Kidney International, Vol. 32 (1987), pp. 187–197

Viscoelastic properties of tubule basement membranes in experimental renal cystic disease

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Viscoelastic properties of tubule basement membranes in experimental renal cystic disease. Hereditary and acquired renal cysts develop from tubule segments that enlarge progressively. We measured the deformability of basement membranes surrounding individual normal tubules and cysts to determine if cysts might develop by simple extension of abnormally–deformable basement membrane in response to normal or increased transtubule hydrostatic pressures. Deformability (cm/dyne) was measured in individual tubules and cysts in vitro by a micropipet aspiration technique that related negative pressures within the pipet to the distance the tubule or cyst wall was aspirated into the pipet. Viscoelastic creep was determined from the time–dependent effect of pipet aspiration on membrane deformation. Proximal and collecting tubules, glomerular capsules and cysts were microdissected from controls and animals with acquired (Diphenylthiazole [rats], Nordihydroguaiaretic acid [rats]), hereditary (C57 BL/6J [cpk/cpk] mice) and spontaneous (CFW_w mice) renal cystic diseases. The major resistance to deformation was localized to the basement membrane since collagenase destroyed the elasticity of tubule and cyst walls. Tubule basement membranes adjacent to cysts appeared abnormal by electron microscopy in the animals fed DPT, but measurements of deformability and viscoelastic creep showed no differences between normal and cystic tubules in any animal model. Deformability values of cysts (7.7 ± 1.1, 10.9 ± 1.1, 11.2 ± 0.6, $9.4 \pm 0.8 \times 10^{-3}$ cm/dyne in DPT, NDGA, C57 BL/6J and CFW_w, respectively) are consistent with the interpretation that high transtubule pressures ranging from 39 to 134 cm H_2O would be required if cysts form by simple stretching of the basement membrane secondary to a transepithelial hydrostatic pressure–gradient. Since in vivo measurements of hydrostatic pressures across cyst walls are not high enough we conclude that cysts do not enlarge due to increased deformability of tubule basement membranes.

As a group, cystic disorders are the most common structural abnormalities affecting the kidneys. Renal cysts are solitary or multiple. They may be inherited or acquired. They derive from nephrons or collecting tubules. A common feature in the cystogenic process is the abnormal proliferation of tubule epithelial cells in specific tubule segments [1–3].

The proliferation of tubule epithelial cells to form a cyst may be primary, that is, due to abnormal growth stimuli arising de novo within the cells or secondary, due to tubule cell responses to extracellular factors. The tubule basement membrane (TBM) is a relatively stiff collagenous structure that forms a scaffolding (sleeve) around all renal tubules [4, 5]. One prominent hypothesis of cyst formation holds that TBM may be abnormally compliant in certain renal cystic disorders [6–8]. According to this view, a primary or secondary process may increase the deformability of the TBM and initiate food dilation of the wall in response to normal or elevated transmural, hydrostatic pressure gradients. If the unique stimulus to cyst formation is an increase in the deformability of TBM, the growth of renal cysts to sizes 1000-fold greater than the original tubule would require extensive thinning of the basal lamina or the continuous synthesis of highly deformable tubule basement membrane throughout the period of cyst enlargement [9].

Prior work in vitro indicates that basement membranes surrounding renal tubules contribute substantial resistance to radial deformation in response to hydrostatic pressure gradients [4, 5]. A technique, developed previously in this laboratory [10, 11], micropipet aspiration of isolated tubule segments, measures several mechanical properties. The extent to which the basolateral surface of a tubule may be sucked into a pipet is determined by three principal factors: 1.) the inherent elasticity, 2.) the resting tension, and 3.) viscoelastic creep. For the basolateral surface the inherent elasticity (deformability) is determined primarily by the molecular structure of the membrane that limits deformation of the TBM. Resting tension may be affected by the transmural hydrostatic pressure–gradient and the extent of macroscopic TBM folding. Prolonged aspiration at constant pressure may cause the basolateral surface of the tubule to "creep" into the pipet. The rate of creep may reflect viscous elements in the TBM.

In the current study we used the micropipet aspiration method to determine directly the mechanical properties of the basolateral surfaces of cyst walls in four different models of renal cystic disease. Two acquired cystic disorders were created in rats by feeding them diphenylthiazole (DPT) or nordihydroguaiaretic acid (NDGA) [1, 6–8, 12–15]. The other cystic disorders occur spontaneously. A unique strain of mice develop recessively inherited, infantile polycystic kidney disease (C57 BL/6J [*cpk/cpk*]) that resembles the recessive human disorder [16, 17]. A second strain of mice (CFW_w) develop renal cysts that are modified by environmental stimuli [18].

The results presented here show that the development of

Received for publication November 21, 1986
and in revised form March 17, 1987

Table 1. Laboratory parameters of control and experimental animals

Animal model	Body weight *g*	Combined kidney weight *g*	kw/body wt × 10^{-3}	Hematocrit	SUN[a] *mg/dl*
DPT rat					
Control	265 ± 17 (3)	2.05 ± 0.11 (3)	7.7 ± 0.01 (3)	48 (1)	NA
Exp	169 ± 7 (6)	3.81 ± 0.18 (6)	19.4 ± 0.03 (6)	50 ± 1.3 (6)	51 ± 4.8 (3)
NDGA rat					
Control	346 (1)	2.33 (1)	6.7 (1)	52 (1)	15 (1)
Exp	219 ± 8.6 (5)	2.16 ± 0.12 (5)	9.9 ± 0.4 (5)	49 ± 1.3 (5)	11 ± 1.2 (5)
C57 BL mouse					
Heterozygote	9.2 ± 1.5 (2)	0.15 ± 0.01 (2)	16.3 ± 0.01 (2)	39 ± 2.1 (3)	21 ± 1.3 (2)
Homozygote	8.6 ± 0.2 (12)	2.52 ± 0.13 (12)	293.0 ± 10.8 (12)	30 ± 1.7 (13)	140 ± 19.3 (8)
CFW_w mouse					
Germ-free	37 ± 0.6 (3)	0.54 ± 0.01 (3)	14.6 ± 0.3 (3)	39 ± 0.7 (3)	9 ± 1.8 (3)
Conventional	30 ± 1.5 (3)	0.56 ± 0.00 (3)	18.7 ± 1.0 (3)	26 ± 2.7 (3)	159 ± 7.8 (3)

Mean values listed ± SE. Number of animals in parenthesis.
[a] Serum urea nitrogen
NA, not available

acquired and inherited cysts is not accompanied by demonstrable changes in the inherent viscoelasticity of tubule basement membranes.

