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Morphometric study of arterioles and glomeruli in the aging kidney suggests focal loss of autoregulation[1]

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Morphometric study of arterioles and glomeruli in the aging kidney suggests focal loss of autoregulation.

Background. In the past it was widely assumed that hyaline afferent arteriolosclerosis was responsible for ischemic glomerulosclerosis in the aging and hypertensive kidney. However, glomerular lesions of focal segmental glomerulosclerosis are now recognized in essential hypertension. Experimentally, such lesions are associated with loss of autoregulation of blood flow and glomerular hyperperfusion, as well as initial glomerular hypertrophy. These observations challenge the notion of ischemia as a unitary explanation for glomerulosclerosis.

Methods. A morphometric study was performed on normal portions of eight kidneys removed for tumors in aging, normotensive patients. Measurements were made of 126 pairs of afferent arterioles and their associated glomeruli. In addition, the amount of extracellular matrix (ECM) in the immediate periglomerular region was quantitated.

Results. Afferent arterioles were divided into three types according to the presence or absence of hyaline deposits and whether these did or did not obstruct the lumen. Arterioles with nonobstructive hyaline deposits had lumens over twice as large as those without deposits ($482 \pm 240\ \mu^2$ vs. $204 \pm 160\ \mu^2$, $P = 0.0000$). Their associated glomeruli had significantly greater total capillary area, particularly the hilar capillaries ($1276 \pm 797\ \mu^2$ vs. $667 \pm 492\ \mu^2$, $P = 0.002$), but with larger individual capillaries elsewhere as well ($P = 0.03$). Arterioles with obstructive deposits differed from those with nonobstructive deposits by their smaller lumens ($P = 0.001$) and walls ($P = 0.004$), with a higher proportion of ECM in the periglomerular region ($P = 0.001$), all consistent with a later stage of lesion. Glomeruli were divided into four basic types: normal, hypertrophic, glomeruli with features of focal segmental glomerulosclerosis (FSGS-type), and ischemic. Compared to normal glomeruli, hypertrophic glomeruli were larger, with greater total capillary area ($P = 0.0005$), particularly the hilar capillaries ($P = 0.0000$), and larger capillaries in the remainder of the tuft ($P = 0.003$), but showed no evident lesions. FSGS-type glomeruli were also larger, with larger hilar capillaries ($P = 0.0005$), but showed an increase in ECM due to sclerotic lesions ($P = 0.004$). The remaining capillaries showed an inverse relation with the amount of mesangial matrix, showing a spectrum of sizes from enlarged to shrunken. As anticipated, ischemic glomeruli were significantly smaller than normal ones in every parameter measured. There was a strong association between hypertrophic/FSGS-type glomeruli and hyaline arteriolosclerosis, found in 90.3% of such glomeruli, versus 29.1% for the remaining glomeruli ($P = 0.0001$). The great majority of hyaline deposits were of the nonobstructive variety (86.2%), but some were obstructive (13.8%), particularly in FSGS-type glomeruli, consistent with a more advanced lesion.

Conclusions. We believe we have demonstrated in the aging kidney of humans the morphologic correlates of loss of autoregulation, occurring on a focal basis, with afferent arteriolar dilatation and increase in glomerular capillary size and subsequent focal segmental glomerulosclerosis. Hyaline arteriolosclerosis of the nonobstructive sort is strongly associated with these changes and may play a role in their pathogenesis.

It is known that in certain animal models glomerulosclerosis is preceded and accompanied by loss of autoregulation. This is true notably for spontaneously hypertensive rats [1, 2], 5/6 nephrectomy [3, 4], steroid [deoxycorticosterone acetate (DOCA)]-induced hypertension [5], and streptozotocin-induced diabetes [6]. This loss of autoregulation is manifested by dilatation of afferent arterioles [3, 7] and hyperperfusion of glomeruli [1, 8–10] from the outset. In almost all of these models, the form of glomerulosclerosis produced closely resembles the lesions of focal segmental glomerulosclerosis (FSGS) in humans. Equally, it can be shown that interruption of autoregulation, as by nifedipine, which preferentially dilates afferent arterioles, leads to the same pattern of glomerular injury [11].

This analysis describes a morphometric study of arteriolar and glomerular lesions in the aging normotensive kidney in humans. We found morphologic lesions consistent with focal loss of autoregulation, with the lesions of hyaline arteriolosclerosis playing a central role. We found that arterioles with nonobstructive hyaline deposits were markedly dilated compared to arterioles without deposits. These arterioles were most prominently associated with hypertrophied glomeruli with larger-than-normal capillary lumens, particularly the hilar capillaries. These glomeruli resembled those in experimental models in which glomerular hypertension and hyperperfusion

[1]**See Editorial by Olson, p. 1162.**

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have been confirmed [8, 12, 13] although clearly this has yet to be directly demonstrated in humans. Such glomeruli form a continuous spectrum with glomeruli with features of FSGS, and these latter are also highly associated with hyaline arteriolosclerosis. (Interestingly, the association between hyaline arteriolosclerosis and FSGS was pointed out nearly 20 years ago, but this was not further pursued at the time [14]). Together, these associations suggest that hyaline arteriolosclerosis may be a rough marker for loss of autoregulation.

METHODS

Patient material

Samples of noninvolved areas of eight kidneys removed for tumor or cysts on the Service of Urology at the St. Joseph Hospital, Paris, were studied. Specimens from eight patients normotensive at the time of operation were selected from a larger group of such specimens gathered for a previous study. Basic clinical data are given in Table 1. All patients were Caucasian and none was clinically diabetic. Serum creatinine at the time of operation was in the normal range in five patients and mildly elevated in two patients. The final patient (patient 7) had a serum creatinine of 278 μmol/L, apparently related to obstruction by a transitional cell carcinoma. The section of kidney uninvolved by tumor, however, revealed only focal cortical atrophy and no lesions attributable to hydronephrosis. Two patients had trace to 1+ proteinuria by dipstick examination. Proteinuria was not further quantified in these patients in whom clinical attention was directed to the neoplastic lesion.

