustrate the gradual decrease in density starting in the proximal portion of the inner medulla. Thus, during Component III, as the radioactivity was removed, the exposure time for equivalent density of the autoradiograph increased (65, 96,
FIGURE 7

A. Autoradiograph from a kidney removed six minutes after Kr$^{85}$ injection showing maximal radioactivity in the inner medulla (exposure time of film 65 hours). B. Tissue section corresponding to A. C. Autoradiograph from a kidney removed 10 minutes following Kr$^{85}$ injection showing maximal radioactivity in the papilla (exposure time of film 96 hours). The border of the kidney is indicated by the dots drawn by hand. D. Tissue section corresponding to C. E. Graph showing times at which the kidneys were removed.
The persistence of radioactivity in the tip of the papilla at 28 minutes after injection (fig. 8) emphasizes the slow rate of "washout" in this region. At 52 minutes (fig. 9) activity was present in the fat only; thus Component IV represents the removal of Kr$^{85}$ from the perirenal and hilar fat.

**B. NUTRIENT RENAL BLOOD FLOW DISTRIBUTION**

Data from 65 experiments in four unanesthetized dogs are summarized in table 1, showing the half-times of the four exponentials, the calculated nutrient flow rates for the cortex, outer medulla, inner medulla, and hilar fat, with the percentage of counts initially distributed to each of these areas. In the 65 experiments (five kidneys) the mean cortical blood flow was 472 ml/100 g/min (Component I); outer medullary and inner cortical flow (Component II) was 132 ml/100 g/min; inner medullary flow (Component III) was 17 ml/100 g/min. Under the conditions of these experiments approximately 80% of the Kr$^{85}$ was initially distributed to the cortex, 16% to the outer medulla and only 2% to the inner medulla. The hilar and perirenal fat blood flow was 21 ml/100 g/min and this area received 2% of the initial counts.

Renal blood flow of these animals was de-
<table>
<thead>
<tr>
<th>Animal number</th>
<th>No. of experiments</th>
<th>Nutrient flow (ml/100 g/min)</th>
<th>Per cent counts distributed initially to each compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Half times (t/2 sec)</td>
<td>mean and range</td>
</tr>
<tr>
<td></td>
<td>Cortex</td>
<td>Outer medulla</td>
<td>Inner medulla</td>
</tr>
<tr>
<td></td>
<td>Cortex</td>
<td>Outer medulla</td>
<td>Inner medulla</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>10.1</td>
<td>50.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>392</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3620</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>9.8</td>
<td>42.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>452</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3670</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>9.7</td>
<td>34.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>315</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2970</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>8.5</td>
<td>39.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>156</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>938</td>
</tr>
</tbody>
</table>

- Mean values: 9.4, 39.0, 331, 2650

*Unless noted, results are for left kidney.*

\[ F = \frac{k \times \lambda \times 100t}{\rho} \]

- \(k\) = slope of line, \(\lambda\) = partition coefficient (a figure of 1 has been used in each case except for fat, in which a \(\lambda\) of 9 has been assumed).
- \(\rho\) = specific gravity of tissue, assumed to be 1.0.

For laboratory identification, dog no. 1 = Canis Minor, dog no. 2 = Radar, dog no. 3 = Quintus, dog no. 4 = Cyrano.
intrarenal nutrient blood flow distribution

**FIGURE 9**

A. Autoradiograph from a kidney removed 52 minutes after Kr\(^{85}\) injection (Component IV) showing radioactivity remaining only in the hilar and perirenal fat (exposure time of film nine days). The border of the kidney is indicated by the dots drawn by hand. B. Corresponding tissue section. C. Graph showing the time at which kidney was removed.

determined repeatedly for periods up to one year, with considerable variation noted from day to day. The widest range of cortical flow observed in one normal dog was 264 to 885 ml/100 g/min (dog no. 2, left kidney, table 1). However, in a well-trained animal, consecutive flow rates determined over a period of three to five hours on a given day frequently were relatively constant, as shown in table 2. Moreover, as experience was gained in the use of the method, it became apparent that rapid, serial determinations of cortical blood flow could be obtained at six-minute intervals. For such measurements, it was found empirically that the slope of the cortical component could be obtained as follows:

1. the number of counts at four and one-half minutes was subtracted from the preceding curve, which yielded two exponentials;
2. the slower or second exponential was subtracted from the initial portion of the curve.

