Direct Determination of Vasa Recta Blood Flow in the Rat Renal Papilla

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SUMMARY. Blood flow in vasa recta capillaries of the exposed renal papilla of young antidiuretic rats (n = 18) was determined by an adaptation of the video-photometric technique of Intaglietta. The erythrocyte velocity and capillary diameter in vasa recta (n = 97) were measured at the same location by means of fluorescence video microscopy, with fluorescein-labeled bovine γ-globulin as a plasma marker. A factor relating erythrocyte velocity to mean cross-sectional blood velocity was determined in vitro to permit the calculation of single vasa recta blood flows from the measured indices, erythrocyte velocity and capillary diameter. Mean blood flow in descending vasa recta was 8.83 ± 0.96 (SE) nl/min, significantly greater than that in ascending vasa recta, 4.82 ± 0.34 nl/min. The total numbers of ascending and descending vasa recta at the base of the exposed papilla were also determined. Over 1500 vasa recta were identified as ascending vasa recta or descending vasa recta in electron micrographs of three papillas. At this level in the papilla (2 mm from the tip), there were four ascending vasa recta for each descending vas rectum. From the total numbers of ascending vasa recta and descending vas rectum, single vessel blood flows were converted to total blood flow. Total blood outflow in all ascending vasa recta, 11.3 μl/min, substantially exceeded total blood inflow in all descending vasa recta, 5.2 μl/min. The difference between outflow and inflow (6.1 μl/min) represents an estimate of water uptake by the papillary microcirculation, and is more than adequate to accommodate the known rate of water reabsorption from the collecting ducts of the exposed papilla. (Circ Res 53: 401-413, 1983)

IN addition to supplying oxygen and substrates and removing metabolic wastes, the renal medullary circulation performs two other functions. It preserves the axial osmotic gradient of the medulla by countercurrent exchange of water and solutes between descending and ascending vasa recta (DVR and AVR, respectively) (Scholander, 1957; Berliner et al., 1958; Gottschalk, 1961; Gottschalk et al., 1962; Ulrich et al., 1962; Thurau and Levine, 1971; Sanjana et al., 1975, 1976). It also removes water reabsorbed from the descending limb of Henle’s loop (including the medullary pars recta) and the medullary collecting duct (Ulrich et al., 1962), but the absolute rate at which AVR removes water from the medulla has not been determined (Sanjana et al., 1975, 1976). The issue of water mass balance in the medulla must be investigated further; medullary blood flow should be analyzed quantitatively. Consider fluid balance in the exposed tip of the renal papilla (Fig. 1). Micropuncture experiments in the young rat have shown that approximately 3 μl/min of water is reabsorbed from the collecting ducts of the exposed papilla in antidiuresis (Oliver et al., 1982) and 5 μl/min in water diuresis (Jamison et al., 1971). Since the rate of water removal from the descending limb in the papilla is unknown (Gottschalk, 1961; Jamison et al., 1973), the aforesaid figures underestimate the rate at which water is added to the papillary interstitium. The papillary tip of the young rat weighs about 2 mg (see below); water uptake by the papillary microcirculation is therefore of the order of 1–3 ml/min per g tissue weight in the antidiuretic rat. Estimates of total papillary blood inflow yield values ranging from 0.4 to 0.7 ml/min per g tissue.

Figure 1. Mass balance for water in the renal medulla. The three kinds of structures in the medulla are depicted: short and long loops of Henle, branched collecting ducts, and descending and ascending vasa recta. Arrows indicate direction of fluid flow. Water is added to the medullary interstitium by absorption from the medullary pars recta and descending limb of the loop of Henle, the collecting duct, and the descending vasa recta. Since the epithelium of the thin and thick ascending limbs is water-impermeable, ascending vasa recta represent the only channels by which the added water can be removed from the medulla.
weight by the isotope-labeled albumin-accumulation technique and from 1.0 to 1.8 ml/min per g tissue weight by the isotope-labeled erythrocyte method (Thurau and Levine, 1971; Wolgast, 1973; Ganguli and Tobian, 1974; Solez et al., 1974; Aukland, 1976; Chuang et al., 1978; Jamison and Kriz, 1982). In other words, the estimated rate of capillary water uptake exceeds the rate of plasma inflow, which seems inherently unlikely. Apparently, the measurements of blood flow or protein concentration involve large errors, or water is removed from the papilla by an extravascular route (Schmidt-Nielsen, 1977).

In addition to the likelihood that these indirect methods for determination of medullary blood flow may be inaccurate (Aukland, 1976; Jamison and Kriz, 1982), they measure either blood inflow or outflow, but not both, and therefore cannot be used to determine whether outflow of blood exceeds inflow by an amount sufficient to satisfy the requirement of mass balance of water in the renal medulla. The purpose of this study was to determine both inflow and outflow of blood to the exposed papilla.

**Methods**

**Preparation of Animals**

Eighteen young (5–6 weeks) Munich-Wistar rats [108 ± 10 (SD) g body weight] were allowed water ad libitum but were deprived of food the night before the experiment. They were anesthetized with Inactin (110 mg/kg body weight), and the left renal papilla was exposed by excision of the ureter, as previously described (Battilana et al., 1978). Body temperature was regulated to 37 ± 1°C with a servocontrolled heating table. The animals received an intravenous infusion of normal saline at 2.5–3% of body weight per hour throughout the experiment. Blood pressure was monitored through a femoral arterial catheter, and urine was collected from the right kidney through a bladder catheter for determination of osmolality. Mean arterial blood pressure exceeded 90 mm Hg in all experiments used.

**Single Vasa Recta Blood Flow, \( Q_{vr} \)**

Single capillary blood flow was calculated according to the following equation:

\[
Q_{vr} = V_{\text{blood}} \cdot A = \left( \frac{V_{\text{RBC}}}{F} \cdot \pi D^2 / 4 \right)
\]

where \( Q_{vr} \) = single capillary blood flow in a vas rectum (pL/sec); \( V_{\text{blood}} \) = cross-sectional blood velocity (mm/sec); \( A \) = capillary cross-sectional area (um²); \( V_{\text{RBC}} \) = erythrocyte velocity in a vas rectum (mm/sec); \( F \) = \( V_{\text{RBC}}/V_{\text{blood}} \) (dimensionless); \( D \) = capillary diameter (um).