Morphometry

Morphometric determinations of the different glomerular domains and parameters were made with the use of an automated image analyzing system, as detailed in a previous communication [15]. A total of 126 glomeruli and their associated arterioles were studied, including only those pairs with recognizable arteriolar and capillary lumens, thereby excluding totally sclerotic glomeruli. Measurements were performed on routine paraffin sections stained with periodic acid-Schiff (PAS) reagent only, without counterstain, which permitted definition of all extracellular matrix (ECM) components and arteriolar hyaline deposits. The image analysis system comprised four elements: (*1*) NS-400 light microscope (Nachet, Evry, France); (*2*) black and white video camera (Sony, Tokyo, Japan); (*3*) Image analysis processor (NS-15000, Nachet) that digitized each microscopic field into a 512 × 512 pixel image, with 256 gray levels; and (*4*) a microcomputer connected to the image processor, piloting all the predefined image transformations according to a personal program written in C language by one of the authors. Two interconnected programs were used.

Table 1. Clinical data and numbers of glomeruli and afferent arterioles measured morphometrically

Patient number	Age/ sex/race	Lesion	BP[a]	Serum creatinine	Urine protein[b]	Type of glomerulus				Arteriolar hyaline deposits			Total glomeruli/ arteriole pairs
						Normal	Hypertrophic	FSGS-type	Ischemic	None	Nonobstructive	Obstructrive	
1	85/M/C	Carcinoma, NOS	Normotensive	150	0	32	7	4	8	26	16	9	51
2	78/M/C	Oxyphil adenocarcinoma	Normotensive	120	Trace to 1+	5	2	3	1	5	2	4	11
3	52/M/C	Clear cell adenocarcinoma	Normotensive	85	Trace	12	1	0	1	11	2	1	14
4	69/M/C	Benign epithelial cyst	Normotensive	97	0	4	3	0	0	4	3	0	7
5	76/M/C	Papillary adenocarcinoma	Normotensive	160	0	10	3	0	0	6	6	1	13
6	37/M/C	Benign renal cyst	Normotensive	74	0	4	1	1	2	4	2	2	8
7	57/M/C	Transitional cell carcinoma, renal pelvis	Normotensive	278	0	6	4	0	0	6	3	1	10
8	60/M/C	Clear cell adenocarcinoma	Normotensive	120	0	9	1	2	0	8	3	1	12
Total						82	22	10	12	70	37	19	126

[a]Blood pressure ≤140/90 mm Hg
[b]Proteinuria measured by dipstick

The first program permitted measurement of all glomerular domains, including overall glomerulus bounded by Bowman's capsule, overall glomerular tuft, urinary space, total capillary surface, hilar capillary surface, number of capillaries, total ECM, surface of the mesangial domain (the mesangium plus overlying glomerular basement membrane (GBM) at points where the two come into contact. At each point, the program permitted the observer to correct provisional observations made by the computer (e.g., provisional division of overall glomerular space into glomerular capillaries versus urinary space), which was followed by correction of this image by transfer of objects from one category to the other (i.e., proposed capillaries into urinary space in the instance of urinary space trapped between adjacent lobes of glomeruli, and vice versa). In addition, the periglomerular space, to a distance of 25 μ circumferentially around the glomerulus was divided into the area of ECM (interstitial tissue plus tubular basement membranes) versus the area of non-ECM (tubular cytoplasm and lumens, arterioles and capillaries), with the two recorded separately and a ratio between the area of ECM and the total area derived.

The second program, analogous to that for glomeruli, collected data for their associated afferent arterioles. Area, length, width, and circumference were measured for the following arteriolar parameters: overall arteriole, lumen, wall both including and excluding hyaline deposits, hyaline deposits, thickness of muscularis in areas of deposits versus areas without deposits.

Morphologic variables

Glomeruli. Only those glomeruli with intact associated afferent arterioles were included in this study. They were initially divided into five types by routine microscopy. First, normal glomeruli that had with a completely patent capillary network, no excess of mesangium (less than three nuclei per mesangial stalk), and no areas of sclerosis, adhesion to Bowman's capsule, or podocyte alterations.

Second, hypertrophic glomeruli were characterized by combinations of two or more of the following features on examination: (*1*) evident increase in size in comparison to most surrounding glomeruli; (*2*) a dilated hilar capillary region in comparison to most surrounding glomeruli, substantially wider than the lumen of its feeding afferent arteriole, usually with evident dilatation of the primary branches; and (*3*) widely patent, often distended capillary loops, in the absence of increase in mesangium or any focal adhesions, sclerosis or podocyte alterations (Fig. 1).

Third, FSGS-type glomeruli had lesions typical of those seen in FSGS in varying stages (Fig. 1). Early lesions, usually perihilar, showed podocyte abnormalities, focal capsular adhesions and thickening of the mesangial matrix and narrowing of capillaries in the associated zones. More advanced lesions showed progressive extension of this process, with a minority of glomeruli showing capillary hyalinosis lesions.

Fourth, ischemic glomeruli showed progressive shrinkage of the tuft, compared with most surrounding glomeruli, with wrinkled capillary walls surrounding narrowed, but still patent capillary loops in most areas, with or without evidence of prior hyalinosis or other focal lesions. Totally sclerotic glomeruli with no patent capillary loops were excluded from consideration, and in fact, where the associated arterioles were identifiable, their lumens were occluded as well.

Fifth, indeterminate glomeruli in which distinctions between categories, normal versus hypertrophic or normal versus ischemic, could not readily be made on initial examination were preliminarily categorized as indeterminate. We then attempted to reclassify these cases, insofar as possible.

