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Cell apoptosis and proliferation in experimental chronic obstructive uropathy

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Cell apoptosis and proliferation in experimental chronic obstructive uropathy. Cell proliferation and apoptosis in kidneys with chronic obstructive uropathy (COU) have not been adequately studied. Whether these fundamental cellular processes play any role in the pathogenesis and evolution of COU remains undetermined. Sprague-Dawley rats with COU induced by unilateral ureteral ligation were sacrificed at postoperative days 1, 6, 9, 15, 25, 34, 43, 60, 75, and 90, and were compared with control, sham-operated rats sacrificed at days 0, 15, 43, and 90. The kidneys with ureteral ligation, the contralateral kidneys, and the control kidneys were submitted to *in situ* end-labeling of fragmented DNAs for the detection of apoptotic cells, and to immunostaining with many monoclonal antibodies directed against the nuclear antigens associated with cell proliferation for the detection of proliferating cells. Additional rats with COU were also submitted to BrdU labeling to detect proliferating cells. The tubular, interstitial, and glomerular cells showing either apoptosis or proliferation were separately quantitated and the obtained data were correlated with dry kidney weight, tubular diameter, glomerular surface area and interstitial volume. Apoptotic tubular cells in kidney with COU increased rapidly, reaching 30-fold that of control at day 25, which was followed by an equally rapid decrease to the control level. During the same period, both the dry kidney weight and the mean tubular diameter decreased markedly. These data suggest that apoptosis may play a significant role in tubular atrophy and renal weight loss. The rapid increase in tubular cell apoptosis was immediately preceded by a 37% gain in the dry kidney weight over the control; just before that increase, there was also an approximate 60-fold increase in the proliferation rate of tubular cells detected by immunostaining for proliferating nuclear antigen or by BrdU labeling. The significance of this intriguing temporal relationship of tubular cell apoptosis and proliferation remains to be elucidated, but it may have pathogenetic implications. In contrast to the rise and fall of the frequency of tubular cell apoptosis and proliferation, the frequency of interstitial cell apoptosis and proliferation displayed continuous increase toward the end of the experiment, with a roughly parallel increase in the interstitial damage. Apoptosis and proliferation of glomerular cells in kidneys with COU did not show any significant changes throughout the experiment. In conclusion, the obtained data suggest that tubular cell apoptosis may be pathogenetically related to the tubular atrophy and renal tissue loss in COU, and that proliferation and apoptosis of interstitial cells may play a role in the observed interstitial changes in this model. This study should provide the impetus for further exploration of the mechanisms of cell death and cell proliferation as a novel venue for understanding the pathogenesis of COU.

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Chronic obstructive uropathy (COU) due to unilateral ureteral obstruction is known to induce characteristic functional and structural changes in the affected kidney [1–3]. In the first few days after ureteral obstruction, the documented changes include a decline in glomerular filtration rate and in renal blood flow, interstitial edema and influx of leukocytes, predominantly macrophages, into the kidney [4–6]. If ureteral ligation is maintained for a longer period of time, the affected kidney develops hydronephrosis and tissue loss, reflected by a markedly reduced kidney weight, tubular atrophy, interstitial fibrosis and interstitial inflammation [1–3]. Although these changes have been uniformly documented in both humans and experimental animals, the pathogenesis is incompletely understood [4–6]. Most early studies using models of acute COU lasting for a few days, have emphasized the role of arteriolar vasoconstriction in response the release of vasoactive compounds from interstitial macrophages and probably from damaged tubular cells [3–6]. More recent studies on COU of longer duration have begun to explore the complex relationship of locally synthesized cytokines and the chronic changes of COU [6–16]. One fundamental aspect of the pathogenesis of COU is the complex changes in the renal cell phenotype and the loss of tissue mass, reflecting an imbalance between cell proliferation and cell death in the affected kidney [1–5]. These changes may potentially represent an important venue through which many pathogenetic factors of COU proceed. Yet, these cellular processes in various compartments of the kidney with COU have never been adequately studied.

Renal cells die through either necrosis or apoptosis. Although necrosis predominates in a few processes, such as renal infarct or acute tubular necrosis, cell death in the normal kidney and the kidney in most renal diseases occurs through apoptosis [17–19]. To the best of our knowledge, there has been only one study by Gobe and Axelsen using routine histologic technique to evaluate apoptosis of tubular cells and to correlate its frequency with renal weight loss in COU [20]. The poor appreciation of apoptosis in various renal diseases, in spite of the potentially crucial pathogenetic role of this fundamental cellular process, may be related to the fact that apoptotic cells are difficult to recognize by routine histologic techniques, due to their short lifespan of only a few hours. Moreover, cells already genetically committed to apoptosis may not yet express recognizable phenotypical changes [17]. It has long been known that the final common pathway of apoptosis is a fragmentation of nuclear DNA into oligonucleotides of several

hundred kilobases. This observation serves as the basis for the recent development of a method to accurately identify apoptotic cells *in situ* by labeling the free ends of the fragmented DNAs with a nucleotide tagged with a histologically recognizable signal [21].

Cell proliferation, an obligatory companion of cell apoptosis [22], has not been well studied in COU. We have found only one study using tritiated thymidine incorporation to demonstrate proliferation of renal interstitial cells in rabbit kidney with COU [2]. Simultaneous evaluation of cell proliferation in different renal compartments is facilitated by the recent availability of many antibodies directed against proliferation-associated antigens, including a group of antibodies against the proliferating cell nuclear antigen (PCNA) consistently expressed in the G_1, S, G_2 and M phases of the cell cycle [23–26].

In the present study, we have used the *in situ* end-labeling technique mentioned above and several antibodies with specificity for proliferating cells to study the frequency of cell apoptosis and cell proliferation in rat kidneys with COU of various durations. We found a significant correlation of the obtained data with some of the morphometrically determined changes in these kidneys, implying an important role for cell apoptosis and proliferation in the pathogenesis of COU.

Methods

Experimental design

Under anesthesia with pentobarbital, male Sprague-Dawley rats weighing 150 to 200 grams were subjected to complete ligation of the left ureter at the ureteropelvic junction using double silk suture. Postoperatively, these rats were given free access to a regular diet and tap water and then sacrificed in groups of three at days 1, 6, 9, 15, 25, 34, 43, 60, 75 and 90. For control, groups of rats with comparable weights were sham-operated and sacrificed at days 0, 15, 43, and 90.