To determine \( Q_{vr} \), values of \( V_{\text{RBC}} \), \( D \), and \( F \) were measured as follows.

**Erythrocyte Velocity, \( V_{\text{RBC}} \)**

Erythrocyte velocities were determined by the video method of Intaglietta et al. (Tomkins et al., 1974; Intaglietta et al., 1975), as described previously (Gussis et al., 1979a, 1979b). Briefly, a silicon-intensified target television camera (COHU 4410 SIT; COHU, Inc.) was mounted on a Leitz intravital microscope (Ernst Leitz GmbH). The video signal from the camera of the image of the microscopic field entered a photoanalyzer (IPM, model 202, Instrumentation for Physiology and Medicine) and a video monitor (COHU, DM 17/C), where the image was displayed. Video recordings of capillary blood flow were made for later analysis. Two electronic “windows,” separated by 11–18 um and generated by the photoanalyzer, were positioned manually upstream and downstream over a capillary. Fluctuations in light intensity resulting from the passage of red blood cells alternating with cell-free plasma past the windows were monitored electronically. The upstream and downstream “window” signals produced by the photoanalyzer were cross-correlated to determine a transit time for the erythrocytes between the windows. Erythrocyte velocities were computed by dividing the distance between windows by the time delay (red blood cell transit time) which maximized the cross-correlation. By replaying the videotape with the windows repositioned, \( V_{\text{RBC}} \) could be measured in several different vessels. The terms \( V_{\text{RBC}} \) and erythrocyte velocity refer strictly to determinations made using this technique and should not be interpreted as necessarily indicating individual RBC velocity or the average velocity of all RBC’s in a given capillary cross-section.

Since our initial investigations were published (Gussis et al., 1979a, 1979b), several changes have been incorporated into the system to increase the accuracy of the velocity measurements. A PDP 11/34 computer (Digital Equipment Corporation) was employed to sample instantaneous red cell velocity. Erythrocyte velocity for each capillary, \( V_{\text{RBC}} \), was the time average of 100 samples over a total period averaging 3 minutes, which has been previously shown to give reproducible values (Gussis et al., 1979a). The computer also modified the raw velocity data from the correlator, using a calibration curve, and corrected for local variations in video magnification. The velocity calibration curve was determined, using the video method, by measurement of apparent velocities of randomly placed spots on the rim of a wheel rotating at a known angular speed. Measurements made by this method were reproducible to within 5% between 0.1 and 1.6 mm/sec wheel rim velocity for window separations ranging from 11 to 18 um.

During an experiment, a field of view was selected in which several vessels were clearly outlined. AVR consistently outnumbered DVR, so fields had to be carefully selected to include at least one DVR.

**Vasa Recta Diameter, \( D \)**

To permit simultaneous determination of capillary diameters, the incident light illumination system previously used was replaced by a Leitz Plomopak (Ernst Leitz, GmbH) through-the-objective illumination system, employing a double dichroic mirror especially designed for fluorescence microscopy (Ploem, 1967) and a DC HBO 200-W mercury arc lamp (Osram). As described in Appendix I, 5–10 mg of fluorescein isothiocyanate (FITC)-labeled \( \gamma \)-globulin, injected intravenously, were employed to enhance contrast of capillary walls for measurement of vessel diameters. Diameter measurements were performed on a previously calibrated video monitor by means of a caliper. Each diameter was measured by two observers, each observer making three measurements.

**Ratio of \( V_{\text{RBC}}/V_{\text{blood}} \)**

As is evident from Equation 1, the measurement of \( V_{\text{RBC}} \) by means of the video microscopy technique is related to
FIGURE 2. Apparatus designed to determine the factor $F$. Blood is added to the perfusion chamber and kept suspended by a stirrer. The rate of blood flow down the quartz capillary is controlled by air pressure. Video microscopy is used to determine red cell velocity, $V_{mc}$. A mercury arc lamp and a condenser provide the source of light. Capillary blood flow is determined by timing the experiment and measuring the volume of effluent blood that accumulates on the plexiglass trough.

mean cross-sectional blood velocity through a proportionality factor which we designate $F$. The range of permissible values for $F$ is $1 \leq F \leq 2$, the lower bound corresponding to single-file RBC flow (a parabolic velocity profile) which occurs when vessel diameter is much greater than erythrocyte diameter. In addition to capillary diameter, $F$ may be a function of mean cross-sectional blood velocity, hematocrit, and the shape, size, and deformability of erythrocytes (Fahraeus, 1928; Barbee and Cokelet, 1971; Gaëtgens et al., 1976). Numerous efforts have been made to examine this complex relationship (Fahraeus, 1928; Barbee and Cokelet, 1971; Gaëtgens et al., 1976), yet $F$ remains ill-defined for the ranges of diameter (8–25 μm) (Jamison and Kriz, 1982), microvessel hematocrit (10–25%) (Ullrich et al., 1961), and $V_{mc}$ (0.1–1.0 mm/sec) (Gussis et al., 1979a, 1979b) which occur in vasa recta. We sought a value of $F$ determined under conditions approximating those of our experiments as closely as possible.

An apparatus was designed to control blood flow through a small-bore quartz capillary (Warden Quartz, Fig. 2). A reservoir supplied blood to a syringe, in which a stirrer kept the red cells dispersed. Blood entered the capillary driven by pressure from a tank of air monitored by a transducer. $V_{mc}$ in the capillary was determined by video microscopy, using a horizontally mounted video microscope focused on the capillary, which was illuminated by light from a DC mercury arc lamp. The apparatus was otherwise identical to that described for in vivo determination of $V_{mc}$.

