Diameter of Afferent Arterioles during Autoregulation Estimated from Microsphere Data in the Dog Kidney

LARS MØRKRID, JAKLE OFSTAD, AND YNGVAR WILLASSEN

**SUMMARY** Afferent arteriolar diameters, relative flow distribution, and flow conductance factors are estimated by nonlinear regression analysis of the sieving effect on microspheres in different vascular structures of the dog renal cortex. The data presented are from experiments in which microspheres of 10-30 μm were injected into the abdominal aorta during normotension and after lowering the blood pressure to the lower limit of autoregulation. Microscopic examination of the spheres trapped in the glomeruli and the renal arteries showed an increasing exclusion of microspheres greater than 15 μm from the afferent arterioles during normotension. This effect was most pronounced for the deeper cortical layers and can be explained mainly as geometrical exclusion of spheres from afferent arterioles. During hypotension, progressively larger microspheres entered glomeruli and afferent arterioles, presumably due to vasodilation of the vessels. There was a significant redistribution of microspheres larger than 15 μm from the outer to the inner cortex during hypotension without a corresponding redistribution of smaller spheres or the estimated blood flow. Approximately the same degree of dilation of afferent arterioles was observed during autoregulatory hypotension in three cortical layers.

IT IS commonly believed that the variation of the preglomerular vascular resistance during autoregulation takes place in the afferent arteriole. There is no direct evidence supporting this; neither hydrostatic pressure in this vessel nor its diameter has been measured during autoregulation. The autoregulatory changes in preglomerular vascular resistance may take place exclusively in the interlobular artery or in addition to the afferent arteriole.

It also is not clear whether the variation in the preglomerular vascular resistance is identical in all of the so-called cortical layers. Lack of autoregulation in the inner medulla (dog) and outer cortex (rat), as well as identical autoregulation in all layers (dog), have been reported by measuring the local blood flow with various indirect methods.

The intension of this study was to measure the variation in diameter of the afferent arteriole in the autoregulating dog kidney. For this purpose we used an original version of the microsphere method, the principle of which is to record the diameter population of the spheres which become trapped in (or pass through) the afferent arterioles and the population of sphere diameters presented to these vessels.

**Methods**

Mongrel dogs of either sex (body weight, 17-25 kg) were anesthetized with sodium pentobarbital (25 mg/kg given intravenously as an initial dose), and N2O was supplied by conventional anesthetic apparatus connected to an endotracheal tube. The arterial oxygen saturation, pH, and Pco2 were within normal limits during the experiments. The dogs were fasted and had free access to water for 12 hours before the experiment.

Both kidneys were exposed by an abdominal mid-line section. After free dissection of the renal arteries, all visible nerves were cut and electromagnetic flow probes were positioned on both renal arteries. A sling made of PE 60 polyethylene catheter and placed around each artery distal to the flow probe was used to zero the flowmeter to stop blood flow transiently or permanently during the experiment. An adjustable clamp was positioned proximal to the renal arteries on the abdominal aorta. For the injection of microspheres, a thin polyethylene catheter was introduced from one femoral artery into the abdominal aorta about 5 cm proximal to the renal arteries. Another catheter was introduced from the other femoral artery into the lower part of the abdominal aorta to record blood pressure. A thin polyethylene catheter was introduced into one brachial vein for infusion of the saline and inulin. A catheter for the collection of urine in the clearance measurements was introduced into each ureter. The arterial blood pressure was measured by a Sanborn 268 B pressure transducer, and the blood flow level in the renal arteries was monitored with a Nycotron square-wave electromagnetic flowmeter, type 372. The flow probes were not calibrated during the experimental procedure and thus the exact renal blood flow was not known, but the degree of autoregulation was satisfactorily controlled. Pressure and flow curves were recorded by a Sanborn 150 recorder.

After the operative procedure, the dogs were allowed to recover for about 45 minutes; during this period, a continuous infusion of isotonic saline was given to obtain a total diuresis of about 1-2 ml/min. Then the clearance of inulin was measured with the collection of the urine for two periods or more.

The autoregulatory capacity of the kidney was then tested by adjusting the aortic clamp. After a recovery...
period of about 15 minutes at normal perfusion pressure, one kidney was excluded from the circulation by tightening its arterial sling, and a dose of microspheres was injected into the abdominal aorta. Immediately after the injection, the arterial sling of the excluded kidney was loosened. The whole period of circulatory arrest in this kidney lasted for less than 2 minutes. About 20 seconds after the injection, the kidney that had been exposed to the injected spheres was permanently excluded from the circulation by the tightening of its arterial sling. Following another recovery period of about 15 minutes, the perfusion pressure was reduced to the lower limit of the autoregulatory range of the remaining kidney by adjusting the aortic clamp. At this lowered perfusion pressure, a second injection of microspheres, identical to the first one, was made. About 20 seconds after the injection, this kidney was also excluded from the circulation by tightening of the arterial sling. Both kidneys were then removed for further examination.