Tissue preparation

Through a catheter placed in the infrarenal aorta, 0.9% saline was infused until the renal vein effuent became clear. Both kidneys were harvested, fixed in 10% buffered formalin and cut in four-micron sections for staining by hematoxylin-eosin, periodic acid-Schiff and Masson's trichrome techniques.

Determination of the dry weight of the kidneys

Each kidney was weighed immediately after harvesting. Dry kidney weight as a percent of wet wt was calculated for each kidney by weighing a representative coronal section of that kidney before and after thorough desiccation. Whole kidney dry weight was calculated from this percentage and the whole kidney wet wt and was expressed as percentage of body wt determined at the time of sacrifice.

In situ end-labeling of fragmented DNAs for the detection of cells with apoptosis

Apoptotic body staining was performed on three-micron tissue sections using the *in situ* end-labeling of fragmented DNAs described by Gavrieli, Sherman and Ben-Sasson [21]. Briefly, paraffin-embedded sections were deparaffinized in xylene for five minutes, rehydrated through graded concentrations of ethanol, and washed in distilled water (DW) twice for five minutes. To inactivate endogenous peroxidase, the tissue sections were incubated for 10 minutes in 2% H_2O_2 in DW and then rinsed in DW twice for five minutes. To facilitate penetration of enzymes and biotinylated deoxyuridine, the slides were subjected to proteinase K digestion by incubation in DNAase-free-proteinase K (20 μg/ml) for 15 minutes at room temperature (RT). The slides were then rinsed in DW four times for two and half minutes. The slides were immersed in terminal deoxynucleotidyl transferase (TdT) labeling buffer (30 mM Trisma-base pH 7.2, 140 mM sodium cacodylate, 1 mM cobalt chloride) for ten minutes. The *in situ* end-labeling of fragmented DNA was performed by incubating the slides with TdT/biotinylated deoxyuridine (UTP) diluted in TdT buffer in a humid chamber at 37°C for one hour [2 μl TdT (UBI, Lake Placid, NY, USA) and 5 μl of biotinylated UTP (Boehringer Mannheim, Indianapolis, IN, USA) diluted in 10 μl of TdT buffer]. The slides were then rinsed in terminating buffer (0.3 M NaCl and 0.03 M sodium citrate) for 15 minutes at RT and then rinsed in DW for five minutes. Blocking was performed by using 2% bovine serum albumin in PBS for ten minutes and rinsing briefly in PBS. The slides were then incubated with the avidin-biotin peroxidase complex (Vector Laboratories, Burlingame, CA, USA) for 30 minutes and then rinsed in PBS twice for five minutes. The slides were developed by using diaminobenzidine/H_2O_2 (50 ml of PBS containing 50 mg of diaminobenzidine and 30 μl of H_2O_2). For negative controls, some slides were incubated in the TdT buffer without TdT. For positive controls, some slides were heated with 1 μg/ml of DNAase (Sigma Chemical Company, St. Louis, MO, USA) in DNA buffer (30 mM Trisma base pH 7.2, 140 mM sodium cacodylate, 4 mM $MgCl_2$). These controls were subsequently subjected to the rest of the same procedure.

Immunolocalization of proliferating cells

Proliferating cells were detected in formalin-fixed, paraffin-embedded kidney tissue by immunohistochemistry, using a standard avidin-biotin peroxidase complex technique and two monoclonal antibodies directed against the proliferating nuclear antigens (PCNA), known to be expressed in most proliferating cells especially the ones in S phase of the cell cycle [23] [Clone $19A_2$ (Coulter Immunology, Hialeah, FL, USA;1:3200 dilution) and clone PC10 (Dako Corporation, Carpinteria, CA, USA; 1:100 dilution)]. A procedure for antigen retrieval using microwave oven heating as originally described by Shi, Key and Karla was used for both antibodies [24].

Since the preliminary evaluation of the PCNA-positive cell revealed a remarkably increased tubular cell proliferation preceding the onset of tubular cell apoptosis (**Results**), other methods of detecting proliferating cells were explored to confirm this interesting observation. For this purpose, renal tissue was subjected to immunohistochemical staining as described above, using both polyclonal and monoclonal MIB-1 antibodies direct against the epitopes Ki-67 known to express in the late G_1, S, G_2 and M phases of the cell cycle [26]. In addition, 5-bromo-2′-deoxyuridine (BrdU; Zymed, San Francisco, CA, USA), which is a thymidine analogue known to be incorporated into proliferating cell nuclei [23], was injected intraperitonealy two hours before sacrifice into groups of two rats with COU at days 0, 1, 6, 9, 15, 25, 34, 43 and 60. The kidneys were subsequently harvested, fixed in 10% buffered formalin, and subjected to immunohistochemical staining for BrdU using a staining kit and a monoclonal antibody against BrdU (Zymed).

Morphometric studies

Morphometry was used to determine the frequency of apoptotic cells, the frequency of proliferating cells, the tubular diameter, the glomerular surface area, and the interstitial volume. All apoptotic cells in a representative sagittal section containing both cortex and medulla of each kidney were counted separately for tubular, interstitial and glomerular compartments. No attempt was made to quantitate apoptotic cells in the vascular compartment since cells in vascular wall may be very difficult or impossible to be distinguished from interstitial cells, especially in the late stage of COU. The frequency of tubular cells undergoing apoptosis was expressed as the number of apoptotic tubular cells in each area covered by a graded ocular grid measuring 1 cm^2 viewed under the ×40 eyepiece of a Nikon microscope (0.0025 cm^2). The frequency of interstitial cell apoptosis was calculated in the same manner, whereas the frequency of glomerular cell apoptosis was expressed as the number of glomerular cells with apoptosis per glomerulus. The corresponding frequencies of cells undergoing proliferation were calculated in the same manner. The tubular diameter (measured in micrometers) was expressed as the mean diameter of ten random tubular cross sections each in cortex and medulla, excluding cross sections with markedly cystic diltation defined as the ones with a diameter at least four times that of adjacent tubular cross sections. These cross sections were rare at any time point (maximally 4% at day 6) and their number was indeed decreased with longer duration of COU (0% at day 90). Since these cross sections were both rare and much larger than the rest of the tubular cross sections, including them in the measurement of tubular diameter would introduce significant bias. The interstitial volume, determined by a point counting technique on tissue sections stained by the Masson's trichrome method, was expressed as the mean percentage of grid points lying within the interstitial area in up to three fields each in cortex and medulla, each of these fields was deliniated by an 1 cm^2 graded ocular grid viewed at ×20 magnification. The glomerular surfaces area was expressed in μm [2] as the mean value of up to ten glomeruli, each of which displayed either a vascular or a tubular pole or both.