A microscope micrometer was recorded on videotape for calibration of magnification. The blood was poured into the reservoir and the chamber pressurized. A pressure of 20–30 mm Hg was used to flush the capillary; it was then reduced until the desired $V_{mc}$ was achieved. During video recordings of velocity, the capillary blood effluent was collected in a plexiglass trough filled with immersion oil. Once a steady state was reached, the capillary was shifted to allow a new drop of effluent to form, and the videotape and timer were started. $V_{mc}$ was measured during 3– to 10-minute periods. At the end of the experiment, the effluent was aspirated into a calibrated pipette to determine the volume. The volume divided by collection time and cross-sectional area of the capillary was used to calculate $V_{blood}$. So that the hematocrit within the capillary could be determined, the capillary was placed inside a microhematocrit tube, sealed twice with epoxy, and centrifuged for 5 minutes. The hematocrit of the effluent was determined in the same way.

Scanning electron microscopy (SEM) was used to measure the capillary diameter for calculation of $V_{blood}$ from volumetric flow rate. Each capillary was glued on a holder with epoxy, sputtered with gold for 2 minutes, and then viewed end-on in the scanning electron microscope.

Human blood, with erythrocytes similar in size to those of the rat, was drawn into a 7-ml Vacutainer containing 10.5 mg ethylenediamine tetraacetic acid (EDTA) (Becton Dickinson). (Attempts to use rat blood failed, due to the formation of microthrombi.) After centrifugation for 10 minutes, plasma was decanted from erythrocytes and spun 12 minutes more to remove platelets. The red cells were resuspended in the platelet-free plasma to a predetermined hematocrit. Capillaries were siliconized with a 7% solution of DRI-Film SC-87 (Pierce), diluted in chloroform, and rinsed twice with toluene and acetone. Just before use, they were checked for patency with saline.

Determination of Number of Ascending and Descending Vasa Recta at the Base of the Exposed Papilla

The measurements described above were used to calculate single vessel blood flows, using Equation 1. To convert single vessel blood inflow to total inflow and single vessel outflow to total outflow of blood from the exposed papilla, the numbers of DVR and AVR, respectively, at the base of the exposed papilla were determined. Three specific capabilities were required: (1) distinguish vasa recta from thin loops of Henle, (2) distinguish AVR from DVR and determine the ratio of AVR to DVR, and (3) determine the total number of vasa recta.

Preparation of Tissue Sections

The rat was anesthetized, and the left renal artery was catheterized and perfused at 100–120 mm Hg with procaine in 0.1 M phosphate buffer solution (pH = 7.3). The kidney was prefixed by perfusion with glutaraldehyde-formaldehyde in phosphate buffer (pH = 7.2) and perfused for 2 minutes with 30% bovine serum albumin in 0.1 M Na2HPO4. The renal vein and artery were clamped, and the kidney was removed and immersed immediately in a glutaraldehyde-formaldehyde solution and refrigerated. (The composition of the immersion solution was 1% paraformaldehyde, 0.01% picric acid, 1.1% glutaraldehyde, and 0.1 M Na2HPO4. The osmolality was 504 M0sm/kg H2O. The perfusion solution was composed of equal volumes of the immersion solution and 0.1 M Na2HPO4 solution.) After 1 hour, the ureter and cortex were cut away, and the exposed papilla was returned to the fixative and refrigerated for 24 hours to complete fixation.

The papilla was prepared for ultrathin sectioning by uranyl acetate and osmium tetroxide staining, dehydration in alcohol, and embedding in epoxy araldite. Several ultrathin sections made 2 mm from the tips of three

* The experiments were done in collaboration with Dr. George Harrell of the Department of Radiology, Stanford University School of Medicine.
papillae were placed on copper grids for transmission electron microscopy. Thick sections from two of these papillae were also made at the 2 mm distance from the tip. They were stained with methylene blue, and the total cross-sectional area of the papilla was determined by planimetry on light microscopic photographs.

Identification of Vasa Recta

Both peripheral and central regions of the cross-section of the papilla were examined in a Hitachi HU-11E-1 transmission electron microscope, and as each vessel was identified, it was labeled on a corresponding low-power electron micrograph. Blood vessels were differentiated from thin limbs of Henle's loop by their albumin-filled lumens (Fig. 3). Descending vasa recta were differentiated from AVR according to their ultrastructure (Schwartz et al., 1976). AVR were distinguished by fenestrated endothelia; DVR had an endothelium without fenestrae (Fig. 3). In addition, DVR, but not AVR, had abundant pinocytotic vesicles along the basement membrane. Identity of AVR and DVR in representative micrographs was confirmed independently by Kriz (personal communication). More than 500 vasa recta were identified in each of three papillae.

We next addressed the question of how many capillaries identified as AVR might actually be communicating capillaries, since their ultrastructure is identical to that of AVR. Communicating capillaries erroneously identified as AVR could spuriously elevate the calculated rate of blood flow out of the papilla. We determined the proportion of transverse flowing (functionally communicating) capillaries among all vasa recta at the base of the exposed papilla from videotapes made of four experiments. Of 148 vessels, there were 105 with ascending flow, 40 descending, three capillaries which described a "loop," and one clearly transverse capillary. We therefore considered the proportion of "AVR" which were actually communicating capillaries to be negligible.

The number of vasa recta per grid on the electron microscope stage was determined for approximately 40–50 grid squares per papilla (corresponding to approximately 500 vasa recta). Using the measured area of the grids, we converted the number of vasa recta per grid to the number of vasa recta per unit area. Those vessels of which only a part lay within a grid were counted only if the visible portion of the cross-sectional area exceeded half the area of the average vessel in that particular grid.

The total cross-sectional area of the papilla 2 mm from the papillary tip was determined by planimetry, using light microscopic photographs of the papilla. The total numbers of AVR and DVR were calculated from the AVR:DVR ratio, the capillary density, and the cross-sectional area.

