Senescence and the Renal Vasculature in Normal Man

By Norman K. Hollenberg, Douglass F. Adams, Harold S. Solomon, Abdur Rashid, Herbert L. Abrams, and John P. Merrill

ABSTRACT
The xenon washout technique and the renal blood flow response to vasoactive agents or alterations in sodium intake were used to characterize the effect of aging on the renal vasculature in 207 normal human subjects ranging in age from 17 to 76 years. A highly significant, progressive reduction in the mean blood flow, the rapid-component flow rate, and the percent of flow into the rapid-flow (cortical) compartment accompanied advancing age. Because $^{133}$Xe measures flow per unit tissue mass, the results indicated a larger reduction in flow than in mass—the anticipated finding if flow reduction is primary in the genesis of atrophy. Age also reduced the vasodilation consequent to administration of acetylcholine or a sodium load; this finding is consistent with a fixed lesion of the vessels. Responses to angiotensin were not modified by age. Thus, offsetting factors of increased ratio of wall to lumen thickness and smooth muscle atrophy are precisely matched. The findings in this study agree with earlier hypotheses based on morphology that suggest a primary vascular process in the development of age-related renal changes.

KEY WORDS acetylcholine mean renal blood flow sodium intake renal function and aging angiotensin atrophy renal cortical blood flow xenon clearance

Few phenomena have been explored more extensively than have the biological effects of aging, perhaps because senescence is our common fate. The kidney participates in this process with a decrease in mass, characteristic morphological changes, and a progressive reduction in blood flow and functional capacity (1-4). The morphological and functional changes have frequently been attributed to the striking local vascular pathology that accompanies aging (5, 6), but doubt has been expressed concerning the central pathogenetic role of reduced renal perfusion (7, 8). There has also been debate about the factors responsible for the decrease in perfusion. Certainly, organic abnormalities of the intrarenal vessels are sufficiently common, extensive, and severe to promote a reduction in renal blood flow. However, it has also been suggested (2, 9) that the reduction is partly due to active renal vasoconstriction.

In this paper the characteristics of renal perfusion and vascular responsiveness with increasing age in normal man were examined. Because xenon washout measures flow per unit tissue mass (10), the relationship between the decrement of mass and flow with age was investigated. The influence of age on renal vascular responses to vasoactive agents and sodium intake provided additional insights.

Methods
The 311 subjects ranged in age from 17 to 76 years. Because potential kidney donors require selective renal arteriography to define their renal arterial anatomy, it was possible to study normal volunteers with techniques demanding arterial catheterization. Each subject received a detailed inpatient evaluation that included a careful history, a physical examination, and an extensive laboratory investigation, which involved a complete blood count and the determination of serum creatinine, urea nitrogen, electrolytes, uric acid, calcium, phosphate, alkaline phosphatase, lactic dehydrogenase, fasting and 2-hour postprandial sugar, proteins with electrophoresis, cholesterol, and triglyceride concentrations. Complete urinalysis and quantitative urine cultures were performed at least twice. Serial 24-hour urine collections were made for the determination of creatinine clearance and sodium, potassium, and protein excretion. Creatinine was determined with the Technicon autoanalyzer, which does not separate non-creatinine chromogens. Each subject also had a chest film, an electrocardiogram, an intravenous pyelogram, and a renal arteriogram. Other laboratory or roentgen tests were performed as required on the basis of the history, the physical findings, or the preliminary

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laboratory evaluation. For example, intravenous glucose tolerance tests were performed in some subjects and pulmonary function tests were performed in others. Blood pressure was measured four times daily in both the recumbent and the standing positions. Mild, easily controlled essential hypertension was not an absolute contraindication to organ donation in our program.

Every potential donor studied with these techniques between August 1965 and November 1972 was included in the analysis unless one of the following criteria for exclusion was fulfilled. The presence of arterial hypertension or other cardiovascular or renal disease was the most common reason for exclusion; over 20% (69 subjects) were excluded on this basis. The age-adjusted criteria described by Pickering (11) were used to diagnose hypertension. Subjects were also excluded if a pharmacological agent which could influence renal perfusion was administered prior to the hemodynamic studies; 30 subjects had received diuretic agents, deoxycorticosterone, or dextran. Three subjects were excluded because of a clinically apparent plasma volume deficit due to diarrhea or overly vigorous use of cathartics and enemas. Another two subjects were excluded because a small thrombotic embolus entered the kidney during selective renal artery catheterization. Neither incidental, apparently unrelated diseases such as silent gallstones or mild chronic bronchitis or an overt emotional reaction during the procedure excluded a subject. After exclusion, data from 207 subjects were available for the analysis; 106 subjects were male and 101 subjects were female. The left kidney was studied in 122 subjects, the right kidney was studied in 73 subjects, and both kidneys were studied in 12 subjects.