The microspheres (specific weight 1.2 g/ml) were taken from two different samples, one with a diameter of 15 ± 5 μm and the other with a diameter of 25 ± 5 μm, as specified by the manufacturer (3M). Equal volumes of 0.4 ml from the two stock solutions were rapidly injected through a special purpose polyethylene catheter (outer diameter = 2.2 mm, inner diameter = 1.2 mm). The injection catheter was supplied with four side holes (diameter = 0.2 mm) arranged around the circumference 5 mm from the distal end which was sealed off. The method involved in preparation of the kidneys for microscopic examination has been described previously. The diameter and location of the spheres in three cortical layers of equal thickness* were determined by microscopic examination of the kidney sections. In the afferent arteriole, the spheres situated in the proximal part and those trapped in the preglomerular part were recorded separately; the vascular hilus was the part involved in preparation of the kidneys for microscopic examination. In the arteriole. In

Theory

Notation:

The following symbols are used:

\[ w(r, f), u(f) = \text{joint p.d.f. of } r \text{ and } f, \text{ marginal p.d.f. of } f \]
\[ U(f) = \text{cumulative probability function of } f \]
\[ \xi, \sigma^2 = \text{expectation value, variance of } r \text{ or } f \]
\[ k = \frac{\xi}{\sigma} \]
\[ x = \frac{r - \xi}{\sigma} \]
\[ z = (r - \xi)/\sigma \]
\[ f(r, f), g(r, f) = \text{microsphere "filtration factor" for entrance into the afferent arteriole and glomerulus, respectively} \]
\[ y = \text{parameter in the "filtration factor"} \]
\[ M(r) = \text{radius frequency distribution of microspheres in tissue under consideration} \]
\[ a = \text{blood flow factor in statistical model} \]
\[ U_i, V_i = \text{theoretical predicted values for the ratios } G(p_i)/A(p_i), A(p_i)/I(p_i), \text{ respectively} \]
\[ \epsilon = \text{error in statistical model} \]
\[ S = \text{sum of squared errors} \]
\[ P = \text{probability in statistical test}; \text{a subscript indicates the type of test used} \]
\[ Y = \text{normal approximation parameter in Wilcoxon's two-sample test} \]
\[ p = \text{renal arterial blood pressure} \]
\[ R = \text{vascular flow resistance} \]
\[ n = \text{number of regression points} \]

A mathematical formulation of the radius distribution of microspheres in a tissue supplied by branches (afferent arterioles) from a larger main stem vessel (interlobular artery) has been reported recently. The normalized relative distribution (i.e., corrected for the injected population and normalized to 1 at radius value zero) was found to be equal to

\[ V = M(r)/M_{max}(r) = \frac{\int_{0}^{\infty} v(r)\Phi(r)\,dr}{\int_{0}^{\infty} v(r)\,dr} \quad (1) \]

Experiments in normotensive dogs indicated that the filtration factor \( f(r) \) maintained a value close to its maximum

\[ f(r) = \begin{cases} 1; & r \leq r_0 \\ 0; & \text{otherwise} \end{cases} \quad (2) \]
The Outer Cortex (OC)

A solution of this problem is to incorporate axial streaming and other effects into the factor \( f(p,r) \) and simply put

\[
M_{\text{max}}(p) = \alpha[\text{Isu}(p) - \text{JSu}(p)]
\]

where \( \text{Isu}(p) \) is some cortical layer subsample of the total cortical microsphere distribution identified in interlobular arteries, afferent arterioles, and glomerular capillaries. In this model, the relative flow factor \( \alpha \) indicates the relative blood flow distributed to the tissue under consideration during the time the microspheres are presented. Hence, \( \alpha \) also must account for the effect of retention of microspheres in the interlobular artery due to microsphere plugging of afferent arterioles or trapping of small microspheres behind larger ones when these occlude the interlobular artery (specially relevant for the outer cortical layer).

The various probable forms of \( f(p,r) \), the theoretically predicted value of \( \alpha \) related to the relative blood flow, and the type of \( \text{Isu}(p) \) for each cortical layer are briefly discussed below.

Juxtamedullary Cortex (JMC)

This layer should be presented with the undisturbed injected microsphere population which should safely be approximated with \( \text{Isu}(p) \). Moreover \( \alpha = \alpha_1 \). To account for possible axial streaming effects in addition to pure geometrical exclusion, \( f(p,r) \) may be chosen somewhere between the geometrical exclusion factor and Ferry’s correction factor.\(^5 \) \( f(p,r) = 1 - p/r \) \( y \); \( 0 < p < r \) (4)

where \( y \) has to be determined; \( y = 0 \) corresponds to the case with pure geometrical exclusion.