Total Papillary Blood Flow

Total inflow of blood to the exposed papilla was calculated as the product of total number of DVR and the mean value of $Q_v$ for DVR. Likewise, total outflow of blood was calculated as the product of total number of AVR and the mean value of $Q_v$ for AVR. A correction which takes into account the possible elliptical configuration of vasa recta in cross-section is discussed in Appendix 1.

These calculations were performed for the region 2 mm from the papillary tip. To convert absolute flows to flows per tissue mass, 19 rats (9 male, 10 female), averaging 110 g, were anesthetized, and the left kidney was removed and weighed. The papilla was quickly excised and the papillary tip severed 2 mm from the tip. The whole kidney and both portions of the papilla were weighed in preweighed capped microcentrifuge tubes.

Statistics

Unless stated otherwise, the data are presented as means ± se. Paired determinations were tested for significance by Student's $t$-test (Snedecor, 1966).

Results

Urinary Osmolality

The mean osmolality was 1250 ± 190 mOsm/kg H$_2$O.

Erythrocyte Velocity, $V_{rc}$

Individual values are illustrated in Figure 4 and summarized in Table 1. In 25 DVR from 18 rats, mean $V_{rc}$ was 1.04 ± 0.10 mm/sec. In 72 AVR from the same 18 rats, mean $V_{rc}$ was 0.38 ± 0.03 mm/sec, significantly lower than in DVR ($P < 0.001$).

Vasa Recta Diameter, D

Individual values are illustrated in Figure 5 and summarized in Table 1. The DVR (mean diameter 15.6 ± 0.5 µm) were narrower than AVR (20.0 ± 0.4 µm) ($P < 0.001$).

Ratio of $V_{rc}/V_{blood}$, F

Table 2 presents the results of the determination of F, and Figure 6 illustrates $V_{rc}$ as a function of
Vasa recta erythrocyte velocity, $V_{\text{m}}$. Individual determinations of $V_{\text{m}}$ in vasa recta were averaged and treated as one value for DVR and AVR per animal. Lines connect the average $V_{\text{m}}$ for DVR (left) and AVR (right) in the same animal. The average value for the 18 animals ± 1 SE is depicted for DVR (left) and AVR (right) by the vertical lines.

$V_{\text{blood}}$. In 25 separate determinations in which $V_{\text{blood}}$ ranged from 0.14 to 1.70 mm/sec, $V_{\text{m}}$ varied from 0.18 ± 0.08 to 2.05 ± 0.10 mm/sec, duration of collection ranged from 2.5 to 11 minutes, "feed" hematocrit ranged from 9 to 21%, and capillary diameters ranged from 12.5 to 28.4 μm; the mean value for F is 1.4 ± 0.3 sp. We were unable to find a significant dependence of F on $V_{\text{blood}}$, hematocrit, or capillary diameter for the range of values for each variable selected in the experiments, a fact that may be attributed to the actual variability of flow in this regime where the transition from single (one red cell per cross-section) to multiple file flow (several red cells per cross-section) takes place, and to unavoidable imprecision inherent in the techniques.

Single Vasa Recta Blood Flow, $Q_{\text{v}}$

Individual values are illustrated in Figure 7 and summarized in Table 1. Blood flow in DVR (8.83 ± 0.96 nl/min) was almost twice that in AVR (4.82 ± 0.34 nl/min) ($P < 0.001$).

AVR:DVR Ratio and Total Numbers of AVR and DVR

The AVR:DVR ratio and the total numbers of AVR and DVR are summarized in Table 3. The total

| Table 1 | Erythrocyte Velocity, Capillary Diameter, and Blood Flow in Individual Vasa Recta |
|--------|---------------------------------|--------|-----------|--------|-----------|
| $V_{\text{m}}$ (mm/sec) | $D$ (μm) | $Q_{\text{v}}$ (nl/min) | DVR | AVR | DVR | AVR |
| Mean | 1.04 | 0.38 | 15.6 | 20.0 | 8.83 | 4.82 |
| SE | 0.10 | 0.03 | 0.5 | 0.4 | 0.96 | 0.34 |
| $P$ | <0.001 | <0.001 | <0.001 | 
| $n$ | 25 | 72 | 18 | 18 |

* Number of vasa recta; ‡ number of animals
† DVR = descending vasa recta; AVR = ascending vasa recta.
TABLE 2
Determination of F*

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<tr>
<th>Experiment no.</th>
<th>D (µm)</th>
<th>Tcoll (sec)</th>
<th>V∞ (mm/sec)</th>
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<th>Htube</th>
<th>Hfriction</th>
<th>Vblood (mm/sec)</th>
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<td>2.05 ± 0.10</td>
<td>13.0</td>
<td>6.0</td>
<td>6.9</td>
<td>1.321</td>
<td>1.54</td>
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<tr>
<td>24</td>
<td>20</td>
<td>142</td>
<td>1.73 ± 0.20</td>
<td>13.0</td>
<td>6.9</td>
<td>9.7</td>
<td>1.696</td>
<td>1.02</td>
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<tr>
<td>25</td>
<td>20</td>
<td>136</td>
<td>1.71 ± 0.10</td>
<td>13.0</td>
<td>8.7</td>
<td>13.6</td>
<td>1.629</td>
<td>1.05</td>
</tr>
</tbody>
</table>

*F = V∞.Vblood; D, diameter of glass capillary; Tcoll, duration of collection; V∞, RBC velocity; H, hematocrit; Vblood, mean cross-sectional blood velocity. — = not determined. n = 25; F = 1.4 ± 0.3 SD.

FIGURE 6. Determination of F = V∞.Vblood. The results of 25 experiments performed using the apparatus depicted in Figure 2 are illustrated. The slope of the line is 1/1.4 (= F⁻¹).
number of vasa recta was the product of the average number of vessels per unit area (1436 mm$^{-2}$) multiplied by the average papillary cross-sectional area at the base determined by light microscopy (2.20 mm$^2$). This was converted to total number of AVR and DVR with the aid of the AVR:DVR ratio established from all grids. More than 1500 individual capillaries were identified in three papillas. The number of AVR exceeded that of DVR by approximately 4:1. As shown in Table 3, there were 593 DVR and 2351 AVR at the base of the exposed papilla 2 mm from the tip.