The glomeruli initially classified as normal, hypertrophic, and ischemic were analyzed. Since the values for almost all of the parameters constituted three clusters with some overlap between, (see Fig. 4 for an example of these clusters), it was thought that only those indeterminate glomeruli with values falling above the means of these parameters for the glomeruli initially characterized as hypertrophic, or below them for glomeruli characterized as ischemic, could be safely be reclassified as hypertrophic or ischemic. The question was which of these parameters was the most important in defining glomerular type. Stepwise multiple regression revealed these to be diameter of associated arteriolar lumens ($P = 0.000000$), area of hilar capillaries ($P = 0.000006$), area of intraglomerular ECM ($P = 0.01$), total area of glomerular capillaries ($P = 0.06$). The 43 glomeruli preliminarily categorized as indeterminate were then analyzed in terms of these parameters. Those indeterminate glomeruli whose values were simultaneously above the means for three of these four parameters for the hypertrophic group were reclassified as hypertrophic. Similarly, those indeterminate glomeruli with values below the means for three of the four parameters for the ischemic group were reclassified as ischemic. Thus, eight initially indeterminate glomeruli were regrouped with hypertrophic (four observations) or ischemic glomeruli (four observations). The remaining 35 initially indeterminate glomeruli were then reclassified as normal.

Afferent arterioles. Only arterioles clearly associated with a glomerulus and having patent lumens were included in this study. They were classified into three types. First, arterioles with no deposits showed no evidence of hyaline deposits in their walls. They were distinguished from efferent arterioles by the presence of a thicker smooth muscle wall and generally larger lumen. In case of doubt, the arteriole and its glomerulus were excluded from study.

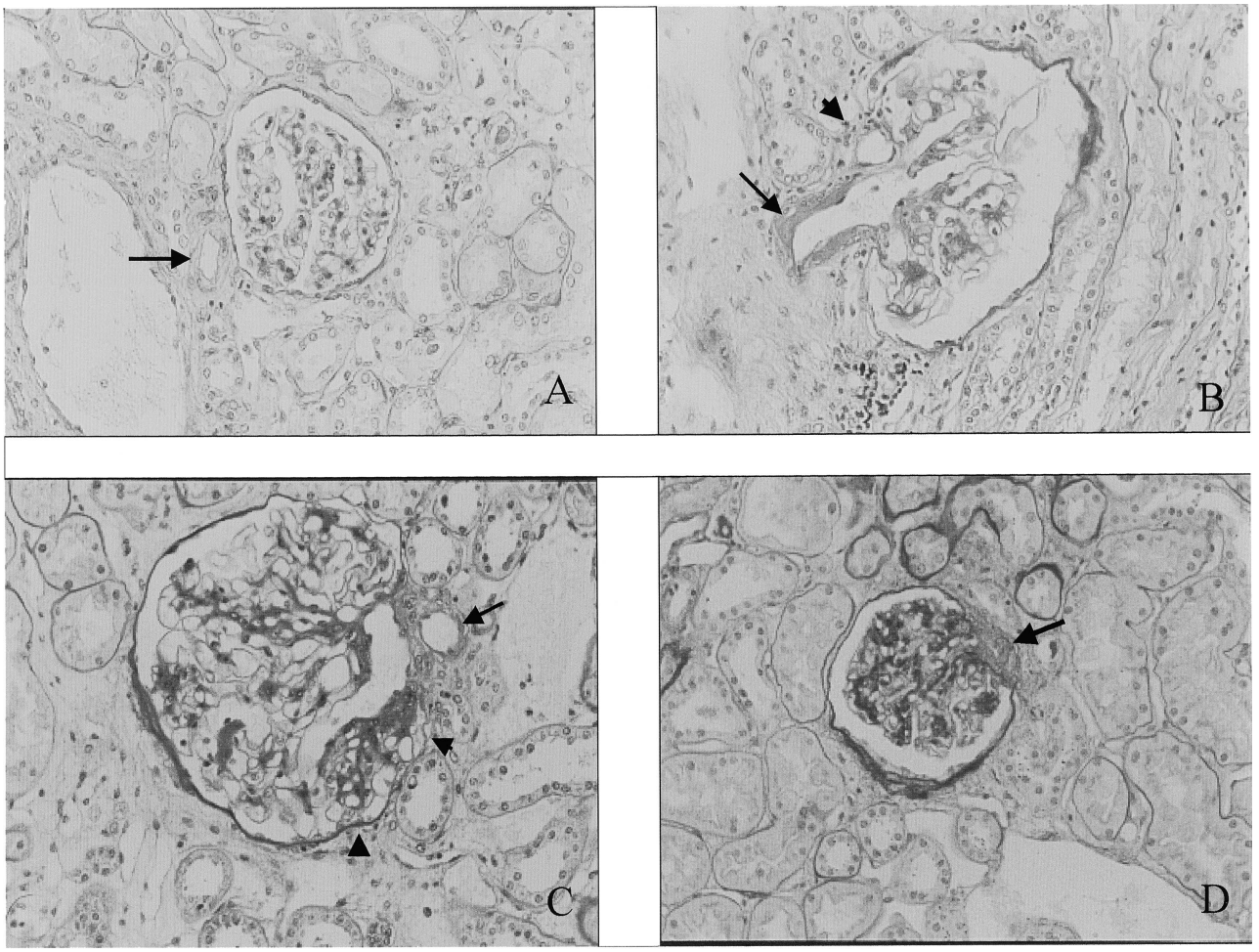

Fig. 1. Glomerular and arteriolar types. (*A*) A normal glomerulus and its associated afferent arteriole (arrow) without hyaline deposits. (*B*) A hypertrophic glomerulus, which although not particularly large in this plane of section, demonstrates the massive dilatation of hilar capillary and its first branches. Peripheral capillaries are dilated and a channel (arrowhead) leading to the efferent arteriole is also dilated. The dilated afferent arteriole (arrow) shows a massive nonobstructive hyaline deposit. (*C*) A focal segmental glomerulosclerosis (FSGS)-type glomerulus shows mesangial increase and sclerosis with capsular adhesions, particularly at hilus (arrowheads). Its associated afferent arteriole (arrow) shows nonobstructive deposits. (*D*) An ischemic glomerulus shows collapsing capillary loops with resulting small capillary lumens. Its afferent arteriole (arrow) is without deposits. All were stained with periodic acid-Schiff, ×250.

