0022-3565/87/2422-0749$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 242, No. 2
Printed in U.S.A.

Alterations in Renal Structure and Function in a Rat Model of Cyclosporine Nephrotoxicity[1]

NANCY M. JACKSON, CHEN-HSING HSU, GEORGE E. VISSCHER, MANJERI A. VENKATACHALAM and H. DAVID HUMES

Departments of Internal Medicine, VA Medical Center and University of Michigan, Ann Arbor, Michigan (N.M.J., C.-H.H., H.D.H.), Sandoz Research Institute, Sandoz Pharmaceuticals, Inc., East Hanover, New Jersey (G.E.V.), and Departments of Pathology and Medicine, University of Texas Health Science Center, San Antonio, Texas (M.A.V.)

Accepted for publication May 1, 1987

ABSTRACT

Adult male Sprague-Dawley rats maintained on a low sodium diet were administered 100 mg of cyclosporine per kg b.wt. per day s.c. for 4 to 10 days. Serum urea nitrogen was significantly elevated by day 4 and continued to rise, whereas serum creatinine was not elevated above control until day 10. Morphologic examination of perfusion-fixed kidneys from cyclosporine-treated rats revealed focal areas of tubular atrophy and interstitial fibrosis in the outer cortex and a generalized increase in interstitial cells in the outer medulla. No areas of acute tubular necrosis were identified. The effect of this dose of cyclosporine on renal hemodynamics was examined in conscious restrained rats. Renal blood flow, measured by microsphere injection, was 70% of control after four daily doses and remained near this level after eight daily doses. The glomerular filtration rate, measured by iodothalamate clearance, was 70% of control after four doses but fell to 34% of control after eight doses. [^{3}H]Thymidine incorporation into renal DNA was used as a sensitive index of renal cell proliferation after cyclosporine administration (100 mg/kg/day). [^{3}H]Thymidine incorporation was increased over control 3-fold in the outer cortex, 7-fold in the inner cortex and 11-fold in the medullary-papillary regions of the kidney after eight daily doses of cyclosporine. Histoautoradiographic examination of renal sections revealed an increase in the number of labeled nuclei in all three regions of the kidney from rats treated with cyclosporine. Morphometric analysis demonstrated that the majority of proliferating cells were located in the interstitium and not in renal tubules. These studies support the hypothesis that cyclosporine administration alters renal excretory function by decreasing renal blood flow and demonstrate a significant proliferative effect of cyclosporine on the cells within the renal interstitium.

Nephrotoxicity is the most frequent and clinically important complication resulting from the use of the new immunosuppressive agent, cyclosporine. This nephrotoxicity may manifest itself either acutely or chronically. An acute decline in excretory function may be observed immediately after initiation of treatment with a gradual rise in both serum urea nitrogen and creatinine concentrations, which are reversible by decreasing the cyclosporine dose (Flechner *et al.*, 1983; Canadian Multicentre Transplant Study Group, 1983; Shell *et al.*, 1983; Rocher *et al.*, 1984). A chronic interstitial nephritis accompanied by focal glomerulosclerosis, which at times leads to irreversible renal insufficiency, has also been described (Myers *et al.*, 1984).

Two studies have found deleterious effects of cyclosporine on the initial function of renal allografts that were subjected to prolonged periods of ischemia (Flechner *et al.*, 1983; Canadian Multicentre Transplant Study Group, 1983). This observation suggests that renal ischemia potentiates drug-induced toxicity. Discontinuation of cyclosporine therapy may rapidly reverse this dysfunction (Shell *et al.*, 1983; Rocher *et al.*, 1984). Murray *et al.* (1985), studying renal hemodynamics in conscious rats, have shown that acute i.v. infusion of 20 mg/kg of cyclosporine can reduce RBF. In addition, i.p. injection of this same dose for 7 days resulted in a significant fall in RBF. Morphologic changes accompanying renal dysfunction include mild tubulointerstitial nephritis, nonspecific vacuolization and giant mitochondria in proximal tubule cells (Kirwan *et al.*, 1981; Mihatsch *et al.*, 1983). Simonton *et al.* (1983) have reported renal interstitial changes in rats dosed with as little as 10 to 40 mg/kg of cyclosporine for 14 days.

