Kidney International, Vol. 62 (2002), pp. 1947–1957

Atubular glomeruli in a rat model of polycystic kidney disease

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Background. Autosomal-dominant polycystic kidney disease (ADPKD) is associated with a progressive decline in glomerular filtration rate (GFR) that often leads to end-stage renal disease. The basis for this decline in GFR is poorly understood.

Methods. Glomeruli in heterozygous Han:SPRD rats with ADPKD and their normal litter mates were studied by light microscopy, using serial sectioning techniques. The connections of the renal corpuscles to proximal tubules were classified as normal, atrophied, or absent (atubular glomerulus). Renal corpuscles also were examined by scanning electron microscopy. Single nephron glomerular blood flows were determined using microspheres.

Results. In the kidneys of six-month-old rats with ADPKD, 50% of the glomeruli were atubular and another 26% were associated with atrophied neck segments; these glomeruli were most often smaller in size than normal. About 16% of the glomeruli were hypertrophied and had normal connections to proximal tubules. Sclerotic changes in cystic kidney glomeruli were usually mild or moderate, and belied the failure of glomerular function. Glomerular blood flow in the cystic kidneys averaged half of normal and was markedly heterogeneous; the majority of small glomeruli displayed very low blood flows and a few showed relatively high blood flows. Fewer glomerular abnormalities were found in rats treated for five months with potassium citrate in their drinking water.

Conclusions. The diminished GFR in the rat with ADPKD can be accounted for largely by the formation of atubular glomeruli. Compensatory glomerular hypertrophy also is present and may contribute to the progression of the renal disease.

Autosomal-dominant polycystic kidney disease (ADPKD) is a common genetic disorder that affects about 500,000 people in the United States and millions more worldwide. In this disease, epithelial cysts form and grow in both kidneys. Glomerular filtration rate (GFR) commonly declines, over many years or decades, to values so low that dialysis or a kidney transplant is necessary to sustain life. The mechanisms involved in the progressive decline in GFR are poorly understood.

During the course of microdissection studies on cystic kidneys of rats with ADPKD [1], we often found glomeruli that were disconnected from proximal tubules (unpublished observations). Some of these "atubular glomeruli" might have been an artifact, since a glomerulus may be separated accidentally from its tubule during microdissection, but these were observed even when meticulous technique was used. In the present study, using serial sectioning techniques, we sought evidence for the existence of atubular glomeruli in cystic rat kidneys. We hypothesized that the presence of atubular glomeruli might explain the reduced GFR in ADPKD.

Key words: atubular glomerulus, autosomal-dominant polycystic kidney disease, Han:SPRD rat, glomerular filtration rate and PKD, progressive renal disease, hypertrophy.

Received for publication March 26, 2002
and in revised form July 9, 2002
Accepted for publication July 31, 2002

METHODS

Animals and protocols

Experiments were done on six-month-old male normal rats (+/+) and heterozygous rats with ADPKD (cy/+) of the Han:SPRD strain. The rats were provided with either tap water or a solution of 55 mmol/L tripotassium citrate/67 mmol/L citric acid (abbreviated "KCitr") to drink beginning at one month of age. For the histological studies, we used kidney tissue samples collected from rats studied earlier [2]. The kidneys had been perfusion-fixed with a 3% paraformaldehyde, 137 mmol/L NaCl, 2.7 mmol/L KCl, 1.5 mmol/L KH_2PO_4, 4 mmol/L Na_2HPO_4, and 2 mmol/L picric acid solution, and stored in 0.1 mol/L cacodylate solution (pH 7.25) in a refrigerator. In these same experiments, kidney GFR was measured from the clearance of polyfructosan, a synthetic inulin.

Three normal rats and three rats with PKD, all six-month-old males, were placed in metabolic cages, and 24-hour urinary protein excretion (sulfosalicylic acid method) was measured.

Glomerular blood flow was measured using microspheres in seven +/+ and four cy/+ six-month-old male rats, as described below. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Evaluation of serial sections

Kidney tissue was routinely embedded in paraffin, and serial sections were cut and stained with hematoxylin and eosin (H&E). The mean section thickness for each kidney was determined using a confocal microscope (LSM 510; Zeiss, Oberkochen, Germany) by measuring section thickness at three different areas on each slide, and then averaging the values for 10 slides per rat kidney. Section thickness averaged 7.3 ± 0.06 μm, with a coefficient of variation in each kidney ranging from 1.1 to 3.6% ($N = 9$ kidneys).

Images of about 40 (range 39 to 45) renal corpuscles from the outer cortex of each animal were recorded with a digital camera (Polaroid DMC, Cambridge, MA, USA), Adobe Photoshop software, and an Apple computer. Glomeruli were identified on one or two slides from four to seven separate regions of the outer cortex of each kidney. The regions were selected by moving the microscope stage two fields at ×50 to 80. Five to ten glomeruli were identified and photographed from the selected region. Then images of one to five of these glomeruli were usually recorded on a digital file at a magnification of ×100 to 160. Images from above and below the initial section were recorded from adjacent sections until the renal corpuscles disappeared.

Individual renal corpuscles were examined for the type of connection between the space of Bowman's capsule and the lumen of a proximal tubule. The connection was classified as either (*1*) atubular (no visible connection), (*2*) atrophied (flattened epithelium, with no connection to a proximal tubule visible), or (*3*) normal (Bowman's capsule continuous with a normal proximal tubule). The areas of both the glomerulus and the entire corpuscle were measured by tracing, using the Scion Image program. From the respective areas and the mean section thickness, we calculated the glomerular and corpuscle volumes (Cavalieri principle).

Calculated volumes were corrected for shrinkage that results from tissue processing (embedding and staining). Shrinkage was determined by photographing and measuring small tissue pieces before and after processing. Shrinkage in one dimension averaged 28 ± 7% ($N = 3$) in the normal kidneys and 26 ± 12% ($N = 3$) in the cystic kidneys. Since shrinkage was not significantly different in the normal and cystic kidneys and averaged 27%, and assuming that shrinkage was equal in all directions, all glomerular and corpuscle volume measurements were multiplied by 2.57 (that is, $1 \div 0.73^3$).

Scanning electron microscopy (SEM)

Tissues from normal and cystic kidneys were processed for SEM. Samples were post-fixed in osmium tetroxide, dehydrated through a series of graded ethanols, critical point dried, and coated with gold-palladium. The specimens were examined using an AMR 1000A scanning electron microscope at an accelerating voltage of 20 kV. We photographed 24 glomeruli (visceral epithelial cells) in normal kidneys and 34 glomeruli in cystic kidneys. The parietal epithelial cell surface also was photographed from 17 renal corpuscles in normal kidneys and 12 renal corpuscles in cystic kidneys. A morphological evaluation was performed on each microphotograph in a blinded fashion.