Second, nonobstructive hyaline deposits of variable size in a primarily subendothelial position were present, rarely with deposits deeper in the wall. They did not impinge on or change the convex contour of the lumen (Fig. 1).

Third, arterioles with obstructive hyaline deposits, generally larger, bulged into the lumen or altered its normal convex contour.

Caveats regarding pathologic material

The presence of tumors in six of the eight specimens studied may have led to glomerular scarring and compensatory glomerular hypertrophy beyond that expected due to simple aging. Thus, the relative percentages of the different types of glomeruli cannot be taken as necessarily representative of the proportions of such glomeruli in typical aging kidneys. Further, since morphometric studies are carried out on only a single section, it seems not unlikely that some glomeruli characterized as hypertrophic might reveal segmental scars in other sections. To the extent that this occurs, however, it would cause differences in statistical comparisons between hypertrophic and FSGS-type glomeruli to be underestimated rather than overestimated.

Statistics

Standard statistical methods were used, with *t* tests or Mann-Whitney tests to determine differences between means and standard deviations for the various parameters, depending on the normality of distribution of the variable. Chi^2 testing was used to determine the differences in distribution of categorical variables, such as arteriolar

Table 2. Arteriolar and glomerular parameters by arteriole type (measurements in μ or μ^2 as appropriate)

	No deposits 70 observations	←P→	Nonobstructive deposits 37 observations	←P→	Obstructive deposits 19 observations	No deposits vs. obstructive
Arteriolar parameters						
Lumen						
Area	204 ± 160	0.0000	482 ± 240	0.0014	268 ± 193	NS
Width	11.7 ± 3.9	0.0000	17.4 ± 4.7	0.0001	12.4 ± 3.2	NS
Circumference	58 ± 32	0.0000	102 ± 50	0.012	68 ± 37	NS
Wall areas						
Muscularis + extracellular matrix	951 ± 571	0.0018	1330 ± 600	0.0038	839 ± 521	NS
Hyaline deposits	0	—	101 ± 94	NS	118 ± 53	—
Total wall	951 ± 571	0.0001	1431 ± 620	0.0061	956 ± 525	NS
Thickness of muscle						
Nondeposit	15.9 ± 5.0	NS	16.5 ± 5.7	NS	14.2 ± 6.0	NS
Beneath deposits	—	—	7.8 ± 3.9	NS	6.7 ± 3.8	NS
Outer circumference	187 ± 86	0.0000	290 ± 104	0.0066	214 ± 75	NS
Wall/lumen ratio	6.4 ± 6.1	0.0018	3.2 ± 1.8	NS	4.4 ± 4.2	NS
Parameters of associated glomeruli						
Areas						
Total tuft	15657 ± 6224	0.04	18424 ± 4761	NS	15993 ± 6400	NS
Extracellular matrix	4788 ± 2855	NS	5285 ± 2301	NS	5065 ± 2282	NS
Total capillaries	10869 ± 5897	0.04	13104 ± 5572	NS	10928 ± 4923	NS
Hilar capillaries (total)	667 ± 492	0.0018	1276 ± 797	NS	1362 ± 799	0.0056
Other capillaries (mean)	83 ± 27	0.03	95 ± 28	0.08	81 ± 27	NS
% Extracellular matrix (periglomerular)	55 ± 16	NS	54 ± 14	0.001	68 ± 15	0.002
Glomerular types						
Normal	58 (82.8%)		11 (29.7%)		13 (68.4%)	
Hypertrophic	2 (2.9%)		19 (51.3%)		1 (5.3%)	
FSGS-type	1 (1.4%)		6 (16.2%)		3 (15.8%)	
Ischemic	9 (12.9%)		1 (2.7%)		2 (10.5%)	

Distribution of glomerular lesions by arteriole type, $Chi^2 = 270.8$; df = 11; $P = 0.000000$.

types, between groups. Pearson product-moment correlation coefficients were used to determine the correlations between continuous variables. Stepwise multiple regression was performed to determine the variables most closely associated with glomerular type.

RESULTS

Afferent arterioles

Differences between arteriolar types. Afferent arterioles with nonobstructive hyaline deposits differed markedly from those without deposits (Table 2). Their lumens were much larger, the area of the lumen being over twice as great (482 ± 240 μ^2 vs. 204 ± 160 μ^2, $P = <0.0000$), with corresponding increases in diameter and outer circumference. The muscle/lumen ratio was reduced as well (3.2 ± 1.8 vs. 6.4 ± 6.1, $P = 0.0018$). By contrast, the arterioles with obstructive hyaline deposits, presumably representing a later stage of lesion, showed no significant differences from arterioles with no deposits.

Glomerular parameters associated with arteriole types. Glomeruli associated with arterioles with nonobstructive deposits showed markedly larger hilar capillary spaces than those associated with arterioles without deposits (1276 ± 797 μ^2 vs. 667 ± 492 μ^2, $P = 0.002$). They also showed significantly larger overall glomerular tufts, larger total capillary area and mean area of individual capillaries (Table 2). The glomeruli associated with arterioles with obstructive hyaline deposits showed only two significant differences from those with no deposits: (*1*) a much larger hilar capillary space ($P = 0.0056$), and (*2*) the proportion of ECM surrounding their glomeruli was greater than for either no-deposit or nonobstructive deposit groups.

