The aging kidney

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CASE PRESENTATION

Patient 1. An 87-year-old woman was admitted to the emergency room of the Alcalá de Henares Hospital after a fall at home. She had a long-standing history of hypertension, which was well controlled with nifedipine and indapamide. A hysterectomy had been performed 30 years ago. Evaluation at the emergency room revealed a femoral neck fracture, and she was prepared for an internal fixation under epidural anesthesia. Pre-operatory evaluation disclosed no significant metabolic alterations, the plasma creatinine concentration was 1.0 mg/dl with a normal urinalysis, and no significant cardiologic problems were detected. The surgical procedure took place without complications, and the patient was treated with intravenous fluids, analgesics, gentamicin, and cefazolin at standard doses. Six days after the surgical procedure, a progressive decrease in the urine volume was detected, and a nephrology consultant was contacted.

A careful analysis of the evolution of the patient's course was unable to detect significant hemodynamic changes after the surgical procedure, and moderate fever was detected only on days 2 and 3. On physical examination, she appeared well, without dyspnea, edema, or clinical signs of volume depletion. A grade II/VI systolic ejection murmur was audible; no pericardial friction rub was heard. The surgical area appeared well, without infection. Neurologic examination was negative; flapping was not detected. The rest of the physical examination was unremarkable.

The hematocrit was 28%; hemoglobin, 9.2 g/dl; white blood cell count, 10,900/mm³. The urea was 143 mg/dl; creatinine, 6.5 mg/dl; sodium, 130 mmol/liter; potassium, 4.3 mmol/liter; chloride, 107 mmol/liter; calcium, 8.7 mg/dl; phosphorus, 5.4 mg/dl; albumin, 2.3 g/dl; glucose, 107 mg/dl; arterial blood pH, 7.33; and bicarbonate, 19.2 mmol/liter. The urine was

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2+ for protein and contained 5 red cells and some granular casts/high-power field. Fractional excretion of sodium was 2.7%. No eosinophils were detected in the urine. An ultrasound examination of the abdomen failed to reveal obstruction of the urinary tract.

Acute tubular necrosis was diagnosed and probably was due to the use of aminoglycosides. She required six hemodialysis sessions. Urine flow progressively increased and she was discharged with a serum creatinine of 2.9 mg/dl. Subsequently the serum creatinine fell to 1.2 mg/dl, with a creatinine clearance of 41 ml/min.

Patient 2. An 80-year-old woman was admitted to the emergency room of the Alcalá de Henares Hospital because of asthenia and muscle weakness. She had had hypertension for a long time, but her blood pressure was well controlled with hydrochlorothiazide, amiloride, and captopril. The patient had no signs of renal disease, and in a recent examination, the serum creatinine was 1.3 mg/dl; urea, 68 mg/dl; sodium, 137 mmol/liter; and potassium, 5.0 mmol/liter, with a normal urinalysis. Because of generalized articular pain, a general practitioner prescribed tenoxicam, a nonsteroidal antiinflammatory agent, 10 days before admission.

Evaluation at the emergency room showed a mildly obese woman who appeared ill, with significant muscle weakness and minimal dryness of the mouth. The hematocrit was 30.9%; hemoglobin, 10.2 g/dl; white blood cell count, 5900/mm³; and platelet count, 164,000/mm³. The urea was 92 mg/dl; serum creatinine, 2.5 mg/dl; sodium, 143 mmol/liter; potassium, 8.5 mmol/liter; chloride, 118 mmol/liter; arterial blood pH, 7.24; and bicarbonate 14.6 mmol/liter. The urine was normal, as was an ultrasound examination of the abdomen. An electrocardiogram revealed widening of the QRS complex with peaked T-waves.

The patient's illness was attributed to the combined treatment with nonsteroidal antiinflammatory drugs, angiotensin-II converting-enzyme inhibitors, and amiloride, with the subsequent hyperkalemia. She was treated with intravenous calcium, glucose, bicarbonate, and cation-exchange resins. All drugs were stopped. Her blood pressure was controlled with calcium antagonists, and the articular pain was treated with acetaminophen. The serum potassium level decreased quickly and the glomerular filtration rate improved. She was discharged with a serum creatinine of 2 mg/dl and a potassium of 5.8 mmol/liter. One month later, the serum creatinine was 1.5 mg/dl and potassium 4.3 mmol/liter.

DISCUSSION

DR. DIEGO RODRÍGUEZ-PUYOL (*Chief, Nephrology Section, University Hospital "Príncipe de Asturias," Alcalá de Henares, and Assistant Professor of Medicine, University of Alcalá, Madrid, Spain*): These patients illustrate some of the clinical problems that challenge nephrologists attending elderly individuals (defined for the purpose of this Forum as older than 70 years). In the first case, an elderly woman with a hip fracture developed severe, acute renal failure that necessitated temporary hemodialysis. She had been well previously and had no evidence of renal disease; the pharmacologic treatment, including dosage, was apparently appropriate; and no major volume depletion was evident. In this setting, the combination of different noxious stimuli, including

minimal degrees of renal hypoperfusion and aminoglyco-
side exposure induced parenchymal damage that practically
abolished the glomerular filtration rate. The second pa-
tient, a stable hypertensive woman receiving pharmacologic
treatment, entered the emergency room with severe hyper-
kalemia after receiving nonsteroidal antiinflammatory
drugs because of articular pain. Her plasma creatinine level
had been in the normal range, and she had tolerated well
therapy with hydrochlorothiazide, amiloride, and captopril.
She had a normal blood pressure and no electrolyte
disturbances. By blocking prostaglandin synthesis, the non-
steroidal antiinflammatory drug blunted the critical equi-
librium in the renin-angiotensin-aldosterone system of the
patient, producing the dangerous hyperkalemia.

In aged individuals, this special sensitivity of the kidney
to a variety of adverse situations is reminiscent of the
biologic responses of patients with chronic renal failure.
Although the changes in renal structure and function that
develop with aging usually are considered "physiologic,"
clinicians must be aware that the renal parenchyma of the
elderly shares some of the characteristics of the chronically
damaged kidney, making necessary a careful analysis and
management of clinical situations as well as judicious
administration of drugs. In this Forum, I will describe in
detail the aging-related morphologic and functional renal
changes to provide a better understanding of the basal
renal situation of elderly individuals. In addition, I will
analyze the possible mechanisms involved in the develop-
ment of the renal parenchymal alterations in these individ-
uals. Before starting this analysis, however, let me review
briefly the available information about the biology of the
aging process in general.

General mechanisms of aging

A careful analysis of the multiple theories proposed to
explain the aging phenomenon is beyond the scope of this
discussion. However, some understanding of the general
mechanisms of aging can help us better understand the
relationship between aging and renal dysfunction.

Theories on aging have evolved according to the devel-
opment of our understanding of biology. General theories
that considered aging a global system-wide process have
been replaced by the idea that the aging of a particular
organism results from the sum of the aging of its individual
cells. This approach to the understanding of aging is
supported by much experimental evidence. Thus, in human
beings, the aging-related dysfunction of organs and tissues,
such as the brain or subcutaneous fat, is closely related to
a reduction in the number of cells. Moreover, the replica-
tive capacity of cells explanted from a variety of mammals
is roughly proportional to the life span of the animals [1];
this finding suggests a relationship between cellular aging
and whole-animal aging. In addition, the WRN gene, a
gene involved in the development of Werner's syndrome, a
disease characterized by the appearance of a precocious

aging phenotype in humans, is homologous to a family of
DNA helicases of *Escherichia coli* [2]. Aging thus might be
a cellular autonomous process. But we must remember
that, in addition to containing individual cells, organisms
also exhibit a complex system of cell-to-cell relationships,
and derangements in these relationships also could be
involved in aging.

Independently of these considerations, two main theories
have been proposed to explain aging. The first hypothesis,
an environmental one, suggests that aging is the conse-
quence of the repetitive action of exogenous factors in a
normal organism, resulting in an accumulated damage that
outstrips the normal repair processes. The second, or
genetic, theory proposes that aging occurs because of a
genetic program that determines the progressive appear-
ance of the different aging-related phenotypic changes.
These two ideas are not mutually exclusive, and the accu-
mulated damage could reflect an environment-dependent
repetitive injury that triggers a genetic program of aging [2, 3].

Organisms must obtain nutrients and, in the case of
aerobic organisms, oxygen from the external media or
environment, to maintain cellular function and homeosta-
sis. During the cellular metabolism of nutrients and oxygen,
different toxic intermediate molecules arise, but cells have
defense systems able to clear these molecules. Sometimes,
however, production of toxic molecules overrides the pro-
tective mechanisms; the resultant damage characterizes
progressive aging. The most important evidence supporting
this hypothesis is that dietary restriction—that is, caloric
restriction without compromising essential nutrients—is
the most reproducible way of slowing aging [4].

Two main groups of toxic molecules likely are involved in
the aging process. Reactive oxygen intermediates (ROI)
probably have been the most widely studied. These mole-
cules, including superoxide anion, hydrogen peroxide, and
hydroxyl radical, among others, are formed during the
progressive reduction of molecular oxygen to form water
within the cell, but also as a consequence of the action of
different cellular enzymes [5]. Reactive oxygen intermedi-
ates can induce chemical changes in many substances
essential for normal cell function, including nucleic acids,
proteins, and lipids, with subsequent structural and func-
tional cell damage. Moreover, the levels of macromolecules
exhibiting oxidative damage increase in certain tissues of
aged organisms [6]. Two recent studies clearly support the
role of ROI in aging. First, drosophila strains bearing extra
copies of genes encoding both superoxide dismutase and
catalase, the main enzymes involved in ROI removal, have
longer life spans than do drosophila without the extra genes
[7]. Second, the age-1 mutant of *C. elegans*, characterized
by an increased life span, also displays higher levels of
superoxide dismutase [8].