Evaluation of glomerular morphology by light microscopy

A pathologist evaluated fixed kidney tissue samples from three normal rats, five rats with PKD that drank tap water, and four rats with PKD that had drunk the KCitr solution from one month of age from our previous study [2]. Three samples from each group had been randomly selected earlier for the more labor- and time-intensive serial section analysis and SEM. Three-micron sections of paraffin-embedded tissue were stained with Jones' silver stain and evaluated for glomerular morphology. The glomeruli were graded from normal to severe changes, depending on the amount and distribution of glomerular matrix, glomerular basement membrane wrinkling and/or capillary collapse, and glomerular size (see Table 3). Adhesions of the glomerular tuft to the capsular wall (synechiae) were counted also. A structure was considered to be a synechia only if the vascular pole was clearly identified and the glomerular basement membrane adhesion was associated with thickening or distortion of Bowman's capsule. Evaluation was done in a blinded fashion, using a Zeiss light microscope at ×250. One hundred glomeruli per rat were studied.

Glomerular blood flow measurements

Rats were anesthetized with Inactin (130 mg/kg body weight IP) and placed on a heated animal board. Rectal temperature was maintained at 37 to 38°C. Surgical procedures included (*1*) a tracheostomy, (*2*) cannulation of the right femoral vein [for infusion of a solution containing 125 mmol/L NaCl, 24 mmol/L $NaHCO_3$, and 2 g/dL bovine serum albumin (BSA) at 3 mL/hr], (*3*) cannulation of the right femoral artery (for measurement of arterial blood pressure with a Statham P23Db transducer and Beckman Dynograph recorder), (*4*) cannulation of the left femoral artery (for reference blood collection), and (*5*) cannulation of the right carotid artery (for injection of microspheres). The animal was heparinized prior to injection of the microspheres.

Non-radioactive carbonized microspheres (3M Co., St. Paul, MN, USA), 8.5 ± 0.8 μm in diameter, were suspended in 10% Ficoll-70 (Pharmacia, Uppsala, Sweden) and 0.08% Tween 80 in 0.9% NaCl. One-half or one milliliter of a freshly sonicated suspension containing 4×10^6 microspheres/mL was injected slowly (1 to 2 min) into the root of the aorta via the carotid cannula.

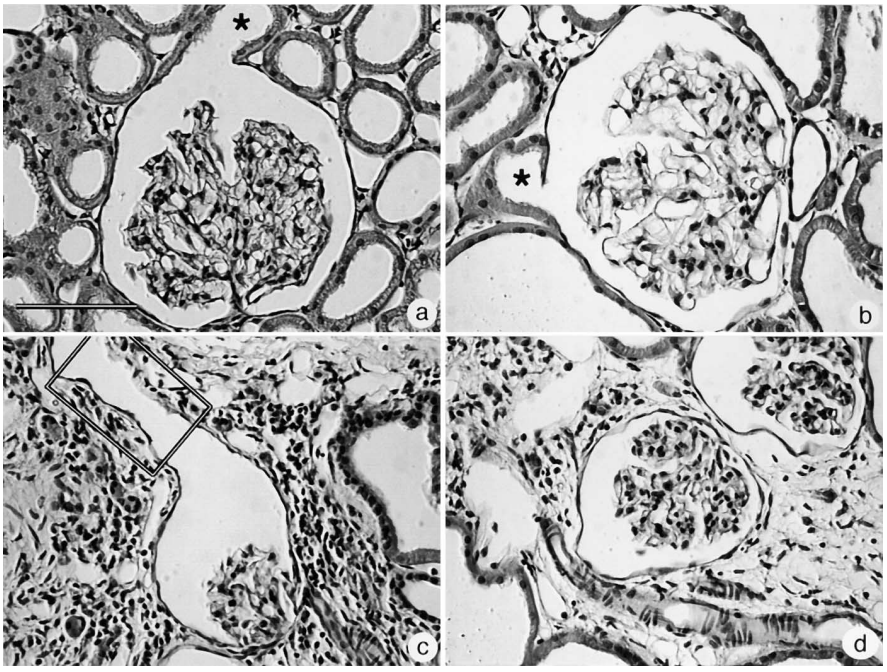


Fig. 1. Photographs of representative hematoxylin and eosin-stained sections of outer cortical glomeruli from normal (*A*) and cystic (*B, C, D*) kidneys. In A and B, Bowman's capsule opens directly into an open proximal tubule (asterisks). In C, a small glomerulus is associated with an atrophied neck segment (see rectangle) lined by indistinct flattened cells. In D, an atubular glomerulus is seen in the center of the field; serial sectioning failed to disclose any connection of Bowman's capsule with an open proximal tubule segment. All figures are at the same magnification. Scale bar, in panel A, equals 100 μm.

Collection of a reference blood sample from the left femoral artery, by free flow, was started 10 seconds before the microsphere injection. The arterial cannula was flushed with 0.9% NaCl for 30 seconds after the microsphere injection, and the reference blood collection was terminated 15 seconds later. Reference blood volume was determined by weighing (assuming a blood density of 1.05 g/mL), and the reference blood flow rate was calculated from the volume and collection time. The blood was hemolyzed and then filtered through a 3-μm-pore-diameter filter (Millipore, Bedford, MA, USA) and the number of microspheres collected was determined by counting under a microscope. The blood flow corresponding to one microsphere was calculated from the reference blood flow rate divided by the total number of microspheres. The left kidney was removed, frozen, and later microdissected after maceration with 8 N HCl at 60°C for 50 minutes. In each experiment, the number of microspheres in 40 glomeruli from the outermost part of the cortex was counted. Glomerular volumes were estimated by photographing the glomeruli using Nomarski optics, measuring the mid-section area, calculating the radius assuming that the glomerular section was circular, and calculating the volume from the radius (assuming that the glomeruli were perfect spheres).

Statistical methods

Data are presented as means ± SD. They were analyzed by linear regression or by analysis of variance (ANOVA) after a preliminary test for homogeneity of variances. Individual groups were compared with the Bonferroni method. If variances were heterogeneous, the Kruskal-Wallis test and Dunn's test were used to compare means. A P value of less than 0.05 was considered significant.

RESULTS

Figure 1 shows representative histological sections with the different types of renal corpuscle–proximal tubule connections. Figure 1A is from a normal kidney, and shows Bowman's space connected to the open lumen of a normal proximal tubule. There is an abrupt transition from the flattened parietal epithelial cells of Bowman's capsule to the cuboidal proximal tubule cells with their characteristic brush border. A similar connection is seen in the cystic kidney corpuscle-tubule junction in Figure 1B. In Figure 1C, Bowman's capsule is continuous with an atrophied tubule segment; the epithelium of the tubule portion is flat. Figure 1D is a section through an atubular glomerulus; serial sections through the renal corpuscle failed to reveal any opening from Bowman's capsule into a proximal tubule. The majority of atubular glomeruli were smaller than glomeruli attached to normal tubules.