Methods

Experimental cyst models

DPT. Six Sprague–Dawley rats, six to eight weeks old were fed pelletized rat chow containing 1.06% diphenythiazole supplied by Baxter Laboratories (Chicago, Illinois, USA) according to a protocol published previously [6–8]. Three control animals were pair-fed a normal rat chow. The experimental animals developed diffuse, renal cystic disease in cortical and medullary tubules associated with bilateral kidney enlargement and mild azotemia (Table 1). Animals were fed the experimental diet for eight weeks and then shipped to Kansas City, Kansas for analysis.

NDGA. Five Sprague–Dawley rats were delivered by caesarean section and raised in a germ-free environment (Charles River Breeding Laboratories, Wilmington, Massachusetts, USA). These animals were shipped to Albuquerque, New Mexico at six weeks of age. At eight weeks of age they were fed a sterilized, pelletized rat chow containing 2% NDGA [15]. The rats consumed approximately 30 grams of chow per day (600 mg NDGA per day). One conventional rat of the same age served as control. After 11 days the germ-free animals were placed in a conventional environment and for one day given water that had been contaminated with stool from the conventional rat. Such "deconditioning" of germ-free rats fed NDGA causes accelerated cystic change within renal tubules of cortex and medulla [15]. After five weeks in the conventional environment, the NDGA and control animals were shipped to Kansas City, Kansas for analysis. The experimental animals developed cysts focally in cortex and medulla. Kidney weight in experimental animals was not greater than in the control animals, although the kidney weight/body weight ratio was increased in the cystic kidneys (Table 1). The experimental animals were not azotemic.

C57 BL/6J (cpk/cpk). Heterozygote mice (C57 BL/6J [*cpk*+]) from the Jackson Laboratory (Mt. Desert Island, Maine, USA) were bred in Kansas City, Kansas. Homozygote *cpk/cpk* mice developed massive bilateral kidney enlargement by three weeks of age associated with marked azotemia [16] (Table 1). Heterozygotes of the same age had normal sized kidneys and normal serum-urea levels.

CFW_w. CFW_w mice raised in a conventional environment developed renal cysts in the cortex and outer medulla by 10 to 12 months of age [18]. By contrast, animals of the same strain and age raised in a germ-free environment did not develop renal disease or azotemia. We raised conventional and germ-free animals in a special facility described previously [18]. Cystic and control mice of the same age were used in this study. The cystic animals weighed less than the age-matched germ-free controls (Table 1). The kidneys of cystic animals did not weigh more than the controls although the kidney weight to body weight ratio was higher in the cystic animals. The cystic animals developed severe azotemia. Recent morphologic studies of the kidneys of these cystic animals show that the cysts develop between 8 to 12 months of age, and that there is a period of pronounced tubular necrosis and interstitial inflammation preceding cyst formation (Evan, A.P. et al, unpublished observation).

Tubule and cyst dissection. Rats and mice were anesthetized with ether to permit blood sampling by retroorbital aspiration into heparinized capillary tubing. The anesthetized animals were euthanized by cervical dislocation. The abdomen was opened with scissors and the kidneys quickly removed and trimmed free of fat. Kidney sections 1 to 2 mm thick were cut with a scalpel and the slices placed in ice-cold dissecting medium. The medium contained, in mmol/liter: NaCl 114, $NaHCO_3$ 25, K_2HPO_4 2.5, $MgSO_4$ 1.2, $CaCl_2$ 2.0, Na acetate 5, dextrose 5.5, and 6 gm/dl bovine serum albumin. pH was adjusted to 7.3 to 7.4 with 20% CO_2/80% O_2. Tubules and cysts were dissected by methods used routinely in this laboratory [19, 20]. Segments of proximal tubule (S_1 and S_2) 0.5 to 1.0 mm long were obtained in all animals (normal and cystic). In some experiments we obtained cortical collecting tubules and intact Bowman's capsules. We were able to dissect proximal and cortical collecting tubules from all control animals except the germ-free CFW_w mice. Cysts were obtained in all experimental

animals with varying degrees of difficulty. Large numbers of cysts of different sizes were easily dissected from C57 BL/6J mice and rats fed DPT. By contrast, rats fed NDGA and CFW_w mice had kidneys from which intact cysts were more difficult to remove.

In cystic kidneys we studied inflated cysts, that is, cysts expanded by fluid in the cavity. In most instances the fluid in the cysts was clear; in some animals fed NDGA and DPT the cysts contained precipitates and cellular debris. We studied only cysts containing clear fluid which had intact epithelial cells that we could easily visualize in the microscope (Fig. 1). Although we did not measure the transmural hydrostatic pressures in the cysts, they indented easily with micropipets indicating that the pressures were relatively low. When we intentionally ruptured a cyst wall, the cavity decreased in size but the cyst did not collapse completely.

Micrurgy methods

Tubules and cysts were transferred to a chamber mounted on the stage of a Unitron inverted microscope as described previously [19–21]. They were viewed through a glass coverslip on the bottom of the chamber at objective magnifications of 10 to 60 ×. The microscope was fitted with a video camera (Panasonic, Model WV 1600) to a television monitor (Panasonic, Model MV 5410) in conjunction with a video cassette recorder (JVC, Model CR 6060u) [22]. With this arrangement experiments could be recorded on video tape for later analysis. At 40 × objective magnification, a 1 μm object measured 2 mm on the video screen; with this system we could easily discriminate changes of 1 μm or greater.

Tubules and cysts were held for positioning by suction applied to a micropipet with a 20 to 40 μm tip. Studies were done at 25°C except when the temperature was raised to 37°C by a heating device embedded in the chamber. Temperature was elevated in one series of experiments to accelerate the enzymatic action of collagenase added to the medium (Fig. 4). The data reported in Tables 1–5 and Figures 1–4 were obtained in specimens incubated at 25°C to decrease proteolysis potentially incurred by microdissection. To measure deformability, we made on a microforge (Stoelting Co, Chicago, Illinois, USA) micropipets with a 30° elbow in the shank so that the tip could be placed flush against the basolateral surfaces of renal tubules and cysts. The tip was broken cleanly (Fig. 1A). The inner pipet diameter ranged between 11 and 30 μm and the inner side walls of the pipet were parallel for about 30 to 50 μm. The pipets were filled with degassed isotonic saline and connected to a water manometer with an overall extension of 120 cm. The pressure at the pipet tip was brought to zero (atmosphere as reference) by adjusting the open end of the manometer to a level at which minute particles neither flowed into or out of the pipet tip.