Figure 2 compares arteriolar diameters versus mean area of individual glomerular capillaries by arteriolar type. Values for arterioles with nonobstructive deposits are generally higher for both parameters than for arterioles with no deposits and there is relatively little overlap between the groups.

Glomerular types associated with arteriolar types. There was a marked difference in the types of glomerular lesions associated with each arteriolar type (Table 2 and Fig. 3). Arterioles with hyaline deposits (obstructive or nonobstructive) were very strongly associated with hypertrophic and FSGS-type glomeruli, representing 28 of 31 (90.3%) of these glomerular lesions. The reverse was true of arterioles without deposits, associated almost exclusively with either normal or ischemic glomeruli [67 of 70 (95.6%) of arterioles].

Arteriolar hyaline deposits

There was a significant correlation between the size of deposits in nonobstructive arterioles and the width of the arteriolar lumen (r = 0.4528, $P = 0.005$), but no other significant correlation with any wall parameter. It

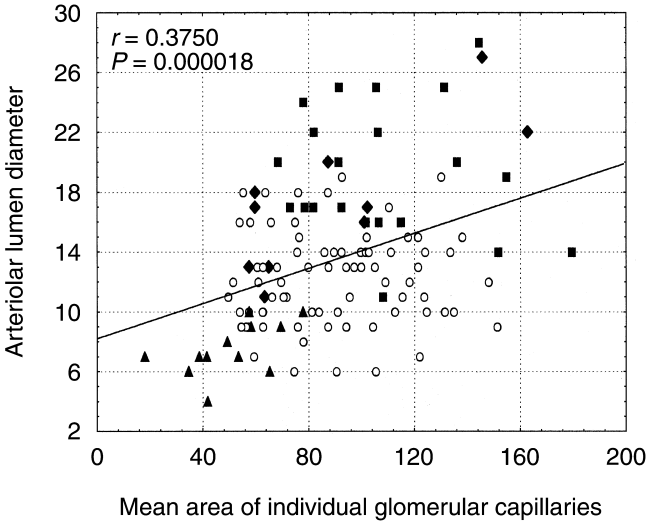

Fig. 2. Arteriolar diameter versus mean area of individual glomerular capillaries, categorized by arteriolar type. The types area normal (○), hypertrophic (■), focal segmental glomerulosclerosis (FSGS)-type (◆), and ischemic (▲). Note that values for arterioles with nonobstructive deposits are generally greater for both parameters, and that there is relatively little overlap with values for arterioles without deposits.

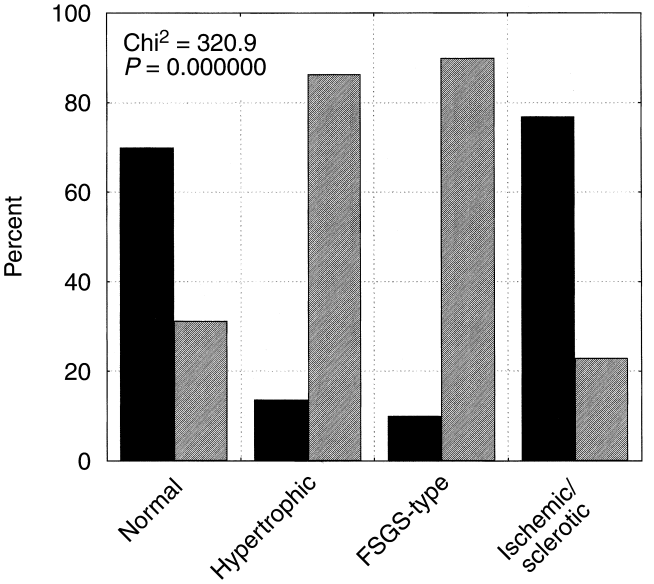

Fig. 3. Proportion of glomeruli of each type served by arterioles with (■) and without (▩) hyaline deposits. Note that the hypertrophic and focal segmental glomerulosclerosis (FSGS)-type glomeruli are served almost exclusively by arterioles with hyaline deposits.

was noted consistently that the hyaline deposits occurred in the subendothelial region, and only rarely in other locations and in those instances almost always with subendothelial deposits as well. A point of pathophysiologic significance was that the wall was almost always thinned out under the deposits, rather than simply being pushed down by the deposit. For all arterioles with deposits, the mean thickness of the wall in portions not involved by deposits was 15.9 ± 5.8 μ as compared to 7.4 ± 3.9 μ beneath the deposits ($P = 0.0000$). Similar results were obtained dividing deposits into obstructive and nonobstructive types.

Glomeruli

Differences between glomerular types. Hypertrophic glomeruli differed most strikingly from normal glomeruli in the size of the hilar capillary space (1756 ± 651 μ^2 vs. 657 ± 404 μ^2, $P = 0.0000$) (Table 3). The overall tuft, total glomerular capillary area and area of individual glomerular capillaries (excluding the hilar capillaries) were also significantly larger.

FSGS-type glomeruli resembled the hypertrophic glomeruli in many respects, differing significantly only in a greater amount of intraglomerular ECM, related to the sclerotic lesions. They also had a significantly greater percentage of ECM in the immediate periglomerular region than hypertrophic glomeruli (67 ± 16% vs. 55 ± 14%, $P = 0.02$). The hilar capillary space was still increased compared to normal glomeruli, but the mean area of individual glomerular capillaries did not differ from that in normal glomeruli. Our impression from routine light microscopy was that there was shrinkage of the glomerular capillary loops in sclerosing areas. Consistent with this observation was the fact that in FSGS-type glomeruli there was an inverse relation between the amount of mesangium and the mean size of individual capillary loops ($r = -0.56$, $P = 0.075$). By contrast, still-intact areas of these FSGS-type glomeruli continued to show obviously dilated capillaries on routine microscopy, at least in the early stages. However, our program does not permit measurements of individual regions of the glomeruli to confirm this impression.