A slope for Component I was thus obtained.

*Circulation Research, Volume XIII, October 1963*
TABLE 2
Consecutive Flow Rates Determined with Kr\(^{38}\) in the Unanesthetized Dog

<table>
<thead>
<tr>
<th>Time</th>
<th>Cortex</th>
<th>Outer medulla</th>
<th>Inner medulla</th>
<th>Renal fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 AM</td>
<td>11.6</td>
<td>70.3</td>
<td>720</td>
<td>4680</td>
</tr>
<tr>
<td>9:30 AM</td>
<td>12.2</td>
<td>43.1</td>
<td>792</td>
<td>6480</td>
</tr>
<tr>
<td>11:00 AM</td>
<td>10.3</td>
<td>60.0</td>
<td>518</td>
<td>4800</td>
</tr>
</tbody>
</table>

Nutrient flow (ml/100 g/min) = k x λ x 100 x P

<table>
<thead>
<tr>
<th>Time</th>
<th>Cortex</th>
<th>Outer medulla</th>
<th>Inner medulla</th>
<th>Renal fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 AM</td>
<td>358</td>
<td>59</td>
<td>8</td>
<td>96</td>
</tr>
<tr>
<td>9:30 AM</td>
<td>341</td>
<td>56</td>
<td>5</td>
<td>94</td>
</tr>
<tr>
<td>11:00 AM</td>
<td>403</td>
<td>69</td>
<td>9</td>
<td>96</td>
</tr>
</tbody>
</table>

\(k = \) slope of line, λ = partition coefficient (a figure of 1 has been used in each case except for fat, in which a λ of 9 has been assumed), P = specific gravity of tissue, assumed to be 1.0.

Discussion

For the measurement of nutrient blood flow by the method described above, the assumption is made that a highly diffusible substance will equilibrate between blood and tissue in a single passage through the capillary bed.\(^3\) Because we have obtained cortical blood flow rates of 1200 ml/100 g/min during intrarenal infusion of acetylcholine, the assumption would appear to be valid. Therefore, the rate of removal of such a substance from the tissue will vary directly with the capillary

\[ \text{Circulation Research, Volume XIII, October 1963} \]
Kety derived an expression for the rate of disappearance of an inert gas from an organ; if the partition coefficient is known, then the nutrient blood flow per unit volume of tissue can be determined.

Krypton qualifies as an ideal substance for such measurements since it is an inert, lipid-soluble, highly diffusible, radioactive gas. Furthermore, because of its low solubility in blood relative to air, 95% or more of the gas is removed in one circulation through the lungs. Thus, the amount of Kr returning to the kidney is negligible.

When a tracer substance is injected into an organ and the rate of removal is a multi-exponential function of time, the various components obtained can be interpreted on the basis of differing parallel rates of blood flow. Dobson and Warner recorded the multi-exponential disappearance of Na after intra-arterial injections in the forearm and developed a theory and a technique of handling the data from a multi-compartmental vascular system. The disappearance of Kr from the kidney is also multi-exponential, with three different rates of blood flow through the parenchyma. The autoradiographs (figs. 4 to 9) have demonstrated that the first exponential describes the rate of nutrient blood flow in the outer cortex, the second component is a measure of outer medullary and inner cortical flow and the third component is an estimate of the inner medullary flow. The values obtained for nutrient flow in cortex and inner medulla agree with those originally reported by Kramer and co-workers using Evans blue transit times as an index of total blood flow. In a more recent review, Ulrich et al reported unpublished observations of Kramer on inner and outer medullary flow which are approximately twice the values found in this study; the discrepancy may be explained in
Histological sections (Mallory stain) of outer medulla (cross-sectional and longitudinal) showing medullary bundles, vasa recta containing erythrocytes, and peritubular capillaries interspersed among the distal portions of the proximal tubules, descending and ascending tubules, and collecting ductus, X 130.