**Total Papillary Tip Blood Flow**

Multiplying the number of DVR (593) times the mean $Q_w$ for the DVR (8.83 nl/min) yielded a value for blood flow entering the exposed papilla of 5.2 $\mu$l/min. The corresponding calculation of blood flow leaving the exposed papilla in AVR was 11.3 $\mu$l/min. Extrapolated values for total blood flows in individual papillas, calculated using the average number of DVR and AVR, are illustrated in Figure 8. In each case, total AVR flow exceeded total DVR flow (mean difference = 6.1 $\mu$l/min; range, 1.0–11.9 $\mu$l/min).

**Papillary Weight**

The mean weight of the left kidney was 465 ± 34 mg. The mean weights of the papilla and the papillary tip were 4.93 mg ± 1.48 and 1.8 ± 0.66 mg, respectively. Thus, the blood inflow to the exposed papilla averaged 5.2 $\mu$l/min per 1.81 mg papillary tip, or 2.9 ml/min per g tissue, and the blood outflow from the exposed papilla averaged 11.3 $\mu$l/min per 1.81 mg papillary tip, or 6.2 ml/min per g tissue.

**Discussion**

We found that at the base of the exposed renal papilla of the rat, blood flow leaving in the AVR, 11.3 $\mu$l/min, is twice as great as that entering in the DVR, 5.2 $\mu$l/min. The difference, 6.1 $\mu$l/min, represents an estimate of net capillary uptake of water. These values were calculated from the total number of AVR and DVR multiplied by mean single vasa recta blood flow ($Q_w$) of AVR and DVR, respectively. Mean $Q_w$ was 4.8 nl/min in AVR and 8.8 nl/min in DVR, but AVR outnumbered DVR by a ratio of approximately 4:1. In each vas rectum, $Q_w$ was calculated from $V_{rec}$, $D$, and $F$, according to Equation 1. Our findings and conclusions are based on several methods with their specific accuracies and certain assumptions, as follows.

The rats were anesthetized with Inactin, which, as recently emphasized (De Lano and Zweifach, 1981), can cause fluctuations in blood pressure. Ex-
posure of the papilla impairs urinary concentrating ability (Jamison, 1970), and may stimulate the release of vasoactive prostaglandins (Chuang et al., 1978).

Erythrocyte Velocity, \( V_{\text{rbc}} \)

The techniques used to determine \( V_{\text{rbc}} \) and their limitations have been discussed previously (Holliger et al., 1975; Gussis et al., 1979a). In brief, the present technique is a substantial improvement over other techniques employed to determine \( V_{\text{rbc}} \), in our opinion. Marsh and Segel (1971) viewed red cells flowing in a vas recta in vivo through a microscope. The animal lay on a movable stage. \( V_{\text{rbc}} \) was determined as that velocity at which the stage was moved manually to maintain the red cells immobile. This relatively crude technique has been abandoned by Marsh in favor of the present video technique. Böttcher and Steinhausen (1976) used a microcine-technique in which the film ran across the objective in an axis perpendicular to that of illuminated red cells flowing in a vas rectum. Images of the red cells in a vas rectum traced parallel lines at an angle to the film centerline. From the angle of the lines and the film speed, \( V_{\text{rbc}} \) was calculated. Variability of \( V_{\text{rbc}} \) during short time intervals would not be easily detected. Another limitation is that the contrast between capillary wall and flowing blood was not enhanced by a plasma marker (like FITC-labeled \( \gamma \)-globulin) to increase the accuracy of the diameter measurements. Because of the improved precision in our methodology, a substantial difference was detected between \( V_{\text{rbc}} \) in DVR and the lower \( V_{\text{rbc}} \) in AVR. Böttcher and Steinhausen (1976) previously reported that erythrocyte velocity is lower in AVR than in DVR.

Our value for \( V_{\text{rbc}} \) in DVR, 1.04 ± 0.10 mm/sec, is higher than that found by Böttcher and Steinhausen (1976) in rats (0.64 ± 0.05 mm/sec) and Marsh and Segel (1971) in hamsters (0.50 ± 0.03 mm/sec). These differences may be attributable to differences in experimental conditions, or to technical improvements in the present study. The latter is suggested by the fact that the value 1.04 mm/sec represents an increase from our own earlier determinations (Gussis et al., 1979a, 1979b), presumably due to an increase in measurable range of \( V_{\text{rbc}} \). The value for \( V_{\text{rbc}} \) in AVR, 0.38 ± 0.03 mm/sec, compares reasonably well with Böttcher and Steinhausen’s 0.49 ± 0.04 mm/sec (1976), but is higher than Marsh and Segel’s value in the hamster, 0.18 ± 0.04 mm/sec (1971).

Vasa Recta and Quartz Tube Diameters, \( D \)

The accuracy of \( Q_r \) also depends on the precision of the capillary diameter measurements, especially since the square of the diameter is used (Eq. 1). The data in Table 4 indicate a small (≤3%) error when the video system is used to measure quartz capillaries of known diameter. Capillary wall contrast was enhanced by the injection of FITC-labeled \( \gamma \)-globulin, together with a Ploemopak fluorescence microscope. We previously tried FITC-labeled high-molecular-weight dextran, but the rats often became hypotensive and edematous. Injection of FITC-labeled albumin was followed by a progressive accumulation of fluorescence in the papillary interstitium. Whether this represents capillary leakage of FITC-albumin (Moffat, 1969; Venkatachalam and Karnovsky, 1972), or of the FITC moiety, alone, is