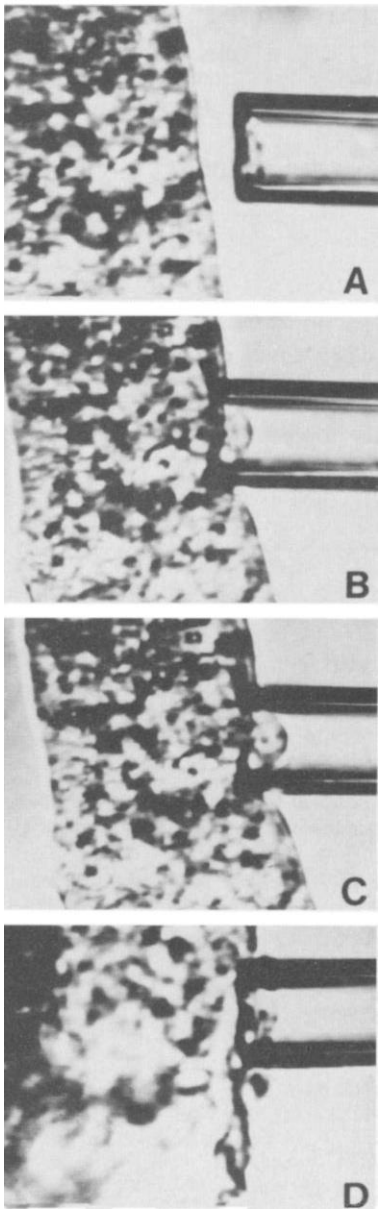

Fig. 1. *Micropipet method to determine basolateral deformability.* **A** Pipet with internal radius of 7.4 μm is aligned perpendicular to the surface of a normal non-perfused rat proximal tubule. **B** Basolateral surface of proximal tubule aspirated into pipet with 40 cm H_2O negative pressure. **C** Basolateral surface of proximal tubule aspirated with 80 cm H_2O negative pressure. **D** Cyst from NDGA-treated rat aspirated into pipet with 40 cm H_2O negative pressure. Distance deformed is equal to the distance from the leading edge of the aspirated bulge to the opening of the pipet.

Viscoelastic deformation

In an experiment a clean area on the basolateral surface of a tubule or a cyst was touched by the tip of the micropipet in which the hydrostatic pressure was zero. The hydrostatic pressure was abruptly lowered thereby sucking the basolateral surface (composed of outermost tubule basement membrane and epithelial cell layer) into the pipet tip. The pressure was held constant for 15 seconds and changed to successively lower levels. The focus of the microscope was adjusted to give a crisp image of the leading edge of the aspirated bulge as it entered the micropipet (Fig. 1B, C, D). The distance between the leading edge and the opening of the pipet is defined as ΔD. This format was used to determine basolateral deformability in all cystic models. In most experiments we used the same pipet for measurements in control tubules and cysts, and when possible we cleaned the pipet between experiments for use in different animals.

Table 2. Viscoelastic properties of nephron segments and cysts from Sprague–Dawley rats fed DPT

Segment	Pipet diameter range μm	Object diameter mean μm	Deformability $cm/dyne \times 10^{-3}$	Initial membrane extention $\mu m/\mu m$	Viscoelastic creep $\mu m/\mu m/min/dyne/cm^2 \times 10^{-6}$
Control					
Proximal	16–17	32	5.4 ± 0.60 (8;4)	0.53 ± 0.04 (8;4)	0.28 ± 0.14 (6;6)
Collecting	16–17	24	6.2 ± 0.88 (10;6)	0.48 ± 0.07 (10;6)	0.27 ± 0.15 (11;6)
Experimental					
Non-cystic tubules					
Proximal	17	39	8.4 ± 2.10 (7;4)	0.35 ± 0.05[a] (7;4)	0.29 ± 0.07 (7;3)
Cysts	17	137	7.7 ± 1.15 (14;7)	0.68 ± 0.09[a] (14;7)	0.40 ± 0.09 (7;7)

Mean ± SE
[a] Significantly different from each other, $P < 0.05$.
(*N*) number of individual measurements; number of tubule segments or cysts

Table 3. Viscoelastic properties of nephron segments and cysts from Sprague–Dawley rats fed NDGA

Segment	Pipet diameter range μm	Object diameter mean μm	Deformability $cm/dyne \times 10^{-3}$	Initial membrane extension $\mu m/\mu m$
Control				
Proximal	14.8	48	8.1 ± 0.97 (4;1)	0.41 ± 0.11 (4;1)
Collecting	14.8	40	15.5 ± 2.26[a] (4;1)	0.48 ± 0.10 (4;1)
Experimental				
Non-cystic tubules				
Proximal	13.6–14.8	43	12.8 ± 0.82 (22;5)	0.44 ± 0.04 (22;5)
Collecting	13.6–14.8	25	17.3 ± 1.91[a] (10;2)	0.55 ± 0.08 (10;2)
Cysts	13.6–14.8	>200	10.9 ± 1.13 (19;6)	0.50 ± 0.05 (19;6)

Mean ± SE
[a] Significantly different from control proximal tubule, $P < 0.05$.
(*N*) number of individual measurements; number of tubule segments or cysts

Table 4. Viscoelastic properties of nephron segments and cysts from non-cystic C57 BL/6J and homozygotic–cystic mice

Segment	Pipet diameter range μm	Object diameter mean μm	Deformability $cm/dyne \times 10^{-3}$	Initial membrane extension $\mu m/\mu m$	Viscoelastic creep $\mu m/\mu m/min/dyne/cm^2 \times 10^{-6}$
Non-cystic mice					
Proximal	15–17	27	8.1 ± 1.2 (11;2)	0.30 ± 0.04[a] (11;2)	0.67 ± 0.13 (4;1)
Collecting	15–17	24	7.9 ± 1.4 (8;2)	0.47 ± 0.05 (8;2)	3.2 ± 0.26[b] (3;1)
Homozygotic cystic mice					
Non-cystic tubules					
Proximal	12.5–20	24	12.0 ± 0.62 (44;10)	0.59 ± 0.02 (44;10)	0.40 ± 0.12 (7;4)
Collecting	17	18	6.8 ± 0.48 (3;1)	0.71 ± 0.05 (3;1)	ND
Cysts	12.5–30	183	11.2 ± 0.57 (53;15)	0.58 ± 0.03 (53;15)	0.75 ± 0.22 (9;5)

(*N*) number of individual measurements; number of tubule segments or cysts; ND, not done. All data are Mean ± SE.
[a] Significantly different from homozygous cystic proximal tubules and cysts $P < 0.05$.
[b] Different from all other segments, $P < 0.05$.