The Middle Cortex (MC)

The microsphere population arriving at this cortical layer is the total injected less that which is trapped in the juxtamedullary cortex. Consequently,

\[
M_{\text{max}}(p) = \alpha[\text{Isu}(p) - \text{JSu}(p)]
\]

where \( \alpha \) is the blood flow fraction of the sum of blood flows to the middle and outer cortical layers that goes to the middle cortex, i.e.,

\[
\alpha = \alpha_2/(1 - \alpha_1)
\]

\( f(p,r) \) is chosen as for the juxtamedullary cortex.

The Outer Cortex (OC)

Reasoning as above, one finds that

\[
M_{\text{max}}(p) = \alpha[\text{Isu}(p) - \text{JSu}(p)] - \text{JSu}(p) = \alpha \text{JSu}(p).
\]

The deviation of \( \alpha \) from 1 expresses the retention of microspheres in the interlobular artery due to the effects previously mentioned. As the interlobular artery terminates in the outer cortical layer, mainly geometrical relationships determine whether a microsphere goes farther into afferent arterioles or lodges in the interlobular artery. Therefore, \( f(p,r) \) may safely be approximated with its maximum value

\[
f(p,r) = \begin{cases} 1; & 0 \leq p \leq r \\ 0; & \text{otherwise} \end{cases}
\]

Similar arguments that led to the establishment of Equation 1 may be used to find the relative distribution of microspheres in glomeruli (normalized with respect to the population that enters into the afferent arterioles). By analogy with Equation 1, one can write

\[
U = \int_0^1 u(f)g(p,f)f(p,r)r^4df/ \int_0^1 u(f)df.
\]

The flow factor \( r^4 \) disappears for the integrals for obvious reasons. The deviation of Equation 9 is based on the assumption that \( r \) and \( f \) are statistically independent; if not, the expression takes the form

\[
U = \int_0^1 \int_0^1 w(r,f)g(p,f)f(p,r)r^4dfdr/ \int_0^1 \int_0^1 w(r,f)f(p,r)r^4dfdr.
\]

In this case, Equation 9 becomes

\[
U = \int_0^1 u(f)df = 1 - U(p)
\]

where \( U(f) \) is the cumulative probability function of \( f \). The experimental equivalent to Equation 9 will be

\[
U(p) = G(p)/A(p)
\]

which is used later on in the regression analysis.

Statistical Tests and Numerical Calculations

The two-samples, two-sided Wilcoxon test (W) corrected for ties\(^6 \) was used to determine significant differences between different microsphere populations. A Hewlett Packard 9820 A desk computer with 423 registers was programmed with intermediate data storage on magnetic tape files to handle a total sum of 720 numbers in the two populations. To examine pooled data exceeding this number, the two-sided Smirnov-Kolmogorov test (S-K) was applied to the experimental cumulated frequency distributions.\(^8 \) Tests for redistribution of microspheres among different cortical layers during hypotension was
performed on grouped numbers of microspheres in three different diameter intervals. The results from the four dogs were pooled together, and the relative number of microspheres in each cortical layer during normotension and hypotension was treated as a Bernoulli pair.6 The one-sided test with normal approximation (B) was used, and the results were compared with those obtained by a one-sided paired t-test (T) with parameters from each of the four dogs.

Regression Analysis

It was assumed that \( \nu(r) \) and \( u(r) \) were normal distributions, mean \( \xi \) and variance \( \sigma^2 \). The parameters \( \alpha, \xi, \) and \( k \) in the theoretical Equations 1 and 9 were then estimated from their experimental counterparts by the following procedure. Assuming large enough (\( \geq 8 \)) sample sizes in each histogram cell, the ratios

\[
Y_i = \frac{A(\rho_i)/I(\rho_i)}{G(\rho_i)/A(\rho_i)}
\]

(15)

were calculated for the normotensive and hypotensive states separately. Assuming that \( \nu(r) \) and \( u(r) \) were normal distributions \( N(\xi,\sigma^2) \), the two nonlinear regression equations

\[
Y_i = \alpha \int r \nu(r) I(\rho_i) r \, dr / \int r \nu(r) r \, dr + \epsilon_i
\]

(16)

\[
Y_i = \int r u(r) r \, dr + \epsilon_i = U_i + \epsilon_i
\]

(17)

were adopted. The sums of squares \( S = \sum _{i=1}^{n} \epsilon_i^2 \) were minimized with respect to the model parameters.

An approximate method to obtain the corresponding normal equations for the method of least squares applied to the model in Equation 17 is shown in Appendix 1. The procedure was more difficult for the complicated model in Equation 16, but noting that the model was linear in \( \alpha, V_i \), was treated as the independent variable and the normal equation solved for \( \alpha \). The set of \( (\xi, k) \), which minimized \( S \) with the found \( \alpha \) as shown in Appendix 2, was chosen. The estimated parameter values thus obtained were used in the statistical models, and these were plotted together with the experimental data.