To understand further the pathogenesis of cyclosporine nephrotoxicity, we undertook a series of experiments to investigate the *in vivo* effects of cyclosporine on renal hemodynamics, renal morphology and renal cell proliferation. The results of these experiments show that cyclosporine treatment in conjunction with a low sodium diet causes minor tubule cell

Received for publication November 21, 1986.
[1] This work was supported in part by the Veterans Administration Research Service, by Public Health Service Grants AM 30879 and AM 37139, by a grant from the National Kidney Foundation of Michigan and by Sandoz Pharmaceuticals Corp. (East Hanover, NJ). Some of the data have been reported in abstract form (Clin. Res. **33**: 487, 1985, and Kidney Int. **29**: 303, 1986).

ABBREVIATIONS: GFR, glomerular filtration rate; RBF, renal blood flow; MAP, mean arterial pressure.

injury, an interstitial infiltrate and a decrease in RBF. The relationship between these three findings is discussed.

Methods

Animals and treatment. Male Sprague-Dawley rats (250–300 g) were housed in suspended cages over wood-chip bedding in climate-controlled rooms on a 12-hr light-dark cycle. Rats were maintained on a modified low-sodium diet (40–60 ppm of Na^+; ICN Nutritional Biochemicals, Cleveland, OH) and given water *ad libitum*. Cyclosporine (100 mg/ml in 10% ethanol in sesame oil) was administered s.c. at a daily dose of 100 mg/kg b.wt. for 4, 6, 8 or 10 days. This high dose was chosen because rats have been shown to be especially resistant to the nephrotoxic effects of cyclosporine. Controls received 1 ml/kg b.wt. of 10% ethanol in sesame oil. Rats were weighed daily and dosages adjusted accordingly.

Biochemical assays. Serum creatinine was measured by the picric acid method, and serum urea nitrogen was measured by the urease method (Tietz, 1976).

Morphologic studies. Rats used for these studies were anesthetized with i.p. injection of sodium pentobarbital (50 mg/kg b.wt). A cannula was inserted into the aorta below the renal arteries. The aorta above the kidneys was occluded immediately after the start of retrograde perfusion with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at a pressure of 130 mm Hg. A successful perfusion was indicated by the complete and immediate blanching of the kidneys. The inferior vena cava and ureters were cut to allow free flow of the perfusate. The perfusion was continued for 5 min, and then the kidneys were removed and a 3-mm-thick longitudinal slice was placed in 4% formaldehyde, 1% glutaraldehyde, 100 mM phosphate buffer (pH 7.2). Tissue was sectioned, mounted and stained with hematoxylin and eosin for examination of histologic alterations.

Renal hemodynamic studies. GFR was measured in conscious, restrained rats. A priming dose of [^{125}I]iodothalamate in normal saline, 1 ml/100 g b.wt., was infused i.v. for 10 min, followed by a sustaining infusion of saline containing [^{125}I]iodothalamate for 50 min at a rate of 0.01 ml/min/100 g. Then, urine was collected from the catheterized bladder for 40 min, and a 1-ml blood sample was taken from the femoral artery at the midpoint of urine collection. RBF was then measured by the infusion of 0.12 ml of ^{85}Sr-microspheres, 15 ± 1 μM in diameter, (1 mg/ml in 10% dextran solution) through a carotid catheter into the left ventricle of the heart (Kurtz *et al.*, 1976; Hsu *et al.*, 1975). Immediately upon injection of the microspheres, approximately 0.2 ml of blood was collected from the femoral artery. The rat was killed with an overdose of pentobarbital and the kidneys removed. Samples of whole blood and each kidney were counted in a Packard gamma counter. Serum urea nitrogen was determined by the urease method (Tietz, 1976). MAP was measured in the femoral artery. GFR was calculated using the standard formula, and RBF was calculated as described previously (Kurtz *et al.*, 1976). Renal vascular resistance was calculated as mean systemic pressure divided by RBF.