Viscoelastic creep

For the purposes of this study we define viscoelastic creep as the change in distance of the tubule wall aspirated into the micropipet between 0.5 and 2 minutes after abruptly lowering the hydrostatic pressure by 100 cm H_2O. This early time point (0.5 minute) was selected because most elastic elements of the basolateral membrane would presumably be at steady state, and subsequent changes in the distance deformed would be due principally to viscous or very slowly, equibrating elastic elements. We measured viscoelastic creep in the DPT, CFW_w and C57 BL/6J animals. This measurement was of lesser priority than the deformability parameters accounting for the fewer tubules and cysts for which viscoelastic creep was analyzed. We had insufficient material to make this measurement in NDGA rats. Distance deformed, ΔD, between 0.5 and two minutes was expressed as a dimensionless fraction of the pipet radius, R_p.

Elastic recoil was determined qualitatively by observing the extent to which the aspirated blister returned to a normal flat configuration after the basolateral surface was blown out of the micropipet.

Collagensase (1 to 2 mg/ml, Type B, Cooper Biomedical, Malvern, Pennsylvania, USA) was added to tubules and cysts to weaken the basement membrane. The activity of the enzyme was increased in some experiments by elevating the tempera-

Table 5. Viscoelastic properties of nephron segments and cysts from CFW_w mice

Segment	Pipet diameter range μm	Object diameter mean μm	Deformability $cm/dyne \times 10^{-3}$	Initial membrane extension $\mu m/\mu m$	Viscoelastic creep $\mu m/\mu m/min/dyne/cm^2$
Germ-free mice					
Proximal	11–18	36	11.8 ± 1.1 (10;5)	0.37 ± 0.04 (10;5)	1.15 (2;1)
Glomerular					
Capsule	11–18	88	11.2 ± 1.4 (6;4)	0.34 ± 0.02 (6;4)	0 (2;1)
Conventional mice					
Non-cystic segments					
Proximal	11–19	46	12.9 ± 2.1 (6;3)	0.61 ± 0.09 (6;3)	0.13 ± 0.13 (3;1)
Glomerular					
Capsule	11–22	88	10.4 ± 1.1 (13;8)	0.41 ± 0.07 (13;8)	0.56 ± 0.32 (6;3)
Cysts	11–22	181	9.4 ± 0.8 (13;7)	0.47 ± 0.07 (13;7)	0.42 ± 0.17 (4;3)

Mean ± SE
(*N*) number of individual measurements; number of tubules, glomerular capsules, or cysts

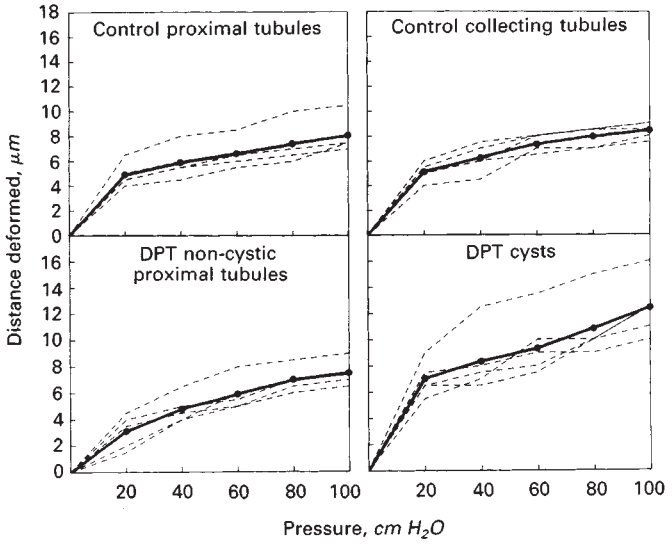

Fig. 2. *Pipet deformation of tubules and cyst walls from DPT-treated rats.* Representative measurements. Dashed lines indicate sequential measurements of individual tubules and cysts. Solid lines are means. Pipet radius, 8.5 μm.

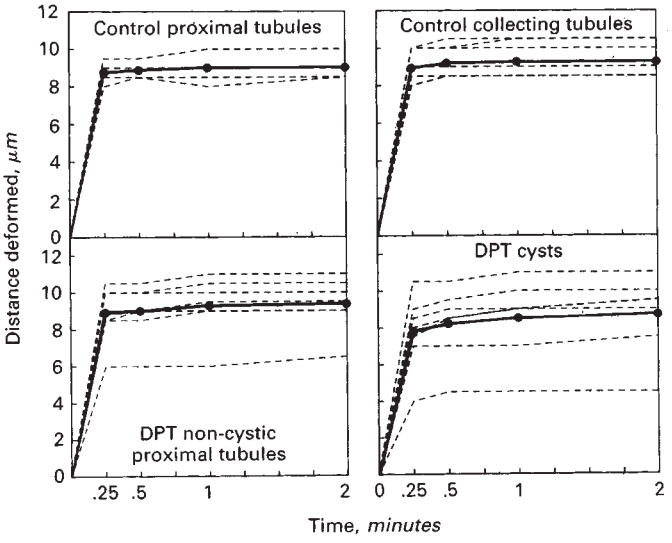

Fig. 3. *Viscoelastic creep during pipet aspiration of tubule and cyst walls from DPT-treated rats.* Representative studies from same animals in Figure 2. Dashed lines reflect sequential measurements of ΔD after the pipet pressure was lowered 100 cm H_2O below atmospheric. Solid lines are means. Pipet radius 8.5 μm.

ture to 37°C. In this way we could evaluate the resistance to deformation offered by the TBM in contrast to the cellular layer.

Serum urea nitrogen was determined colorimetrically [23].

Differences between experimental and control measurements were evaluated by one way ANOVA. Significant differences between means were determined by Tukey's test. A level of 0.05 was accepted as significant.