Correlative studies and statistical analysis

The morphometric data were plotted against the experimental durations and were expressed as mean ± SE. Statistical difference was assessed by analysis of variance. $P < 0.05$ was considered to be significant.

Results

Pathologic changes

The kidney with COU displayed a spectrum of changes including tubular dilatation and interstitial edema in the early phase, tubular atrophy and interstitial fibrosis in the later phase, and mononuclear inflammatory cell interstitial infiltrate of progressive severity. Necrosis, defined as involvement of a large number of cells with variable degrees of disintergration of cell membrane and nuclei was not seen. Individual cells, especially tubular cells, undergoing apoptosis, characterized by cytoplasmic condensation and nuclear fragmentation, were easily identified. More accurate detection of these apoptotic cells by *in situ* labeling of fragmented DNAs is described below. The glomeruli were initially normal, but subsequently displayed mild glomerular collapse associated with an enlarged urinary space. No significant histologic changes were noted in the contralateral kidneys or kidneys of the sham-

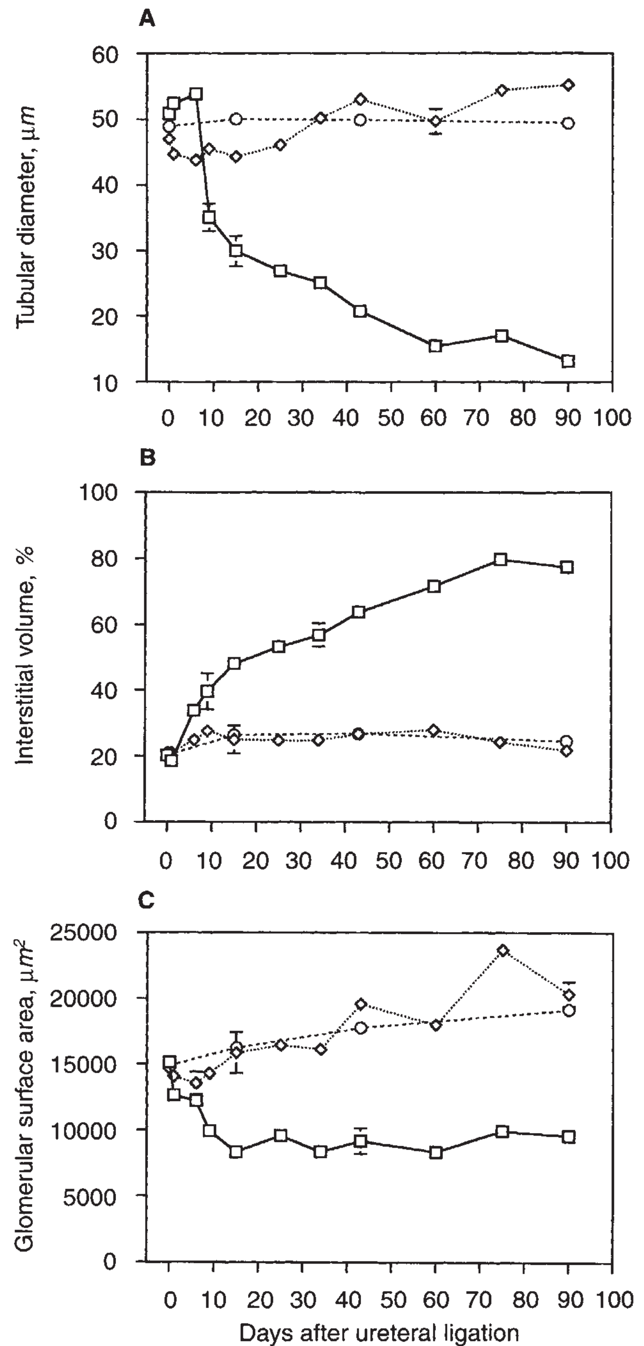

Fig. 1. *Morphometric data for kidneys with ureteral ligation (□), contralateral kidney (◇) and control kidneys (○).*

operated control rats. The correlation of the mean tubular diameter, the glomerular surface area and the interstitial volume of kidneys in each group with experimental duration is summarized in Figure 1. The differences between the kidneys with ureteral ligation and the contralateral as well as control kidneys were highly significant ($P < 0.001$).

Dry kidney weight

The data are summarized in Figure 2. After ureteral ligation, the dry weight of the kidneys with COU, expressed as percent of body wt, increased rapidly, and peaked at day 6 when it was about

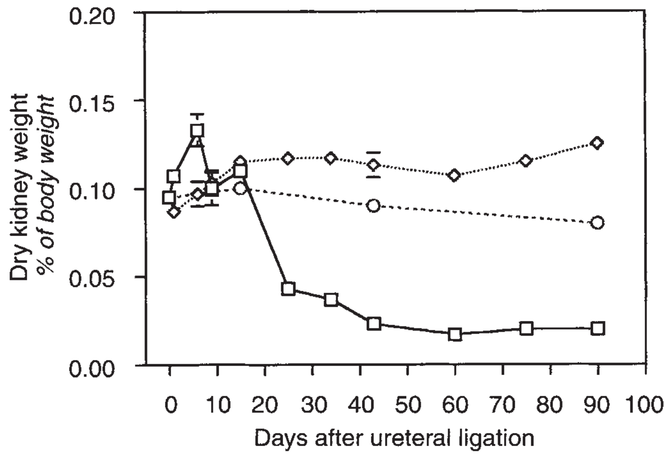

Fig. 2. *Dry weight (expressed as percentage of body wt) of kidneys with ureteral ligation (□), contralateral kidneys (◇) and control kidneys (○).*

37% above that of contralateral kidneys (0.133 ± 0.03% vs. 0.097 ± 0.003%), and about 24% above that of control kidneys (0.133 ± 0.003% vs. 0.107 ± 0.003%). Subsequently, the dry weight decreased rapidly to about 47% of the contralateral kidneys at day 25, and stabilized at about 16% of the weight of the contralateral kidneys throughout the rest of the experiment. There was a minor weight gain by the contralateral kidneys toward the end of the experiment, whereas a steady decrease was noted for the weight of the control kidneys. The observed difference was highly significant ($P < 0.001$).