Table 1 summarizes the different types of renal corpuscle-proximal tubule connections observed in three normal kidneys and three cystic kidneys. In the normal kidneys, only 4 out of 123 renal corpuscles had no apparent opening to a tubule and no atrophied tubule segments were seen. By contrast, in the cystic kidneys, 50% of the glomeruli were atubular, and another 26% of the

Table 1. Numbers of different types of connections between Bowman's capsule and the proximal tubule in three 6-month-old normal (+/+) and three heterozygous cystic (cy/+) Han:SPRD rats

Rat	GFR *μL/min/kidney/ 100 g body weight*	Atubular	Atrophied tubule	Normal tubule	Total
Normal 1	405	1	0	42	43
Normal 2	378	1	0	39	40
Normal 3	390	2	0	38	40
Mean ± SD	391 ± 14	1 ± 0.6	0 ± 0	40 ± 2	41 ± 2
Cystic 1	137	23	14	8	45
Cystic 2	109	18	11	10	39
Cystic 3	105	22	8	11	41
Mean ± SD	117 ± 17	21 ± 2.6	11 ± 3	10 ± 1.5	42 ± 3

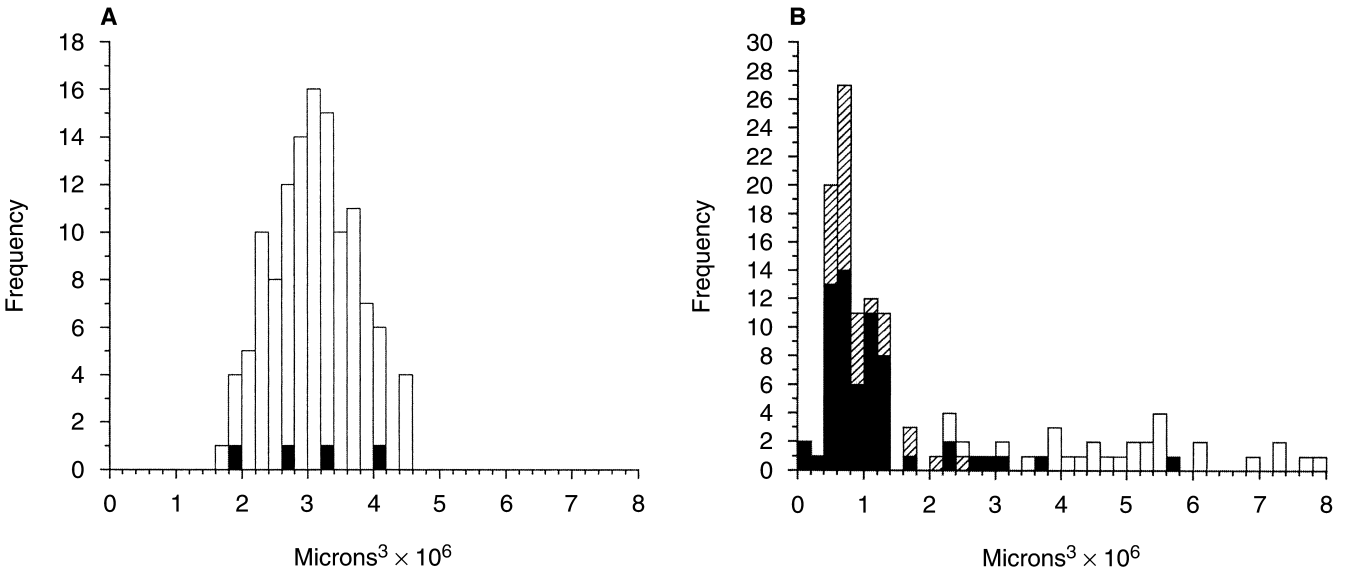


Fig. 2. Frequency histograms of glomerular volumes from three normal (*A*) and three cystic (*B*) rat kidneys. The ordinate gives the number of glomeruli in each class and the abscissa is the glomerular volume. The nature of the connection of the glomerulus to the proximal tubule (normal, atrophied, or atubular) is also indicated. Symbols are: (■) atubular; (□) tubular; (▨) atrophied.

glomeruli were associated with atrophied tubule segments. Kidney GFR in the cystic kidneys was reduced to 30% of normal. These results suggest that the reduced GFR in the cystic kidneys has a structural basis, and is mainly due to the formation of atubular glomeruli.

Figure 2 shows frequency histograms of glomerular volumes from normal rats and rats with PKD. The histograms indicate the types of connections between renal corpuscles and tubules. The glomerular volumes of normal (Fig. 2A) and cystic (Fig. 2B) kidneys differ strikingly. Glomerular volumes in normal kidneys were distributed in a bell-shaped fashion over a range of 1.8 to 4.5 × 10^6 μm^3, and averaged 3.1 ± 0.6 × 10^6 μm^3 (N = 123). In cystic kidneys, the glomerular volumes were more broadly distributed (ranging from 0.1 to 8.0 × 10^6 μm^3) and skewed to the right, and averaged 1.9 ± 2.0 × 10^6 μm^3 (N = 125). The majority (69%) of glomeruli had volumes below the smallest glomerulus seen in the normal kidneys; these small glomeruli were invariably atubular or connected to atrophied tubules. Sixteen percent of the glomeruli in cystic kidneys were larger than any glomeruli in the normal kidneys. With one exception, these large glomeruli were connected to open proximal tubules. Overall, the results suggest that the kidneys in the six-month-old rats with PKD are mainly populated by small glomeruli, while normal-sized glomeruli and glomeruli that have undergone compensatory hypertrophy are present in roughly equal numbers.

Figure 3 shows frequency histograms of renal corpuscle volumes from normal and cystic kidneys. The pattern seen is similar to that observed for glomerular volumes. Corpuscle volumes in normal kidneys were more uniform and averaged 4.9 ± 1.0 × 10^6 μm^3, with a range from 2.3 to 7.9 × 10^6 μm^3. By contrast, corpuscle volumes in cystic kidneys averaged 4.1 ± 3.2 × 10^6 μm^3, with a range from 0.4 to 18.7 × 10^6 μm^3. A large number of unusually small renal corpuscles and some unusually large renal corpuscles were found in the cystic kidneys.