Results

The resistance to deformation (deformability) was estimated from the relationship between the applied negative pressure and the distance the basolateral surface was aspirated into the micropipets, a plan adopted from the studies of Mitchison and Swan [24] and Evans and La Celle [25]. We determined the explicit relationship between pressure (ΔP, cm H_2O) and distance deformed (ΔD, μm) in a series of pilot experiments in proximal tubules from normal rats and in tubules and cysts from the same strain of rats fed DPT (Fig. 2). The same pipet was used (8.5 μm radius) for all of the measurements in these pilot studies. From the mean values for distance aspirated into the pipet in response to 20 cm H_2O stepwise pressure increments spanning a range from 20 to 100 cm H_2O, we inscribed a biphasic curve with an initial steep portion followed by a relatively linear section. The initial steep portion between zero and 20 cm H_2O is ascribed to the initial membrane extension that includes the seating of the material within the pipet orifice in addition to basolateral deformability, whereas the more linear portion between 40 and 100 cm H_2O reflects primarily the deformability of the basolateral surface [25, 26]. The linear relationship between 40 and 100 cm H_2O observed in Figure 2 indicates that the *elastic constant* does not change appreciably as the basolateral barrier is stretched during the measurement in normal and DPT-treated tubules [26]. These pilot experiments, which defined the shape of the plotted data, showed that sufficient tongues of basolateral surface could be aspirated to permit reasonably precise measurements of deformability. (In this paper we measure the resistance to deformation, a term

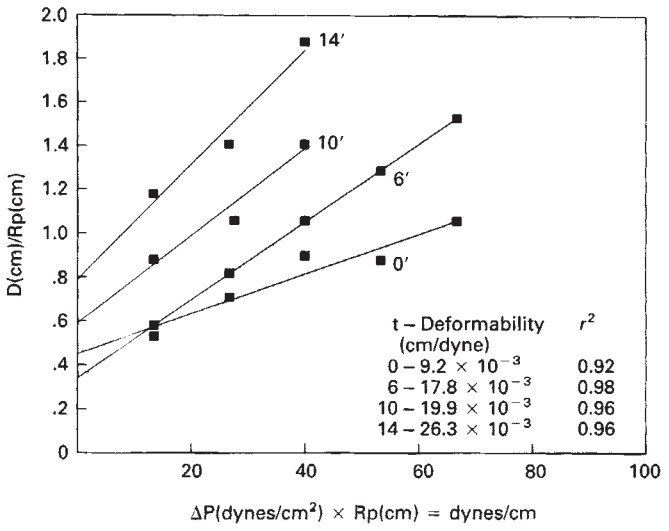

Fig. 4. *Effect of collagenase on deformability.* Normal rat proximal tubule at 37°C. Collagenase, 2 mg/ml, added to medium. Deformability measured at zero time and 6, 10 and 14 minutes after adding collagenase. Regression lines (least squares) are shown for each set of data points. In contrast to Figure 2, deformability was determined from normalized values for ΔD and ΔP. Pipet radius was 6.8 μm. The inset lists the deformability values and the correlation coefficients of each linear regression. Pressure could not be reduced more than 60 cm H_2O on the 10 and 14 minute examples due to continuous aspiration of the cells into the pipet.

used interchangeably with deformability. In our convention deformability is expressed graphically as strain [ΔD]/stress [ΔP]. In conventional physics elasticity is preferred and is expressed as stress/strain. The elastic constant of the basolateral surfaces can be obtained from the reciprocal of the deformability values.)

The mean correlation coefficients (R^2) for the deformability studies exceeded 0.94 in each of the four examples in Figure 2. The intercept with the ordinate of the relation between ΔP and ΔD between 40 and 100 cm H_2O reflects the initial membrane extension. We performed similar pilot analysis on tubules and cysts from all of the cyst models to confirm the applicability of the pipet deformability method in all cases (data not shown).

Visoelastic creep was determined from the distance the tongue of basolateral tissue was aspirated into the pipet between 0.5 and 2.0 minutes after the pipet pressure was decreased 100 cm H_2O below atmospheric. In pilot studies (Fig. 3) we determined the relationship between aspiration time and the distance deformed for the DPT treated tubules and cysts shown in Figure 2. In all cases, there was an abrupt increase in distance deformed; after 0.25 minutes there was a very slight increase in distance deformed beyond that seen during the initial 0.25 minutes. These results proved that the basolateral surface behaved mechanically as a solid rather than a liquid. When the aspirated tissue was released, the bulge receded to the original shape in less than a minute in tubules and cysts. This latter result indicates that our estimation of viscoelastic creep probably reflects slowly equibrating elastic elements, rather than viscous behaviour beyond the elastic limit.

We measured deformability, initial extension and viscoelastic creep in control renal tubules, unaffected tubules from cystic kidneys and individual cysts in the different experimental models. We used a normalization technique to permit comparisons among pipets of different internal radii [25, 26]. For deformability measurements, the relation between pressure and membrane extension (ΔD) was normalized to be independent of pipet radius (R_p). The units for deformability, the slope of the relationship between $\Delta D/R_p$ and $\Delta P \times R_p$, are cm/dyne $\times 10^{-3}$. Stiffness is the reciprocal of deformability, dynes/cm $\times 10^3$ [24]. For measurements of viscoelastic creep the change in distance deformed between 0.5 and 2.0 minutes of aspiration was normalized for R_p. Initial membrane extension ($\Delta D/R_p$) was obtained from the calculated intercept of the relation between $\Delta D/R_p$ and $\Delta P \cdot R_p$.

DPT rats

DPT causes cysts to form primarily in distal and collecting tubule segments. The deformability values of control Sprague–Dawley proximal and collecting tubules, 5.4 and 6.2 $\times 10^{-3}$ cm/dyne, were not different (Table 2). The deformability of non-cystic proximal tubules in rats treated with DPT, 8.4 $\times 10^{-3}$ cm/dyne, was not different from control proximal or collecting tubules. The deformability of the cysts (7.7 $\times 10^{-3}$ cm/dyne) was not different from non-cystic proximal or collecting tubules. The initial membrane extension was greater in the cysts than in the non-cystic proximal tubules, but was not different from normal proximal and collecting tubules. Viscoelastic creep was not significantly different among the normal tubules, non-cystic tubules in DPT-fed rats and the cysts.

In summary, there was no evidence in DPT-treated renal cysts that deformability or viscoelastic creep were increased above control or non-cystic tubule values.

NDGA rats

NDGA causes cysts to form primarily in distal and collecting tubule segments. The deformability values of control proximal and collecting tubules were 8.1 and 15.5 $\times 10^{-3}$ cm/dyne which are significantly different from each other (Table 3). The deformability of non-cystic collecting tubules in rats fed NDGA (17.3 $\times 10^{-3}$ cm/dyne) was also different from control proximal tubules. The deformability of cysts, however, was not greater than non-cystic tubules from animals fed NDGA or tubules from control animals. The initial membrane extension was not different among tubules and cysts. Viscoelastic creep was not measured in these animals.