<table>
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<th>Capillary Diameter Measurements</th>
<th>Quartz Capillary</th>
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<tr>
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<td>1</td>
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<td></td>
<td>2</td>
</tr>
<tr>
<td><strong>Observer 1</strong></td>
<td><strong>Observer 2</strong></td>
</tr>
<tr>
<td><strong>Capillary 1</strong></td>
<td><strong>Capillary 2</strong></td>
</tr>
<tr>
<td><strong>8.4 ± 0.2</strong></td>
<td><strong>16.8 ± 0.2</strong></td>
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<tr>
<td><strong>8.6 ± 0.3</strong></td>
<td><strong>17.1 ± 0.2</strong></td>
</tr>
<tr>
<td><strong>8.6 ± 0.3</strong></td>
<td><strong>17.4 ± 0.3</strong></td>
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</tbody>
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* Values in \( \mu \text{m} \); mean ± SD.
† SEM, scanning electron microscopy; values in \( \mu \text{m} \).
unknown. Injection of FITC-labeled γ-globulin was found to be a much improved method for outlining capillary lumina. Not only was the interface between plasma and capillary walls well demarcated, but the cross-correlation of the upstream and downstream video signals was improved, due to the sharper contrast between erythrocytes and plasma.

The use of fluorescent γ-globulin made it possible to measure the capillary diameter at the same site at which \( V_{bc} \) was measured. Previous methods involved measurement by microscopic ocular of the same vessel (Böttcher and Steinhausen, 1976) or determination from photographs of a separate group of vasa recta (Marsh and Segel, 1971).

Two limitations to this method of determining capillary diameters persist. First, the image quality or sharpness of the contrast between the plasma and the capillary wall decreases with time. At present, this restricts measurements to a period 30-60 minutes after infusion of the FITC-labeled γ-globulin. The second limitation is the assumption that vasa recta in vivo have circular cross-sections (see Appendix I). Capillary diameters determined from our videotapes necessarily are those of an image through a single plane. If the elongate or roughly elliptical configuration of vasa recta suggested in Figure 3 and elsewhere (Jamison and Kriz, 1982) is verified by a morphological study, then true capillary cross-sectional areas, especially of the more elongate AVR, are overestimated using the formula, \( A = \pi D^2/4 \). We calculated that the single vessel flows would be reduced in DVR by 9.5%, from 8.8 to 8.0 nl/min, and in AVR by 15.8%, from 4.8 to 4.1 nl/min (see Eq. 1 and Appendix I). These numbers should be considered preliminary, subject to correction later on the basis of a more extensive morphological study.

**Ratio of \( V_{bc} / V_{blood} \): F**

Despite several attempts to define the ratio between red cell velocity, \( V_{bc} \), and mean cross-sectional blood velocity, \( V_{blood} \) (Barbee and Cokelet, 1971; Gaethgens et al., 1976), \( F \) remains ill-defined for the composite range of diameter, microvessel hematocrit, and blood velocity encountered in vasa recta. For that reason, we determined \( F \) for this range in in vitro experiments.

The in vitro experiments, which represent, to our knowledge, the first attempt to estimate \( F \) for capillaries in the 15–25 \( \mu m \) diameter range, had several limitations. First, we used quartz capillaries, not mammalian capillaries. However, Fenton et al. (1982), in experiments in which monolayers of human endothelial cells were grown over the inner surface of glass tubes, demonstrated that (compared with unlined tubes) the presence of an endothelial lining had no effect on the flow of either plasma or red cells suspended in plasma. Second, we used platelet-free EDTA-treated human blood, not rat whole blood. Under normal conditions, however, platelets or EDTA are unlikely to affect \( F \) in flowing blood, and human red cells are nearly the same size as rat red cells. Third, we used isotonic, not hypertonic fluid; whether hypertonicity might have stiffened red cells and possibly reduced \( F \) remains speculative. Fourth, there was considerable scatter in some individual determinations, as can be seen by inspecting Table 2. Nevertheless, the individual determinations do not exceed the theoretical limits of \( 1 < F < 2 \) (actual range, \( 0.94 < F < 1.96 \)), and represent a normal distribution about the mean of 1.4. Moreover, we were able to encompass the range of values of capillary diameter (13–56 \( \mu m \)), erythrocyte velocity (0.18 to 2.05 mm/sec), and hematocrit (6–21%) known for vasa recta in vivo. The values obtained for \( V_{bc} / V_{blood} \) in 25 experiments (Fig. 6; Table 2) suggest that 1.4 is a reasonable estimate in this range of values for these variables, but, for any experiment considered separately, this ratio may vary from 0.7 to 1.41 times the mean value given. As mentioned above, an important point is that this value of \( F \) is a calibrating parameter that permits measurements made, using our video microscopy techniques, to be converted into estimates of capillary blood flow; i.e., this value of \( F \) is specific for these techniques and may not be applicable to experiments employing other means to determine \( V_{bc} \).

**AVR:DVR Ratio**

The principal assumption in determining the ratio of AVR to DVR is that ultrastructural differences of capillary endothelia correspond to differences in direction of blood flow. Only two types of blood vessels exist in the medulla (Kriz et al., 1976; Schwartz et al., 1976): one type has a relatively thick continuous nonfenestrated endothelium, whereas the other possesses a thin attenuated endothelium with numerous fenestrae (Schwartz et al., 1976). Schwartz et al. (1976) were able to distinguish AVR from DVR by partial injection of carbon, or by tracing individual vasa recta from their origins. The endothelium of DVR was found to be continuous, whereas AVR had fenestrated endothelium like the peritubular capillaries of the renal cortex.

We considered the possibility that some vessels counted as AVR were actually "communicating" capillaries between AVR and DVR. Determination of direction of blood flow in 148 capillaries from 60 \( \mu m \)-wide strips of the papillary surface on the video monitor revealed only four vessels that were not unambiguously ascending or descending. Assuming the papillary surface is representative of the underlying papilla, the number of connecting capillaries in a histological section 12 times thinner (<5 \( \mu m \)) probably can be neglected. This neglect does not mean that the medulla contains no communicating capillaries—only that, in a section less than 5-\( \mu m \) thick, their frequency is probably very low. In fact, Jamison and Kriz (1982) have suggested that AVR originate very near DVR, without a large intervening
segment of connecting capillaries.