Advanced glycosylation end products (AGEs) comprise
the other group of molecules (formed as a consequence of
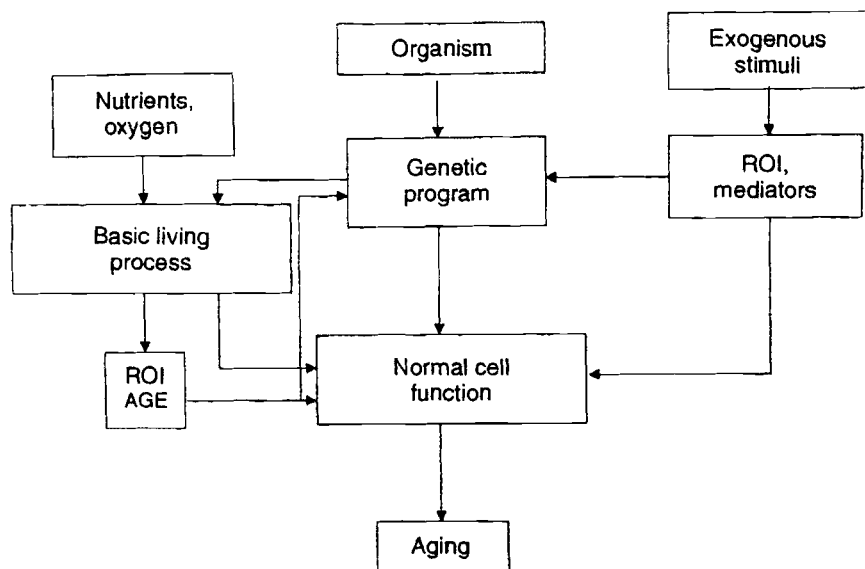the basic living process) that seems to play a pathogenetic

Fig. 1. Schematic representation of the aging process, considering the organism as the sum of its different cells. Abbreviations are: ROI, reactive oxygen intermediates; AGE, advanced glycosylation end products; mediators, other possible mediators.

role in aging development [9]. Formed by the long-term interaction of reducing sugars, such as glucose or fructose, with the amino groups of intracellular or extracellular proteins [9], AGEs are increased in several pathologic situations, particularly diabetes, and in aged organisms. Protein glycosylation or the interaction of AGEs with specific cell receptors is associated with the development of functional and structural changes similar to those characterizing aging [9]. A close relationship exists between AGE and ROI, as it seems that ROI are generated in cells after the interaction of AGEs with their receptors [10]. Moreover, glycated proteins can undergo oxidative damage, with the subsequent accumulation of glycoxidation products such as N-epsilon (carboxymethyl) lysine, which are considered good markers of aging-related tissue damage [11].

The genetic theory of aging proposes that the life span of a particular organism is determined by a specific genetic program, which culls older individuals from the general population. One interesting piece of evidence supporting this theory is the observation that Pacific salmon undergo rapid senescence after spawning [2]. Moreover, a limited number of genes, including age-1, daf-2, and clk in *C. elegans* and WRN in human beings, have been related to the development of aging-related phenotypic changes [2,8]. The exact mechanism of this programmed cell death has not been adequately defined; several possibilities have been proposed [2,3]. The "telomere shortening theory" proposes that cells do not completely replicate their chromosomes during a cell division cycle, so that very late replicating DNA sequences are lost. If certain nonessential repetitive DNA sequences were at the ends of replicative units, essential genes would not be lost until a number of divisions were achieved. The theory of "terminal differentiation" posits programmed cell senescence as the consequence of

the activation or inactivation of particular genes after a certain number of cell divisions. Other hypotheses envision aging as the result of minimal but repetitive DNA injuries, or progressive loss of copies of important genes. Nevertheless, these theories only partially explain the aging phenomenon, particularly at a cellular level, and a universal mechanism of cell aging has not yet been determined.

Figure 1 summarizes the aging process. Living organisms are provided with a genetic program, including perhaps a specific aging program, which controls their different functions. To maintain these functions, organisms must obtain nutrients and oxygen from the external medium and, in the processing of these metabolites, toxic molecules are formed. Although organisms can disarm most of these molecules, a small number of them can interfere with normal basic functions, or even with the genetic program, thus determining a particular rate of aging. Other external stimuli also might influence the aging process.

Aging-related renal changes

Morphologic changes. Although assessment of specific aging-related morphologic renal changes in the elderly is not easy because of the high prevalence of superimposed vascular or inflammatory diseases, studies in apparently disease-free individuals have provided valuable information (Table 1) [12, 13]. Renal mass increases progressively from about 50 g at birth to over 400 g at the fourth decade, and then declines to under 300 g by the ninth decade. The loss of renal mass mainly depends on progressive atrophy of renal cortex, with relative sparing of the medulla. The cortical atrophy roughly reflects a decreased number of functioning nephrons. Under age 40, few glomeruli appear sclerosed; in contrast, by the eighth decade, between 10% and 30% of the glomeruli are completely sclerosed, the

Table 1. Morphologic changes in the kidneys of elderly individuals

Macroscopic changes	Reduced size and weight
	Relative cortical atrophy
Vascular changes	Hyalinosis of arterial walls
Glomerular changes	Increased number of sclerosed glomeruli
	Hypertrophy of the remnant glomeruli[a]
	Increased thickness of basal membrane
	Mesangial matrix expansion
	Irregular fusion of foot processes
Tubular changes	Reduction in the number of tubules
	Atrophy of the tubular epithelium
	Tubular dilation
	Increased thickness of basal membrane
Interstitial changes	Interstitial fibrosis

[a] Well documented in rats, but controversy exists in humans.

glomeruli of the outer cortex being especially affected. The remaining functioning glomeruli appear to increase in size, although recent measurements performed by computer-assisted image analysis suggest that after the fourth decade glomerular size declines slightly [14]. The glomerular changes that determine the development of sclerosis have been studied previously [12, 13]. Mesangial matrix increases progressively, and glomerular basement membrane undergoes progressive thickening; free intraglomerular anastomoses appear and functioning capillary loops are reduced (so-called "glomerular simplification"). Eventually, the increased extracellular matrix condenses into hyaline material and collapses the glomerular tuft, finally inducing complete glomerular sclerosis. Degeneration of glomeruli in the renal cortex in turn results in atrophy of the afferent and efferent arterioles; in the juxtamedullary area, glomerulosclerosis seems to cause the formation of a direct channel between these two arterioles. These channels could contribute to the maintenance of medullary blood flow as cortical perfusion declines. Tubular structures also decrease with aging. Although some studies suggested a dissociation between glomerular and tubular atrophy, this hypothesis has not been confirmed, and a close relationship appears to exist between degenerative changes in glomeruli and those in tubules [12, 13]. Interstitial changes, with increased fibrosis, also frequently occur in the aging kidney [12, 13].

Studies of aging-related renal changes in experimental models, performed mostly in albino rats, are consistent with the pathologic findings in humans [15]. Two strains of rats, Fisher 344 and Sprague-Dawley, are especially prone to the development of age-related nephropathy, but other albino strains also develop variable degrees of renal damage with aging [15]. In these animals, glomerular sclerosis is readily demonstrated after 24 months, but increased glomerular basement membrane thickness and progressive expansion of mesangial matrix are detected as early as 3 months [15]. Glomerular size of the intact glomeruli increases with age [14, 16]. It is interesting that Hayashida et al noted an increase in the number of mesangial cells with age [17]. Intratubular casts occur more frequently in old rats, with

flattening and atrophy of the tubular epithelia. Interstitial fibrosis, a constant characteristic of these animals [15], has been detected as early as after 8 months in Lewis rats [18]. In fact, some authors believe that the interstitial changes precede glomerular sclerosis in the renal aging process [19].

Recently, our group has performed morphologic studies in 24-month-old Wistar rats. The morphologic changes observed were similar to those previously described (Fig. 2). After staining with Syrius red, we used a computer-assisted planimetric procedure developed at the Pathology Department of the University of Granada, which allowed us to accurately measure mesangial matrix expansion and interstitial fibrosis. Mean increases of 273% and 181% were detected, respectively, for these two parameters, when compared to 3-month-old animals [20].

To complete the analysis of aging-related morphologic changes, let me comment on the biochemical nature of the extracellular matrix accumulation in the aging kidney. Studies performed both in human beings and in rats have confirmed that the chemical composition of the glomerular basement membrane differs between young and old individuals. Several changes have been detected in the latter, including increased non-enzymatic glycosylation of proteins and changes in the degree of sulfation of glycosaminoglycans, but the most widely found biochemical change is increased collagen content [14]. However, Abrass et al recently questioned the hypothesis of collagen accumulation by performing immunofluorescence studies in Fisher 344 rats with a wide panel of antibodies [19]. These authors demonstrated a moderate increase of collagens I and III only in areas with interstitial fibrosis, but detected no changes in collagens I, III, and IV at the glomerular level. The changes observed in the glomerular tuft, particularly in the glomerular basement membrane, were related to an increased content of various laminin isoforms, whereas in the interstitial compartment, they observed a generalized immunostaining for fibronectin and thrombospondin. The relationship between interstitial fibrosis and collagen I accumulation also seems to be supported by the demonstration of increased levels of type-I collagen mRNA in the cortex of old rats [21]. In contrast to the results from Abrass, preliminary results from our laboratory demonstrate an increased collagen type-IV mRNA (alpha-1 chain) in the renal cortex of 24-month-old Wistar rats (Fig. 3). This finding suggests that accumulation of this collagen plays a role in the genesis of the morphologic renal changes observed in aged rats. Differences in rat strain, age of the rat at the time of the study, or sensitivity of the techniques might account for the apparent discrepancies detected in the different studies.