Results

Hemodynamic Data and Microsphere Populations

The kidney weights, blood pressure, inulin clearance, and relative blood flow in the hypotensive kidney in three different situations are given in Table 1.

In the individual dogs, no significant difference was found between the total microsphere diameter distribution of the normotensive and hypotensive kidneys (\( P_w > 0.2 \); pooled data from all dogs for the two types of kidneys (Fig. 1) also showed that the two diameter distributions were in excellent agreement (\( P_{s,a} = 0.40 \), Table 2). Despite this fact, in order to minimize sampling errors, only data from normotensive kidneys were used to normalize “normotensive” microsphere subsamples, as shown in Equations 1 and 14, and likewise for “hypotensive” samples.

The distribution of microspheres to different anatomical structures is shown in Table 2. The diameter subpopulations of microspheres along the afferent arteriole (paraglomerular, juxtaglomerular, and glomerular hilus positioned) were not significantly different (\( P_w > 0.1 \)) for all parts of combinations within the same blood pressure group. Consequently, these three subpopulations of each blood pressure group were pooled together and taken to represent the afferent arteriole microsphere populations. The mean diameter and standard deviations of the individual populations are listed in Table 3.

From Table 2 it is seen further that transition from the normotensive to the hypotensive state decreased the relative amount of microspheres recovered in the interlobular artery. This occurred in all four dogs, and a paired two-sided t-test gave \( P_T = 0.08 \) for the mean relative decrease. The effect could be related to a significant (\( P_T = 0.03 \)) increase in the mean relative number of microspheres recovered from glomeruli, whereas the mean relative number of microspheres lodged in afferent arterioles actually was in fact significantly decreased (\( P_T < 0.01 \)).

The amount of larger spheres which had entered afferent arterioles and glomeruli during hypotension was significantly larger than in normotension (\( P_w < 0.001 \)). This effect is illustrated further in Figure 2, which depicts the relative number of microspheres recovered in afferent arterioles and glomeruli \( A(\rho_i)/I_{TOT}(\rho_i) \) of the different cortical layers. The effect of lowered arterial pressure is to increase the number of larger spheres in \( A(\rho_i) \), but this effect becomes less pronounced as one moves from the juxtamedullary to the outer cortical layer when normalizing with respect to the total identified cortical population \( I_{TOT}(\rho_i) \). Normalizing with respect to the population that is actually presented to each cortical layer, as indicated in the theory, gives a more homogeneous picture as demonstrated in Figure 3.

Tests on glomerulus microsphere populations in the individual dogs revealed a significantly larger amount of larger particles in the hypotensive kidney (\( P_w < 0.05 \)). Pooling the data only increased the significance level (\( P_{s,a} < 0.0001 \)).

Diameter of the Afferent Arteriole

Distal (Glomerular) End

The regression model in Equation 17 was applied to the relative distribution of microspheres recovered in the glomerular capillaries beyond the vascular hilus normalized against the population of microspheres that had entered the afferent arterioles: \( Y_i = G(\rho_i)/A(\rho_i) \). The dimension parameters thus obtained should represent the narrowest region of the afferent arteriole or the entrance opening into glomerular capillaries and are given in Table 4 both for the total cortex and for the different cortical layers. Experimental values of \( G(\rho_i)/A(\rho_i) \) and the regression curves are shown in Figure 4. Note that, since the model is nonlinear in the parameters, the results from the regression run for the total cortex will not in general be the arithmetic mean of the individual cortical layers.
TABLE 1  Kidney Weight, Blood Pressure, Inulin Clearance, and Relative Blood Flow in Four Dogs

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Kidney weight (g)</th>
<th>Blood pressure (mm Hg)</th>
<th>Inulin clearance (ml/min per g)</th>
<th>Blood flow (relative)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NT</td>
<td>hT</td>
<td>NT</td>
<td>hT†</td>
</tr>
<tr>
<td>1</td>
<td>50.0</td>
<td>51.0</td>
<td>125</td>
<td>127</td>
</tr>
<tr>
<td>2</td>
<td>45.7</td>
<td>45.4</td>
<td>132</td>
<td>127</td>
</tr>
<tr>
<td>3</td>
<td>46.2</td>
<td>46.9</td>
<td>105</td>
<td>110</td>
</tr>
<tr>
<td>4</td>
<td>57.5</td>
<td>58.8</td>
<td>145</td>
<td>150</td>
</tr>
<tr>
<td>Mean</td>
<td>49.9</td>
<td>50.5</td>
<td>127</td>
<td>129</td>
</tr>
</tbody>
</table>

NT = normotensive; hT = hypotensive.
* Control state just before aortic constriction.
† At reduced pressure before the microsphere injection.
‡ Before interruption of blood flow.
§ Not significantly different from 1.