Urine protein excretion, in mg/day per 100 g body weight, averaged 1.8 ± 0.6 in three normal six-month-

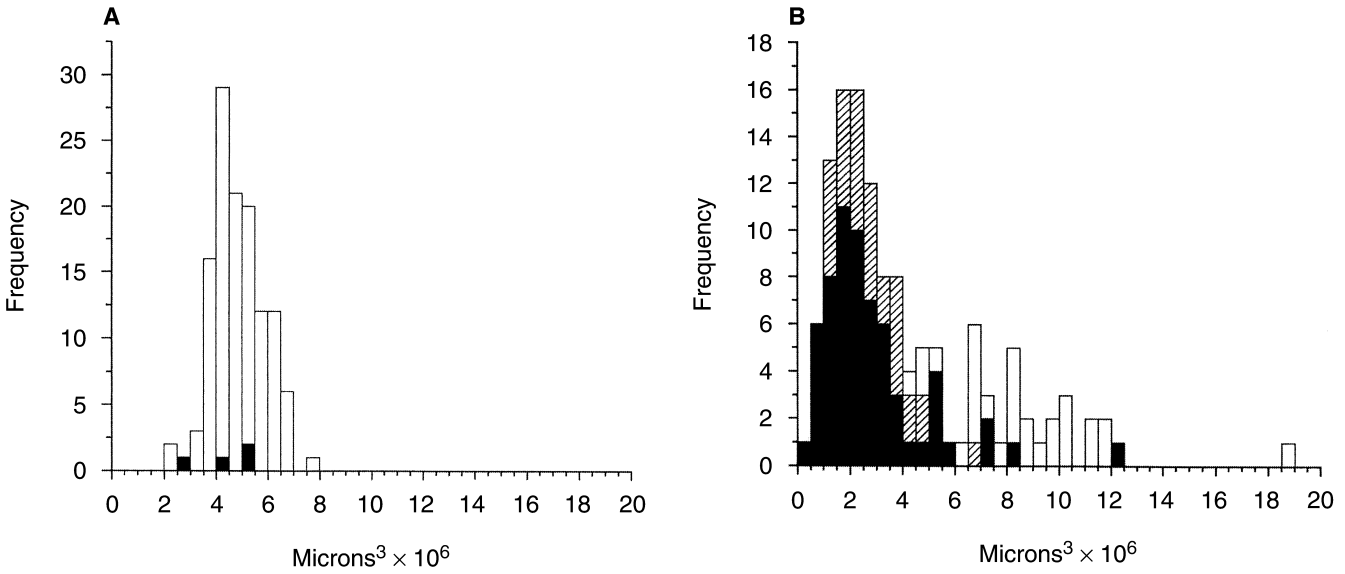


Fig. 3. Frequency histograms of renal corpuscle volumes from three normal (*A*) and three cystic (*B*) rat kidneys. Symbols are: (■) atubular; (□) tubular; (▨) atrophied.

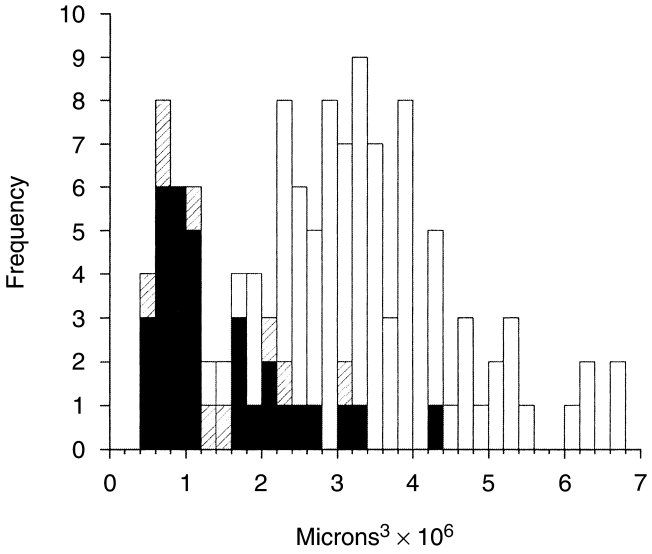


Fig. 4. Frequency histogram of glomerular volume from three six-month-old rats with PKD that had been treated with potassium citrate/citric acid in their drinking water from age of one month. Symbols are: (■) atubular; (□) tubular; (▨) atrophied.

old male rats, and 13.6 ± 2.6 in three rats with PKD ($P < 0.005$). The rats weighed 508 ± 34 g and 516 ± 19 g, respectively.

Figure 4 shows frequency histograms of glomerular volumes from three six-month-old cy/+ rats that had been treated with KCitr in their drinking water beginning at one month of age. Kidney GFR averaged 423 ± 55 μL/min per 100 g body weight, a value not significantly different from normal. In these kidneys, of 121 glomeruli examined, 25% of the glomeruli were atubular, 7% were connected to atrophied segments, and 68% were connected to normal proximal tubules. The distribution of glomerular sizes appears bimodal, a composite of the untreated cystic kidneys and the normal kidneys (Fig. 2). Twenty-three percent of the glomeruli are smaller and 14% are larger than any glomeruli in the normal kidneys.

Table 2 summarizes results from experiments in which we measured glomerular blood flow (GBF) with microspheres. GBF in cystic kidneys (232 ± 71 nL/min) averaged about half of that in normal kidneys (455 ± 98 nL/min). The patterns of distribution of GBF in normal and cystic kidneys appear very similar to the patterns for glomerular sizes (compare Fig. 5 with Fig. 2). GBF in both normal and cystic kidneys was positively correlated with glomerular volume; the equations of the least squares lines were, respectively, GBF (nL/min) = 37 × glomerular volume (in $\mu m^3 \times 10^6$) + 390 ($r = 0.20$, df = 276, $P < 0.001$) and GBF (nL/min) = 187 × glomerular volume (in $\mu m^3 \times 10^6$) + 19 ($r = 0.61$, df = 155, $P < 0.001$).

Glomerular pathology was evaluated by light microscopy (Table 3 and Fig. 6). In normal kidneys, the majority (71%) of glomeruli looked healthy (Fig. 6A); they had uniform glomerular basement membranes, open capillary loops, and a normal amount of matrix. In all groups, about 1/4 of the glomeruli had mild changes, that is, minor wrinkling of the glomerular basement membrane and increased mesangial matrix involving less than 1/3 of the glomerulus (Fig. 6B).