Functional changes. Elderly kidneys manifest significant functional as well as morphologic, changes (Table 2). In human beings, renal blood flow (RBF) decreases about 10% per decade after the maximum reached in young
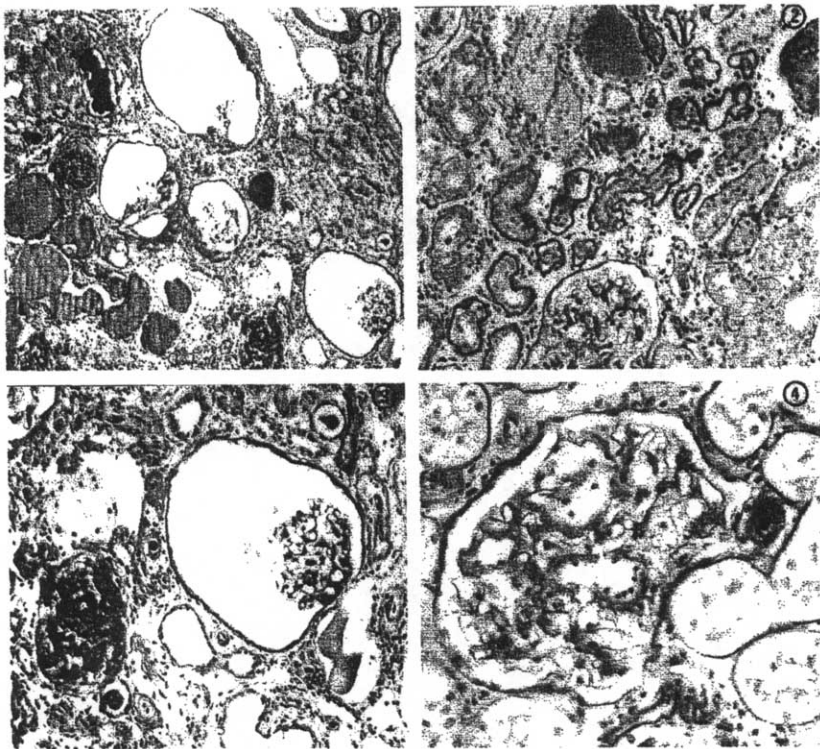
Fig. 2. Morphologic changes in the renal cortex of 24-month-old Wistar rats. (1) Diffuse glomerular and tubular changes, with cystic appearance and atrophy of the glomerular tuft of some glomeruli, glomerulosclerosis, tubular dilation, and intratubular casts (PAS × 100). (2) Tubular atrophy, reduplication of basal membranes, and interstitial expansion (PAS × 400). (3) Magnification of a sclerosed glomerulus, near another glomerulus with cystic appearance, in an area with interstitial expansion (PAS × 400). (4) Arteriolar hyalinosis (PAS × 600).
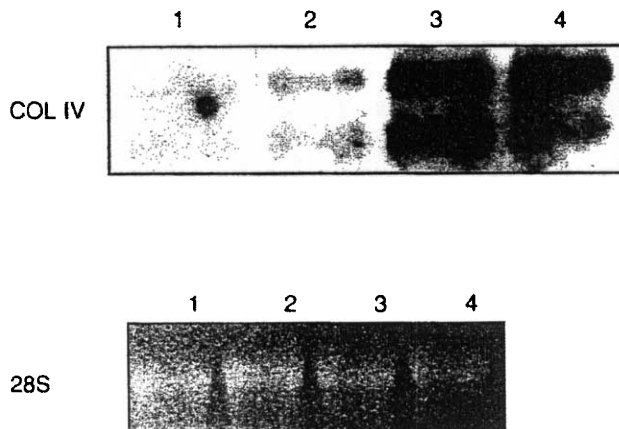
Fig. 3. Expression of the collagen type-IV mRNA (α-1 chain) in the renal cortex from 3-month-old (lanes 1 and 2) and 24-month-old (lanes 3 and 4) rats.

Table 2. Functional changes in the kidneys of elderly individuals

Renal blood flow	Decreased[a]
	Relative increase of medullary blood flow
Glomerulus	Decreased glomerular filtration rate[a]
	Increased filtration fraction[a]
	Increased permeability to macromolecules[b]
Tubule	Impaired ability for sodium handling
	Deranged tubular transport
	Impaired concentration and dilution
	Impaired acidification
Other	Decreased synthesis of renin
	Decreased 1-alpha-hydroxylase activity

[a] Generally accepted in humans but not in rats.
[b] Increased prevalence of microalbuminuria in humans. Over proteinuria in rats.

adulthood. Thus, renal plasma flow (RPF) of approximately 600 ml/min/1.73 m^2 during the third decade decreases to about 300 ml/min/1.73 m^2, a 50% reduction, in the ninth decade [12, 13]. The decrease in RBF is associated with significant increases in afferent and efferent arteriolar resistances [12, 13]. The decline in RBF cannot be explained as a secondary phenomenon associated with the previously described renal mass reduction, as specific studies designed to answer this question demonstrated that the decreased RBF was accompanied by a real decline in

blood perfusion per unit of renal tissue mass [22]. Neither can changes in cardiac function account for the reduction in this parameter, as the minimal reduction in the percentage of cardiac output directed to the kidney (that occurs in the elderly) does not explain the observed decline in RBF [23]. Studies utilizing the xenon washout technique have demonstrated that the reduction in RBF is not uniform throughout the kidney. In fact, and according to the anatomic descriptions, cortical blood flow is preferentially decreased in the elderly, with a relative sparing of the blood flow in juxtamedullary glomeruli [22]. As these glomerular structures have a higher filtration fraction than do the cortical glomeruli, the observation that filtration fraction increases with advancing age could be explained by this observation.

Changes in RBF in experimental animals differ from those observed in human beings: the absolute values of RBF remain stable between 3 and 20 to 24 months [16, 24, 25], and even slight increases in this parameter have been observed in 15- to 18-month-old Sprague-Dawley rats [24, 26]. When RBF was factored by kidney weight, however, it significantly decreased with aging [16, 24]; these data have been sometimes interpreted as indicative of an aging-related significant derangement of RBF. The analysis of preglomerular and postglomerular resistances by micropuncture has yielded different results, depending on the rat strain. Thus, these resistances were increased in old Munich-Wistar rats [16], but were decreased in Sprague-Dawley animals [25, 27].

Glomerular filtration rate (GFR) has been studied extensively in the elderly. Cross-sectional studies have demonstrated that GFR decreases progressively after age 30 to 40 years [12, 13]. This decreased GFR was not only detected in cross-sectional studies but also in longitudinal studies. The Baltimore Longitudinal Study of Aging also found a progressive decline of glomerular filtration with aging. The rate of decline was 0.8 ml/min/1.73 m^2/year, a rate similar to that previously reported in cross-sectional studies [28]. Interestingly, GFR did not change in approximately one-third of the patients included in this longitudinal study [28].

In contrast to the decline in GFR, plasma creatinine does not change with increasing age. As it happens, muscle mass, from which creatinine is derived, decreases with age at approximately the same rate as does GFR. In consequence, the age-related loss of GFR is not reflected by an increased concentration of plasma creatinine. Thus, this parameter must be used with caution in aged populations to assess GFR, as it underestimates renal function; the commonly used formulas for estimating creatinine clearance from plasma creatinine values always take into account the age of the patients.

Another aspect of glomerular function that has been extensively studied is the permselectivity of the filtration barrier. Although some reports describe an increased prevalence of proteinuria in a population of persons over 65 years [29], only a minority of disease-free patients over 80 years show clinical proteinuria [13]. Moreover, when the glomerular permeability to macromolecules has been studied, no differences were detected between young and old individuals [30]. Thus, it seems that the permselectivity of the glomerular filtration barrier in human beings is only minimally altered in the elderly.

Again, glomerular function in old rats differs from that in older humans. Assessing different reports on GFR is difficult because data are frequently expressed corrected for the body and kidney weight, and these two variables increase in old animals, but it does seem that GFR remains stable in albino rats until 18 to 24 months of age [16, 24] and then declines progressively. On the other hand, pro-

teinuria is a constant manifestation of renal dysfunction in old rats in strains that develop glomerulosclerosis [15, 31]. Although the exact nature of this selectivity defect has not been elucidated, some evidence points to a combined charge and size defect as responsible for the aging-related increased proteinuria [32].

Tubular absorption of sodium seems to be impaired as a consequence of aging. When old individuals are sodium-deprived, sodium excretion progressively declines, but it takes longer to achieve equilibrium than when younger people undergo the same deprivation. The mean half-time for reduction of sodium excretion is 17 hours in individuals less that 30 years old, but is prolonged to 31 hours in subjects more than 60 years old. Nevertheless, the elderly can achieve a sodium equilibrium even when given diets with a very low sodium content [33]. Old subjects also have problems with sodium overloads, and edema and hypertension frequently occur in this population. Short-term sodium-loading studies show distinct age-related sodium excretion patterns: after a 2-liter normal saline load, individuals older than 40 years show a lower 24-hour sodium excretion (with a significantly greater portion of the sodium excreted at night) than do their younger counterparts [34].

Perhaps one of the best-known aspects of tubular dysfunction in the elderly is their relative inability to adequately concentrate and dilute the urine. Studies comparing the maximal urinary density or osmolarity after water deprivation in young and old individuals have clearly demonstrated that kidneys from old subjects do not form urine as concentrated as that of young people [35]. Renal diluting ability also is impaired in elderly individuals. During water diuresis, urine osmolarity in old subjects is significantly higher than that in young subjects, and solute-free water clearance is lower [33]. The same defects in urinary concentration and dilution also have been described in laboratory rats [15].

Other disorders of tubular transport widely studied are decreases in sodium-hydrogen exchange and in sodium-coupled phosphorus reabsorption. The latter defect also has been demonstrated in laboratory animals and even in preparations of brush-border vesicles [15, 36]. This age-related decline in sodium-dependent phosphate transport precedes the effect of age on sodium-hydrogen exchange in brush-border membrane vesicles [37]. These data suggest that all membrane transport functions at the proximal tubule are not similarly affected during the aging process. As in the case of the other tubular functions, under normal physiologic conditions older individuals maintain normal acid-base excretion. However, the elderly do have a decreased time-referred acid excretion (when compared with young controls) when challenged with an acid load; in addition, young subjects excrete a significantly higher proportion of the acid load as ammonium [38].