In seeking anatomical correlations between the three exponentials and the three zones observed autoradiographically, it became apparent that these components represented flow in the three major histological divisions of the canine kidney (fig. 6C). The decay curves obtained may be the sum of a larger series of exponentials but the three main components undoubtedly represent physiologically significant averages referable to the three divisions of the renal parenchyma. The rapid cortical flow, with a turnover rate of four to five times a minute, represents the major fraction of blood flow in the kidney. This high rate of blood flow supplies the large volume of plasma for filtration. Although, in the normal animal, krypton is uniformly distributed in this area to both tubular and vascular elements, only a relatively small amount of the glomerular filtrate leaves the cortex. Therefore, the urine removes an insignificant proportion of the krypton compared to the large amount removed by the blood.* Assuming that the cortex is 75% of the kidney volume, the outer medulla 15% and the inner medulla 10%, then the average flow rates/100 g of total kidney for the three areas are 354, 20 and 2 ml/min respectively. Thus nutrient cortical flow is approximately 17.7 times as great as outer medullary flow, and 177 times that of inner medullary flow, or, relative flows are 177, 10, and 1. Using the average figures given above, a value for total renal blood flow of 376 ml/100 g/min is obtained, a figure which agrees with the average renal blood flow of 400 ml/100 g/min obtained with PAH.**

Although the indicated nutrient flow to the outer medulla is only 5% of total nutrient blood flow in the dog kidney, the actual volume flow through this region (nutrient plus vasa recta) is undoubtedly higher, as sug-

*In vitro equilibration experiments in this laboratory have shown the urine/blood partition coefficient to be 0.68 ± 0.06 (sd).

**Circulation Research, Volume XIII, October 1963
gested by the observations of Kramer. This region is supplied solely by the efferent blood vessels of the juxtamедullary glomeruli which divide to form the vasa recta and the peritubular capillaries. The bundles of vasa recta, which extend from the juxtamедullary area of the cortex (figs. 11 and 14) to the outer portion of the inner medulla, have been shown by Trueta to be comprised of hairpin loops of vessels which penetrate the outer medulla to different depths. Thus, as the number of vessels decreases progressively, the bundles taper as they approach the inner medulla (fig. 11). The short loops at the top of the vasa recta, with their apparent low resistance, may act as arteriovenous anastomoses with a flow rate so fast that the shunt flow is not detected by the K^+ washout. Moreover, with such a vascular arrangement only a small percentage of the juxtaglomerular flow will reach the inner medulla.

Trueta and Longley have emphasized the presence of a large surface area available for intercapillary exchange within the bundles of vasa recta which facilitates rapid movement of diffusible substances from the descending limb to the ascending portion (fig. 12). Hence, the vasa recta act as efficient countercurrent exchangers for diffusible substances. As Seholander and Lassen have indicated, whenever a gradient for a diffusible substance exists between the two limbs, a countercurrent exchanger acts as a barrier to the net transport of that substance along the long axes of the vessels; the effectiveness of the barrier is directly proportional to the diffusibility of
the material. Thus, as Lassen, and the above observations have demonstrated, the counter-current exchange in the vasa recta retards the deposition in the papilla of Kr, an inert, lipid-soluble and diffusible substance (fig. 15). Moreover, the effectiveness of the exchange is indicated by the slow medullary deposition of antipyrine, a much less lipid-soluble material with an olive oil/water partition coefficient only 1/300th that of krypton.  

Although the vasa recta have a large inter-capillary surface area for exchange within the bundles, the capillary surface area exposed to the tubules of the outer medulla is comparatively small (figs. 11 and 12). Therefore, the vasa recta are relatively ineffective as a nutrient blood supply to the tubular cells of the outer medulla (figs. 14 and 15). The peritubular capillaries in this region also arise from the efferent vessels of the juxtamedullary glomeruli. They penetrate to the outer border of the inner medulla (figs. 5 and 6), and then return to the arcuate veins in the juxtamedullary area. The nutrient flow supplying the tubular cells in the outer medulla is represented by Component II of the Kr disappearance curve, and averages 132 ml/100 g/min.

The arterial blood supply to the inner medulla and papilla is derived from the terminal branches of the vasa recta which divide into longitudinal capillaries in intimate contact with the tubular elements (figs. 13 and 15). Available evidence indicates that the venous blood returns through the ascending limb of the vasa recta in the bundles of the outer medulla.  