Apoptosis

Tubular cells. Data related to apoptosis are summarized in Figures 3 and 4. The *in situ* end-labeling technique enabled easy recognition and accurate quantitation of apoptotic cells. Starting from day 6 (Fig. 3A), apoptosis of tubular cells increased rapidly, peaked at day 25, subsequently decreased rapidly until day 43, and then decreased gradually until the end of the experiment. Although the frequency of apoptotic tubular cells was not separately quantitated for different segments of the nephron due to the difficulty in identifying them in obstructed kidney, especially in the late phase, it was recognized that tubular apoptosis started at the inner medulla on day 1, spread to the entire medulla on day 15, and involved both cortex and medulla on day 25. Tubular apoptosis of the contralateral and control kidneys remained low throughout the experiment. The differences between the kidneys with ureteral ligation and the contralateral as well as the control kidneys were highly significant ($P \leq 0.001$).

Interstitial cells. Apoptotic interstitial cells gradually increased throughout the entire experimental period. They were about 10% as frequent as apoptotic tubular cells at day 25, when tubular apoptosis peaked, but became more numerous than the latter starting at around day 60 (Fig. 3B). Although interstitial fibroblasts, vascular wall cells and interstitial inflammatory cells could all potentially contribute to the apoptotic cell population, it was not possible to quantitate them separately because apoptosis, regardless of cell origin, is morphologically similar. Nevertheless, inflammatory cells were the most frequent cell type in the interstitium toward the end of the experiment and probably accounted for the high frequency of interstitial cell apoptosis seen in this period. Interstitial cell apoptosis of the contralateral and control kidneys was similar and stable throughout the experiment (Fig. 3B). The observed difference between the ligated kidneys and the contralateral as well as the control kidneys were highly significant ($P \leq 0.001$).

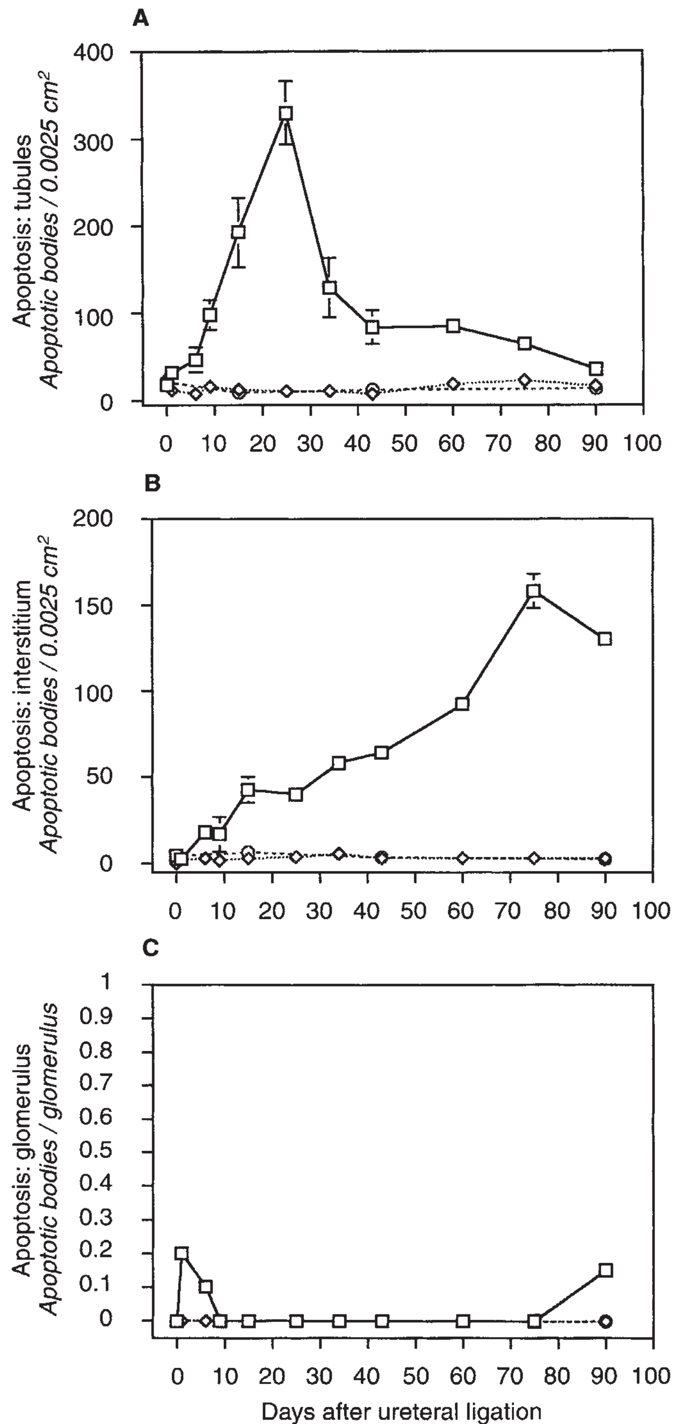

Fig. 3. *The frequency of apoptotic cells in the tubular (**A**), interstitial (**B**) and glomerular (**C**) compartments of kidneys with ureteral ligation (□), contralateral kidneys (◇) and control kidneys (○).*

Glomerular cells. Apoptosis of glomerular cells was rare (0 to 0.03 cells per glomulus) and did not show any statistically significant differences among the three groups of kidney tissue ($P \geq 0.149$; Fig. 3C).