In cystic kidneys of untreated rats (rats that drank tap water), the percentage of healthy glomeruli was significantly lower than in normal kidneys, and there were many moderately abnormal glomeruli, that is, glomeruli

Table 2. Microsphere (MS) measurements of glomerular blood flow (GBF)

Rat expt.	Body wt *g*	MAP *mmHg*	MS in ref.[a]	Ref. flow *mL/min*	Flow/MS *nL/min*	MS/glomerulus	GBF *nL/min*
Normal (+/+) rats							
8/20/00	461	107	1868	0.151	81	6.3 ± 3.0	509 ± 240
9/26/00	492	106	2595	0.124	48	10.5 ± 4.0	503 ± 192
9/29/00	492	113	2490	0.086	34	14.7 ± 4.6	508 ± 160
10/4/00	468	109	4088	0.143	35	9.6 ± 3.9	334 ± 136
10/26/00	433	101	4080	0.159	39	7.8 ± 3.5	304 ± 137
10/27/00	469	118	2319	0.192	83	6.8 ± 2.9	563 ± 240
12/21/00	403	112	5856	0.168	29	16.2 ± 4.9	466 ± 140
Mean ± SD	460 ± 32	109 ± 6	3328 ± 1410	0.146 ± 0.034	50 ± 23	10.3 ± 3.9	455 ± 98
Rats with PKD (cy/+)							
8/8/00	468	120	1973	0.168	85	1.7 ± 2.6	145 ± 223
9/12/00	490	128	2134	0.155	73	4.1 ± 4.3	299 ± 310
9/22/00	467	109	3001	0.141	47	4.4 ± 5.3	204 ± 250
9/28/00	505	123	3640	0.191	52	5.3 ± 5.5	278 ± 287
Mean ± SD	482 ± 18	120 ± 8	2687 ± 779	0.164 ± 0.021	64 ± 18	3.9 ± 1.5	232 ± 71
P	NS	<0.05	NS	NS	NS	<0.02	<0.01

[a] The reference (ref.) blood sample was collected by free-flow from the left femoral artery; the blood flow per MS (nL/min) was calculated by dividing the reference blood flow by the number of MS in the reference sample

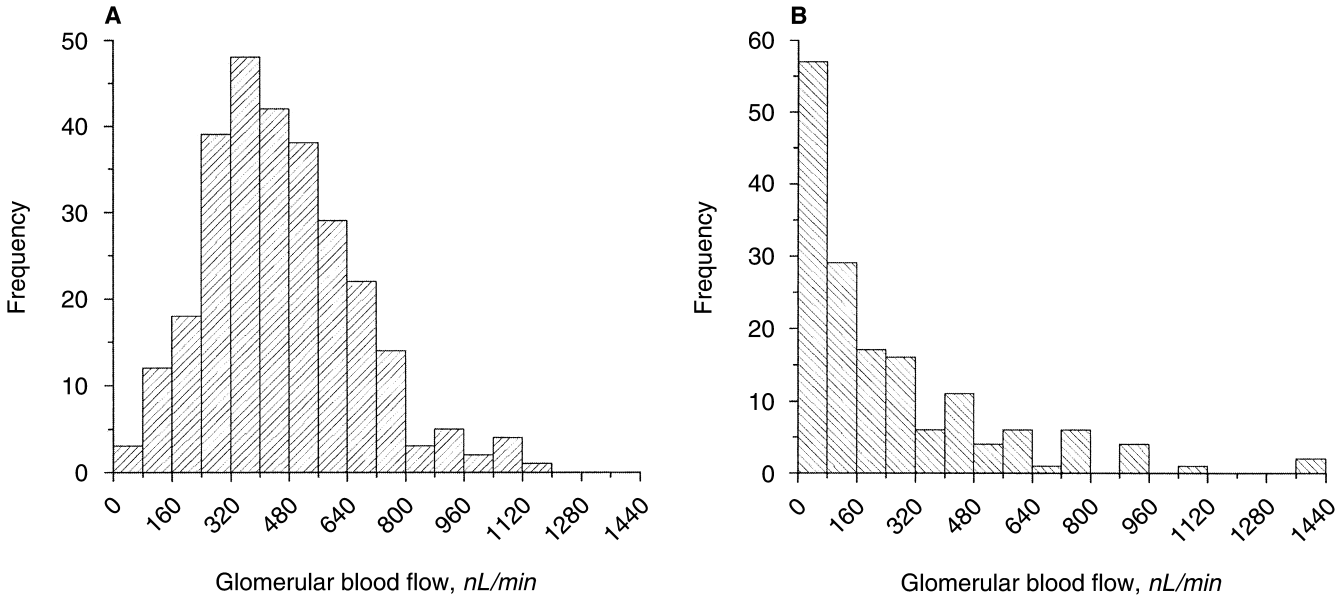


Fig. 5. Frequency histograms of glomerular blood flow, measured with the microsphere method, from seven normal (*A*) and four cystic (*B*) rat kidneys.

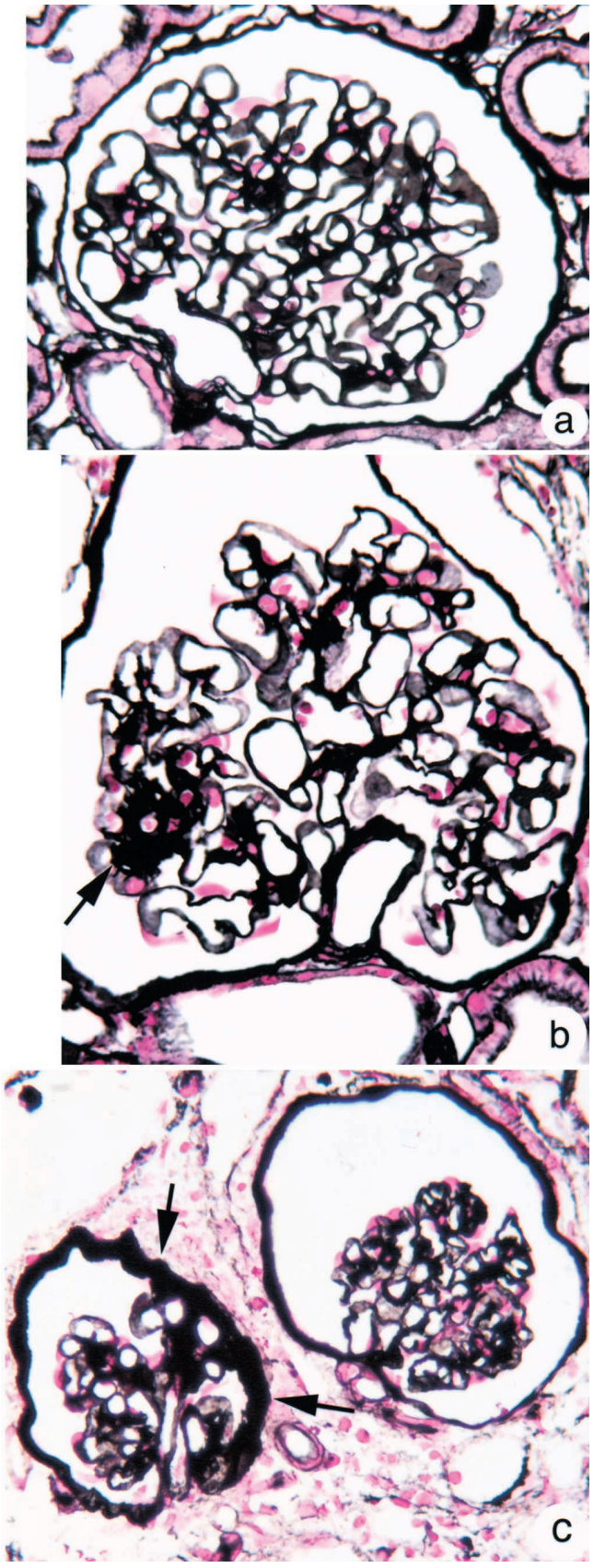
Table 3. Glomerular pathology evaluated by light microscopy[a]