Moreover, the results from the former will be more reliable due to the larger sample size. It is seen that during hypotension there is a relative diameter increase of about 0.21–0.28, and the most prominent change is found in the outer cortex. The conductance ratio increases 2.3–2.8 times, and the value obtained for the total cortex is somewhat larger than the pressure ratio between normotension and hypotension p/p' = 1.81. The corresponding ratio for the pressure gradients along the afferent arteriole Δp/Δp', which is the proper parameter to compare with the conductance ratio, is of course unknown.

Proximal End

In the outer cortex the regression model in Equation 16 with f(r,t) given by Equation 8 was used with Y1 = A(ρi)/I(ρi), i.e., the relative distribution of microspheres that had entered the afferent arteriole normalized with respect to the distribution of microspheres which had reached this cortical layer. The estimates of ξ and k listed in Table 5 give a conductance ratio somewhat lower than that obtained for the distal end, but are still higher than the ratio p/p'. It is also to be noted that the two α values in normotension and hypotension are in excellent agreement. Regression curves are shown in Figure 5.

Due to much smaller samples, the middle and juxtamedullary cortex were pooled together (cf. Equations 4 and 5) with

\[ A(\rho) = A_{MC}(\rho) + A_{MC}(\rho) \]

\[ I(\rho) = I_{MC}(\rho) + I_{MC}(\rho) - I_{MC}(\rho) \]

The estimated α value should then be

\[ \dot{\alpha} = \alpha_1 + \alpha_2 \]

Note that \( \dot{\alpha} > \alpha_1 + \alpha_2 \). The resulting values obtained in the regression analysis are shown in Table 5.

It was, however, difficult to obtain a good fit to the regression model in Equation 16 both with f(r,t) equal to the pure geometrical factor which gave too low ξ and k values and when including the Ferry correction (too high ξ and k values). The conductance ratio was high in both cases, and with the Ferry correction the lack of fit to the

Figure 1  Frequency distribution of all microspheres recovered from normotensive and hypotensive kidney in four dogs. The histogram with solid lines represents the combined data.
TABLE 2  Anatomical Positions of the Microspheres Found by Microscopy

<table>
<thead>
<tr>
<th>Anatomical position</th>
<th>Total or relative number</th>
<th>Diameter population statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NT</td>
<td>hT</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1183</td>
<td>1246</td>
</tr>
<tr>
<td>Unrecognized</td>
<td>0.073</td>
<td>0.063</td>
</tr>
<tr>
<td>TOTAL IDENTIFIED</td>
<td>1097</td>
<td>1167</td>
</tr>
<tr>
<td>Interlobular artery</td>
<td>0.319</td>
<td>0.211</td>
</tr>
<tr>
<td>Afferent arteriole</td>
<td>0.287</td>
<td>0.214</td>
</tr>
<tr>
<td>Paraglomerular</td>
<td>0.188</td>
<td>0.132</td>
</tr>
<tr>
<td>Juxtaglomerular</td>
<td>0.030</td>
<td>0.032</td>
</tr>
<tr>
<td>Glomerular hilus</td>
<td>0.069</td>
<td>0.051</td>
</tr>
<tr>
<td>Glomerulus</td>
<td>0.393</td>
<td>0.570</td>
</tr>
</tbody>
</table>

For the various anatomical structures, except for the total populations, relative content of microspheres is given. Statistical tests on diameter difference between normotensive (NT) and hypotensive (hT) groups: Z<sub>α</sub> = normal approximation parameter in Wilcoxon’s two-sample test, S-K = Smirnov-Kolmogorov’s test.

TABLE 3  Characteristics of Microsphere Populations Located in Afferent Arterioles

<table>
<thead>
<tr>
<th>Vascular position</th>
<th>Normotension</th>
<th>Hypotension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (µm)</td>
<td>d (µm)</td>
</tr>
<tr>
<td>Paraglomerular</td>
<td>206</td>
<td>18.40</td>
</tr>
<tr>
<td>Juxtaglomerular</td>
<td>33</td>
<td>17.37</td>
</tr>
<tr>
<td>Glomerular hilus</td>
<td>76</td>
<td>18.32</td>
</tr>
<tr>
<td>Pooled data</td>
<td>315</td>
<td>18.27</td>
</tr>
</tbody>
</table>

n = number of microspheres; d = mean diameter; s = standard deviation.

Redistribution of Microspheres

Redistribution during hypotension was examined in the three different cortical layers for the total population of microspheres in three diameter ranges. The percent changes in the relative number of microspheres are listed in Table 6. A significant increase in the relative number of microspheres was found only for diameters greater than 15 µm in the juxtamedullary cortex with a corresponding significant decrease in the outer cortex. Since the outer cortex contains a higher number of microspheres, the magnitude of the percent change is smaller here than for the juxtamedullary cortex.

Discussion

The mathematical model used is based upon several assumptions which have been discussed earlier. The effect of three factors (the anatomy of the interlobular artery, repeated sieving of microspheres along this vessel, and the pressure drop over the afferent arteriole) are of particular importance to the present study.