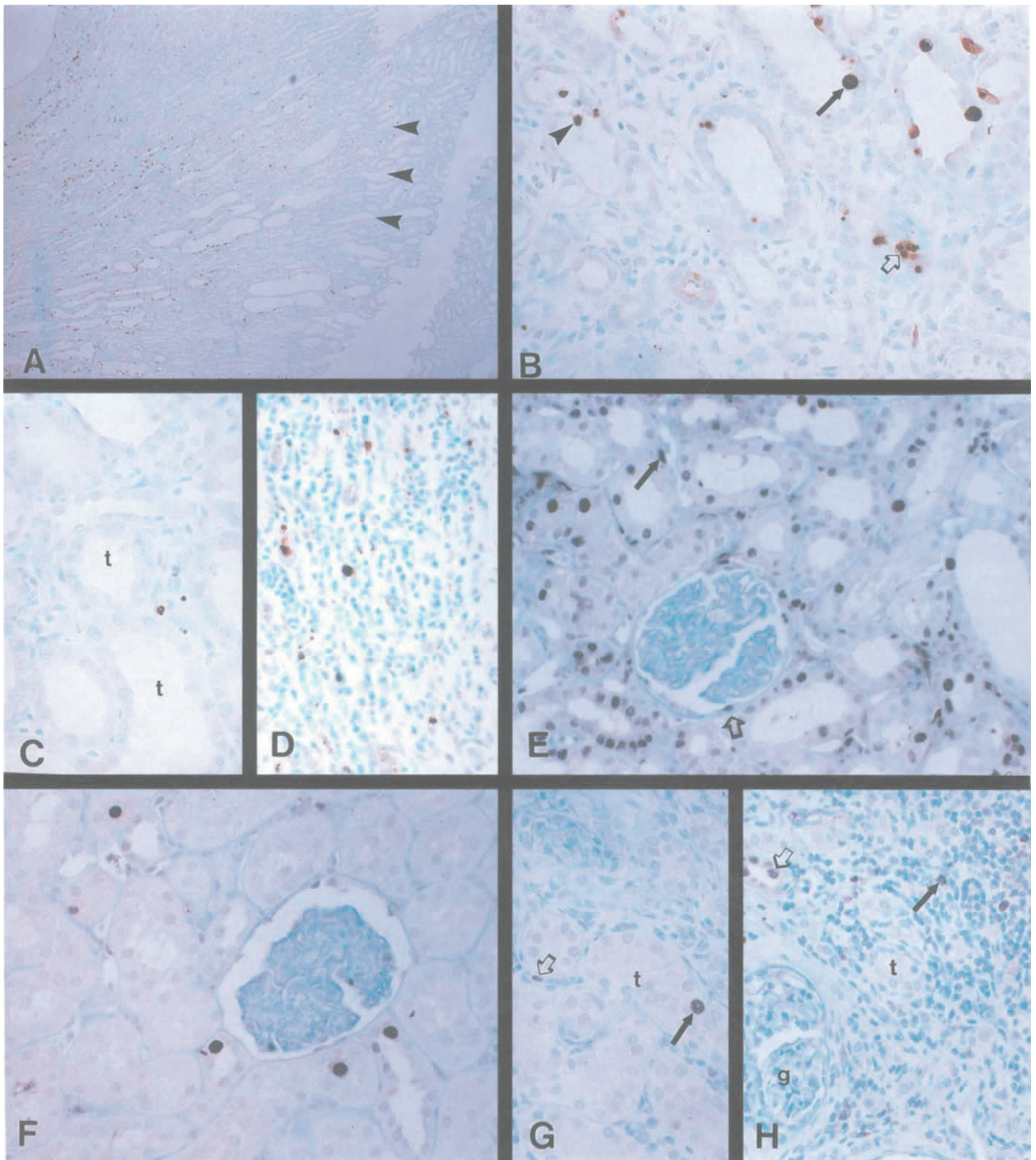

Fig. 4. *Demonstrations of apoptotic cells (A-D).* (**A**) A kidney nine days after ureteral ligation displays many apoptotic cells seen mostly along the dilated collecting ducts in the inner medulla (arrowheads; magnification ×100). (**B**) A kidney after 25 days of ureteral ligation. The nuclei of tubular apoptotic cells appear as a single body intensely stained (arrow), are fragmented (open arrow), or expelled into the tubular lumen (arrowhead). Tubular atrophy, interstitial fibrosis and inflammation are also seen (×1,000). (**C**) A kidney after 60 days of ureteral ligation. Apoptosis is noted in some interstitial cells, the cell type of which cannot be determined with certainty. Tubular dilation (t) and interstitial changes are also seen (×1,000). (**D**) A kidney after 90 days of ureteral ligation. An area of heavy interstitial mononuclear inflammatory cell infiltrate showing some apoptotic cells (×1,000). Demonstrations of proliferating cells (E-H). (**E**) A kidney six days after ureteral ligation. Nuclei of many tubular cells are positively stained for proliferating nuclear antigen. Some proliferating interstitial cells (solid arrow) and parietal epithelial cells (open arrow) are also noted (×500). (**F**). The contralateral kidney of the same rat shown in E displays only a few proliferating tubular cells (×500). (**G**) A kidney 43 days after ureteral ligation displays many atrophic tubules (t) with an apoptotic tubular cell (solid arrow) and an apoptotic interstitial cell (open arrow; ×1,000). (**H**) A kidney 70 days after ureteral ligation with atrophic tubules (t), proliferating tubular cells (open arrow) and proliferating interstitial cells (solid arrow), probably representing inflammatory cells. An intact glomerulus is seen (g) (×1,000). Publication of this figure in color was made possible by a grant from Hoffmann-La Roche, Inc., Raritan, New Jersey, USA.