Measurements of [^{3}H]thymidine incorporation into DNA. One day after the last injection of cyclosporine and 1 hr before sacrifice, rats were given i.p. injections of 250 μCi of [*methyl*-^{3}H]thymidine (78 Ci/mmol) diluted in 0.25 ml of normal saline. After the rats were killed by decapitation and exsanguination, the kidneys were quickly removed and cut longitudinally. Portions (100 mg) of outer cortex, inner cortex and outer medulla and the inner medulla and papilla were placed in tared glass tubes and snap-frozen in liquid nitrogen. Samples were stored at −20°C for a maximum of 3 days before processing. Weighed tissue samples were homogenized in 49 volumes of ice-cold distilled water using two 30-sec pulses of a Tekmar Tissumizer (speed set at 60%). DNA was purified from 2 ml of homogenate (40 mg of tissue) following the method of Munro and Fleck (1966) as modified by Laurent *et al.* (1983). Extracted DNA was assayed by the method of Burton (1956) using calf thymus DNA (type 1) as a standard.

Radioactivity was measured on 0.1 ml of sample in 10 ml of Safety-Solve (Research Products International, Mt. Prospect, IL) in a Beckman 9000 scintillation counter. Counting efficiency was estimated by an internal standard. Serum samples were counted to assure consistent i.p. absorption of the [^{3}H]thymidine. The amount of acid-soluble radioactivity in the tissue homogenates was also measured. Samples with low acid-soluble counts (1300 dpm/mg of tissue) were omitted from the study because this indicated that insufficient [^{3}H]thymidine was available to the kidney. Samples of the DNA extract were counted and corrected for sample DNA content.

Histoautoradiography of the kidney. The half-kidney not used for DNA extraction was fixed in 4% formaldehyde, 1% glutaraldehyde, 100 mM phosphate buffer (7.2). Specimens were dehydrated and embedded in methacrylate. Tissue sections (4 μ) were dipped in Kodak NBT-2 nuclear emulsion and stored in the dark at 4°C for 21 days. The sections were developed and counterstained with Lee's methylene blue-basic fuchsin. For quantitative evaluation, a computerized operator-interactive system was utilized (Visscher *et al.*, 1983). Analysis was performed with a Zeiss Videoplan in conjunction with a standard light microscope. Sections were examined at 63× magnification, and 25 fields were counted each in the outer cortex, inner cortex and medullary-papillary regions for a total of 75 fields/kidney. The cell type of the labeled cells was identified as either tubular or interstitial.

Reagents. All reagents used were of the highest grade commercially available. All organic reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated. [^{125}I]Iodothalamate was obtained from Iso Tex Diagnostic (Friendswood, TX), and ^{85}Sr-labeled microspheres were obtained from 3M Co. (St. Paul, MN). [*Methyl*-^{3}H] Thymidine was obtained from New England Nuclear (Boston, MA). Cyclosporine was kindly donated by R. Voges of Sandoz Ltd. (Basel, Switzerland).

Statistical analysis. The Student's *t* test was used to determine statistical significance.

Results

Rats treated with cyclosporine lost weight compared with controls during the first 6 days of dosing (fig. 1). Weight loss then stabilized in cyclosporine-treated rats but did not recover to control weights. A mild hair loss in the cyclosporine-treated group was the only other notable change.

Renal Function

A significant increase in serum urea nitrogen was observed after four daily injections of 100 mg/kg of cyclosporine (fig. 2), and the values continued to increase during the 10-day course of treatment. The maximum elevation of serum urea was 3 times control levels. Serum creatinine levels were only significantly elevated after 10 daily injections of cyclosporine. The urea nitrogen/creatinine ratio thus increased with cyclosporine treatment.