Rats, treatment	Percent of glomeruli in each grade				Synechiae per 100 glomeruli
	Normal	Mild	Moderate	Severe	
+/+, H_2O (N = 3)	71 ± 9	28 ± 9	0 ± 1	0	0 ± 0.6
cy/+, H_2O (N = 5)	15 ± 12[c]	25 ± 6	59 ± 17[b]	0	15 ± 5[b]
cy/+, KCitr (N = 4)	44 ± 11[e]	27 ± 7	29 ± 6[d]	0	9 ± 1

[a] Glomeruli were graded in a blinded fashion by C.L.P. Definitions are: normal, no significant increase in mesangial matrix, no wrinkling of glomerular basement membrane (GBM), open capillary loops; mild, increased mesangial matrix involving less than one-third of the glomerulus, minor wrinkling of GBM (less than three loops), open capillary loops, glomerular volume not decreased; moderate, increased mesangial matrix involving greater than one-third of the glomerulus but less than total obsolescence (some capillary loops remain open), moderate wrinkling of GBM (more than 3 loops), glomerular volume decreased; severe, obsolescence (scarring of entire glomerulus) with decreased glomerular volume and no open loops.

[b] $P < 0.01$ and [c] $P < 0.001$ compared to +/+ rats

[d] $P < 0.05$ and [e] $P < 0.01$ compared to cy/+ rats drinking water



with a widespread increase in mesangial matrix, wrinkling of glomerular basement membranes, and decreased glomerular volume (Fig. 6C). Consumption of a solution of potassium citrate and citric acid (KCitr) by rats with PKD led to less severe glomerular damage (Table 3).

Synechiae were rare (1 out of 300 glomeruli) in the normal kidneys, but were seen in 15% of glomeruli of untreated rats with PKD. Treatment with KCitr resulted in a lower average number of glomeruli with synechiae, but the difference was not statistically significant (Table 3).

Scanning electron microscope observations on the morphological appearance of renal corpuscles from the outer cortex were determined from micrographs at a magnification of ×500 to 5000. Most of the glomeruli from the cystic kidneys were indistinguishable from those of normal kidneys. Thick and stubby podocyte foot processes were found in both groups, but tended to be present more often in the cystic kidneys (Fig. 7).

The cell bodies of podocytes were more rounded than normal in the small glomeruli of cystic kidneys (Fig. 8A), possibly due to a greater reduction in capillary surface area than in podocyte number. The parietal epithelium consisted of flat, polygonal cells and had a normal appearance in the cystic kidneys. In one glomerulus from a cystic animal, however, we observed podocyte processes extending onto the parietal layer of Bowman's capsule (Fig. 8); such a structure would be counted as a synechia under the light microscope. In earlier studies [1], we found that macrophage-like cells were attached to the luminal surface of some cysts, no such cells were seen on top of the parietal or visceral epithelial cells. In summary, the differences between glomeruli from normal and cystic kidneys, as revealed by SEM, appeared to be minor.

DISCUSSION

Atubular and hypertrophied glomeruli

The main finding in the present study is that the kidneys of adult rats with ADPKD contain large numbers of atubular glomeruli. The presence of these nonfunctional units must contribute in a major way to the reduced GFR.

We also found appreciable numbers of glomeruli associated with proximal tubule segments having a flattened, indistinct epithelium. This is in contrast to the normal rat kidney where the cells of the initial portion of the

Fig. 6. Sections of representative glomeruli stained with Jones' silver stain. (*a*) Normal glomerulus, from an unaffected kidney. (*b*) Mild changes, from a cystic kidney. (*c*) Moderate changes in two glomeruli from a cystic kidney. Note the increased mesangial matrix in one segment (arrow) of the glomerulus in panel b, but in most tufts in panel c. Also note the synechia (delimited by two arrows) in panel c. All figures are ×400.

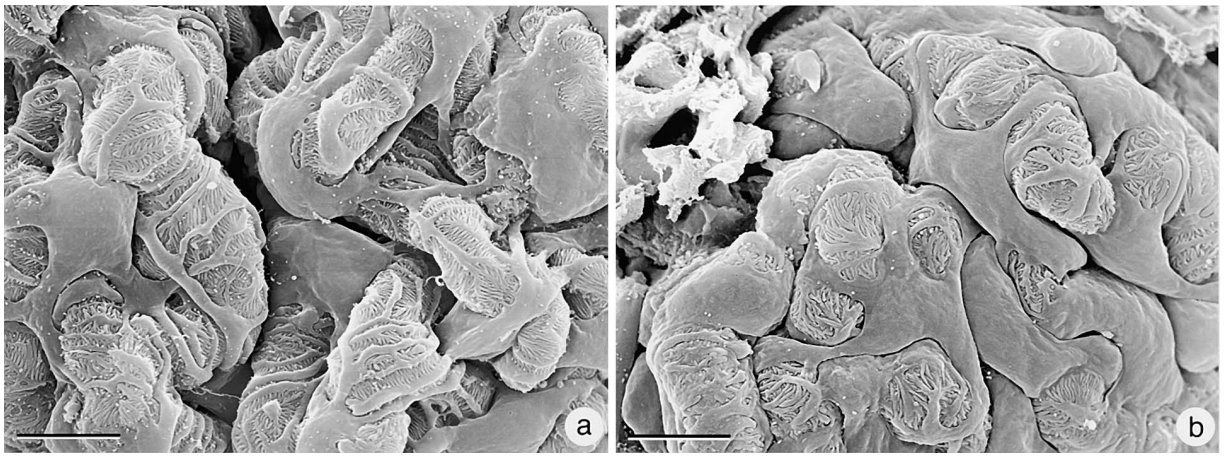


Fig. 7. (*a*) Scanning electron microscope photograph of podocytes (visceral epithelial cells) from a normal kidney. Numerous slender foot processes are evident. (*b*) SEM of podocytes from a cystic kidney. The foot processes appear broader and stubbier and fewer in number. Bars = 10 μm.