![Schematic illustration of renal circulation in the dog.](http://circres.ahajournals.org/)

Circulation Research, Volume XIII. October 1968
Figure 15

Schematic illustration of the movement of K+-ps in the kidney of the normal dog.
are the sole nutrient supply to the region, they function as effective countercurrent exchangers because of their length and density. Thus the rate of accumulation of a diffusible substance in the inner medulla, and its removal from this part of the kidney are both extremely slow in the antidiuretic state. Because of the slow entrance of Kr\(^{85}\) into the inner medulla the number of counts initially distributed to this region is difficult to determine; the uncertainty in the calculation of \(A_{c0}\) is indicated by the use of a dotted line in figure 2. However, variations in \(A_{c0}\) do not alter significantly the calculated flow rates and distribution of Kr\(^{85}\) in Components I and II.

During the "washout" of the inner medulla, the concentration and total amount of Kr\(^{85}\) progressively decreases, necessitating increasing exposure time for the autoradiographs, as noted in the legends of figures 7 and 8. In addition, since the effectiveness of the countercurrent exchange for retarding the outflow of a diffusible substance is proportional to its length, the activity is lost first in the proximal part of the inner medulla, while the papilla is the last area to be cleared. The countercurrent exchange introduces some error in the calculation of the nutrient flow; for this reason it may be more appropriate to designate the estimated "washout" rate in this region as effective flow. However, since the movement of Kr\(^{85}\) in the medulla may serve as a model for that of water, \(O_2\) and \(CO_2\), the physiological importance of this method of measuring capillary flow is apparent. Moreover, the low effective inner medullary flow is ideally suited for the maintenance of the high solute concentration observed in this region in the antidiuretic state.

In the antidiuretic state the urine appears to play a minor role quantitatively in the movement of krypton within the kidney, or the clearance of krypton from the organ. The outer medulla effectively "traps" highly diffusible substances from the tubular urine as well as from the blood, retarding their deposition in the inner medulla. As indicated above, preliminary experiments show that a negligible amount of Kr\(^{85}\) is carried into the pelvic urine at urine flow rates of less than 0.5 ml/min. At the present time insufficient data are available to indicate whether the lymphatics play any significant role in the removal of Kr\(^{85}\) from the kidney.

**Summary**

A method has been described for the measurement, by means of Kr\(^{85}\), of intrarenal nutrient blood flow distribution in the unanesthetized dog. Injection of the isotope into the renal artery is followed by a multi-exponential disappearance curve which can be obtained by external monitoring with a scintillation detector. In acute experiments autoradiographs have demonstrated that the first exponential component represents cortical blood flow; the second, outer medullary blood flow; the third, inner medullary blood flow; and the fourth, hilar and perirenal fat blood flow. The average cortical blood flow in 65 experiments in five kidneys of four unanesthetized dogs was 472 ml/100 g/min, the outer medullary 132 ml/100 g/min, and the inner medullary 17 ml/100 g/min. Eighty per cent of the radioactivity was distributed initially to the cortex, 16% to the outer medulla, and 2% to the inner medulla. The hilar and perirenal fat, which receives approximately 2% of the initial radioactivity, was estimated to have a flow rate of 21 ml/100 g/min. In addition, a method for the rapid determination of serial cortical blood flow rates has been described. The importance of these findings has been discussed with reference to the anatomy of the kidney, and to the countercurrent concept as it applies to passive reabsorption of lipid soluble substances, and to the maintenance of an osmotic gradient.

**Acknowledgment**

We express our thanks to Mr. Franklin Smith for his devoted care of the dogs, and for his technical assistance.

**Addendum**

Since this paper was submitted for publication, a number of the Kr\(^{85}\) curves have been analyzed on the IBM 7090 computer at the Massachusetts Institute of Technology.
INTRARENAL NUTRIENT BLOOD FLOW DISTRIBUTION

of Technology with the program developed by Berman and co-workers. Excellent agreement was observed between the values obtained by graphical analysis and those determined by the computer. We express our sincere appreciation to Drs. Momes Berman and Marjory F. Weiss of the National Institutes of Health, Dr. Frank Perkins of the Massachusetts Institute of Technology, Dr. Walter Scheider, and Gordon Nordby of the University of Michigan for their advice and aid.

References

Intrarenal Distribution of Nutrient Blood Flow Determined with Krypton$^{85}$ in the Unanesthetized Dog

GEOFFREY D. THORBURN, HUGH H. KOPALD, J. ALAN HERD, MILTON HOLLENBERG, CHARLES C. C. O'Morchoe and A. CLIFFORD BARGER

doi: 10.1161/01.RES.13.4.290

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1963 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/13/4/290

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/