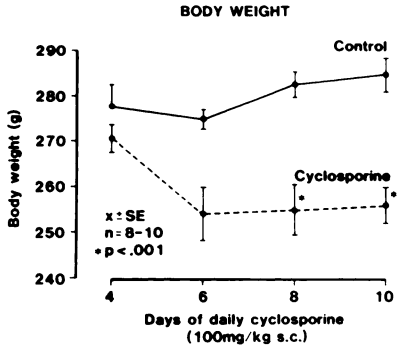

Fig. 1. Effect of cyclosporine treatment on body weight.

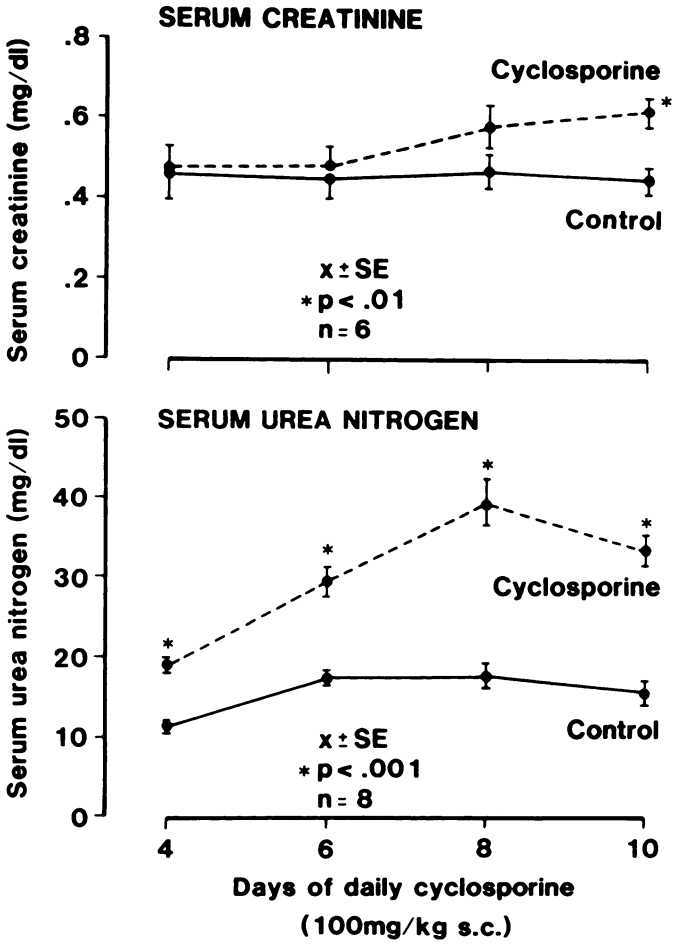

Fig. 2. Effect of cyclosporine treatment on serum creatinine concentration and serum urea nitrogen.