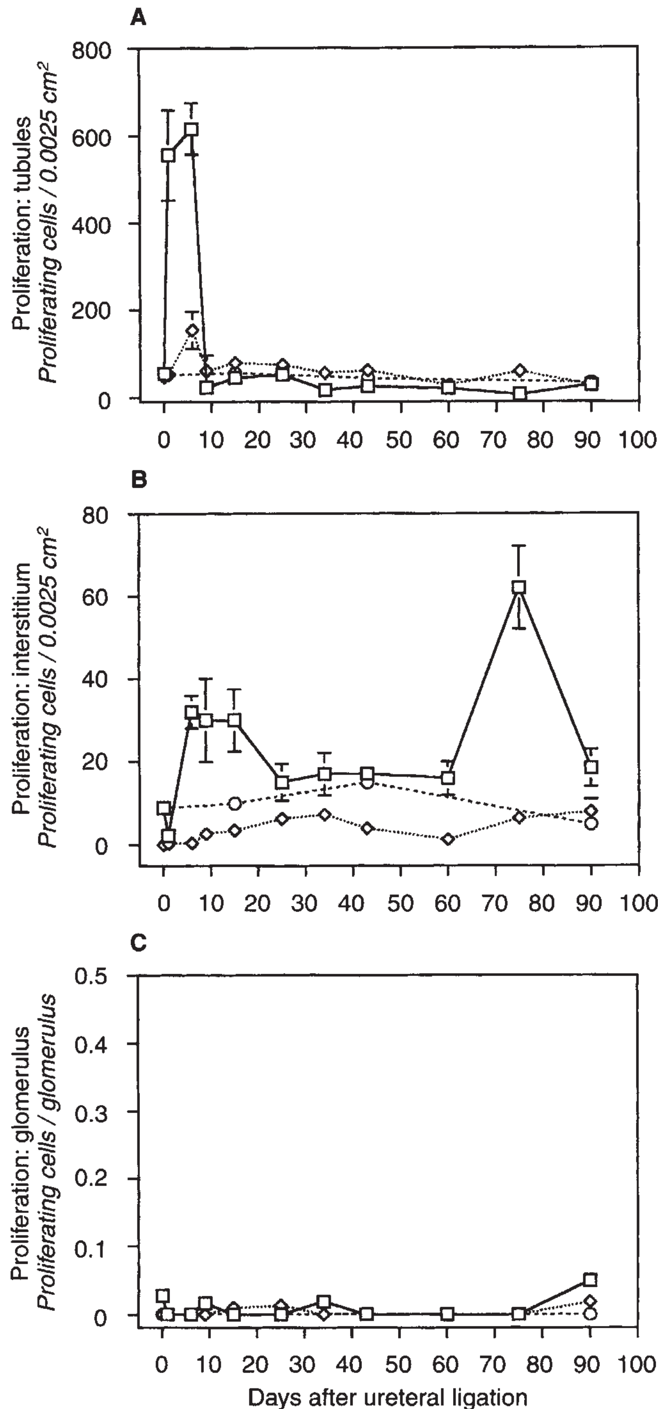

Fig. 5. *The frequency of proliferating cells in the tubular (**A**), interstitial (**B**) and glomerular (**C**) compartments of kidneys with ureteral ligation (□), contralateral kidneys (◇) and control kidneys (○).*

Cell proliferation

Tubular cells. The two antibodies against PCNA crisply decorated nuclei of proliferating cells in a similar manner, and were used for quantitation of cell proliferation. Data related to cell proliferation are summarized in Figures 4 E-H and 5. Marked increase in tubular cell proliferation (Fig. 5A) was noted in the affected kidney immediately after ureteral ligation, peaked by day 6 at a level almost 60 times that of control, and thereafter decreased drastically to reach the control level starting at day 9, where it remained throughout the rest of the experimental period. The tubular cell proliferation seemed to be most pronounced at the outer medulla, involving mostly collecting ducts, thick ascending limbs of Henle loop and, to a much lesser extent, S3 segments of proximal tubules. A parallel but much lower peak was noted in the contralateral kidneys, whereas the frequency for the control kidneys remained stable throughout the experiment. The differences noted above were highly significant ($P < 0.001$). Both the polyclonal and monoclonal antibodies MIB-1 failed to detect proliferating cells. The BrdU labeling technique, however, clearly identified proliferating cells. The frequencies of proliferating tubular cells at days 0, 1, 6, 9, 15, 25, 34, 43 and 60, detected by PCNA immunostaining and BrdU labeling, respectively, are listed in Table 1. The obtained data demonstrate the same frequency and the temporal pattern of variation for proliferating tubular cells detected by these two techniques.

Interstitial cells. Increased proliferation of interstitial cells in the kidneys with COU (Fig. 5B), compared to the contralateral and control kidneys, was noted throughout the experimental period, with two peaks, one between day 1 and 25, and the other between days 60 and 90 ($P \leq 0.009$). Although interstitial fibroblasts, vascular wall cells and interstitial inflammatory cells, as in the case of apoptosis, participated in the proliferative process, the relative contribution of each cell type to this process could not be accurately quantitated.

Glomerular cells. Glomerular cells in proliferation were rare (0 to 0.066/glomerulus; Fig. 5C) and did not show any statistically significant differences among the three groups of kidney tissue ($P \geq 0.541$).

Discussion

Our study clearly documents distinctively different patterns of cell apoptosis and cell proliferation for tubular, interstitial and glomerular cells in kidneys with COU. In each of these compartments, these distinctive patterns reflect the experimental duration, and, in conjunction with the morphometrically determined morphological changes of the affected kidneys, may provide some insight into the pathogenesis of COU.

Gobe and Axelsen were the first to draw attention to tubular cell apoptosis as a cause of tubular atrophy in COU [20]. However, they employed routine histologic technique to identify apoptosis, and the sampling for apoptosis in their study was somewhat limited. Moreover, pertinent factors such as tubular cell proliferation and cell kinetics of other renal compartments were not addressed. Our approach, using a sensitive and specific technique to detect apoptotic cells *in situ*, and a morphometric method that reduced sampling error to a minimal level, clearly documented the frequent occurrence of apoptosis in tubular cells, implying a significant role of this process in the pathogenesis and evolution of COU. Our data support Gobe and Axelsen's suggestion that tubular apoptosis mediates tubular atrophy and renal weight loss in COU [20]. The supporting evidence includes the presence of a remarkable inverse relationship of renal weight and the frequency of tubular cell apoptosis between days 6 and 25, during which period the magnitude of these changes was at a maximum. Subsequent to this peak activity, tubular cell apoptosis stabilized and was associated with a stable, albeit markedly reduced, renal weight. The relationship of tubular diameter with tubular cell apoptosis remains incompletely understood. The

Table 1. Frequency of proliferating tubular cells (cell number/0.0025 cm^2) by PCNA immunostaining and by BrdU labelling[a]

	Day 0	Day 1	Day 6	Day 9	Day 15	Day 25	Day 34	Day 43	Day 60
PCNA	55 ± 11.3	55.6 ± 1.04	661.3 ± 59.6	23 ± 1.5	45 ± 1.5	51.6 ± 88	17.3 ± 6.3	24.6 ± 6.7	21.6 ± 2.3
BrdU	11.3 ± 3.1	219.6 ± 9.9	591.8 ± 167.8	31.9 ± 6.2	17.7 ± 7.2	35.9 ± 11.3	21.1 ± 10.9	20.7 ± 9.9	21.1 ± 2.4

[a] Correlation analysis was performed and a P value less than 0.002 was found, indicating that the data from PCNA immunostaining BrdU labelling are comparable.

tubular diameter depends on at least three parameters, that is, tubular cell volume, numbers of tubular cells per cross section and the diameter of tubular lumen. Although attempts to quantitate these parameters, especially the cell volume, were unsuccessful, careful light microscopic observation suggests that the increased tubular diameter between day 0 and day 6 was probably related to both dilatation of tubular lumens and tubular cell proliferation (Fig. 5A). The rapid decrease of tubular diameter between days 6 and 15 was probably related to a decrease of both tubular cell volume and tubular lumen diameter, and an increased tubular cell apoptosis. The gradual decrease of tubular diameter occurring after day 25 was probably related only to apoptosis since a significant change in the tubular cell volume was not appreciated during this period. There are many other factors which theoretically may control renal mass, such as apoptosis of glomerular or interstitial cells, and proliferation rate of tubular, glomerular or interstitial cells. Our data, however, indicate that these factors play no significant role in the weight loss in kidney with COU since they either did not significantly deviate from control, or when there were changes they were mild, and indeed, occurred at a time when renal weight was already stabilized.