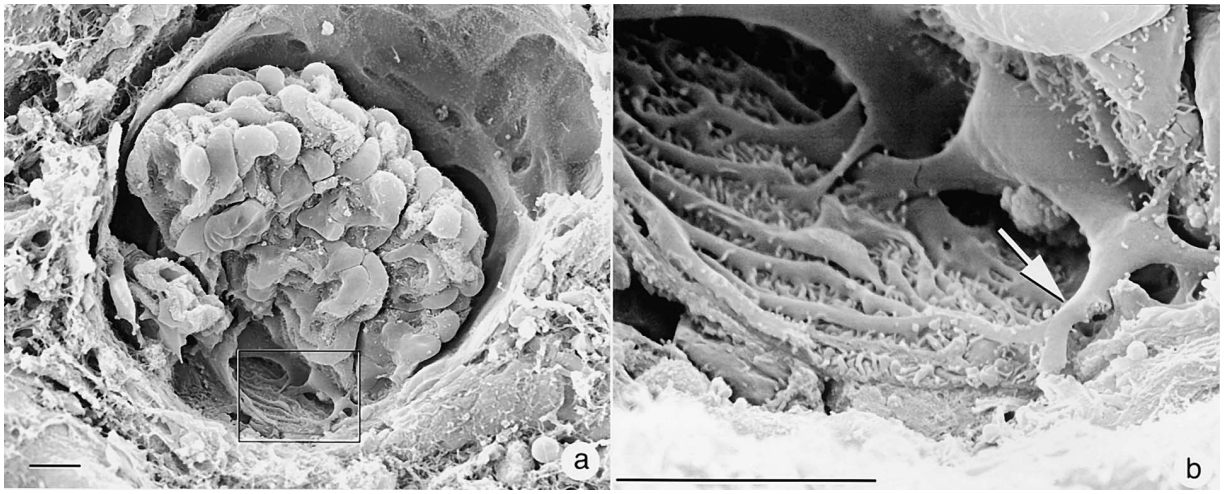


Fig. 8. (a) Scanning electron microscope photograph of a renal corpuscle with a small glomerulus from a cystic kidney. (*b*) Higher power view of the same renal corpuscle (see rectangle in panel a). The foot processes have extended onto the parietal wall of the renal corpuscle, forming a synechia (arrow). Bars = 10 μm.

proximal tubule are cuboidal with a distinct apical brush border. Capsule-tubule junctions may be lined by flattened, indistinct epithelium and may be narrowed for a variable length in some renal diseases; these are referred to as neck segments [3, 4]. We consider it unlikely that neck segments opened downstream into normal, patent proximal tubules beyond the plane of section, but we cannot exclude this possibility.

Normal-looking proximal tubule segments adjacent to glomeruli with atrophied neck segments were usually absent. In a previous study (Fig. 7B of [1]), we illustrated a dissected nephron with a 100-μm-long narrow neck segment from a cystic kidney; although the lumen of the proximal tubule was open for a short distance beyond the neck, most of the tubule had collapsed and was atrophied. Development of a narrowed neck segment most likely represents an intermediate stage between the normal nephron and the atubular glomerulus [3, 4].

Assuming that the atubular glomeruli and glomeruli associated with atrophied tubule segments are nonfunctional, our data suggest that the remaining functional nephrons, on average, have a higher single nephron GFR than normal. The GFR in cystic kidneys of six-month-old rats was 30% of normal, but the number of functional nephrons was only 23% of normal (Table 1). To the extent that the selection of glomeruli was biased by the serial sectioning approach [5], we may have overestimated the number of functional (large) glomeruli in the cystic kidneys and underestimated the number of nonfunctional (small) glomeruli. Therefore, we would conclude that there was even greater hyperfiltration in the remaining functional nephrons.

The additional findings in cystic kidneys that appreciable numbers of glomeruli are hypertrophied (Fig. 2) and that at least a few glomeruli may have abnormally high blood flows (Fig. 5) also support the idea that individual glomeruli show hyperfiltration. Enlarged glomeruli were almost always connected to open proximal tubules (for example, Fig. 1B), suggesting that they are hyperfiltering as a compensation for the major loss of functioning nephrons.

The conclusion that hyperfiltration may occur in cystic kidney disease is at odds with the conclusion of Kang et al [6]. They demonstrated that in the Han:SPRD rat with PKD there is virtually no compensatory increase in GFR in the remaining kidney after uninephrectomy or infarction of 50% of kidney mass. However, their animals had significant hypertension and proteinuria after these operations, suggesting that accelerated renal injury may have impaired a compensatory response that is present in the absence of superimposed stresses.

Numerous studies have suggested that glomerular hypertrophy or hyperfiltration in diseased kidneys plays a significant role in the progression of renal disease [7–11]. These abnormalities may lead to glomerular injury and progressive glomerulosclerosis. Early in ADPKD, GFR may be higher than normal, at least in a subgroup of patients (abstract; Chapman A, *Kidney Int* 35:203, 1989). Also, glomerular hypertrophy appears to be present in human ADPKD kidneys. Pfaltz and Briner observed glomerular enlargement in seven of nine patients with ADPKD [12]. Likewise, Zeier et al reported that the upper range for glomerular diameters is higher in kidneys from patients with ADPKD than in kidneys from healthy individuals or even diabetic patients [13].

Glomerular filtration rate of cystic rats treated with KCitr was normal at six months of age, but 25% of the glomeruli were atubular and hence nonfunctional. Fourteen percent of the glomeruli in these treated rats were larger than normal, so it is reasonable to conclude that these glomeruli were hyperfiltering and maintaining the GFR. In our earlier study [2], KCitr-treated rats with PKD developed end-stage renal disease at an average age of 17 months, instead of the usual 10 months in untreated rats. It is tempting to speculate that hypertrophy and/or hyperfiltration contribute to the eventual glomerular demise and the unstoppable progression of cystic disease.

Pathological changes in the renal corpuscles

Pathological changes in the glomeruli of cystic rat kidneys were generally mild to moderate, and there were no obsolescent or completely scarred glomeruli (Table 3). The relatively well-preserved structure of atubular glomeruli in the cystic kidney obscures the facts that these glomeruli must have filtration rates that are virtually nil and they do not contribute to the final urine. Urinary protein excretion was about eight times higher in rats with ADPKD than in normal rats. Although such an increase might be due to glomerular injury, it is also possible that the proteinuria is mainly due to changes in tubular handling of filtered proteins [14]. Moderate proteinuria is often observed in patients with ADPKD, even at a young age [15].