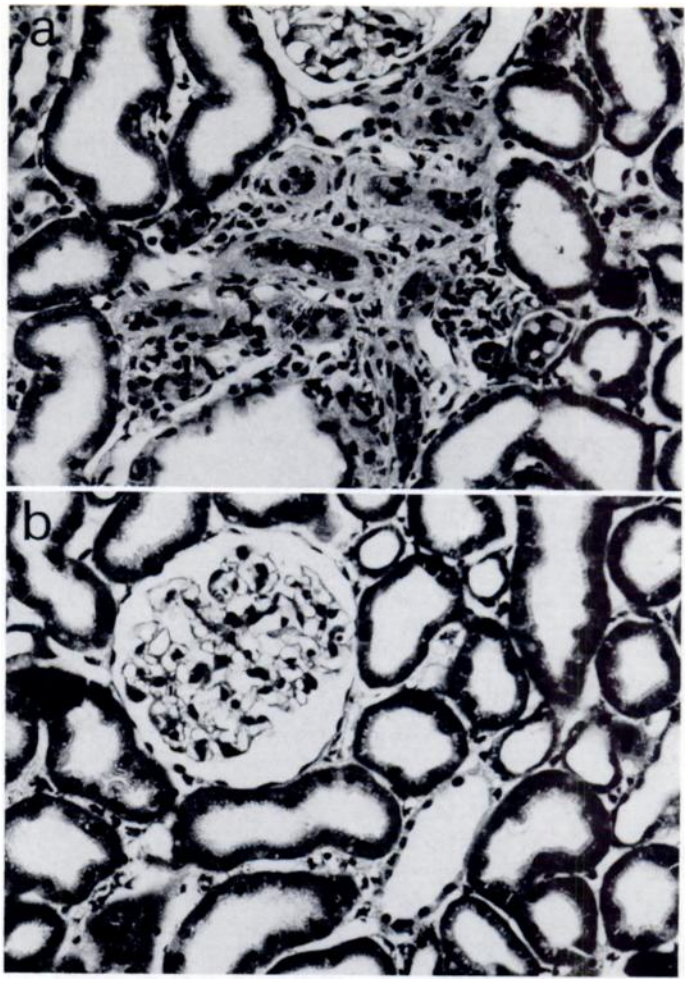

Fig. 3. Panel A: photomicrograph of outer cortex of kidney from rat that received daily cyclosporine (100 mg/kg s.c.) for 10 days. A focal area of tubular atrophy and surrounding interstitial proliferation and fibrosis is displayed. This area is well demarcated from normal surrounding tissue. (Magnification is 390×.) Panel B: photomicrograph of outer cortex of kidney from rat that received daily vehicle (sesame seed oil) injection for 6 days. No abnormalities were found. Control preparations at 8 and 10 days of vehicle treatment were similar in histologic appearance. (Magnification is 390×.)

Histologic Observations

Cortex. The morphologic changes in the cortex were confined to the outer cortex and consisted of focal areas of tubular atrophy and interstitial fibrosis (fig. 3a). These areas were few in number, were found only in the outer cortex and not in the inner cortex and were sharply demarcated from surrounding areas of normal histologic appearance. An increase in overall number of interstitial cells appeared to be present. Neither glomerular abnormalities nor vacuolization of proximal tubule cells was observed. No areas of acute tubular necrosis were present.

Medulla. The morphologic changes in the medulla were confined to the inner stripe of the outer medulla and consisted of widening of the interstitium and marked and generalized increase in the number of cells in the interstitium (fig. 4a). Focal areas also demonstrated an increase in the amount of connective tissue within the interstitium. An occasional mitotic figure was observed in the interstitium (fig. 5). The identity of the proliferating cells could not be determined by light microscopy, but they were mononuclear in nature and irregular in size and shape. Occasional collecting ducts contained casts (fig. 4a). No changes were observed in the inner medulla.

Effect of Cyclosporine on Systemic and Renal Hemodynamics

MAP monitored in the femoral artery was 133 mm Hg in control rats and 130 mm Hg in rats treated with four daily doses of 100 mg/kg of cyclosporine (table 1). After eight doses of cyclosporine, MAP was 114 mm Hg, representing a significant decline compared with control. GFR measured by iodothalamate clearance was decreased to 70% of control after 4 days of cyclosporine. GFR was further decreased to 34% of control after 8 days of cyclosporine (fig. 6). RBF was reduced in cyclosporine-treated rats to 70% of control after 4 days and 66% of control after 8 days (fig. 6). The net effect of these alterations yielded no apparent change in filtration fraction after 4 days of dosing, but there was a significant decrease in this parameter after eight daily doses (table 1). Renal vascular resistance was significantly increased in cyclosporine-treated rats (table 1).