The model is appropriate for the entrance of microspheres from the afferent arteriole into the glomerulus and for the sieving of spheres into the arterioles of a very short segment of the interlobular artery. The statistical treatment of microsphere sieving does not include the process of repeated sieving which occurs along the interlobular artery. To compensate for the enrichment of larger particles in the interlobular artery due to repeated sieving along its course, the cortex should be divided into a series of distinct layers which should be treated individually. For this reason, the regression run for the total cortex should be interpreted with some caution.

Furthermore, the model is based on the assumption that the interlobular artery passes from the inner border of the juxtamedullar cortex all the way up to the outer border of the outer cortex. If this is the case, the microsphere distribution in the middle and the juxtamedullar cortex should be affected similarly when the afferent cortex.
arteriolar diameters are changed. The fact that the middle
cortex did not show significant sphere redistribution
during hypotension (Table 6) may indicate that some inter-
lobular arteries terminate here, i.e., that the middle
cortex plays an intermediate role between the outer and
the juxtamedullar cortex. The assumed normal distribu-
tion of afferent arteriolar diameters also may be ques-
tioned. It is known that small arteries with an anotomical
structure similar to the afferent arterioles supply more
than one glomerulus; these vessels, which presumably
have their own diameter distribution, may have been
diagnosed as true afferent arterioles. The resulting total
distribution may therefore be biphase and this, in turn,
will elevate the $M(p)/M_{\text{max}}(p)$ curve at higher diameter
values above the course predicted by the simple normal
uniphasic model.

As there is no information on the pressure drop over
the afferent arteriole, the true relationship between the
entrance opening diameter of the afferent arteriole and
the blood flow running into it is unknown. The total renal
vascular resistance calculated from the entrance opening

\[
R_{\text{TOT}} = R_{\text{pre}} + R_{\text{post}}
\]

where

\[
R_{\text{pre}} = c/r^4.
\]

The corresponding flow conductance is $1/R_{\text{TOT}}$, and
from Equations 21 and 22,

\[
c/R_{\text{TOT}} = r^4/(1 + Kr^4)
\]

where

\[
K = R_{\text{post}}/c.
\]

If the conductance factor (proportional to Equation 23) is
approximated with $r^4$, as done in this investigation, either
the preglomerular resistance dominates ($Kr^4 \ll 1$) or the
factor $1 + Kr^4$ must be kept constant when $r^4$ varies. The
use of $r^4$ instead of the more correct value in Equation 23
will tend to give estimated afferent arteriole diameter
values that are somewhat lower than the correct values.
The error in the estimates must in any case be less than
the shift due to changing the conductance factor from $r^4$ to
the constant value, which could occur in the unlikely
case of zero afferent arteriolar flow resistance. From the
general theory presented earlier, it is possible to show
that this maximal relative shift in $\xi$ ranges from 0.1 to 0.2
when $k$ lies in the interval 4 to 6. A constant factor $Kr^4$ in
practice means that all glomeruli have the same blood
pressure and that Poiseuille's law can be applied. The

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Vascular Dimension Parameters for Glomerular Capillary Entrance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical layer</td>
<td>Normotension</td>
</tr>
<tr>
<td></td>
<td>$\xi$</td>
</tr>
<tr>
<td>Total cortex</td>
<td>17.1</td>
</tr>
<tr>
<td>Outer cortex</td>
<td>16.8</td>
</tr>
<tr>
<td>Middle cortex</td>
<td>17.2</td>
</tr>
<tr>
<td>Juxtamedullary cortex</td>
<td>18.8</td>
</tr>
</tbody>
</table>

* According to the statistical model in Equation 17.
$\xi$ = estimated mean diameter in $\mu$m.
flow in each afferent arteriole then varies as $r^4$. To manage this during autoregulatory hypotension, there must be a compensatory constriction of postglomerular vessels, a phenomenon that already has been described in the rat. However, without knowledge of the afferent arteriolar pressure gradient, the change in $r$ predicted by autoregulatory vasomotion of the afferent arterioles cannot be assessed properly, nor can the postulated counteraction of postglomerular vessels with respect to vascular resistance be tested.

The form of the filtration factor appeared to have great influence on the magnitude of the estimated $\xi$, $k$, and $\alpha$. However, the conductance ratio (cf. Appendix 3) seems to be more insensitive to these changes. The present study also confirms the suggestion that factors in addition to pure geometrical exclusion of spheres are present. The model of pure geometrical exclusion (Table 5) cannot account for the findings both in normotension and hypotension, and there seems to be a good reason that the same model should apply to steric restriction in both experimental conditions. The best experimental fit to the statistical models requires a $f(p,r)$ that is near its maximum value, i.e., $y \ll 1$. The true geometrical exclusion factor is $y = 0$. It might be difficult to conceive how this could be the case with an opening whose radius is equal to that of the nontapered branch. A funnel-shaped entrance opening or a funnel-shaped hydrodynamical inflow tract will however favor Equation 2 when $r$ is taken to be the radius of the branch and not that of the inflow tract.