Ischemic glomeruli were, as anticipated, smaller in every parameter measured and showed much the highest percentage of ECM in the periglomerular area.

Arteriolar parameters associated with glomerular types. The arterioles associated with both hypertrophic and FSGS-type glomeruli had lumens roughly twice the size of those of normal glomeruli, with corresponding increases in width and circumference (Table 3). Equally, the size of arteriolar hyaline deposits was much greater in both types of glomeruli. Further, the wall/lumen ratio was markedly reduced in both types of glomeruli. By contrast, the arterioles associated with ischemic glomeruli had significantly smaller lumens, and the overall wall was smaller than that associated with normal glomeruli. Here, the wall/lumen ratio increased dramatically (10.3 ± 12.9 vs. 5.3 ± 3.2, $P = 0.003$) compared to arterioles associated with normal glomeruli.

Types of arterioles associated with glomerular lesions. The vast majority (90.6%) of arterioles associated with hypertrophic and FSGS-type glomeruli had hyaline de-

Table 3. Glomerular and arteriolar parameters by glomerular yype measurements in μ or μ^2 as appropriate

	Normal 82 observations	←P→	Hypertrophic 22 observations	←P→	FSGS-type 10 observations	P vs Nl	Ischemic 12 observations	P vs Nl
Glomerular parameters								
Areas								
Total tuft	16478 ± 7324	0.012	20840 ± 6368	NS	21229 ± 6279	0.05	6550 ± 3614	0.0000
Extracellular matrix	4995 ± 2592	NS	5491 ± 2446	0.005	7170 ± 2440	0.01	2561 ± 869	0.003
Total capillaries	11471 ± 5282	0.002	15349 ± 4577	NS	14058 ± 4551	NS	3899 ± 2891	0.0000
Hilar capillaries (total)	738 ± 522	0.0002	1520 ± 758	NS	1227 ± 876	0.05	419 ± 325	NS
Other capillaries (mean)	87.2 ± 25.6	0.012	102.8 ± 24.8	NS	86.7 ± 34.6	NS	49.7 ± 18.1	0.0000
% Extracellular matrix (periglomerular)	53 ± 14	NS	53 ± 14	0.01	67 ± 17	0.003	80.0 ± 6.1	0.0000
Arteriolar parameters								
Lumen								
Area	237 ± 159	0.0000	542 ± 253	NS	450 ± 255	0.0004	103 ± 107	0.0000
Width	12.3 ± 3.2	0.0000	19.1 ± 4.4	NS	17.4 ± 4.7	0.0000	7.6 ± 1.9	0.0000
Circumference	63.6 ± 30.6	0.0000	115 ± 62	NS	87.0 ± 33.2	0.03	41.7 ± 26.7	0.02
Wall								
Area of muscle + extracellular matrix	982 ± 474	0.0000	1584 ± 783	0.03	940 ± 611	NS	585 ± 303	0.005
Area of deposits	30.5 ± 58.5	0.0008	90.0 ± 109.5	NS	120.2 ± 63.5	0.0000	23.8 ± 59.6	NS
Area total	1012 ± 486	0.0000	1674 ± 785	0.04	1059 ± 624	NS	609 ± 300	0.0000
Outer circumference	188 ± 69	0.0000	302 ± 141	0.05	204 ± 86.8	NS	133 ± 73	0.01
Wall/lumen ratio	5.28 ± 3.19	0.01	3.45 ± 2.34	0.06	1.98 ± 0.44	0.002	10.76 ± 13.37	0.002
Arteriolar types								
No deposits	58 (70.7%)		2 (9.1%)		1 (10%)		9 (75.0%)	
Nonobstructive deposits	11 (13.4%)		19 (86.1%)		6 (60%)		1 (8.3%)	
Obstructive deposits	13 (15.8%)		1 (4.5%)		3 (30%)		2 (16.7%)	

Distribution of arteriolar types by glomerular lesion type, $Chi^2 = 270.8$; df = 11; $P = 0.000000$.

posits, predominantly of the nonobstructive type (Table 3 and Fig. 3). By contrast, arterioles without deposits were associated primarily with normal glomeruli (68%) and those showing ischemia and sclerosis (75%) (Table 2, Fig. 3). (These differences were so striking that they raised the possibility that perhaps the categorization of glomeruli as normal versus hypertrophic might have been unconsciously influenced by the type of associated arteriole. However, combining normal and hypertrophic glomeruli into a single group and dividing them strictly according to arteriolar type produced results that showed much less separation of values than did division by type of glomerulus. For example, for mean area for individual capillaries, division by glomerular type gave a split of 89.3 ± 25.8 μ^2 vs. 109.5 ± 30.3 μ^2, $P = 0.003$, compared to 92.4 ± 25.8 μ^2 vs. 97.8 ± 30.9 μ^2, $P = 0.39$ for division by arteriolar type.)

Correlations between glomerular capillary and arteriolar lumen parameters

Both arteriolar luminal area and diameter showed good correlations with the mean area of individual glomerular capillaries, but better with diameters since the measurement of areas depends more on the plane of section. For the series overall, there was a very good correlation between area of individual glomerular capillaries and arteriolar lumenal diameter (r = 0.3750, $P = 0.00002$), and yet better when only arterioles with nonobstructive hyaline deposits were considered (r = 0.4741, $P = 0.004$).