The renal aging process is also characterized by decreased renin synthesis. Studies in humans and in rats have

demonstrated decreased concentrations and activities of plasma renin despite normal plasma concentration of renin substrate, as well as a decreased renal renin content in elderly individuals. In these subjects, maneuvers designed to stimulate renin secretion amplified the differences in plasma renin levels with respect to the young population [39]. Jung et al demonstrated decreased renin mRNA content in renal tissue in 12-month-old Sprague-Dawley rats, even in the absence of significant changes in renal renin [40]. In contrast, Corman et al detected a significantly decreased renin content in 30-month-old female WAG/rij rats, without changes in renin mRNA expression [41]. A deficit in 1-alpha-hydroxylase activity is another characteristic of aged subjects. As a consequence of this defect, plasma levels of 1,25-dihydroxycholecalciferol decrease in this population, with a subsequent derangement in calcium homeostasis [42].

Mechanisms responsible for renal changes during aging

Analysis of the mechanisms involved in the development of aging-related renal changes has been performed at two levels. Most studies have looked for the immediate reasons that explain the changes detected in renal structure and function in old human beings or animals. However, these studies have not established a relationship between the specific mechanisms studied and the aging process. In consequence, a second set of studies or hypotheses have tried to establish a casual link between the aging process itself and the possible mechanisms involved in the genesis of the renal changes. In this second part of this discussion, I will focus on mechanisms responsible for aging-related morphologic and functional renal changes, and links between the aging phenomenon and these mechanisms.

Morphologic. Aging-related morphologic renal changes are similar to those detected in renal disease and experimental models characterized by progressive chronic renal failure, including glomerulonephritis, diabetes, and surgical reduction of renal mass [43]. Although the exact biochemical composition of the expanded extracellular matrix in aging kidneys is not fully comparable to that in any of those pathologic situations [19], one could hypothesize that aging shares some of the pathogenetic mechanisms proposed for these diseases. Table 3 lists the most widely accepted mechanisms of extracellular matrix expansion and changes in cell numbers in the kidney in progressive renal diseases. Some of these mechanisms have been widely explored in experimental models of glomerulonephritis or diabetes, as well as in rats with surgical renal mass reduction [43–48]. Four main aspects of the table must be stressed. First, the number of cells at a particular time in disease progression is regulated by the balance between cell proliferation and apoptosis (programmed cell death). Second, increased cell numbers can precede glomerulosclerosis and interstitial fibrosis, even in situations in which cell proliferation is not readily detected. Third, changes in the complex equilibrium

Table 3. Mechanisms and factors involved in the expansion of extracellular matrix and changes in cell numbers in progressive renal diseases

General mechanisms
  Changes in the proliferation rate of resident or infiltrating cells
  Changes in the apoptosis rate of resident or infiltrating cells
  Increased synthesis of normal or abnormal extracellular matrix components
  Decreased degradation of normal or abnormal extracellular matrix components
Factors involved in the regulation of these mechanisms
  Growth factors: PDGF, EGF, TGFβ, FGF, IGF-1[a]
  Cytokines Il-1, Il-13, TNF
  Vasoactive peptides: AII, ET, ANP
  Lipid mediators: PGE_2, PGI_2, TxA_2, PAF
  Others: NO, ROI

[a] Abbreviations: PDGF, platelet-derived growth factor; EGF, epidermal growth factor; TGFβ, transforming growth factor β; FGF, fibroblastic growth factor; IGF-1, insulin-like growth factor 1; Il-1, interleukin-1; Il-13, interleukin-13; TNF, tumor necrosis factor; AII, angiotensin II; ET, endothelin; ANP, atrial natriuretic peptide; PGE_2, prostaglandin E_2; PGI_2, prostacyclin; TxA_2, thromboxane A_2; PAF, platelet-activating factor; NO, nitric oxide; ROI, reactive oxygen intermediates.

between matrix synthesis and degradation are, perhaps, the main critical point in fibrosis development. Fourth, from a functional point of view, a wide overlap exists between the classical growth factors and different vasoactive factors, as the former may induce significant hemodynamic effects, whereas the latter may modify the rate of proliferation and protein synthesis in different cells.

These mechanisms have been scarcely explored in aging. The number of mesangial cells increases in the early stages of aging in rats [17], but no additional studies have confirmed this finding. It would be very important to assess the rates of proliferation and apoptosis of the different renal cells as a function of age to ascertain the importance of these phenomena in the genesis of the progressive replacement of cells by extracellular matrix. In addition, glomerular hypertrophy frequently occurs in elderly individuals and old rats [12–14]. Although this hypertrophy has been frequently considered the consequence of intrarenal hemodynamic changes [49], some authors believe that the hypertrophy depends on the release of local mediators, including growth factors [50], and that it could be an indirect marker of the increased local production of growth-promoting metabolites. Only one recent report has analyzed the levels of one of these growth factors in elderly individuals. Chou et al demonstrated an inverse relationship between urinary epidermal growth factor excretion and age, and suggested that a reduced production of this growth factor retards the repair process at the kidney level [51]. In contrast, different vasoactive autacoids have been studied as possible mediators of the functional changes of the aging kidney, and it is now a well-recognized fact that autacoids regulate cell proliferation and/or extracellular matrix synthesis. Thus, angiotensin II and endothelin have well-defined effects on cell proliferation, whereas nitric oxide (NO) and atrial natriuretic peptide (ANP) possibly inhibit cell growth and
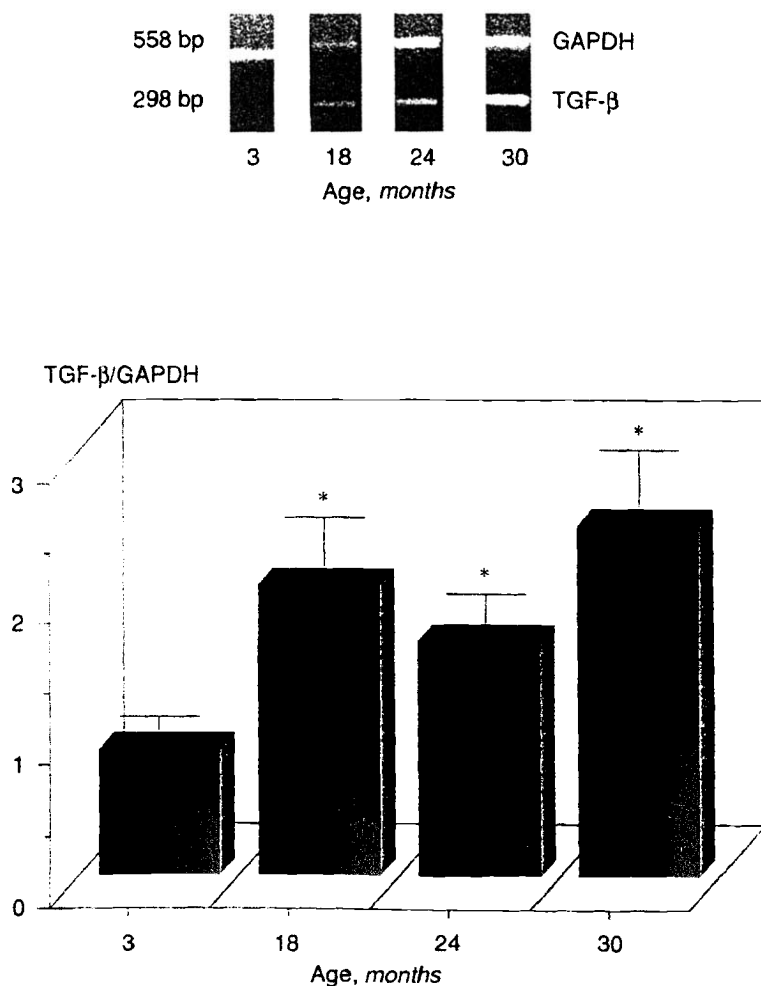
Fig. 4. Expression of the TGF-β mRNA in the renal cortex from 3-month-old, 18-month-old, 24-month-old, and 30-month-old rats. *Upper panel*, the simultaneous amplification of the TGF-β1 and GAPDH (housekeeping gene) mRNAs by using RT-PCR, in samples from rats of different ages. *Lower panel*, the ratio between the two amplification products (TGF-β1/GAPDH) were calculated and the mean $\pm$ SEM of 6 different rats are given. *$P < 0.05$ versus 3-month-old rats. (Published with permission of *J Am Soc Nephrol.*)

extracellular matrix synthesis [44]. Changes in these vaso-active, growth-promoting metabolites could be involved in the development of the aging-related morphologic changes. I will return to this topic. Finally, data about the possible importance of the extracellular matrix degradation in the genesis of the structural changes related to aging have been provided by Schaefer et al [52] and Reckelhoff et al [53], who found that glomerular and tubular proteinase activities decreased in aging rat kidneys. According to the previously discussed criteria, the possible consequences of this decreased proteinase activity would be increased extracellular matrix and subsequent morphologic changes in the kidney.

Some of these mechanisms are likely influenced by gender, an important determinant of the rate at which the kidney is damaged with age. Morphologic alterations are less severe in old women than in old men [12, 13], and male rats have a higher mortality rate due to renal failure [16]. These gender differences seem to depend on the presence of androgens rather than on the absence of estrogens; castration of older animals prevented the development of

renal dysfunction in male rats without modifying the pattern of renal damage in female rats [16].

Studies from our laboratory suggest that TGFβ is involved in the development of aging-related morphologic changes, particularly interstitial fibrosis [20]. Using semi-quantitative reverse transcriptase-polymerase chain reaction techniques, we demonstrated that expression of TGFβ mRNA in renal cortex of Wistar rats increased progressively with aging (Fig. 4). The blockade of TGFβ expression by long-term treatment with captopril partially prevented the development of interstitial fibrosis but not of glomerulosclerosis. One of the growth factors most widely studied for its role in the structural changes in different pathologic conditions, TGFβ modulates the proliferation rate of different renal cell types, as well as matrix synthesis and degradation. Thus, the progressive glomerulosclerosis associated with experimental glomerulonephritis, diabetes, or renal mass reduction could depend on TGFβ mRNA overexpression [48]. We expected to find significant changes in this growth factor in our experiments in old rats.

Although we demonstrated a direct temporal relationship between aging and TGFβ mRNA expression [20], only interstitial fibrosis seemed to depend on this overexpression, in contrast with previous results in other pathologic situations. Perhaps, as suggested by Abrass et al [19], the nature and pathogenesis of aging-related renal dysfunction are not fully comparable to those of glomerulonephritis, diabetes, or renal mass reduction.