All but about 15% of the subjects were admitted to a metabolic ward for the evaluation. Sodium and potassium intake was controlled in 147 subjects; 95 subjects received 10 mEq of sodium daily and 52 subjects received 200 mEq of sodium daily. The diet also provided 100 mEq of potassium daily with a fluid intake of 2,500 ml. The other 60 subjects were studied on a self-selected intake which generally provided an intermediate sodium intake and a similar consumption of potassium and water. Nothing was allowed by mouth for 8–12 hours prior to study, so that all subjects were in a state of mild hydropenia.

**TECHNIQUES**

The techniques used for selective renal arterial catheterization, renal arteriography, assessment of renal blood flow and its intrarenal distribution with radioxenon, subject monitoring in the catherization laboratory, and drug infusion have been described previously (12, 13). In brief, the renal artery was catheterized percutaneously under fluoroscopic guidance and local anesthesia. The transit of radioxenon through the kidney after intra-arterial injection was monitored with a probe-mounted scintillation detector. The peak count in all curves exceeded 90,000 counts/min. Mean flow was calculated from the initial slope and the intrarenal distribution of blood flow by compartmental analysis with graphical techniques. The percent of flow entering each compartment was calculated from its zero-time intercept, and the compartmental flow rates were calculated from the slopes. Arterial blood pressure, heart rate, and the electrocardiogram were recorded continuously and monitored with an oscilloscope during the last 200 studies.

The effect of age on renal vascular responses to a number of vasoactive agents was assessed in 31 subjects. Acetycholine chloride was administered intra-arterially in log-dose increments from 1 to 100 μg/min in 19 subjects (14). Angiotensin amide (Hypertensin, Ciba) was administered intra-arterially at 30 ng/min in 12 subjects (15). A coaxial catheter system was used for the intra-arterial administration of vasoactive agents. The acetylcholine and angiotensin were infused continuously through a small internal catheter (0.99 mm, o.d.), and the larger outer catheter (2.20 mm, o.d., 1.40 mm, i.d.) made it possible to monitor arterial blood pressure throughout the infusion. The inner catheter occupied only 35% of the outer catheter’s lumen, and damping was rarely a problem. Subjects were generally stable with respect to arterial blood pressure, heart rate, and electrocardiogram throughout the hemodynamic study, which was performed at least 45 minutes after the aortogram.

Replicate studies to assess stability were performed sequentially on the same kidney in 18 subjects, and flow in both kidneys was compared in an additional 12 subjects. When multiple measurements were made, only the first was used in the analyses of regression on age.

Written consent was obtained prior to each study after a detailed description of the procedure was explained. All protocols were approved by the Human Experimentation Committee of the Peter Bent Brigham Hospital. For subjects under the age of 21 years, consent was obtained from both the subject and from a legal guardian.

Data were expressed as mean values and the SE was used as the index of dispersion. Regression analysis was performed with the statistical package of a PDP15 computer. Statistical significance was assessed by Student’s t-test, analysis of variance, or the Wilcoxon rank sum test for nonparametric data. The null hypothesis was rejected when $P < 0.05$.

**Results**

Increasing age was associated with a significant progressive reduction in every index of renal perfusion and function assessed in this investigation. The relationships are listed in Table 1 and displayed in Figure 1, where they have been arranged according to age by decade for convenience of presentation. A linear reduction in mean blood flow/g kidney ($y = 4.39 - 0.026x$, $F = 24.4$, $P < 0.001$) was
associated with a parallel reduction in the rapid-component flow rate \( (y = 5.84 - 0.030x, F = 22.5, P < 0.001) \), where \( x \) is age in years. The percent of flow entering the rapid component also fell \( (y = 83.5 - 0.21x, F = 8.3, P < 0.005) \); the rapid component is thought to provide an index of cortical flow (12, 16). Creatinine clearance fell with age \( (y = 135 - 0.84x, F = 33.1, P < 0.001) \). Although the analysis of creatinine clearance provided an apparently excellent linear least-squares fit, the data in Figure 1 suggest an alinear relationship with a gradual fall to the fourth decade and a more precipitous fall thereafter. Neither mean renal flow nor the intrarenal perfusion patterns were influenced by sex or by location of the kidney (right or left) tested. As a further test the average difference in mean blood flow between the right and the left kidney measured in 12 subjects was only \( 9.0 \pm 4.8 \text{ ml} \text{/} \text{100 g min}^{-1} \). In 18 subjects sequential flow determinations performed on the same kidney showed a similar random difference of \( 15.9 \pm 9.8 \text{ ml} \text{/} \text{100 g min}^{-1} \).