In summary, there was no evidence in NDGA-treated renal cysts that viscoelastic parameters were increased above control or non-cystic tubule values.

C57 BL/6J (cpk/cpk)

In these animals cysts form primarily in collecting tubules. The deformability values of proximal and collecting tubules from control mice, 8.1 and 7.9 $\times 10^{-3}$ cm/dyne, were not different (Table 4). In homozygous mice the deformability of cysts was not significantly greater than the non-cystic tubules, or tubules from non-cystic animals.

Initial membrane extension differed among several segments. The value for cysts was greater than in non-cystic control proximal tubules, but not different from control non-cystic collecting tubules. This parameter was also greater in proximal

tubules from non-cystic homozygous mice than in proximal tubules from heterozygotes.

In heterozygotes, viscoelastic creep was unusually high in collecting tubules in comparison to proximal tubules, but in homozygotes the values in cysts were not greater than in non-cystic segments in heterozygotes or homozygotes.

In summary, there was no evidence in renal cysts from C57 BL/6J animals that any viscoelastic parameters increased significantly above heterozygote or non-cystic homozygote tubule values.

CFW_w

In these animals cysts develop in proximal tubules and glomerular capsules. The deformability values of control (germ–free) proximal tubules and glomerular capsules were not different from each other, or from the same non-cystic structures in animals with cystic kidneys (Table 5). The deformability values of cysts, $10.4 \pm 1.1 \times 10^{-3}$ cm/dyne, was not different from the control values. Initial membrane extension and viscoelastic creep were not different among control and non-cystic nephron segments and cysts.

In summary, there was no evidence in renal cysts from CFW_w animals that any viscoelastic parameters were increased above control or non-cystic tubule values.

Effect of removing the tubule basement membrane with collagenase

Previous studies showed that the lumens of renal tubules treated with collagenase cannot be perfused with fluid because the enzyme removes the scaffold against which the highly–deformable epithelial layer rests [21]. To verify that we could detect changes in the various viscoelastic parameters by the micropipet method we exposed normal tubules and cysts to different concentrations of collagenase and determined the sequential changes in deformability. One example of time–dependent changes in deformability and initial membrane extension is shown in Figure 4. Collagenase increased deformability of a normal proximal tubule from 9.2 to 26.3×10^{-3} cm/dyne, and initial membrane extension was increased from 0.36 to 0.79. The change in deformability was faster at 37°C than at 25°C, and the enzyme increased the deformability of cysts as well as control tubules (data not shown). After several minutes in collagenase measurements of viscoelastic creep could not be quantified because the highly-deformable epithelial cells flowed continuously into the pipets due to removal of the basement membrane. When the pipet pressure was returned to zero, the deformed cells did not return to a normal tubular configuration.

Morphology of tubule basement membranes

Transmission electron micrographs were obtained of normal and non-cystic tubules and cysts in each animal model of cystic disease (Fig. 5). In DPT rats the basement membranes were strikingly thickened adjacent to cysts, but they appeared normal adjacent to control or non-cystic tubules. Basement membranes in NDGA-treated animals were uncommonly thickened slightly and focally split adjacent to cysts, but adjacent to normal and non-cystic tubules the basement membranes appeared normal. In homozygous C57 BL/6J animals the basement membranes adjacent to cysts did not appear different from those adjacent to non-cystic and control tubules. In CFW_w mice, there was extensive interstitial fibrosis, but the basement membranes next to cysts did not appear different from those adjacent to non-cystic and control tubules.

Discussion

These results support the view that in vitro, and perhaps in vivo, basement membranes provide the principal resistance to mechanical deformation in renal tubules, glomerular capsules and renal cysts [4, 11]. The results prove that basement membrane of normal and cystic tubular structures behaves mechanically as a solid rather than a liquid. The intrinsic elastic parameters measured in the current study (deformability and viscoelastic creep) depend to a major extent on the tensile strength of the molecular structures comprising the basement membranes. It is not unreasonable to suppose that Type IV collagen components, by virtue of cross–linking, accounts to a major extent for the mechanical strength of tubule basement membrane [4, 27–29]. In this sense the viscoelastic parameters measured in the current study may provide an indirect measurement of the contribution of basement membrane collagen molecules to the tensile strength of normal and cystic renal tubules.

The examination of renal tubules from two rat and two mouse strains revealed that in control animals basement membrane viscoelasticity values were variable. For example, the deformability of CFW_w mouse proximal tubules was 11.8 in contrast to 5.4×10^{-3} cm/dyne in Sprague–Dawley rats. Deformability of control Sprague–Dawley rat, cortical collecting tubules in the NDGA study was 15.5 in contrast to 6.2×10^{-3} cm/dyne in the same strain of animals on a control pair–feeding regimen with DPT-treated animals. Viscoelastic creep was highly variable among control animals as well (Tables 2–5). Some of the variability in control values may be accounted for by differences in basement membrane thickness, differences in the relative abundance of Type 4 collagen in the basement membrane and differences in ages of the animals. We doubt that variability was contributed by the dissection technique. In all species the proximal tubules were easily dissected in the control animals, and yet several–fold differences in deformability and viscoelastic creep were observed. We tried to keep dissection conditions and time constant to avoid introducing variable effects, and in some species in which segments could not be dissected in reasonable time (CFW_w germ–free mouse, cortical collecting tubules) we made no measurements. In the last analysis, we found no consistent patterns in the normal tubules that would distinguish among species and strains of animals on the basis of viscoelastic properties.

Microdissection of renal tubules obtained from kidneys of humans with autosomal dominant and acquired polycystic kidney disease and non-hereditary simple cysts provide strong evidence that renal cysts develop in nephrons and collecting ducts [7, 30, 31]. All renal cysts, hereditary or acquired, are lined by epithelium that in some instances appears to function in many respects like the intact tubule from which the cyst derived [9, 32–34]. The cysts appear to begin as localized dilations of tubules. In some reports basement membranes surrounding human renal cysts appears abnormal, although a consistent morphologic pattern has not been observed [3, 35, 36]. As in human cystic diseases, the cysts that develop in laboratory