Functional. Aging-related functional changes are closely related to the morphologic alterations previously described. However, they are not only the consequence of structural changes, as a deranged regulation of different aspects of normal renal function also seems to be involved in the genesis of these changes. Moreover, a complex network of functional relationships between the different renal structures exists, and changes in the function of a particular structure can depend on the dysfunction of others.

Renal vessels in healthy elderly humans or rats do not show structural changes significant enough to completely explain the reduction in RBF, except when other pathologic situations such as arteriosclerosis are superimposed on the basic aging process. In consequence, authors have looked for the intrinsic causes of the decreased renal plasma flow in the disease-free elderly individual. It is generally accepted that the basic defect in the vessels of these individuals is an impaired ability to relax in the presence of some well-defined vasorelaxant stimuli. From the initial description of decreased renal vasodilation after pyrogen injection in human beings 4 decades ago, authors have demonstrated, in human beings or in animals, that the normal vasodilatory responses induced by acetylcholine, amino acids, glycine, or after food ingestion are blunted in the elderly [22, 24, 54]. However, some discrepancies exist in this area. Hill and coworkers described a normal, unblunted renal vasodilatory response to acetylcholine and L-arginine infusion in older rats [55]. On the other hand, the agonist-induced renal vasocontrictive response in the aging kidney might not be the same in human beings and rats. Hollenberg and colleagues demonstrated that the angiotensin II-induced reduction of RBF in elderly humans was independent of the age of the subjects [22], and the renal vasodilatory response to acute angiotensin converting enzyme inhibition persisted even in old people [56]. In contrast, Tank et al found that kidneys of aged rats exhibited an exaggerated response to systemic vasoconstrictor stimuli [26]. Taken together, these data suggest that the combination of defective vasodilation with normal or increased vasoconstriction accounts for the reduced RBF that characterizes aging.

The basis for these altered vascular responses in the aging kidney is not understood. Pyrogen injection, acetylcholine, and amino acid infusion share a common mechanism of inducing renal vessel relaxation, that is, the local release of NO, one of the most important vasodilator mechanisms. Defective synthesis or activity of the NO

system might be involved in the impaired renal vascular vasodilatory response observed in old individuals. Three mechanisms could account for this possible defective response: reduced production of NO in response to different stimuli, increased degradation of the NO released, or increased synthesis of this metabolite with a decreased response of the target cells. The first of these three hypotheses is supported by the fact that the 24-hour urinary excretion of nitrites plus nitrates (considered an indirect index of NO synthesis in the kidney [57]) as well as the glomerular synthesis of nitrites [58] are decreased in older rats. However, in studies of aged rats with NO synthesis blockade, Reckelhoff and Manning demonstrated that the dependence of renal blood flow on nitric oxide is greater in old than in young individuals. They suggested that increased synthesis of this compound is necessary to maintain renal perfusion [25]. As a consequence of the increased and maintained basal NO synthesis, the response to stimuli such as acetylcholine or amino acids would decrease. Unfortunately, neither direct measurements of local NO synthesis in renal vessels nor a detailed analysis of the expression and activity of NO synthases in renal cortex is available at present. However, a report from Hwang et al demonstrated that cultured proximal tubule epithelial cells from kidneys of old donors express significantly higher amounts of constitutive NO synthase [59]; this report opens new perspectives in the study of this problem. The reasons for a possible increase of basal NO synthesis in renal cortex of the aging kidney are unclear. Nitric oxide could act as a counteracting mechanism of some vasoconstrictor mediators that might be increased in aging. High circulating endothelin levels have been described in plasma of aging men [60], endothelin mRNA expression is increased in cultured vascular endothelial cells from old compared with young individuals [61], and increased endothelin-1 secretion was detected in aged cultured human umbilical vein endothelial cells [62]. Endothelin, via its ET-B receptor, might increase NO synthesis. Our group, in collaboration with the Department of Pathology of the University of Granada, has found that mRNA expression of preproendothelins 1 and 3 increases in old rats (unpublished data) (Fig. 5); this work thus supports a possible role for this peptide in the vascular renal changes that characterize aging.

Nitric oxide and endothelin are not the only vasoactive systems that have been studied as possible mediators of aging-related functional changes. Impaired arterial baroreflex, with a subsequent increased renal sympathetic activity, has been proposed as a possible mechanism for increased renal vascular resistance [54]. The previously mentioned data concerning renin synthesis in elderly human beings and rats [39–41] would lead us to expect that angiotensin II decreases with aging, and some studies support this contention [41]. However, one recent report proposing that this peptide actually increases with age [63], and the
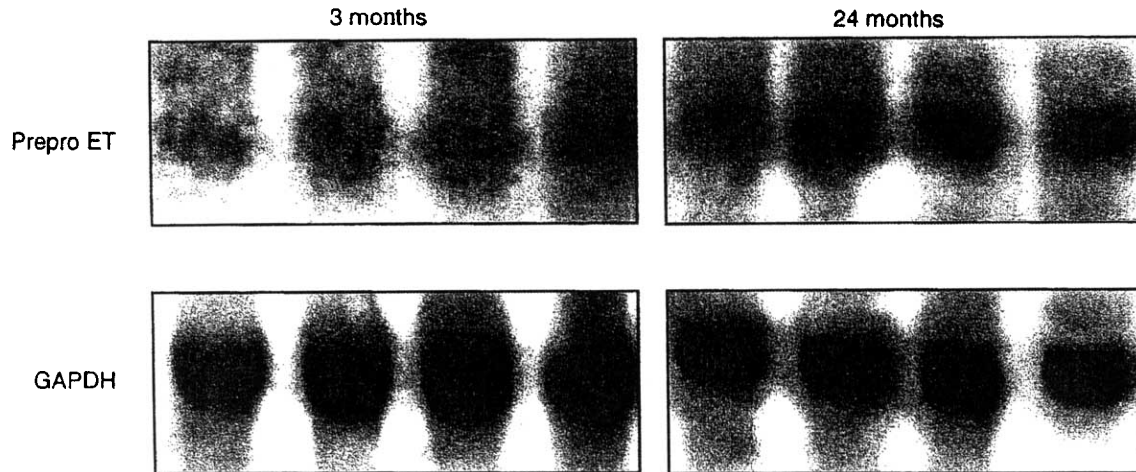
3 months   24 months

Prepro ET

GAPDH

Fig. 5. Northern blot analysis of the expression of the preproendothelin-1 and GAPDH (housekeeping gene) mRNAs in 3-month-old and 24-month-old rats.

significant renal vasodilation with increases in renal plasma flow observed in older rats in response to the acute angiotensin II blockade [64], suggest that intrarenal angiotensin II is activated in senescent rats. Synthesis of platelet activating factor (PAF), a lipid mediator with well-recognized vasoconstrictor ability, seems to be increased in isolated glomeruli of aged rats [31]; this finding suggests a pathogenetic role for this autacoid in aging-induced altered vascular responses. The equilibrium between prostacyclin and thromboxane also is deranged with aging, and the ratio of prostaglandin I_2 to thromboxane A_2 decreases in the urine of older humans, as well as in the glomeruli and inner and outer medulla of older rat kidneys [62, 65]. Finally, impaired ANP-induced relaxation of renal arteries in rats and monkeys [66] provides yet another way of impairing renal vasodilation.

Another possible explanation for the impaired renal vasodilatory response is defective activation of intracellular second messengers that mediate vascular smooth cell relaxation. A blunted cAMP response to β-adrenergic agonists has been described in blood vessels with aging, and Lakatta has proposed that this alteration modifies vascular responses to stress and exercise [67]. Further study reveals that this response could depend on impaired guanine nucleotide regulatory protein (G protein) function [68]. However, it was not possible to detect changes in the amount of these G proteins in aged rats, particularly Gs and Gi, at the renal level [69]. On the other hand, the defective response to ANP in blood vessels from elderly individuals might be due to accelerated degradation of cGMP by phosphodiesterases [66]. Additional studies are needed, including evaluations of the different intracellular systems involved in vascular smooth muscle cell relaxation, to clarify the importance of these mechanisms in the development of aging-related decreased renal plasma flow.

Changes in GFR in the elderly are generally attributed to progressive glomerular sclerosis and decreased renal plasma flow. However, formation of the glomerular ultrafiltrate is a finely regulated phenomenon, and the reduction in GFR occurs more slowly than does the fall in RPF, the result being an increased filtration fraction. Two mechanisms might account for the relative GFR maintenance, even in the presence of significant hemodynamic and structural changes. First, as I said before, the aging process produces a non-homogeneous derangement of renal blood flow, with a preferentially decreased cortical flow [22]. As the filtration fraction of the juxtamedullary glomeruli seems to be higher than that of the cortical glomeruli, the GFR would diminish less than in the case of a homogeneous reduction in renal perfusion. Second, it is well known that the remaining glomeruli undergo hemodynamic changes to compensate for the lack of function of the sclerosed glomeruli [49]. This phenomenon, known as secondary hyperfiltration, could maintain adequate GFR even in the presence of a significant reduction of functioning nephrons. The mechanisms involved in the development of hyperfiltration have been extensively studied in different experimental models. In most cases, hyperfiltration seems to depend on increased glomerular plasma flow and increased hydrostatic pressure in the glomerular capillary network as a consequence of selective vasodilation of the afferent arteriole [49]. Micropuncture studies in aged rats have analyzed the determinants of glomerular ultrafiltration. Most of these studies demonstrate decreased resistance of the glomerular afferent arteriole with an increased glomerular plasma flow [25-27]. Controversy exists, however, with respect to changes in the glomerular capillary pressure in these old rats, because normal [16, 25, 27] or increased values [70] have been detected. Moreover, an increased age-related ultrafiltration coefficient also has

been described [25, 27]. A recent micropuncture study by Baylis, performed in Munich-Wistar rats, failed to demonstrate the previously described changes in old rats, as glomerular plasma flow decreased and afferent arteriole resistance increased, whereas the ultrafiltration coefficient did not change [16]. This study points to a possible inter-strain variability in the mechanisms involved in the development of aging-induced GFR changes. In any case, we must remember that these studies were performed in rats as old as 2 years, when glomerulosclerosis and proteinuria are readily detected [15] but when absolute values of GFR are maintained [16, 24].