Our observations in this study (Tables 2 and 6, Figures 2 and 3) support our earlier findings that there is a considerable sieving effect on the microspheres presented to the afferent arterioles and also a significant redistribution of large microspheres from the outer to the juxtamedullary cortex during hypotension. Microspheres smaller than 15 $\mu$m in diameter, presumably indicative of blood flow distribution, showed a relative increase of 11.9% and 15.8% in the middle and juxtamedullary cortex, but the change was not significant ($P_B > 0.05$). This value is even larger than the 10-11% relative increase in the flow factor $a$ in the statistical sieving model used on combined data from the middle and juxtamedullary cortex (models C and D, Table 5). Due to its form expressed by Equation 20, it can be shown that the change in $a$ in general will be slightly greater than the mean relative increase in blood flow to these cortical layers. Redistribution of blood flow during autoregulation cannot be ruled out, but, if present at all, it must be considerably smaller than the values of about 40% reported from redistribution of radioactivity in comparable experiments. This study therefore supports our earlier suggestions that results based on measurements of tissue radioactivity and interpreted as redistribution of blood flow can be explained as due to altered steric restriction, i.e., as a methodological artifact.

The discrepancy between the estimated mean diameter values for the afferent arteriole presented here and that derived from the diameter of the microsphere trapped in the afferent arterioles may be explained by the presence of some small nonoccluding microspheres trapped behind larger ones. The present regression model is not as sensitive to this special effect as the method published earlier which gave slightly lower diameter values.

While the present investigation was in progress, Chenitz et al. published a similar method for calculation of afferent arteriolar diameter and ratios between preglomerular resistances based upon the 50% recovery point of microsphere in the glomeruli. Their method differs from ours in several respects.

1. They did not normalize the identified population of microspheres in the glomerular capillaries with respect to...
The presence of microspheres of identical diameters along different parts of the afferent arterioles in the outer, middle, and juxtamedullary cortical layers indicates that the tapering of the afferent arterioles is minimal. This seems to be incompatible with arteriolar contraction according to a string-sausage model; the findings also indicate that different parts of the arteriole are equally involved in the autoregulatory vasomotion. It is possible that the spheres in the hilar position were stopped by the first division of the arteriole. The fact that spheres with diameters larger than that of the glomerular capillaries were located far from the glomerular hilus indicates, however, that the postarteriolar structures offer very little resistance against luminal expansion.

The finding of an identical arteriolar dilation in all cortical layers during autoregulation shows that all cortical layers were involved in the autoregulation. This is in correspondence with results of studies in the dog by Loyning who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggesting that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex. Taking into consideration that the blood flow measured by Thrau et al.1 who applied the dye dilution method and indicated that the blood flow in the inner medulla of the dog kidney was not autoregulated, suggests that this may be the case also for the juxtamedullary cortex.

Combining the data on glomerular pressure and the glomerular blood flow measured by the microsphere technique and measurements of the hydrostatic pressure in the subcapsular part of the interlobular artery in the Sprague-Dawley rat kidney, Kjällskog et al.19 calculated the variation of vascular flow resistance in the interlobular artery and in the afferent and efferent arterioles during

---

**Table 6** Percent Number of Microspheres and Their Relative Change in Three Cortical Layers of Equal Thickness during Hypotension

<table>
<thead>
<tr>
<th>Diameter range (µm)</th>
<th>No. of microspheres</th>
<th>Outer cortex</th>
<th>Middle cortex</th>
<th>Juxtamедullary cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;15</td>
<td>302</td>
<td>377</td>
<td>322</td>
<td>295</td>
</tr>
<tr>
<td>15-20</td>
<td>549</td>
<td>574</td>
<td>577</td>
<td>572</td>
</tr>
<tr>
<td>≥20</td>
<td>332</td>
<td>377</td>
<td>572</td>
<td>572</td>
</tr>
</tbody>
</table>

Pooled data from four dogs. € = percent relative number of microspheres in normotension (NT) and hypotension (hT), respectively. ∆e/€ = (€ - e)/e given in percent was significantly changed where indicated by asterisks (* = P< 0.002, ** = P< 0.0001). The other values were not altered significantly (P> 0.05).
autoregulation. They concluded that the variation of the renal vascular resistance during autoregulation in the rat was restricted mainly to the interlobular artery and that the outer cortex exhibited almost no autoregulation. This is in variance with the findings of this study. Our results are quite compatible with the model of autoregulation of glomerular filtration in the mutant Wistar rat introduced by Brenner’s group; in their estimated resistance variation in comparable autoregulation experiments corresponds well with the change in the afferent arteriolar diameter estimated by our microsphere method.