Effect of Cyclosporine on Renal Cell Proliferation

Incorporation of tritiated thymidine into the DNA of replicating cells was used to determine the effect of cyclosporine treatment on renal cell proliferation. Various control measurements were made to assure that the differences in DNA-specific radioactivity were really due to differences in [^{3}H]thymidine

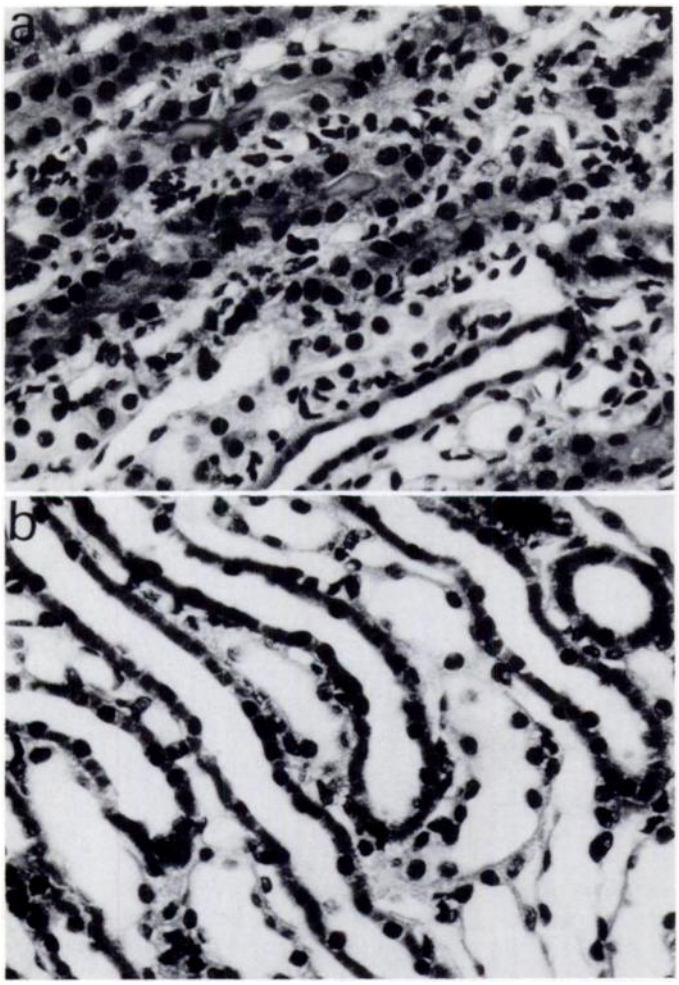

Fig. 4. Panel A: Photomicrograph of inner stripe of outer medulla of kidney from rat that received daily cyclosporine (100 mg/kg s.c.) for 10 days. An increase in interstitial cell number with widening of the interstitium is clearly discernible. The interstitial cells are mononuclear but irregular in shape and size. Casts in the collecting ducts are also present. (Magnification is 625×.) Panel B: Photomicrograph of inner stripe of outer medulla of kidney from rat that received daily (sesame seed oil) vehicle for 6 days. No abnormalities were found. Control preparations at 8 and 10 days of vehicle treatment were similar in histologic appearance. (Magnification is 625×.)

incorporation and not due to changes in the availability of the radiolabel. Treatment with cyclosporine did not alter serum [^{3}H]thymidine levels (mean = 1750 dpm/μl) or the amount of acid-soluble radioactivity in the kidney homogenates (nonspecific labeling mean = 1756 dpm/mg of tissue). In addition, the amounts of DNA extracted from the different sections of kidney examined (outer cortex, inner cortex and medulla-papilla) were not significantly altered by cyclosporine treatment compared with appropriate control.