As in the case of RBF, changes in GFR in the elderly might be due, at least partially, to an imbalance among the autacoids that regulate intraglomerular hemodynamics. However, no studies have analyzed independently these possible local mediators. As regulation of intraglomerular hemodynamics depends on the regulation of the afferent and efferent arterioles, the autacoids involved could be the same in both processes. But additional studies are needed to clarify the specific mediators that lead to the reno-vascular and glomerular changes of aging.

What accounts for the relative inability of elderly individuals to reabsorb sodium normally? First, aging-associated structural renal alterations, such as interstitial fibrosis or a decreased number of tubules, might play a part in the homeostasis. Second, decreased GFR and its attendant hyperfiltrating glomeruli also might explain some aspects of the deranged sodium homeostasis. Thus, a decreased GFR could produce, on a short-term basis, a relative inability to excrete a sodium load. On the other hand, the hyperfiltering nephrons excrete a solute load significantly higher than do normal nephrons, with a subsequent osmotic diuresis and natriuresis; these hyperfiltering nephrons are unable to readily reabsorb sodium. Third, hormonal changes also might account for changes in sodium homeostasis. When plasma aldosterone was measured in elderly individuals, values were significantly lower than those in young people [71]. This decreased aldosterone synthesis could contribute to the relative inability of the aging kidney to conserve sodium. The hormone proposed to account for this deranged sodium excretion is ANP; plasma levels of this hormone significantly increase in elderly subjects [72]. However, the natriuretic response after the infusion of exogenous ANP seems to be decreased in healthy elderly men [73]. These data have been interpreted as a relative decreased responsiveness of the aging kidney to ANP.

Decreased concentrating ability in the elderly has been attributed to changes in the functional status of the hypo-thalamic-pituitary axis. However, when the release of arginine vasopressin (AVP) was analyzed under different phys-iologic stimuli, elderly subjects exhibited increased AVP release with respect to young individuals [74]. Not all studies have had similar results, however, and decreased aging-related AVP release also has been demonstrated

[75]. Moreover, non-osmotic AVP release also might be deranged in the elderly [74, 75]. In any case, it is generally accepted that the most important mechanism involved in the elderly's renal concentrating defect is an inadequate renal response to endogenous AVP. In humans, this lack of response has been attributed to aging-related tubulointer-stitial structural changes as well as to a derangement in the intrarenal mechanisms responsible for the maintenance of medullary hypertonicity, including solute transport by the thick ascending limb of the loop of Henle and relatively slow medullary blood flow [22, 33]. Studies in rats also suggest that impaired responsiveness of the collecting duct cells to AVP is involved in the concentrating defect in old animals. The basis of this defect could be a decreased number of V2 receptors, but a recent report failed to demonstrate such a decrease. In consequence, defective coupling of this receptor to the adenylate cyclase system likely explains the lack of response to AVP in older rats [76, 77]. The intrinsic mechanisms of the renal diluting defect in the elderly have been less studied, but they might relate to the aging-related decrease in GFR and decreased solute transport in the thick ascending limb of the loop of Henle.

Other aging-related tubular defects, including defective renal acidification and phosphate management, have been analyzed less extensively. Impaired acidification seems to be a consequence of reduced renal mass [38], although some studies suggest an intrinsic acidification defect, pos-sibly associated with impaired ammonium excretion [78]. Defective phosphate reabsorption by the proximal tubule partially depends on the increased PTH concentration associated with decreased GFR [36]. But parathyroidec-tomy only partially prevents this defect, so alternative mechanisms of deranged phosphate reabsorption must be at play. Phosphate transport is decreased in cultured renal tubular cells [79], and Levi et al have suggested that this decreased transport results from changes in the chemical composition of cell membranes [80]. Moreover, the expres-sion of the type-II Na-Pi cotransporter decreases with age in tubular cells [81].

Aging and activation of the mechanisms involved in the renal changes in the elderly

As I stated before, most studies dealing with the mech-anisms involved in the development of aging-related mor-phologic and functional renal changes have focused on a particular deranged aspect of renal structure or function. However, these studies have not provided enough clues about how aging determines the activation of these mech-anisms. For instance, some authors have proposed that reduced RBF in elderly individuals depends on changes in the local synthesis of NO [25, 75], but we still do not know how aging induces these changes. Our knowledge of the general mechanisms of aging and of the direct mediators of renal dysfunction may help us to better understand the aging process at the renal level.
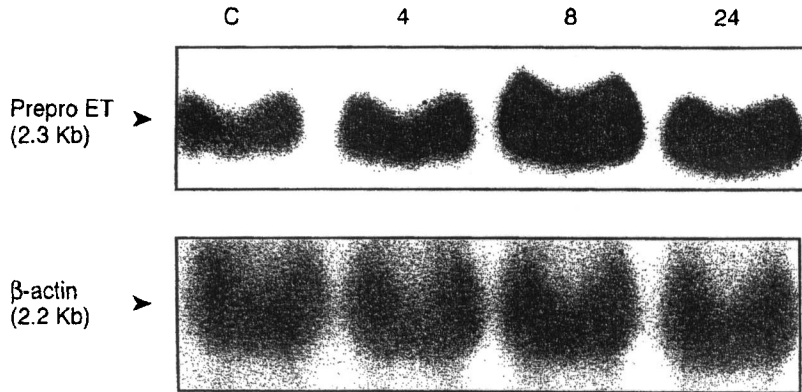
Fig. 6. Northern blot analysis of the changes in the preproendothelin-1 mRNA expression in cultured bovine aortic endothelial cells incubated with hydrogen peroxide (100 μM) for different times (4 to 24 hours).

Alterations in the genetic program, induced by turning on the cell death program (apoptosis) or by exogenous damage to DNA may explain the activation of particular pathogenetic mechanisms in the aging kidney. Thus, changes in the synthesis of certain growth factors, vasoactive mediators, or cell transporters might be associated with the genetic changes that induce aging. Unfortunately, a detailed analysis of the genetic changes in senescent kidney cells, unlike in senescent fibroblasts [1], has not been performed.

One attractive hypothesis might explain how the kidney ages. Anderson and Brenner suggest that aging-related, progressive renal damage is the consequence of a continuous exogenous stimulus, the diet, on renal structure and function [49]. This hypothesis supports the general mechanisms of aging that I already discussed. Long-term low-protein feeding and chronic angiotensin II converting-enzyme inhibition have a protective effect on the glomerulus in the aging rat [15, 49, 70]. Because these two maneuvers lower intraglomerular pressure in other experimental models of renal disease, these data have been interpreted as indicating that glomerular hypertension, induced by a high-protein diet, causes age-induced nephropathy. The mechanisms connecting intraglomerular hypertension with progressive damage of glomerular structures are currently being investigated, and it seems that changes in the mechanical forces acting on the cells might induce significant phenotypic changes in resident glomerular cells, thereby modulating the release of local mediators [82, 83].

The critical point for validating this theory would be the direct demonstration of increased intraglomerular pressure in elderly experimental animals. Anderson et al reported increased intraglomerular pressure in 24-month-old Munich-Wistar rats [70]. However, Baylis did not find significant changes in glomerular pressure in males up to 20 months of age of the same rat strain, even though the animals had significant glomerular structural damage [16]. Moreover, intraglomerular pressures in castrated male and

in female Munich-Wistar rats, which do not develop glomerulosclerosis, did not differ from values in intact male rats [16]. Other rat strains that develop glomerulosclerosis earlier, for example, Sprague-Dawley, show small increases of intraglomerular pressure at 13 to 18 months but not at 20 to 22 months of age [25]. All these data suggest that intraglomerular hypertension is a relevant, but not the sole, factor in aging-related renal damage. Nor is it likely the initial mechanism that triggers progressive renal dysfunction in the elderly.

Reactive oxygen intermediates also might be a link between aging and renal damage. The first argument supporting a role for ROI in the progressive renal damage of aging comes from the observation that these metabolites likely are involved in the pathogenesis of other renal diseases characterized by progressive extracellular matrix expansion and decreased GFR, such as experimental renal mass reduction or glomerulonephritis [84]. Moreover, the cellular biology of resident glomerular cells is clearly influenced by ROI. Although high concentrations of these metabolites usually induce cell necrosis, under particular experimental conditions, they induce proliferation of mesangial cells [85]. This fact could be related to tyrosine phosphorylation of the platelet-derived growth factor receptor and the pp60c-src protein [86]. Further, ROI promote changes resembling apoptosis in tubular cells [87]. By promoting cell proliferation or apoptosis, ROI might play different pathogenetic roles during different stages of aging. In early stages, increased cell proliferation associated with increased synthesis of extracellular matrix might induce matrix expansion. In more advanced stages, apoptosis could reduce the number of cells in different parts of the nephron as well as in the interstitium.