Modification of sodium intake produced a striking change in the relationship between age and renal hemodynamics (Fig. 2). The relationship between mean blood flow and age was significantly

| TABLE 1 |

<table>
<thead>
<tr>
<th></th>
<th>( F )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean flow (ml/g min(^{-1}))</td>
<td>( 4.39 - 0.026x )</td>
<td>( 24.4 )</td>
</tr>
<tr>
<td>Component I flow (ml/g min(^{-1}))</td>
<td>( 5.84 - 0.030x )</td>
<td>( 22.5 )</td>
</tr>
<tr>
<td>Component I (% total flow)</td>
<td>( 83.5 - 0.21x )</td>
<td>( 8.3 )</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>( 135.0 - 0.84x )</td>
<td>( 33.1 )</td>
</tr>
</tbody>
</table>

Component I represents the most rapid component defined by compartmental analysis of xenon washout from the kidney: it probably reflects cortical perfusion; \( x \) = age in years.
flatter in subjects on a sodium-restricted diet (y = 3.76 - 0.019x, F = 17.6, P < 0.001) than it was in subjects on a high-sodium diet (mean flow = 4.94 - 0.038x, F = 38.6, P < 0.001) (t = 2.57, P < 0.02). The group on an unrestricted diet showed intermediate values. The result was a striking difference in mean blood flow for younger subjects on different sodium intakes and a progressive reduction in the influence of sodium intake with increasing age. Regression of blood flow with age was significant in the subjects receiving the sodium-restricted diet (P < 0.001). The relationship between age and renal hemodynamic characteristics was similar in the subjects with a well-defined sodium balance on a daily intake of 10 mEq of sodium and those who did not achieve a balance but whose sodium excretion was less than 35 mEq/day.

The effect of age on renal vascular responsiveness to vasoactive agents is shown in Figures 3 and 4 and Table 2. Acetylcholine induced a dose-related increase in mean blood flow that was influenced significantly by the age of the subjects. The increase in blood flow was greater at all doses in younger subjects. Each dose increment to 30 μg/min induced a significant increase in the slope relating blood flow to age. The maximum dilation achieved, previously demonstrated to occur with a dose of 100 μg/min (14), was significantly smaller in the older subjects (P < 0.01).

Responses to angiotensin infused into the renal artery were not influenced by age. The relationship between blood flow during the infusion of angiotensin (30 ng/min) and age was precisely parallel (mean flow = 3.88 - 0.023x vs. 3.29 - 0.022x) to the control run (Figs. 3 and 4 and Table 2). For obvious reasons maximal vasoconstrictor responses were not defined. There was a striking similarity in the characteristics of the control regression relationships in the two small groups prior to the infusion of the vasoactive agents.

Discussion

The assessment of the effects of aging on any process in man raises two major problems. The first problem involves the selection of an appropriate population, and the second problem involves the definition of normality. Shock (2), who has had the largest experience in this field, has stressed the importance of the sampling problem in the assessment of phenomena relating to senescence. In many studies, young individuals, frequently students, have been compared with elderly subjects drawn from an institutionalized population. A much more comparable sample of the older population is one drawn from that segment still residing and functioning in the community. The potential kidney donors in this study satisfactorily fulfilled this criterion. Typically, well-being is defined by exclusion, i.e., by ruling out unsuspected, potentially relevant disease. As potential donors, it was necessary to assess suitability for major surgery and the loss of a kidney with a thorough, expensive diagnostic evaluation in each subject. Thus, a population was defined in which well-being had been assured. For example, in most studies intravenous pyelography is not routine; however, it was not rare for us to discover unsuspected, potentially relevant renal abnormalities such as calyceal blunting or medullary sponge kidney. Similarly polycystic kidney disease was diagnosed by renal arteriography when even the excretory urogram was negative. Thus, an unusual circumstance, the existence of a living donor transplant program, made it possible to examine the aging process in an ideal population.