Treatment with cyclosporine increased [^{3}H]thymidine incorporation into DNA in the kidney, indicating an increase in cell turnover (fig. 7). [^{3}H]Thymidine incorporation increased in the outer cortex from 25.7 ± 3.8 for control to 64.4 ± 20.6 × 10^3 dpm/mg of DNA for cyclosporine-treated animals after four doses and from 20.3 ± 2.1 for control to 60.4 ± 11.1 after eight doses. In the outer cortex, this increase was significant only after 8 days of cyclosporine administration. In the inner cortex-outer medulla, cell proliferation was increased 2-fold (control: 17.2 ± 2.7 *vs.* cyclosporine: 40.4 ± 9.1) after four daily doses

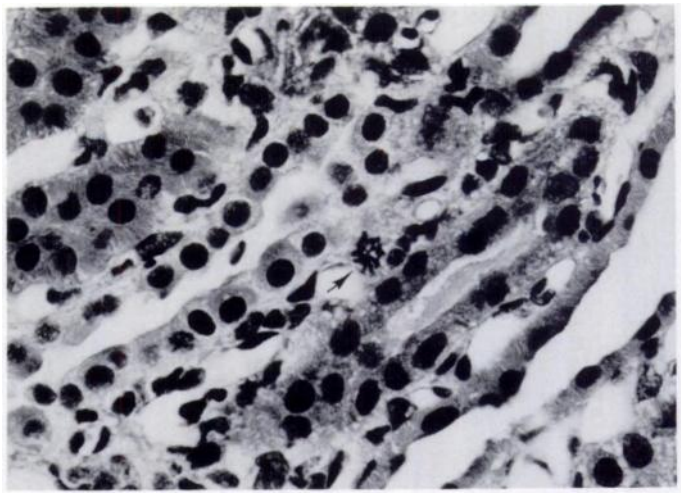

Fig. 5. Photomicrograph of inner stripe of outer medulla of kidney from rat receiving daily cyclosporine (100 mg/kg s.c.) for 10 days. In the middle of the picture, there is a mitotic figure within the renal interstitium (arrow). (Magnification is 925×.)

TABLE 1

Effect of cyclosporine on systemic and renal hemodynamics

		Cyclosporine (100 mg/kg/day)	
	Control (n = 15)	4 days (n = 8)	8 days (n = 7)
Systemic blood pressure (mm Hg)	132.0 ± 13.0	130.0 ± 10.0	114.0 ± 6.0**
Renal plasma flow (ml/min/100 g b.wt.)	4.90 ± 0.8	3.18 ± 1.27*	2.99 ± 1.07**
Renal vascular resistance (mm Hg/ml/min/g kidney wt.)	16.2 ± 3.8	24.8 ± 8.0*	23.6 ± 10.3*
Filtration fraction	23.3 ± 1.7	27.7 ± 3.9	13.6 ± 1.6**

* Significantly different from control by Student's t test, $P \leq .05$.
** Significantly different from control by Student's t test, $P \leq .01$.

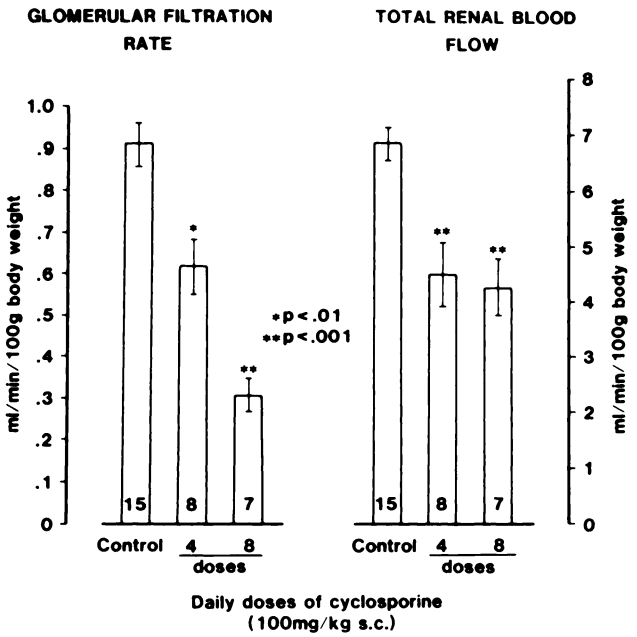

Fig. 6. Effect of cyclosporine on GFR and RBF.