Reactive oxygen intermediates also might modulate synthesis of the vasoactive factors involved in the dysfunction of the aging kidney. Reactive oxygen intermediates increase prostanoid synthesis in varying renal structures [84]. Moreover, PAF synthesis by mesangial cells also might be increased in the presence of ROI [88]. In addition, ROI
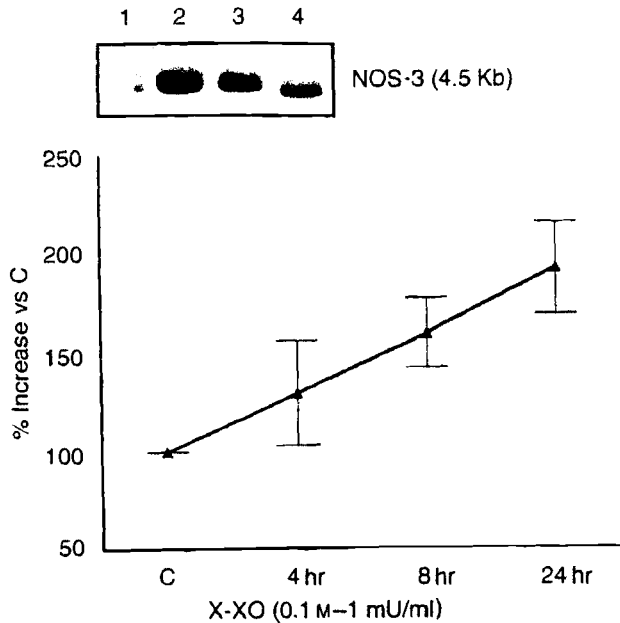
Fig. 7. Northern blot analysis of the changes in the endothelial constitutive nitric oxide synthase (NOS-3) mRNA expression (*upper panel*) and in the activity of this enzyme, measured as the conversion of arginine to citrulline (*lower panel*), in cultured bovine aortic endothelial cells incubated with xanthine (X, 0.1 M) plus xanthine oxidase (XO, 1 mU/ml) for different times (4 to 24 hours).

stimulate endothelin production in cultured human mesangial cells [89]. In our own laboratory, expression of pre-proendothelin-1 mRNA increased in cultured bovine aortic endothelial cells incubated with hydrogen peroxide (Fig. 6) [90]. The relationships between NO and ROI are less well documented. Superoxide anion may inactivate NO, thereby inhibiting this vasodilatory system [91]. However, recent results from our laboratory point to alternative possibilities [92]; messenger RNA expression of one of the enzymes involved in NO synthesis, the endothelial constitutive nitric oxide synthase, as well as its activity, might be increased in cultured bovine aortic endothelial cells incubated with a ROI generating system such as xanthine-xanthine oxidase (Fig. 7). The relationship between these in-vitro findings and the in-vivo results in aged individuals must be evaluated carefully in the future.

Data concerning the ability of ROI to modulate cytokine synthesis by different glomerular cells are scarce. Synthesis of tumor necrosis factor seems to be stimulated by ROI [93], and deferoxamine, an iron chelator with well-defined antioxidant properties, might regulate tumor necrosis factor release in mesangial cells [94]. On the other hand, ROI might be an intermediate metabolite in the release of monocyte chemoattractant protein and monocyte colony-stimulating factor induced by tumor necrosis factor [95]. The possible role of these cytokines in the aging kidney has not been studied.

Two recent reports from our laboratory stressed the

importance of ROI in the pathogenesis of the changes that characterize the aging kidney. In the first of these studies. we demonstrated increased ROI synthesis by isolated glomeruli and cultured mesangial cells from old rats, as well as increased oxidative damage in the renal cortex of these animals [31]. In the second, probucol, a drug with antioxidant properties used for hypercholesterolemia, prevented protein accumulation in the glomeruli of aged rats without modifying cholesterol levels [96]. These data indicate that increased age-related ROI synthesis induces well-defined effects in different renal structures, and triggers the functional and morphologic changes that characterize aging.

The role of AGEs in the development of diabetic complications has been studied extensively [9]. Increased in elderly individuals [9], AGEs might induce some of the changes involved in the development of aging-induced renal dysfunction. Receptors for AGEs have been described in macrophages and monocytes [97]. These cells, which transiently infiltrate the renal parenchyma, might synthesize interleukin-1, insulin-like growth factor I, tumor necrosis factor, and granulocyte-macrophage colony stimulating factor in response to receptor binding by AGEs [98]. In addition, AGE receptors have been identified on glomerular mesangial cells, where they seem to play a role in the modulation of PDGF-induced extracellular matrix synthesis [99]. Prolonged administration of AGEs to normal rats induced glomerular hypertrophy and extracellular matrix expansion [100]; this finding underscores the importance of these metabolites in the development of glomerulosclerosis. Finally, the functional properties of several important matrix components are altered by AGE formation, disrupting the normal matrix-to-matrix and cell-to-matrix interactions [101, 102], thus favoring the progressive matrix expansion of aging kidneys.

Before I finish this discussion, I would like to mention a particular point of view regarding the pathogenesis of renal dysfunction in the aging kidney. The finding that about one-third of the subjects included in the Baltimore Longitudinal Study of Aging did not show any change in the glomerular filtration rate [28], and the existence of rat strains that do not develop any aging-related renal damage [103], suggest that the renal dysfunction of the elderly is due to an accumulation of damage induced by minimal, clinically undetected, renal disease, and is not the consequence of the aging process itself. Although it is a well-recognized fact that aged patients with superimposed diseases, such as hypertension or diabetes, show a more rapid decrease of renal function, healthy human beings and laboratory rats with well-controlled disease develop these renal changes in the absence of any detected renal disease. It is likely that a complex relationship between external influences, including diet, and the genetic program of a particular individual determines the variability observed in aging humans.

QUESTIONS AND ANSWERS

Dr. John T. Harrington (*Dean, Tufts University School of Medicine, Boston, Massachusetts, USA*): Thank you, Diego, for a wonderful summary and synthesis of a great deal of data regarding aging of the kidney. My question is, are rats a good model for these studies? Are there comparable studies in mice, hamsters, or dogs?

Dr. Rodríguez-Puyol: Most information has been obtained in rats and mice, perhaps because of the wide availability of these animals. But discrepancies do exist with respect to the adequacy of the model. In contrast to human beings, rats are characterized by the early appearance of proteinuria and the maintenance of a normal GFR until very advanced ages. In some rat strains, almost no changes are detected in the kidney even in very old animals [103]. In consequence, some authors believe that rodents, particularly rats, are not the most suitable model for studies of aging. The available information about the aging phenomenon in other animal models is scarce, and no definite data point to a particular species as the best model for these studies. In fact, efforts are underway to develop rat strains that could constitute better models for analyzing aging [104].

Dr. Harrington: My second question is more specific. Can you block the production of mesangial matrix, specifically laminin?

Dr. Rodríguez-Puyol: Strategies for blocking mesangial matrix synthesis have been directed to the blockade of TGFβ, as this growth factor is likely responsible for the increased extracellular matrix observed in a variety of pathophysiologic conditions. ACE inhibitors, anti-TGFβ antibodies, and decorin have been the most widely used tools for blocking TGFβ. To my knowledge, no studies have been designed that specifically block laminin synthesis in experimental models of renal disease, because this matrix protein has not been considered as pathophysiologically relevant as collagens or fibronectin. The interesting observations from Abrass and colleagues demonstrating the accumulation of laminin in the glomerular basement membrane of old rats suggest that this extracellular protein plays a role in the genesis of the aging-related renal changes [19]. In consequence, experiments directed at specifically blocking particular laminin isoforms could provide interesting information regarding the mechanisms involved in the aging process.

Dr. Angel De Francisco (*Professor of Nephrology, University Hospital "Marqués de Valdecilla," Santander, Spain*): I have a question concerning the hemodynamic actions of ACE inhibitors in aging. Aging is accompanied by renal vasoconstriction. Taking into account the importance of angiotensin II in the regulation of renal blood flow, do you believe that ACE inhibitors could be useful in preventing the aging-related reduction in renal blood flow?

Dr. Rodríguez-Puyol: The importance of angiotensin

II in the aging-related reduction in renal blood flow reduction is not known. Different experimental approaches, such as measuring the circulating levels of renin or angiotensin II or measuring the angiotensin II turnover, have provided conflicting results [39–41, 63, 64]. The conflicting results are not too surprising, as the critical question—the degree of activation of the local renin-angiotensin system—cannot be directly explored. When ACE inhibitors are used to block the synthesis of angiotensin II, renal plasma flow in older rats increases [64], thus suggesting that, at least partially, renal vasoconstriction may depend on increased local synthesis of angiotensin II in rats. Young and old human beings show a comparable hemodynamic response to angiotensin II blockade [54, 64].

Dr. Manuel Arias (*Head, Nephrology Department, University Hospital "Marqués de Valdecilla"*): We have some data in patients with advanced chronic renal failure showing a predominance of FAS and a decrement of bcl-2, a pattern of increased programmed cell death. Do you have any experience in the analysis of renal tissue for the apoptosis genes? Do you think that apoptosis might be responsible for aging of the kidney?

Dr. Rodríguez-Puyol: That is a very interesting question. We have considered the hypothesis that a change in the apoptosis rate is a pathogenetic mechanism in the aging kidney. Using Northern blot analysis, we were unable to detect changes in the mRNA expression of bcl-2, whereas bax slightly but significantly decreased in the renal cortex from old rats. However, these results are preliminary and incomplete, as bcl-2/bax are not the only the genes involved in the regulation of apoptosis. Additional studies are needed to adequately answer your question.

Dr. José María Morales (*Assistant Professor of Medicine, Hospital "12 de Octubre," Madrid, Spain*): Do you have information regarding cyclosporine's nephrotoxicity in aging kidneys?

Dr. Rodríguez-Puyol: As I said earlier, our group believes that reactive oxygen species seem to be involved in the pathogenesis of aging-related renal changes. Moreover, we have recently demonstrated that cyclosporine A increases the synthesis of ROS by cultured human mesangial cells [105]. In consequence, it is possible that cyclosporine A is more nephrotoxic in the elderly. The influence of age in cyclosporine A-induced nephrotoxicity has been studied in rats [106], but rats are not a good model for analyzing cyclosporine-induced damage because the dose that must be used to induce renal damage is much higher than the usual doses in human beings (50 mg/kg/day). In patients receiving a renal graft, cyclosporine A nephrotoxity does not seem to be increased with increasing donor age [107]. To my knowledge, however, no clinical studies have been designed to answer this question specifically. These studies should be performed in patients with heart, liver, or lung transplants, or in patients receiving cyclosporine because of extrarenal diseases.

DR. JAVIER DÍEZ (*Professor of Medicine, Head, Vascular Pathophysiology Unit, University of Navarra, Pamplona, Spain*): Frohlich and colleagues have shown that blockade of the endothelial constitutive nitric oxide synthase in young SHR rats causes morphologic and functional renal changes similar to those present in untreated, old SHR rats [108]. This group also demonstrated that ACE inhibitors can prevent these renal changes. Can hypertension accelerate the aging process by amplifying the alterations due to diminished nitric oxide availability? Is there a role for ACE inhibitors in preventing renal aging in hypertensive patients?