An increase in mesangial matrix in one part of the glomerulus (segmental sclerosis) was observed in about 1/4 of the glomeruli in all groups of rats (Table 3). The presence of mild mesangial lesions and focal glomerular sclerosis in the normal adult laboratory rat is common [16]. In the cystic rat kidneys, many cases were found where glomeruli showed increased mesangial matrix throughout the glomerulus, but the changes were not severe and the glomeruli were not globally sclerotic (completely scarred or obsolescent, with no open capillary loops). By contrast, in kidneys from ADPKD patients, Zeier et al reported that segmental glomerulosclerosis was rare (4% of glomeruli) and global glomerulosclerosis was frequent [13]. The percentage of glomeruli considered to be globally sclerotic was 29% in patients with early renal failure and 49% with terminal renal failure. One explanation for this difference between the rat and the human patient with PKD may be that the disease process is much more prolonged (years vs. months) in the patient with PKD, allowing more time for severe glomerular changes to develop.

The parietal epithelium of Bowman's capsule appeared normal in cystic kidneys. No parietal podocytes lining the entire capsule were seen, as was reported in a study by Gibson et al, in atubular glomeruli of end-stage rejected human kidney transplants [17]. In one case, however, we observed by SEM that podocytes of a small glomerulus were splayed out onto the parietal surface of Bowman's capsule (Fig. 8). Such a structure would be identified as a synechia by light microscopy. Synechiae were essentially absent from the normal kidneys, but were seen in about 15% of glomeruli from cystic kidneys. Synechiae may be significant in terms of development of tubular atrophy and atubular glomeruli (see below).

Glomerular blood flow

Single nephron GBF averaged half of normal in cystic kidneys; this agrees with the 50% reduction in whole kidney blood flow we previously reported in six-month-old rats using the PAH clearance and extraction method [2]. Some glomeruli in the cystic kidneys had quite normal or even higher than normal blood flow (Fig. 5). The majority of glomeruli, however, had very low blood flows. The reduced GBF probably reflects narrowing of small arteries and arterioles in the kidney, or possibly lengthening of renal vessels due to stretching of vessels by expanded cysts [18]. Moreover, a reduction in the

glomerular capillary bed could contribute to the reduced blood flow, since the glomeruli with low blood flows were smaller in size than normal. Blood that leaves the glomerulus provides nourishment for the tubular structures in the kidneys. Hence, our GBF measurements suggest that regional blood flow to tubules and cysts in the cystic kidneys may vary widely and is often greatly reduced.

We injected a large dose (2 to 4 $\times$ 10^6) of microspheres to enable study of the variance of individual glomerular blood flows. With such a dose, the scale (that is, nL/min blood flow per trapped microsphere) was small. Our previous study showed, by monitoring renal blood flow continuously with an electromagnetic flowmeter during injection of 2.5 $\times$ 10^6 microspheres into smaller rats (210 g body wt), that this injection does not decrease whole kidney blood flow [19]. In the present study, GBF may have been reduced by the trapped microspheres in glomeruli with very high blood flows, resulting in an underestimation of the number of hyperperfused glomeruli. Despite this caveat, the highest individual glomerular blood flows were found in cystic kidneys (Fig. 5B).

Mechanisms of atubular glomerulus formation

Atubular glomeruli have been reported in many experimental models of renal disease and in human diseased kidneys [20–24]. Interestingly, they are more common in those diseases that are classified as tubulointerstitial (this would include ADPKD) and less common in diseases that are primarily glomerular in origin [21]. How or why atubular glomeruli are formed is not certain, although, based on our current knowledge, three mechanisms are attractive:

First, in elegant studies, Kriz et al demonstrated that there is often attachment of the podocyte cell layer to the outer wall of Bowman's capsule (a synechia) in glomerular diseases [25, 26]. This leads to misdirected filtration, segmental glomerulosclerosis, and degeneration of the attached proximal tubule. The tubule segment closest to the renal corpuscle undergoes degeneration first. We found that synechiae were much more frequent in cystic kidneys than in normal kidneys (Table 3). Segmental sclerosis was present also in many glomeruli of cystic kidneys (Table 3 and Fig. 6 B, C). Therefore, misdirected filtration may lead to formation of atubular glomeruli in the cystic kidney.

A second possible mechanism for the development of the atubular glomerulus is related to tubule obstruction. In young (2- to 4-month-old) Han:SPRD rats with PKD, an appreciable number of cystic nephrons are obstructed [1]. Furthermore, chronic obstruction of the lumen of single proximal tubules in normal kidneys leads to both tubular atrophy and a small and sclerotic glomerulus [27]. The blocked proximal tubules become thin, with small cells and collapsed lumens, and they are surrounded by interstitial fibrosis [27, 28]. Although we did not focus on the junction of Bowman's capsule and proximal tubule in these earlier studies, it is likely that atubular glomeruli were formed as a consequence of tubule obstruction.

A third possible mechanism involved in formation of atubular glomeruli is ischemia. In the present study, we observed very low blood flows in the small, atubular glomeruli. A decreased glomerular blood flow may produce glomerular damage, proteinuria, and tubular damage. Abnormal release of growth factors, chemokines, and vasoactive mediators, as well as local hypoxia may all contribute to the injury [29]. Since the initial portions of the proximal tubule would encounter the highest concentrations of filtered proteins, the damaging effects of proteins [30] may be expressed most strongly here. It has been shown that ischemic injury, induced by temporary occlusion of a renal artery in the rat [23] or produced by renal artery stenosis in patients [24], leads to the formation of atubular glomeruli. All three of the above mechanisms (misdirected filtration, tubular obstruction, ischemia) probably contribute, in varying degrees, to the formation of atubular glomeruli in the cystic kidney.

Conclusion

The glomerulus in human ADPKD has received scant attention, perhaps because the tubular cystic changes are so much more impressive. Here we demonstrate in a rat model that there are significant structural changes in glomeruli and their connections to proximal tubules. The formation of atubular glomeruli clearly leads to functional failure. Glomerular abnormalities are probably a relatively late, downstream event in the disease process. Nonetheless, these changes are important because it is the failure of glomerular function that produces end-stage renal disease and the need for dialysis or renal transplantation. Better understanding of the processes that lead to glomerular filtration failure and prevention of these changes may lead to treatments that benefit patients with ADPKD.

ACKNOWLEDGMENTS

We thank the Polycystic Kidney Disease Foundation for grant support. M.A. Tielker was awarded a travel grant from the Physiology Department in a graduate student competition, and presented portions of this work at the American Society of Nephrology meeting, Toronto, Canada, October 2000. We thank Ms. Jennifer Stashevsky for preparing the histological sections and Mr. Philip Blomgren for assistance with the photography.

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