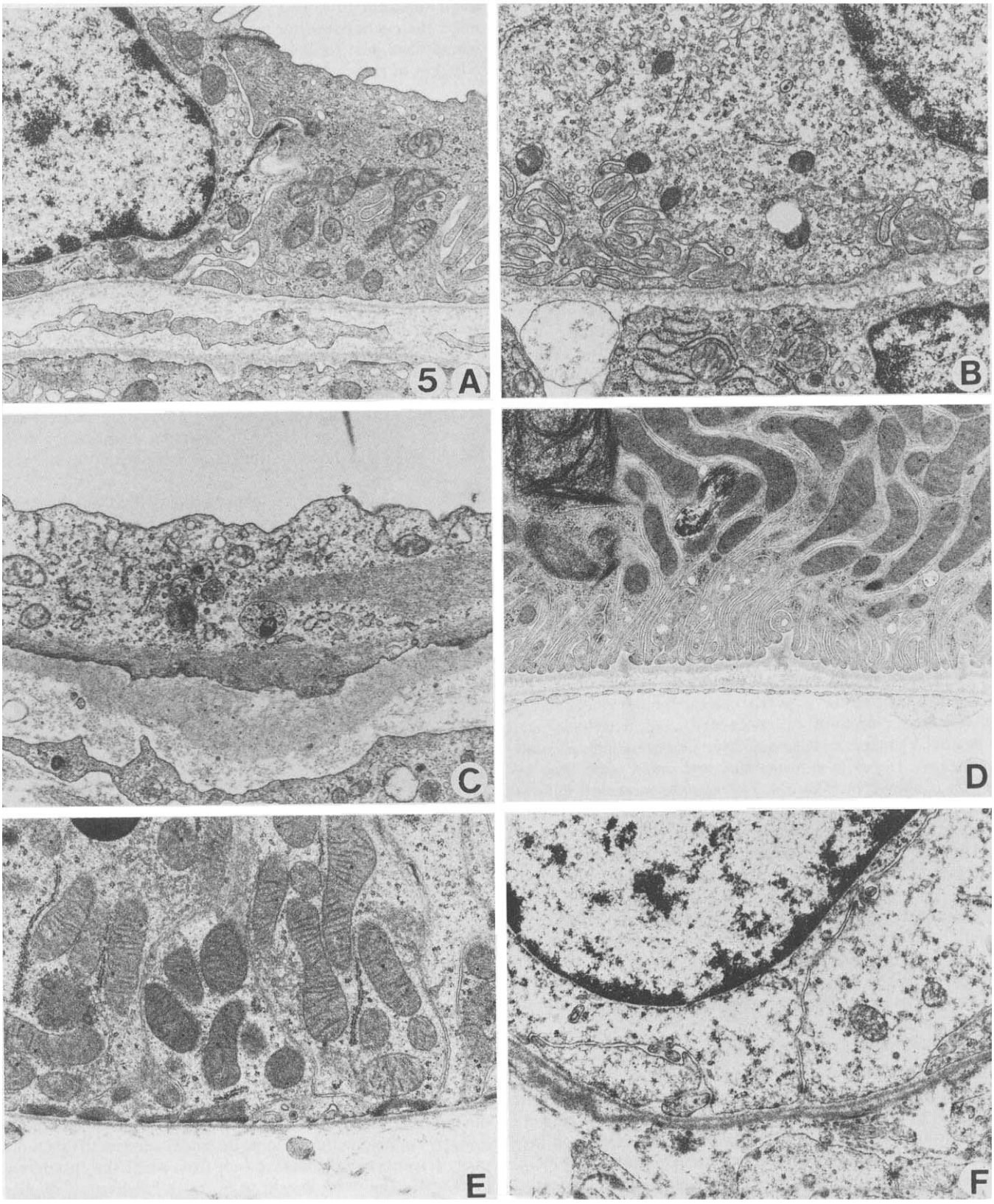

Fig. 5. *Basolateral surfaces of control tubules and cysts.* Transmission electron micrographs of: **A** Control Sprague–Dawley collecting tubule; **B** Non-cystic Sprague–Dawley collecting tubule; **C** DPT-treated Sprague–Dawley collecting tubule. **D** Control Sprague–Dawley proximal tubule. **E** Non-cystic Sprague–Dawley proximal tubule. **F** NDGA-treated Sprague–Dawley collecting tubule. (Continued on next page.)