Although our study clearly indicates that tubular cell apoptosis mediates weight loss in kidneys with COU, whether it has additional roles in the pathogenesis of COU is not known and cannot be answered from this study. It has been recognized that apoptosis can be induced by contact, mediated by a ligand-receptor system called Fas, between target cells and cytotoxic T lymphocytes [27], and that apoptotic cells are rapidly removed by macrophages [17, 28], or adjacent native cells [29]. Whether or not tubular cell apoptosis represents one of the mechanisms by which inflammatory cells are recruited to the area of injury is not known. Nevertheless, the Fas receptor mRNA has been documented in renal tubular cells in ischemic injury [30]. Moreover, in our model and that of Schreiner and coworkers [6], influx of macrophages was limited to the inner medulla during the first few days, a time when tubular cell apoptosis was found almost exclusively in this zone.

In addition to apoptosis, our study revealed an unexpected pattern of tubular cell proliferation characterized by a rapid rise and subsequent fall of tubular cell proliferation, which was associated with a parallel change, albeit of a lesser magnitude, in dry weight of the COU kidneys (Figs. 2 and 5A). This proliferation peak also immediately preceded the surge in tubular cell apoptosis. This interesting pattern of tubular cell proliferation was initially observed by immunostaining for PCNA and was subsequently confirmed by BrdU labeling. Although this finding has never been documented in kidneys with COU, a similar trend has been noted in some experimental models, including silver nitrate-induced renal hyperplasia [31] and the repair phase of ischemic tubular damage [19, 32]. Cell death and cell proliferation are obligatory companions [17]. Indeed, it has been noted that the genetic programming for both apoptosis and proliferation, at least in the early phase, is similar with subsequent divergence depending on the participation of additional factors promoting cell proliferation or cell death, respectively [17, 33]. Some elements of this concept may be applicable to our COU model. For example, the early proto-ongogene *c-myc* is known to induce both cellular apoptotic and proliferative commitment [17, 33]. Activation of proto-oncogenes, including *c-myc* [7, 15], known to occur immediately after ureteral ligation, can potentially induce tubular cell proliferation followed by apoptosis mediated by the same oncogenes, but now under a modifying influence of a loss of epidermal growth factor. This phenomenon is well documented in kidneys with COU [10]. Regardless of whether the intriguing relationship of tubular cell proliferation and apoptosis is mediated through this or other mechanisms, this relationship probably has some pathogenetic significance and deserves further study.

Our study further demonstrates that apoptosis and proliferation also occur in interstitial cells, albeit in a temporal pattern distinctly different from that of tubular cells. Data summarized in Figure 3 showed that apoptosis and proliferation, which were active in the tubular compartment in the early phase of COU, were subsequently switched to the interstitial compartment, where there was a gradual but continuous increase in apoptosis and proliferation to the end of the experiment. During this period, the tubular cell apoptosis and proliferation returned to control level. The interstitial cell apoptosis increased in parallel with the increased interstitial volume. However, the relationship between interstitial cell proliferation and interstitial volume was more complicated since although the interstitial volume progressively increased toward the end of the experiment, two peaks of interstitial cell proliferation were noted at days 9 and 75. Although our model and those of others demonstrate an increased interstitial cellularity including myofibroblasts, inflammatory cells and probably vascular wall cells [1–4], data from our study do not allow an accurate determination on the relative contribution of each cell type in the pool of interstitial cell apoptosis or proliferation. Nevertheless, the continuous increase in interstitial cell apoptosis and proliferation at a time when these processes had subsided in the tubules clearly reflects the high turnover rate of interstitial cells as well as the dynamic role of the interstitium in the late phase of COU. By what mechanism these processes mediate known interstitial changes in COU such as fibrosis, inflammation and vascular wall remodeling remains to be determined.

Previous studies have emphasized the absence of glomerular changes in COU [1–3]. In our model, although the glomerular surface area was mildly decreased, no other significant changes were noted. While the reason for this structural integrity is currently not known, the absence of any deviation of glomerular cell apoptosis and proliferation of kidneys with COU from those of control kidneys (Figs. 3C and 5C), indicating a low cell turnover rate, may be a part of the explanation.

The study of the mechanisms controlling apoptosis is active but

is still in its infancy. Many factors have been implicated in the promotion of apoptosis [reviewed in 17], which may be intrinsic (such as target cell expression of clusterin, oncogenes, Fas receptor, wild type P_{53} gene, c-*myc* oncogene), or extrinsic (such as tumor necrosis factor, transforming growth factors-β, ischemia). Factors inhibiting apoptosis include expression of the gene *bcl-2, bcl-x,* and P_{53} mutant gene by the target cells or the extrinsic effect of estrogen, epidermal growth factor, hepatic growth factor and antioxidants. Although none of these factors has been tested for their pathogenetic role in the apoptosis seen in COU, it is noteworthy that many of these factors have been implicated in the development of COU. In this regard, our study, recognizing completely different proliferative and apoptotic patterns for the tubular and interstitial cells, suggests the existence of distinct pathogenetic mechanisms for these fundamental processes in various renal compartments.

In summary, the current study documents, in detail, the distinctive proliferative and apoptotic patterns of cells in various compartments of kidneys at different stages of experimental COU. The obtained data suggest that tubular cell apoptosis plays a role in the development of tubular atrophy and renal tissue loss in COU; furthermore, they suggest that proliferation and apoptosis of interstitial cells may be related to the observed interstitial changes. This study should set the stage for further investigation of the mechanisms of cell death and cell proliferation as a novel venue for understanding the pathogenesis of COU.