Graphically, cases plotted by glomerular type show distinctive clusters, with ischemic glomeruli at the lower end for both capillary area and arteriolar diameter, normal glomeruli clustered in the midrange, and hypertrophic glomeruli at the upper end (Fig. 4). FSGS-type glomeruli, by contrast, are more disperse. Some show high values, in the range of hypertrophic glomeruli, while others, presumably representing more advanced lesions, are found in the lower ranges occupied by normal and ischemic glomeruli.

Periglomerular ECM

Not surprisingly, there is a progressive increase in the proportion of ECM in the immediate periglomerular area with increasing severity of lesions. Figure 5 compares the proportion of ECM with type of glomerular lesions. To be noted, although most normal glomeruli have a proportion of ECM below 0.60, some ostensibly normal glomeruli have a very high proportion of surrounding ECM, as do many hypertrophic glomeruli. However, glomeruli with overt FSGS-type lesions have a higher threshold of ECM and a higher general proportion of ECM than normal glomeruli (0.67 ± 0.17 vs. 0.55 ± 0.15, $P = 0.05$). Virtually all ischemic glomeruli are associated with high proportions of periglomerular ECM (Fig. 5). Equally, there is a strong inverse correlation between the proportion of periglomerular ECM and total glomerular capillary surface (r = −0.4351, $P = 0.000000$) or alternately mean area of individual capillaries (r = −0.4665, $P = 0.000000$).

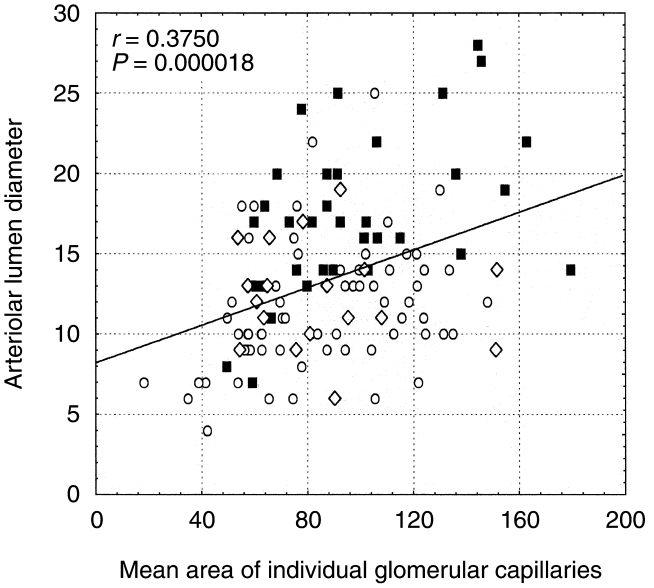

Fig. 4. Arteriolar diameter versus mean area of individual glomerular capillaries, categorized by glomerular type. Types are no deposits (○), nonobstructive deposits (■), and obstructive deposits (◇). Note that values for normal and hypertrophic glomeruli tend to cluster, those for hypertrophic glomeruli being greater for both parameters. As anticipated, the values for ischemic glomeruli cluster in the lower ranges. By contrast, the values for focal segmental glomerulosclerosis (FSGS)-type glomeruli are quite disperse. Some lie in the range of hypertrophic glomeruli, while others, presumably with more advanced lesions, lie within the range for normal glomeruli.

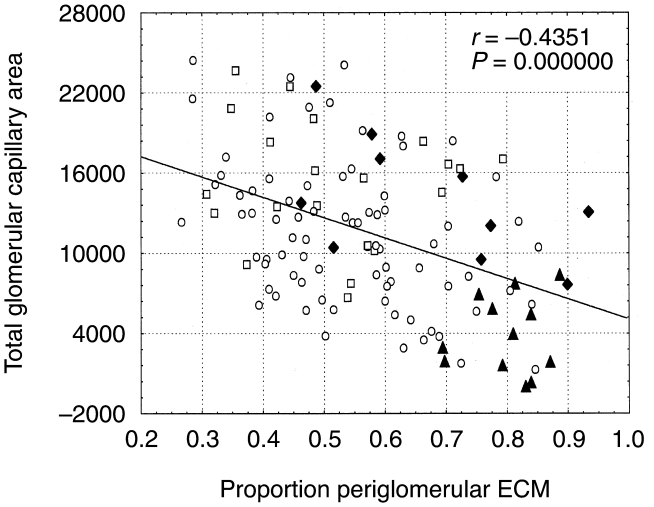

Fig. 5. Percentage of extracellular matrix (ECM) in the periglomerular region compared to total glomerular capillary area. Types of glomeruli are normal (○), hypertrophic (□), focal segmental glomerulosclerosis (FSGS)-type (◆), and ischemic (▲). Values are quite disperse, with even normal glomeruli occasionally having a high proportion of ECM in the periglomerular region. However, values for FSGS-type glomeruli start at higher levels and are generally displaced to the right. Ischemic glomeruli have a very high proportion of ECM in the periglomerular region.

DISCUSSION

Arterioles

The single most striking observation in this study is that nonocclusive hyaline deposits occur in arterioles with dilated lumens. Comparison with lumens of arterioles without deposits revealed the lumens to be well over twice as large on average (Table 2), with corresponding increases in the width and circumference of the lumen. These markedly dilated lumens have implications for the pathogenesis of the hyaline lesions. Deposits occur almost invariably in areas where the smooth muscle is thinner (55 of 56 observations). LaPlace's Law indicates that the circumferential mural tension is directly proportional to the radius of the vessel and inversely proportional to the thickness of the wall. Circumferential tension would thus be increased in such dilated arterioles, and the ability of the thinned smooth muscle to contract against this tension would be diminished. Using average values for the arterioles with nonobstructive deposits in this study, it can be calculated that the tension would theoretically be increased by roughly twofold in areas of deposits, even assuming the underlying muscle was normal. And, in fact, prior electron microscopic studies indicate that the muscle under the deposits is usually atrophic [16]. Clearly, cause and effect relationships between the atrophic smooth muscle and the deposits remain to be determined. What is clear is that the size of the deposits and the dilatation of the lumen go hand in hand. There is a very good correlation between the size of the hyaline deposits and the width of the arteriolar lumen ($r = 0.4306$, $P = 0.000000$).