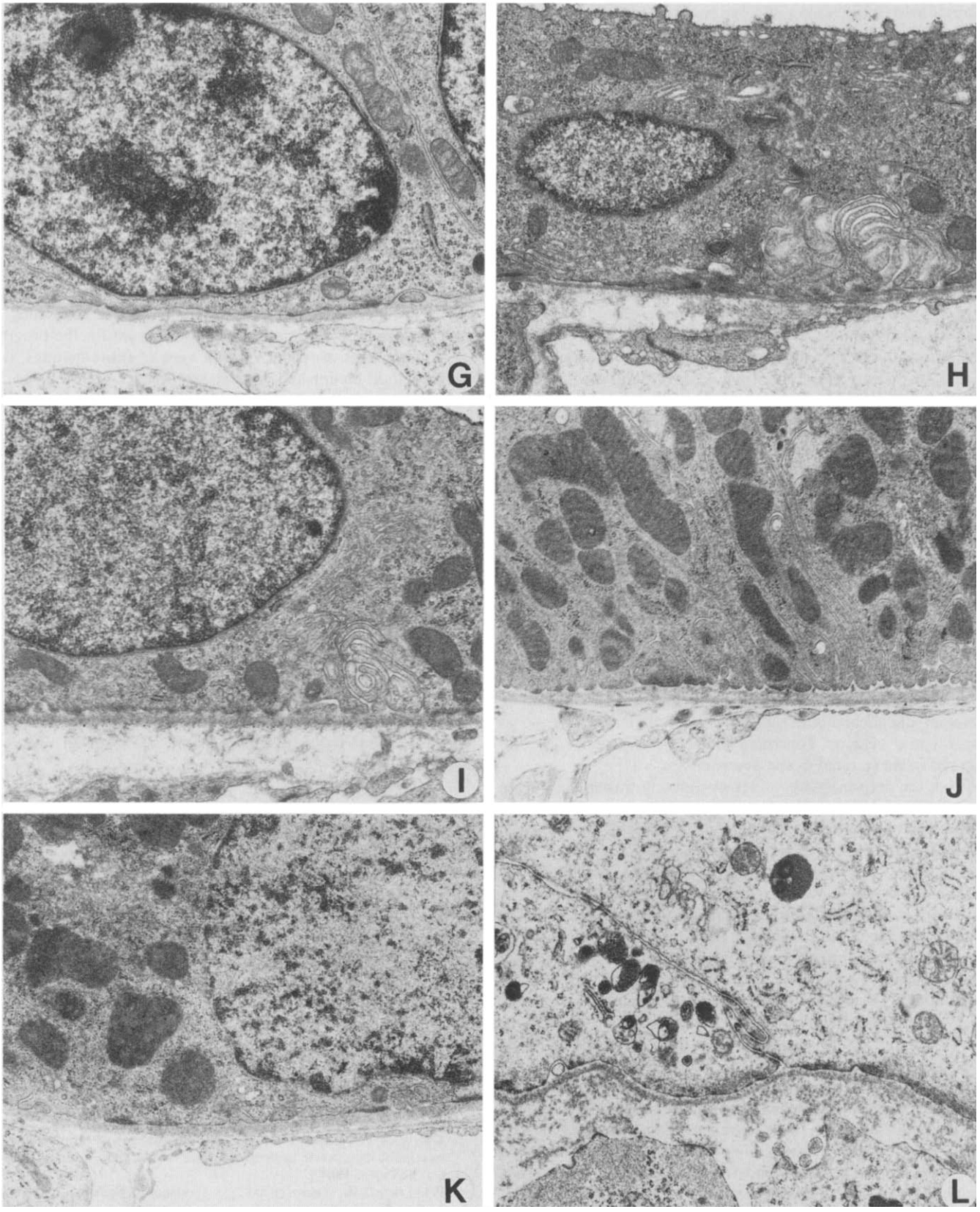

Fig. 5G Control collecting duct, C57 BL/6J. **H** Non-cystic collecting duct, C57 BL/6J. **I** Collecting duct cyst, C57 BL/6J. **J** Control proximal tubule CFW_w germ–free. **K** Non-cystic proximal tubule CFW_w cystic mouse. **L** Cyst, CFW_w. Kidneys were perfusion–fixed in glutaraldehyde, post-fixed in osmium tetroxide and processed for electron microscopy as described previously [1, 14, 15]. Magnification ×12,000.

animals arise from renal tubule segments. In the experimental models the cysts may develop as focal or saccular dilations of renal tubule [2, 7, 15, 31], yet their study here provides no direct support for the view that renal cysts develop in tubules secondary to increased deformability of the tubule basement membrane. Indeed, the results are consistent with the view that in cystic kidneys the tensile strength of the tubule basement membrane surrounding cystic and non-cystic renal tubules is relatively normal.

The conclusions of this study rest on the elastimer method for evaluating membrane viscoelastic properties. To increase the accuracy of the method we measured the effect of several sequential hydrostatic pressures (stresses) on membrane deformation in each tubule, glomerular capsule or cyst. The relation between stress (pressure) and strain (deformation) was linear between 40 and 100 cm H_2O, indicating that the elastic constant of the structure principally resisting deformation did not change during the measurement in normal nephrons and cysts [25, 26]. Consequently, we determined a unique value for deformability in each of the objects studied, obviating the need to use larger numbers of tubules and cysts. The fact that collagenase increased deformability, together with earlier direct measurements of plasma membrane and basement membrane deformability [11], adds additional support to the view that in our hands the elastimer method registers primarily the deformability of basement membranes.

To assess the potential role of viscous elements in the tubule basement membrane we applied a large static head of hydrostatic pressure (100 cm H_2O) and measured viscoelastic creep in cystic and non-cystic tubules. In most of the tubules from normal and cystic animals creep was barely detectable, consistent with the finding that certain basement membrane molecules, most likely Type IV collagen, are highly interconnected by covalent chemical bonds [27–29]. We found no evidence to suggest that viscous, basement membrane elements were increased in these renal cystic disorders.

From the deformability values obtained in this study we can estimate the steady-state transepithelial hydrostatic-pressure that would be required to form cysts by simple dilation of renal tubules and stretching of the tubule basement membranes. For this analysis we assume that the number of cells and quantity of TBM remains constant. Therefore, the relative extension of the basement membrane of tubules forming cysts is reflected by the relative change in circumference from the normal value. If we also assume that the deformability values for the cysts pertain from the start of the cystogenic process we calculate that mean transtubule pressures of 82, 134, 80 and 39 cm H_2O would be required to dilate the tubules to the extent observed in the cysts from DPT (collecting tubule), NDGA (collecting tubule), C 57 (collecting tubule), and CFW_w (proximal tubule) animals, respectively. In two animal models, DPT and NDGA, intratubular pressures in cystic tubules measured in situ were less than 53 cm H_2O, and 90% of the values were less than 32 cm H_2O [1, 7, 14]. Thus, on the basis of this analysis in situ intracystic pressures are not high enough to account for cyst formation by simple dilation of tubules in response to an increase in transepithelial hydrostatic pressure. Welling and Welling [9] reached a similar conclusion based on tubule compliance measurements obtained in normal, renal tubule segments perfused in vitro.

The data obtained in this study can be examined in a different way to assess the possible role of TBM in the cystogenic process. One can ask how great the change in deformability would have to be to account for the observed change in diameter from a tubule to a cyst were the net transepithelial hydrostatic pressure constantly increased by no more than 20 cm H_2O, the nominal maximal pressure in normal renal tubules in these experimental animals [1, 7, 14]. Under these conditions deformability would have to be 1.9 (CFW_w) to 6.7 (NDGA) times greater than the measured deformability in Tables 2–5 for the cysts to develop from renal tubules without an increase in transepithelial hydrostatic pressure above 20 cm H_2O. These calculations, the published pressure measurements [1, 7, 14], and the observation that the tubule cells and basement membranes lining the cysts in all of these model systems do not appear thinned (Fig. 5), indicate that passive stretching of tubule basement membranes does not account for the progressive enlargement of renal cysts. In view of these findings, it is unlikely that diminished tensile strength of TBM Type IV collagen is a central factor in the genesis of hereditary and acquired renal cysts.

The findings in this study lead to the supposition that cysts form because the epithelial cells proliferate and synthesize additional basement membrane material with normal tensile strength. In an earlier report, Welling and Welling [9] considered this to be the most likely mechanism of sustained cyst enlargement. Recent evidence suggests that an increase in the number of cells (cell proliferation) may be a central process in the formation of all renal cysts. In view of the current study, it is reasonable to suggest that proliferating epithelial cells synthesize and insert into the existing basal lamina basement membrane material with relatively normal tensile strength. This view is consonant with recent observations that changes in some of the non-collagen components of the basement membrane–extracellular matrix may be involved in the increased epithelial proliferation observed in the DPT model of cystic disease [37, 38].

Acknowledgments

This work was supported by a program project grant from HHS AM P01 33003. The Polycystic Kidney Research Foundation, Kansas City, Missouri provided assistance. We thank James Kornhaus, Bonnie Danley and Alice Algie for assistance.

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