A decrement in renal perfusion with increasing age was first demonstrated by Shock (1). Recently, Wesson (3) summarized 38 renal hemodynamic studies in 634 normal subjects over a wide age range. Total renal blood flow was well maintained to approximately the fourth decade; thereafter there was a progressive decline of about 10% per decade. A number of questions raised by this observation can partly be resolved by the present analysis. What is the relationship between the reduction in renal blood flow and the renal mass? What mechanisms are responsible for the flow reduction? What are the implications for renal function? Can the hemodynamic changes account for the functional and the morphological characteristics of renal senescence?

A 10–20% decrease in renal mass occurs between the fourth and the eighth decade (5, 17). If the reduction in blood flow is secondary to parenchymal atrophy, either a proportional decrease in flow and mass or a reduction in flow less than the reduction in mass would be anticipated. Thus, flow per unit mass would be unchanged or increased but certainly not reduced. Total renal blood flow and renal mass have obviously never been measured simultaneously. The technique we used for flow measurement provided an alternative approach to the assessment of the relative reduction in renal perfusion and renal mass: xenon washout measures blood flow per unit tissue mass.
Agreement progressive reduction in mean blood flow per unit mass with advancing age was demonstrated in this study. The blood flow reduction exceeded the reduction in mass, as would be expected if the morphological and functional involution of the kidney was due to a limited blood supply. Therefore, the flow reduction probably was primary.

A frequently documented, progressive reduction in creatinine clearance with age (1-3) was corroborated in this study. Several factors probably contribute to the reduced glomerular filtration rate. First, to the extent that plasma flow is a determinant, the decreased cortical perfusion rate should limit filtration. Second, examination of available morphological data suggests that age-related atrophy involves the renal cortex more than it involves the medulla and that the glomeruli are especially affected (18). Third, the vascular lesions primarily involve small arteries rather than arterioles (5, 6), which results in a distribution that

### Table 2

<table>
<thead>
<tr>
<th>Dose</th>
<th>Mean Renal Blood Flow (Qm)</th>
<th>r</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine (μg/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Qm = 3.93 - 0.021x</td>
<td>-0.610</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>Qm = 6.10 - 0.029x</td>
<td>-0.767</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10</td>
<td>Qm = 7.67 - 0.046x</td>
<td>-0.716</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30</td>
<td>Qm = 8.32 - 0.055x</td>
<td>-0.744</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>100</td>
<td>Qm = 8.50 - 0.050x</td>
<td>-0.679</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Angiotensin (ng/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Qm = 3.88 - 0.023x</td>
<td>-0.696</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>30</td>
<td>Qm = 3.29 - 0.022x</td>
<td>-0.630</td>
<td>&lt;0.95</td>
</tr>
</tbody>
</table>

*Probability that the slope of a drug-related regression line differs from the control slope.
favors the simultaneous reduction of filtration pressure in large numbers of glomeruli (19). Why then does the fall in filtration rate not exceed the fall in plasma flow? In fact, filtration is relatively preserved, so that the calculated filtration fraction rises (3). This finding has been attributed to an increase in postglomerular resistance; however, no morphological basis for such an increase exists (5, 6). An alternative explanation can be found. Glomeruli in the outer cortex have a higher perfusion rate and a lower filtration rate than do inner cortical glomeruli (20, 21). Thus, preferential obliteration of outer cortical nephrons would raise the filtration fraction. Our xenon-washout data are compatible with this hypothesis. The percent of flow in the most rapid component, which is generally attributed to outer cortical perfusion, fell with age. Lesions of small arteries that are characteristic of the aging process (5) are situated so as to especially influence perfusion in the outer cortex. If better maintained perfusion of inner cortical nephrons participates in the increase in filtration fraction with age, the many similarities in the function of the senescent and the neonatal kidney (22) should have a similar basis—preferential perfusion and filtration in the inner cortex.

Are the morphologically evident vascular lesions adequate to account for a reduction in renal perfusion? Because vascular resistance is a function of the fourth power of the radius, by the time a reduction in vascular lumen is apparent with histologic techniques a rather striking reduction in blood flow should have occurred. It is difficult, however, to relate the morphological features of vessels studied in vitro when they are not distended by intravascular pressure to their in vivo state. Perhaps the most compelling evidence for an influence of fixed renal vascular changes during perfusion has come from the studies of Cox and Dock (23), who perfused kidneys at necropsy and demonstrated that the capacity of the renal vasculature to accept blood flow fell with increasing age: any functional influence specific for age must have been minimized post-mortem.