DR. RODRÍGUEZ-PUYOL: I have tried to communicate my particular point of view, which is that aging is an evolutionary process in which the equilibrium between the deleterious vasoconstrictor/profibrotic local mediators and the beneficial vasodilator/antifibrotic autacoids is progressively displaced towards the former. For instance, nitric oxide could counterbalance the effects of angiotensin II for long periods, until the nitric oxide system fails and renal dysfunction develops. If this hypothesis were right, the answers to your two questions would be yes. Hypertension, by inducing endothelial dysfunction, could decrease the activity of NOS-3, and subsequently accelerate renal aging. On the other hand, the decreased local concentration of angiotensin II that occurs after ACE inhibitor treatment could slow the aging process. But as I said, neither the exact role of angiotensin II nor the nature of the changes in the nitric oxide system has been adequately clarified in the pathogenesis of aging in the kidneys. More studies are needed.

DR. MANUEL PRAGA (*Assistant Professor of Medicine, Hospital "12 de Octubre"*): As you know, several studies have shown that ACE inhibitors can slow the progression of renal disease in different clinical settings [109]. However, patients over 65-70 years old generally have been excluded from these studies. Do you think that ACE inhibitors also could slow the progression of the age-related decline in renal function?

DR. RODRÍGUEZ-PUYOL: We believe that ACE inhibitors can slow the progressive decline of renal function in the elderly. Our own results in rats, which have been presented previously [20], support this contention. However, no studies in elderly human beings (older than 65–70 years) have been performed. It would be very interesting to analyze, on a long-term basis, the role of ACE inhibitors on the progression of aging-related renal changes.

DR. CARLOS QUEREDA (*Chief, Clinical Section of Nephrology, University Hospital "Ramón y Cajal," Madrid*): As the kidneys are mainly vascular organs, to what extent do you think that aging-related functional changes depend on structural vascular damage? In other words, do you believe that most of the renal dysfunction is a consequence of arteriosclerosis?

DR. RODRÍGUEZ-PUYOL: That very interesting question was specifically addressed some years ago [22]. Although it is difficult to separate intrinsic renal damage from the vascular process in patients with severe vascular disease, renal dysfunction cannot be solely explained by structural arterial changes in older healthy humans. Several investigations have demonstrated that the renal circulation in older individuals has characteristic patterns of response to the administration of different drugs. These responses were defective when compared to those in young subjects, and I do not believe that the differences can be explained by arteriosclerotic damage [22, 24, 54].

DR. ARMANDO TORRES (*Professor of Nephrology, University Hospital, Tenerife, Spain*): Insertion-deletion polymorphism of the ACE might contribute to progression of chronic renal failure. As you mentioned, about 30% of older individuals do not have an age-related decrease in GFR. Has any evidence disclosed an association between ACE polymorphism and an age-related decrease in GFR?

DR. RODRÍGUEZ-PUYOL: The lack of renal dysfunction in a substantial minority of the aged population has been explained by different mechanisms. It has been attributed to environmental differences and to the absence of any overt renal disease. Obviously, genetic factors would be important; in this sense, ACE polymorphism could be involved. Unfortunately, this hypothesis has not been tested yet.

DR. NELIDA ELENO (*Associate Professor, Department of Physiology, University of Salamanca, Spain*): Did you try to give antioxidant treatment to those old rats that you presented in your talk?

DR. RODRÍGUEZ-PUYOL: We tried to determine whether vitamin E could block the expression of the TGFβ mRNA in the renal cortex of older rats, but we did not obtain conclusive results. We are now working with taurine, an amino acid with antioxidant properties. It seems that taurine could block the mRNA expression of some collagen types in the renal cortex. However, these results are very preliminary.

DR. MANUEL MARTÍNEZ-MALDONADO (*Professor of Medicine, Emory University, Atlanta, Georgia, USA*): Data suggest a beneficial effect of ACE inhibitors in reducing the aging-related morphologic and functional renal alterations in rats [110]. Are there studies of a similar nature, or studies examining other functional aspects, such as urine concentration and dilution, in humans? What is the effect of nutrients on renal function as aging proceeds? Could this be the reason patients were separated into three groups in the Baltimore aging study [28]?

DR. RODRÍGUEZ-PUYOL: Unfortunately, there are no definite answers for your questions. The ability of ACE inhibitors to prevent the aging-related morphologic and functional renal changes has not been tested in long-term studies in humans. With respect to the ability of ACE inhibitors to modify the non-hemodynamic, non-glomerular alterations that characterize renal aging in men, no data are available. ACE inhibitors might prevent interstitial

fibrosis in aging rats. Likewise, these agents might improve tubular function in humans, but the functional improvement would be very difficult to distinguish from the hemodynamic modifications. Finally, one of the possible explanations for the existence of three different groups in the Baltimore study could be diet, but other alternatives exist, as I said before.

DR. FRANCISCO ORTEGA (*Head, Health-Related Quality of Life Unit, Hospital Central de Asturias, and Nephrology Research Institute "Reina Sofia," Oviedo, Spain*): In our studies, patients over 65 years undergoing chronic hemodialysis had a worse quality of life than did those in the normal population randomly selected, but elderly patients with a functioning kidney graft had a similar quality of life, even better in some dimensions, than this normal population [111]. Do you think that a functioning kidney transplant can delay the consequences of general aging on quality of life?

DR. RODRÍGUEZ-PUYOL: I am not familiar with your data on quality of life, but I do not believe that renal transplantation specifically delays the consequences of aging. I believe that a renal graft improves the quality of life in every patient, including the elderly, in such a significant way that usual quality-of-life tests cannot detect significant differences between the grafted and normal populations.

DR. ALBERTO MARTÍNEZ-CASTELAO (*Chief, Dialysis Division, Bellvitge C.S.U.B., Barcelona, Spain*): In our experience, more than 50% of patients with nonsteroidal antiinflammatory drug nephrotoxicity are diagnosed after age 65. Do you have any evidence that relates ROI overproduction with this increased sensitivity?

DR. RODRÍGUEZ-PUYOL: To my knowledge, nonsteroidal antiinflammatory drugs do not induce ROI overproduction. In fact, cyclo-oxygenase and lipoxygenase activation might be linked to ROI synthesis. The deleterious effect of these agents in elderly individuals must be due, as in other renal diseases, to the importance of arachidonic acid derivatives in the maintenance of renal function.

DR. FERNANDO VALDERRÁBANO (*Professor of Medicine, University Hospital "Gregorio Marañón," Madrid*): Diego, congratulations for your excellent review. Nowadays, we frequently transplant organs from donors older than 65 to young patients. Is there any evidence regarding changes in the process of aging in these cases?

DR. RODRÍGUEZ-PUYOL: Transplantation of old kidneys into young patients could be a very interesting model for analyzing the relative importance of genetic and ambient factors in the development of aging-related renal changes. This question has not been systematically considered, and I believe that taking into account the complex situation of the transplant recipient, it will be very difficult to reach significant conclusions from these kind of grafts.

DR. SANTOS CASADO (*Head, Nephrology Department, Fundación Jiménez Díaz, Madrid*): Dr. Rodríguez-Puyol, in your presentation. you mentioned that ROI and different cyto-

kines could be involved in the genesis of the renal changes that characterize aging. You also reviewed the role of endothelial constitutive NOS and endothelin. Did you test iNOS expression?

DR. RODRÍGUEZ-PUYOL: No, we have not tested iNOS expression in aging kidneys. However, we believe that this NOS isoform must be involved in the aging process, at least in the development of the functional changes, although we are not sure about the relationships between iNOS and aging. One of the first demonstrations of aging-related impaired renal vasodilation was performed in rats treated with pyrogen [13], and it is now a well-recognized fact that some of the components of pyrogen induce iNOS expression.

DR. DÍEZ: Diego, at the vascular level, one of the most important sources of ROI is the NADP-NADPH oxidase system. An overproduction of ROI has been reported in the aorta from spontaneously hypertensive rats [112]. Have you any information concerning possible alterations in the expression of this enzyme in the kidneys from old normotensive rats?

DR. RODRÍGUEZ-PUYOL: We have not performed this kind of analysis and, to my knowledge, this specific information has not been published. But it would be very interesting to analyze the NADP-NADPH oxidase system in aging, as some vasoactive factors, particularly angiotensin II, stimulate ROI synthesis by activating this system. The NADP-NADPH oxidase system could constitute the link between an increased synthesis of local mediators and ROI overproduction.

DR. JULIO PASCUAL (*Division of Nephrology, Hospital "Ramón y Cajal," Madrid*): Looking at aging as a slowly progressive death process, do you think that ROI play a relevant role in high morbidity-mortality conditions such as acute renal failure with multiorgan failure, or even chronic dialysis in elderly patients?

DR. RODRÍGUEZ-PUYOL: From a theoretical point of view, the answer is yes. Reactive oxygen intermediates might be increased in the patients that you have mentioned, and aging could increase local synthesis of these mediators. However, the Madrid Acute Renal Failure Study Group determined the prognosis of acute renal failure in older patients to be similar to that in younger patients [113]; these data suggest that age is not a particularly poor prognostic sign.

DR. HARRINGTON: Let me ask the last question. The patients you presented both had iatrogenic renal problems. How should we deal with our increasing aging population to prevent the type of problems seen in your patients?

DR. RODRÍGUEZ-PUYOL: If you consider the two patients whom I presented, the problems began in two different settings. In the second case, nephrotoxic drugs were prescribed by general physicians on an outpatient basis. General physicians must be extensively and repeatedly informed about the potential nephrotoxicity of three kinds of

drugs. nonsteroidal antiinflammatory drugs, ACE inhibitors, and diuretics, particularly if they are prescribed together. Within the hospital, as was the case with the first patient, the problem is more complex. The situation of the patient is more critical, more potentially nephrotoxic drugs are used, and intravenous fluid therapy frequently is needed. In addition to adequate monitoring of the extracellular volume status and biochemical parameters, as well as to judicious use of drugs and fluids, early contact with the nephrology team may avoid unnecessary complications.

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