Appendix 1

The error \( e_i \) in the model

\[
Y_i = (2\pi \sigma^2)^{-1/2} \int^{\infty}_{0} \exp \left[ -(r - \xi)^2 / (2\sigma^2) \right] \, dr + e_i
\]

can be written

\[
e_i = (Y_i - U_i)
\]

where

\[
U_i = (2\pi)^{-1/2} \int^{\infty}_{0} \exp \left[ -(r - \xi)^2 / (2\sigma^2) \right] \, dr
\]

Appendix 2

The regression model

\[
Y_i = \alpha V_i + e_i, \quad i = 1, 2 \ldots n
\]

where \( U_i \) is a complicated function of the two parameters \( \xi \) and \( k \) can, in principle, be solved by treating \( V_i \) as the independent variable and solving the normal equation for \( \alpha \). By trial and error, a series of combinations between \( \xi \) and \( k \) are tested. The combination chosen is the one that gives the minimal value to the sum of squared errors:

\[
S = \sum_{i=1}^{n} (Y_i - \alpha V_i)^2
\]

Using the mean value theorem, this can be expressed as

\[
e_i = (2\pi)^{-1/2} [\rho_i - z \sigma - \xi] \exp \left( -z^2 / 2 \sigma^2 \right) \]

where \( z \) lies somewhere between \( (\rho_i - \xi) / \sigma \) and \( \tilde{z} \).

Since the sum of squared errors is

\[
S(\xi, \sigma) = \sum_{i=1}^{n} e_i^2
\]

one finally has the normal equations

\[
\frac{\delta S}{\delta \xi} = 0 \quad \text{or} \quad \sum_{i=1}^{n} [\rho_i - z \sigma - \xi] \exp \left[ -z^2 / 2 \right] = 0
\]

and

\[
\frac{\delta S}{\delta \sigma} = 0 \quad \text{or} \quad \sum_{i=1}^{n} \left[ (\rho_i - z \sigma - \xi) \exp \left[ -z^2 / 2 \right] \right] = 0.
\]

Solving Equation A.1.7 for \( \sigma \), one obtains

\[
\sigma = \sum_{i=1}^{n} (\rho_i - \xi) E_i / \sum_{i=1}^{n} z_i E_i
\]

and inserting this into Equation A.1.8, one has

\[
\tilde{\xi} = [\Sigma \rho_i^2 E_i \Sigma z_i E_i - \Sigma \rho_i E_i \Sigma z_i \rho_i E_i] / [\Sigma \rho_i E_i \Sigma z_i E_i - \Sigma E_i \Sigma z_i \rho_i E_i]
\]

The regression can now be performed as an iterative process, first with

\[
\tilde{z}_i = z_i
\]

and subsequently with

\[
\tilde{z}_i = [ (\rho_i - \tilde{\xi}) / \tilde{\sigma} + z_i ] / 2
\]

where \( \xi, \sigma \) are the parameter values found in the previous run. For not too large \( e_i \), the process rapidly converges.

Appendix 3

Calculation of Conductance Ratio between Hypo- and Normotensive States

According to Poiseuille’s law, the hydrodynamical flow conductance in a cylindrical tube of radius \( r \) is proportional to \( r^4 \). When \( r \) is statistical distributed \( u(r) \), the equivalent expression is

\[
\int_{0}^{\infty} u(r) r^4 \, dr
\]

The assumptions

\[
u(r) = (2\pi \sigma^2)^{-1/2} \exp \left( -(r - \xi)^2 / (2\sigma^2) \right)
\]

and the transformation \( z = (r - \xi) / \sigma \) give the following expression for Equation A.3.1:

\[
(2\pi)^{-1/2} \int_{0}^{\infty} \exp \left( -z^2 / 2 \right) \cdot (z^2 / k + 1)^4 \, dx
\]

By expanding the factor \((z + k)^4\) in powers of \( z \), noting that uneven powers give no contribution to the integral, and even powers can be transformed into gamma func-
tions, it is readily shown that Equation A.3.4 reduces to
\[ \xi' \cdot (3/k^4 + 6/k^2 + 1). \quad (A.3.5) \]
Denoting the parameters of the hypotensive state by primes, the conductance ratio becomes
\[ (\xi'/\xi) \cdot (3/k'^4 + 6/k'^2 + 1)/(3/k^4 + 6/k^2 + 1). \quad (A.3.6) \]
The advantage of the formula in Equation A.3.6 instead of the commonly used \( (\xi'/\xi)^4 \) becomes clear when noting that small errors in the estimation of \( \xi' \) and \( \xi \) may give rise to great errors in the conductance ratio. In the regression analysis, however, these errors will be counter-balanced by corresponding shifts in the \( k', k \) values.

References
Diameter of afferent arterioles during autoregulation estimated from microsphere data in the dog kidney.
L Mørkrid, J Ofstad and Y Willassen

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