Conversely, a number of observations have led to the suggestion that vasoconstriction participates in the fall in renal perfusion (2). Cardiac output and renal blood flow both fall with advancing age in man, suggesting that the reduction in renal blood flow and glomerular filtration rate represent a response to the reduction in cardiac output. It is equally plausible, however, that the reduction in cardiac output reflects a reduced capacity and requirement for flow in peripheral tissues. Also, pyrogen, a renal vasodilator, increases renal blood flow in older subjects (1). It is important to recognize that an increase in blood flow induced by pyrogen in this earlier study (1) and by acetylcholine in the present study only shows that some degree of vascular smooth muscle tone is still present. This finding does not, per se, provide evidence that the flow reduction is due to vasoconstriction or even the degree of vasomotor tone still present. To draw the latter conclusion one would have to show that the maximal flow achieved with a vasodilator is unchanged. Thus, the flow increase would be larger and the vascular bed would appear to be sensitized to vasodilators (24). Therefore, analysis of the data as a percent change is inappropriate; we must be concerned with the absolute flow level achieved rather than with normalized changes. Dose-response curves have not been defined for pyrogen in earlier studies. The characteristics of the response to acetylcholine in this study makes active vasoconstriction unlikely; the response fell with advancing age. The age-related effects of dopamine that we reported earlier (25) were similar but are open to a number of interpretations because the drug was administered intravenously. Acetylcholine, on the other hand, was administered intra-arterially and must have acted locally. Active renal vasoconstriction potentiates the vascular response to vasodilators, including acetylcholine (24). It is difficult to reconcile active vasoconstriction and a reduced response to dilators in the senescent kidney; most of the evidence suggests a blood flow reduction due to fixed organic vascular changes. Similar, but less marked age-related vascular changes occur in other vascular beds. For example, in skeletal muscle, advancing age reduces the maximal flow that the vascular bed will carry (26). The number of capillaries per unit tissue mass of skeletal muscle is the same in young and old subjects (27); the decrease in maximal flow, therefore, does not reflect a loss of vascular bed but rather a reduction in each vessel's lumen.

The morphological characteristics of aging blood vessels must account for these functional characteristics. The dominant features are especially marked in the renal vasculature, including hyperplastic intimal thickening, hyalinization of the media, an increase in the amount of connective tissue, smooth muscle atrophy, and destruction of the elastic fibers (5, 6). Aging not only results in a reduction in radius and an increase in wall thick-
ness—factors that make a vessel stiffer—but also results in a restructuring with an increase in the elements that contribute to indistensibility; the amount of fibrous connective tissue is proportional to the requirement for maintenance of tension without a major energy expenditure (28–30). Perhaps the changes are accentuated in the kidney because the renal arterial tree delivers an unusual level of hydrostatic pressure to the glomerulus. Hydrostatic pressure must also be unusually high in more proximal arterioles and small arteries. If arterial blood pressure is traumatic, with prolonged exposure the development of a stronger supporting jacket would be anticipated even in normal man. To the extent that hypertension increases the trauma, the process is accentuated in rate and degree.

Age did not modify renal vasoconstrictor responses in this study. This finding was surprising in view of the profound effects of an increase in the ratio of vascular radius to wall thickness on responsiveness (31). Smooth muscle atrophy that accompanied the increase in wall thickness and the reduction in lumen size may have reduced the response, but with remarkable precision. Perhaps the smooth muscle atrophy is due to the enhanced contractile efficiency of a thickened vessel? Such atrophy of disuse would account for the remarkable consistency of contractility despite the presence of two offsetting factors that probably influence the contractile response.

The effects of age on renal vascular responses to sodium restriction were not anticipated. The well-sustained vasoconstrictor response to angiotensin with increasing age ruled out a reduced capacity for constriction. Perhaps the problem of interpretation lies in our being trapped by perspective: sodium restriction does not induce a sodium deficit, a concept developed by Strauss et al. (32) almost 20 years ago. According to this concept (32), dietary sodium in excess of the minimal requirements to replace skin and other losses represents a surfeit. On this basis the relationship between blood flow and age in the subjects on a low-sodium diet was unexpected. The unrestricted and high-sodium diets then result in volume expansion with its renal vascular consequence, vasodilation. The response to sodium, accordingly, is strikingly similar to the responses to nonspecific vasodilators; aging blunts the response. A similar phenomenon recently has been recognized in the rat, whose responses to changes in sodium intake are strikingly blunted by increasing age (33). The factors responsible for renal vasodilation with a sodium load are ill-defined (13); perhaps we should be searching for a vasodilator substance rather than the suppression of a vasoconstrictor system. Certainly, sodium intake must be specified in any study of the renal vasculature, as we have learned to do for the assessment of the renin-angiotensin-aldosterone system.

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