From 200 capillaries on the surface of the exposed rat papilla in vivo, Böttcher and Steinhausen (1976) determined the ratio of AVR to DVR to be 2.2, lower than the ratio of 4 in the present study. Aside from differences in methods employed, the main difference between their study and ours is that they determined the ratio 0.5 mm from the tip of the papilla, while we determined the ratio 2 mm from the tip. In light of the implication that the ratio of AVR to DVR approaches unity toward the tip of the papilla (Jamison and Kriz, 1982), the difference between our results and theirs is perhaps not surprising.

**Total Papillary Tip Blood Flow**

Total blood flow was calculated by multiplying Qvr by the number of AVR or DVR. Two implicit assumptions are that Qvr was determined exactly 2 mm from the tip of the papilla in every vessel, and that the number of AVR and DVR 2 mm from the tip of the papilla is reasonably constant. A definitive test of the latter assumption awaits a systematic study of the number of AVR and DVR in a larger series of animals.

Taking the physiological variability and the technical limitations together, we estimated the possible range of these determinations for single vessel flow and total blood flow. Based on confidence limits of 95.4% (given by mean ± 2 se) for each contributing parameter, we obtain a worst case range for single vessel flows in DVR of 5.6–12.4 nl/min and in AVR of 3.6–6.8 nl/min. The true variability of numbers of AVR and DVR 2 mm from the tip of the papilla is difficult to estimate; if the whole range of actual numbers determined is used, the number of DVR ranged from 0.71 to 1.46 of the mean value, and the number of AVR ranged from 0.73 to 1.31 of the mean value. Combining these limits with those for single vessel flow yields the following worst case bounds: for inflow, 2.4–10.7 µl/min; for outflow, 6.2–20.9 µl/min. Corrected for the weight of the exposed papillary tip, these values convert to 1.3–5.9 ml/min per g tissue weight inflow and 3.4–11.6 ml/min per g tissue weight outflow.

**Comparison with Other Measurements of Papillary Blood Flow**

Plasma flow entering the renal papilla determined by the Lilienfield 131I-labeled albumin-accumulation technique (Lilienfield et al., 1961) ranges from 0.29 to 0.38 ml/min per g tissue weight (Solez et al., 1974; Chuang et al., 1978; Buerkert et al., 1981) or, in terms of blood flow, 0.39–0.51 ml/min per g tissue weight, assuming a capillary hematocrit of 25%. This is lower than our estimated lower bound of 1.3 ml/min per g tissue weight. The recent modification of the albumin-accumulation technique (Rasmussen, 1978) also yields values lower than ours, 0.44–0.72 ml/min per g tissue weight.

As Chuang et al. (1978) have noted, exposure of the papilla may increase blood inflow by as much as 40%. Using this figure to adjust for papillary exposure, our values would correspond to 0.8–3.5 ml/min per g tissue weight with the ureter intact. Blood flow per gram of tissue to the extrarenal papilla may be greater than to the papilla as a whole, because it is more vascular than the rest of the papilla (Knepper et al., 1977).

Since the Lilienfield technique requires several assumptions that may be incorrect (Aukland, 1976; Rasmussen, 1978; Jamison and Kriz, 1982), a fundamentally different method for measuring papillary blood flow is useful. The concentration profile of 125I-labeled albumin in the plasma entering the recta is assumed to be the same as that in systemic plasma, whereas a more plausible assumption is that the radioactivity profile of plasma by the time it arrives at the papilla is considerably dispersed compared with the radioactivity profile in systemic plasma. The technique assumes no loss of labeled albumin from the papilla during the accumulation period. Within an accumulation time even as short as 10 seconds, however, labeled albumin may already begin to be carried out by AVR, judging from the time course of disappearance of lissamine green from the papilla (unpublished observations). In view of these considerations, the Lilienfield technique may underestimate true plasma flow to the papilla. A more rigorous comparison of values obtained by our method with those obtained by the Lilienfield method must await the performance of experiments in which both techniques are used simultaneously.

Values obtained for inner medullary and papillary blood flow rate by other methods, such as the dye dilution technique and diffusive isotopes [summarized by Thurau and Levine (1971) and Jamison and Kriz (1982)], are also lower than the flow rates reported herein. Recently, however, using an isotope of radium, Hermansson and Wolgast found values reasonably close to our inflow values (Wolgast, personal communication). The findings of Meier et al. (1964) are of particular interest. They determined medullary blood flow by inserting the photoelectric cells directly into the renal medulla of anesthetized dogs. Average plasma velocity was 2 mm/sec. On the basis of differences between transit times determined by diffuse illumination of the medulla and that determined by local illumination, Meier et al. concluded that the velocity of blood in AVR is lower than that of DVR, that the blood volume of AVR is 1.7 times that of DVR, and that the higher AVR volume reflects net fluid uptake.

Our findings indicate that the rate of blood flow to the exposed papilla is about 2.9 ml/min per g tissue weight (DVR) (range 1.3–5.9), whereas outflow in AVR is about 6.2 ml/min per g tissue weight (range 3.4–11.6). Thus, the estimated capacity for water uptake of 3.3 ml/min per g tissue weight (range 2.1–5.7) is adequate to account for the re-
moval of water reabsorbed from the papillary collecting duct (1.5–2.6 ml/min per g) (Oliver et al., 1982).