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References

1. NAGLE RB, BULGER RE: Unilateral obstructive nephropalthy in the rabbit. II. Late morphologic changes. *Lab Invest* 38:270–278, 1978
2. NAGLE RB, JOHNSON ME, JERVIS HR: Proliferation of renal intestitial cells following injury induced by ureteral obstruction. *Lab Invest* 35:18–22, 1976
3. WILSON D, KLAHR S: Urinary Tract Obstruction, in *Diseases of the Kidney* (5th ed), edited by SCHRIER RW, GOTTSCHALK CW, Boston, Little Brown and Co., 1993, pp 657–687
4. KLAHR S, PURKERSON ML: The pathophysiology of obstructive nephropathy: The role of vasoactive compounds in the hemodynamic and structural abnormalities of the obstructed kidney. *Am J Kid Dis* 23:219–233, 1994
5. HARRIS KPG, KLAHR S, SCHREINER G: Obstructive nephropathy: From mechanical disturbance to immune activation? *Exp Nephrol* 1:198–204, 1993
6. SCHREINER GF, HARRIS KPG, PURKERSON ML, KLAHR S: Immunological aspects of acute ureteral obstruction: Immune cell infiltrate in the kidney. *Kidney Int* 34:487–493, 1988
7. WALTON G, BUTTYAN R, GARCIA-MONTES E, OLSSON CA, HENSLE TW, SAWCZUK IS: Renal growth factor expression during the early phase of experimental hydronephrosis. *J Urol* 148:510–514, 1992
8. KANETO H, MORRISSEY J, MCCRACKEN R, MCCRACKEN R, REYES A, KLAHR S: Enalapril reduces collagen type IV synthesis and expansion of the interstitium in the obstructed rat kidney. *Kidney Int* 45:1637–1647, 1994
9. CHEVALIER RL, THORNHILL BA, GOMEZ RA: EDRF modulates renal hemodynamics during unilateral ureteral obstruction in the rat. *Kidney Int* 42:400–406, 1992
10. STORCH S, SAGGI S, MEGYESI J, PRICE PM, SAFIRSTEIN R: Ureteral obstruction decreases renal prepro-epidermal growth factor and Tamm-Horsfall expression. *Kidney Int* 2:89–94, 1992
11. DIAMOND JR, VAN GOOR H, DING G, ENGLEMYER E: Myofibroblasts in experimental hydronephrosis. *Am J Pathol* 146:121–129, 1995
12. KANETO H, MORRISSEY J, KLAHR S: Increased expression of TGF-β1 mRNA in the obstructed kidney of rats with unilateral ureteral ligation. *Kidney Int* 44:313–321, 1993
13. SHARMA AK, MAUER SM, KIM Y, MICHAEL AF: Interstitial fibrosis in obstructive nephropathy. *Kidney Int* 44:774–788, 1993
14. CONNOR J, BUTTYAN R, OLSSON CA, D'AGATI V, O'TOOLE K, SAWCZUK S: SGP-2 expression as a genetic marker of progressive cellular pathology in experimental hydronephrosis. *Kidney Int* 39: 1098–1103, 1991
15. SAWCZUK S, HOKE G, OLSSON CA, CONNOR J, BUTTYAN R: Gene expression in response to acute unilateral ureteral obstruction. *Kidney Int* 35:1315–1319, 1989
16. SCHLEGEL PN, MATTHEWS GJ, CICHON Z, AULITZKY WK, CHENG CY, CHEN CLC, SASO L, GOLDESTEIN M, BARDIN CW, VAUGHAN ED JR: Clusterin production in the obstructed rabbit kidney: Correlations with loss of renal function. *Am J Nephrol* 3:1163–1171, 1992
17. SAVILL J: Apoptosis and the kidney. *J Am Soc Nephrol* 5:12–21, 1994
18. GOBE GC, AXELSEN RA: The role of apoptosis in the development of renal cortical tubular atrophy associated with healed experimental renal papillary necrosis. *Pathology* 23:213–223, 1991
19. GOBE GC, AXELSEN RA, SEARLE JW: Cellular events in experimental unilateral ischemic renal atrophy and in regeneration after contralateral nephrectomy. *Lab Invest* 63:770–779, 1990
20. GOBE GC, AXELSEN RA: Genesis of renal tubular atrophy in experimental hydronephrosis in the rat: Role of apoptosis. *Lab Invest* 56:273–281, 1987
21. GAVRIELI Y, SHERMAN Y, BEN-SASSON SA: Identification of programmed cell death in situ via specific labelling of nuclear DNA fragamentation. *J Cell Biol* 119:493–501 1992
22. MARX J: Cell death studies yield cancer clues. *Science* 259:760–761, 1993
23. CONNOLLY K, BOGDANFFY M: Evaluation of proliferating cell nuclear antigen (PCNA) as an endogenous marker of cell proliferation in rat liver: A dual-stain comparison with 5-bromo-2′ deoxyuridine. *J Histochem Cytochem* 41:1–6, 1993
24. SHI SR, KEY ME, KARLA KL: Antigen retrieval in formalin-fixed paraffin-embedded tisues: An enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem* 39:741–748, 1991
25. KELLEHER L, MAGEE HM, DERVAN PA: Evaluation of cell proliferation antibodies reactive in paraffin sections. *App Immunohistochem* 2:164–170, 1994
26. SCOTT RJ, HALL PA, HALDANE JS, VAN NOORDEN S, PRICE Y, LANE DP, WRIGHT NA: A comparison of immunohistochemical markers of cell proliferation with experimentally determined growth fraction. *J Pathol* 1165:173–178, 1991
27. ITOH N, YONEHARA S, ISHII A, YONEHARA M, MIZUSHIMA SI, SHAMESHIMA M, HASE A, SETO Y, NAGATA S: The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 66:233–243, 1991
28. SAVILL J, HOGG N, REN Y, HASLETT C: Thrombospondin cooperates with CD36 and the vitronectin receptor in macrophage recognition of neutrophils undergoing apoptosis. *J Clin Invest* 90:1513–1522, 1992
29. WYLLIE AH, KERR JFR, CURRIE AR: Cell death: The significance of apoptosis. *Int Rev Cytol* 68:251–306, 1980
30. NOGAE S, KOJI T, NAKANISHI Y, ABE K, NAKANE PK: Induction of apoptosis in ischemia-reperfusion kidney model: Appearance of DNA strand breaks and expression of Fas mRNA. (abstract) *J Am Soc Nephrol* 5:905, 1994
31. LEDDA-COLUMBANO GM, COLUMBANO A, CONI P, FAA G, PANI P: Cell deletion by apoptosis during regression of renal hyperplasia. *Am J Pathol* 135:657–662, 1989
32. SHIMIZU A, YAMANAKA N: Apoptosis and cell desquamation in repair process of ischemic tubular necrosis. *Mol Pathol* 64:171–180, 1993
33. EVAN GI, WYLLIE AH, GILBERT CS, LITTLEWOOD TD, LAND H, BROOKS M, WATERS CM, PENN LZ, HANCOCK DC: Induction of apoptosis in fibroblasts by c-myc protein. *Cell* 69:119–128, 1992