The implication of this dilatation is that there would be less diminution of pressure by the arteriole and that this greater pressure would be transmitted to the glomerulus. The glomerular parameters are all consistent with this interpretation, particularly the area of the hilar pole capillary, well over twice as large, with lesser increases in the area of other glomerular capillaries as well (Table 2).

Glomeruli

Before commencing this study, it was our intuitive view that there is a spectrum of glomerular types progressing from normal through hypertrophic to glomeruli with frank lesions of the type seen in FSGS. We believe that the results obtained support that notion.

Hypertrophic glomeruli appear to represent a distinct category, rather than simply being the far end of the spectrum of normal glomeruli. They have larger glomerular tufts and much larger hilar capillaries, as well as somewhat larger capillaries in the remainder of the tuft (Table 3). More important, the arterioles serving them are vastly different in caliber of lumen and the great majority of them have nonobstructive hyaline deposits, as opposed to normal glomeruli, which are served for the most part by arterioles without deposits. (This difference in associ-

ated arterioles is very striking. However, grouping normal and hypertrophic glomeruli and dividing them simply by arteriole type produces much less separation between groups than division by glomerular type.)

Additional support for the notion of hypertrophic glomeruli as a separate category comes from their similarities with FSGS-type glomeruli, in terms of overall size, overall capillary area, and size of hilar capillaries. They differ only in ways that might be anticipated: overall amount of ECM, both intraglomerular and extraglomerular, and the mean size of the individual glomerular capillaries (though the latter in FSGS glomeruli appear to be composed of two subpopulations, shrunken in areas of sclerosis and dilated in still-intact areas).

This spectrum of lesions from normal through hypertrophic to FSGS-type, with initial increase in size followed by decrease as the glomeruli become sclerotic, parallels exactly that shown by Yoshida et al [17] in experimental models of FSGS. Experimentally, glomerular hypertrophy and glomerular capillary hypertension have been associated with one another in a number of models [8, 12, 13], as well as with increased single-nephron glomerular filtration rate (SNGFR) (summarized in [8]). Further, it is well-established in experimental models that such glomerular hypertrophy and hyperperfusion leads to glomerulosclerosis of the FSGS-type [8, 18].

In humans, the same sequence of glomerular hypertrophy followed by sclerosis with FSGS features exists in diabetes and in FSGS, both of the primary and secondary forms [19–22]. Thus, it is logical to assume that glomeruli of the FSGS-type in humans often are, or have been, victims of the same sort of hyperperfusion and hyperfiltration seen experimentally, although this has only been formally demonstrated in diabetes [21, 22], and there, only on a global basis, not at the level of the individual glomeruli. We believe the present study offers morphologic data consistent with the occurrence of this phenomenon on a focal basis in the aging kidney.

Loss of autoregulation

The association of dilated afferent arterioles with hypertrophic and FSGS-type glomeruli implies that these glomeruli have suffered loss of autoregulation. Experimentally, most models of loss of autoregulation show afferent arteriolar dilatation from the outset [3, 5, 11, 23, 24], with variable efferent arteriolar dilatation or constriction. This is attended by increased glomerular capillary pressure [1, 8], and in other studies these models have been shown to have an increased glomerular filtration rate (GFR) and increased protein excretion. In all of the models in which there is impairment or loss of autoregulation, there is glomerular enlargement and eventual glomerulosclerosis of the FSGS-type [1, 6, 9, 25, 26].

In this morphometric study we have demonstrated a population of arterioles and glomeruli in the aging kidney of humans with features comparable to those seen with loss of autoregulation experimentally, dilatation of afferent arterioles and increase in the size of the glomerular tuft and glomerular capillaries (Tables 2 and 3). It seems likely that they are associated with focal loss of autoregulation in humans as well.

Loss of autoregulation has indeed been demonstrated in humans on a global, whole-kidney basis in two clinical settings, diabetes and proteinuria of various nondiabetic etiologies [27, 28]. In these studies, lowering of the blood pressure with clonidine led to marked diminution of the GFR, indicating loss of autoregulation, in those with diabetic nephropathy or proteinuria, whereas diabetics without nephropathy and control subjects showed much lesser changes in GFR. This phenomenon has yet to be demonstrated in essential hypertension or normotensive subjects and will pose more of a challenge in these patients in whom only a small percentage of glomeruli are likely hyperfiltering at any one time, as compared to near-universal hyperperfusion/hyperfiltration in diabetics.

CONCLUSION

In this study we have demonstrated nonobstructive arteriolar hyaline deposits are associated with marked dilation of the arteriolar lumen. These arterioles are in turn strongly associated with hypertrophic glomeruli, closely resembling glomeruli demonstrated in experimental models to have augmented glomerular capillary pressure and GFR. These hypertrophic glomeruli are larger in size, have markedly dilated hilar capillaries and larger individual capillaries. They form a continuum with glomeruli showing FSGS-type lesions. By contrast, normal glomeruli and those associated with ischemic sclerosis are associated predominantly with arterioles without hyaline deposits.

These dilated arterioles and hypertrophic and FSGS-type glomeruli exactly parallel the changes seen experimentally in situations of loss of autoregulation and likely represent the same phenomenon in humans. If so, in human's hyaline, arteriolosclerosis appears to serve as a rough marker for this loss of autoregulation and may well participate in the pathogenesis of the associated glomerular lesions.

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