Appendix I

Cross-sectional Area of Vasa Recta

Introduction

To convert mean cross-sectional blood velocity, \( V_{\text{blood}} \), to single vasa recta blood flow, \( Q_w \), we need to determine the cross-sectional area, \( A \), of the vas rectum. True vasa recta diameter, \( D \), often exceeds the diameter of the red blood cell column because of the existence of a peripheral layer of cell-free plasma (Holliger et al., in press). The interface between cell-free plasma and capillary endothelium is not visible by ordinary video microscopy. Therefore, our intention was to enhance the contrast between plasma and the capillary endothelium/interstitial background. Fluorescein isothiocyanate (FITC)-labeled high-molecular-weight dextrans have been used previously as a plasma marker. Intravenous FITC-dextran administration, however, caused acute hypotension and edema. We tried FITC-labeled bovine serum albumin (66,000 daltons). Unexpectedly, the FITC-albumin seemed to extravasate into the interstitium within about 15 minutes. To avoid extravasation, we next used bovine \( \gamma \)-globulin (150,000 daltons). The plasma contrast enhancement achieved was stable over the measurement period, and intravenous administration was not associated with visible edema or with a change in systemic blood pressure (105 ± 3 mm Hg before, vs. 106 ± 4 mm Hg 15–20 minutes after).

Preparation of FITC-Labeled \( \gamma \)-Globulin

Sixty milligrams of bovine \( \gamma \)-globulin (Cohn Fraction II, Sigma Chemical Company) were dissolved in 5 ml of carbonate-buffered saline at pH 9.9 (Rasmussen, 1978). After the protein dissolved, 40 mg of 10% fluorescein isothiocyanate (FITC) on celite (Sigma) were added and swirled gently. After 2 hours of reaction at room temperature, the solution was centrifuged at 5000 rpm for 10 minutes to remove the celite. The supernatant was passed through a 23-cm Bio-Gel P-4 (Bio-Rad Laboratories) column to remove free, unreacted FITC. The elution fluid for the column was phosphate-buffered saline (0.1 M, 0.85% at pH 7.4). The labeled \( \gamma \)-globulin (approximately 1% solution) was collected and stored at 4°C. An aliquot of the solution (0.5–1 ml) was warmed to room temperature and injected intravenously 30–60 minutes before velocity and capillary diameter determinations.

Measurement of Vasa Recta Diameter, \( D \)

Vessel dimension (\( V_s \)) were determined directly from the frozen-frame capillary image on the television monitor. Owing to the inhomogeneity inherent in video systems, the measurements were converted to absolute capillary dimensions in terms of local magnification. The monitor was partitioned into 20 regions in which local horizontal and vertical microfins were established with a Leitz stage micrometer. The accuracy and reproducibility of measurements of vessel diameters were investigated using FITC-filled quartz capillaries. Diameters determined with a scanning electron microscope were the standard with which the video measurements were compared. Table 4 summarizes the results, where the standard deviation of the much more accurate SEM method documents the variability of inner diameters along the test capillary; mean values from the video system agreed with the electron microscope values to within 3%.

As shown in Figure 3, vasa recta may be more accurately regarded as elliptical in cross-section. [Please see also, for example, Figure 16-3, p. 238, in Jamison and Kriz (1982).] Therefore, capillary dimensions measured using the video monitor are not necessarily the diameters of circular vasa recta.

Assuming a uniform distribution of ellipse orientations, an average capillary cross-sectional area \( A \) for the purpose of blood flow calculations can be computed according to the formula:

\[
A = A' \int_0^\pi \frac{\beta u(\beta)}{\sqrt{1 + (\beta^2 - 1)\sin^2 \theta}} d\theta
\]

where \( A' \) is the apparent cross-sectional area as determined by video microscopy (\( A' = \pi D^2/4 \)), \( \beta \) is the ellipse aspect ratio (ratio of major axis length to minor axis length), \( u \) is a normalized AVR or DVR-specific distribution function for \( \beta \), and \( \theta \) is an angle that is zero when \( D \) equals the minor axis length, and is 90° when \( D \) equals the major axis length.

The elliptical model itself is a simplification of the actual shapes of the vasa recta cross-sections suggested by Figure 3. Its main advantage lies in the fact that it can accommodate a continuum of vessel shapes without becoming mathematically intractable. Still unresolved is the question of whether or not the noncircular shapes of the vasa recta cross-sections in the electron micrographs are artifacts or reflect the true state of affairs in vivo. We know of no a priori reason that the vasa recta should be cylindrical tubes, although it is conceivable that DVR—with their thicker endothelium—may tend to be more nearly cylindrical than AVR.

It is unlikely that the angle of sectioning of the papilla could account for the eccentric shapes of vasa recta cross-sections. The chance of fixation artifact is more troublesome, and will require further morphological studies. Because of the possibility that vasa recta are elliptical, rather than circular, the “corrected” values for blood flow given below represent possible lower bounds set by this uncertainty.

Aspect ratios were determined for AVR and DVR from electron micrographs of two papillas. Ellipses were hand-drawn to approximate capillary cross-sections for all those vasa recta which lay entirely within electron microscope grids in which no fixation distortion was evident. The major and minor axes then were measured, and \( \beta \) calculated as their ratio. A total of 143 AVR and 63 DVR were analyzed. The median value for \( \beta \) in the AVR was 1.9; that in the DVR was 1.6. Replacing the continuous distribution of \( \beta \) in Equation 2 by a discrete, step-function distribution, the following "corrected" values were obtained: single vasa recta blood flow in AVR is 4.06 nl/min (compared to 4.82 nl/min), in DVR 7.99 nl/min (compared to 8.83 nl/min). Total blood flow into the exposed papilla in DVR is 4.7 \( \mu \)l/min (compared to 5.2 \( \mu \)l/min), and from the exposed papilla in AVR 9.6 \( \mu \)l/min (compared to 11.3 \( \mu \)l/min); the difference (which is a

† The authors will provide a complete derivation of Equation 2, upon request.
We are grateful to Kristina Blouch and Jim Knapp for technical assistance and Ditter Peschke-Koedt for secretarial assistance.

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Holliger et al. / Direct Determination of Blood Flow in Vasa Recta


INDEX TERMS: Vas recta blood flow • Renal medulla • Papilla • Fluorescence video microscopy
Direct determination of vasa recta blood flow in the rat renal papilla.
C Holliger, K V Lemley, S L Schmitt, F C Thomas, C R Robertson and R L Jamison

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