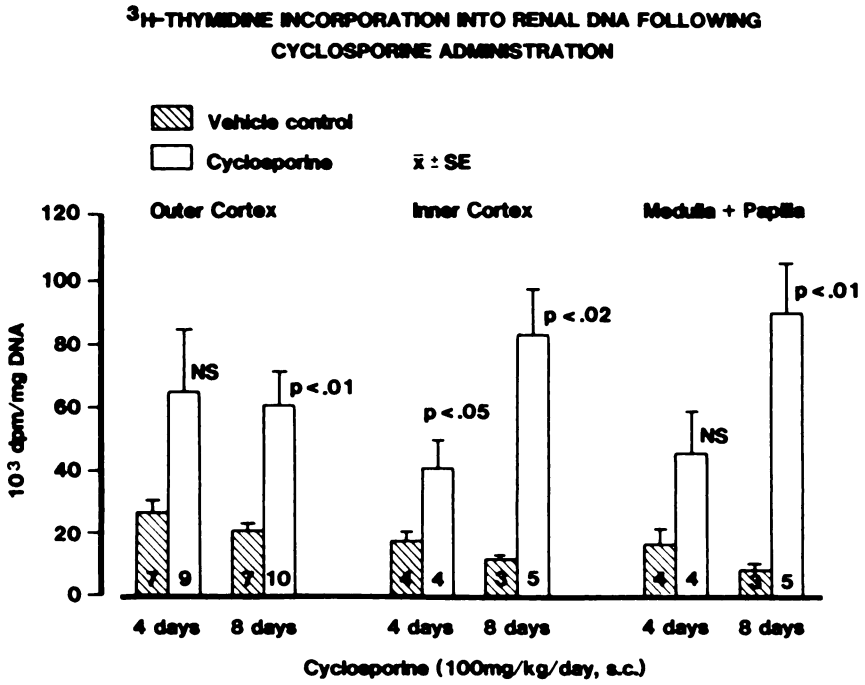

Fig. 7. [^{3}H]Thymidine incorporation into renal DNA after cyclosporine administration.

and 7-fold (control: 11.1 ± 0.8 *vs.* cyclosporine: 82.7 ± 14.9) after eight daily doses. In the inner medulla-papilla section of the kidney, [^{3}H]thymidine incorporation was increased from 16.1 ± 5.3 for control to 44.7 ± 13.9 after four doses of cyclosporine and was significantly increased 11-fold over control (7.8 ± 2.1) after eight daily doses of cyclosporine (89.4 ± 15.5 × 10^3 dpm/mg of DNA) (fig. 7).

Histoautoradiographic studies were performed to determine the location of the cells labeled with [^{3}H]thymidine. Formaldehyde-gluteraldehyde immersion-fixed sections were used from the kidneys with the highest levels of [^{3}H]thymidine per mg of DNA incorporation for the development of histoautoradiographs. Figure 8 is representative of the labeling at the corticomedullary junction. Labeling was confined to the nuclei. In tissues from vehicle-treated rats, the label was evenly distributed between tubule and interstitial cells. After cyclosporine treatment, there was a dramatic increase in the number of labeled nuclei in all three regions of the kidney: the outer cortex, the corticomedullary area and the inner medulla-papilla. From these figures, it is obvious that a greater proportion of labeled cells after cyclosporine treatment is in the interstitium rather than the tubules (fig. 8). Dark field exposures of inner cortical sections highlight the differences in [^{3}H]thymidine incorporation in vehicle- and cyclosporine-treated rats (fig. 8).

This impression was confirmed by morphometric analysis of the labeled sections with an operator-interactive system utilizing semiautomated computer-based planimetry. The results of this study are detailed in table 2. Labeled interstitial or tubule cells were counted in 25 fields in each area, outer cortex, corticomedullary junction and inner medulla-papilla. Cyclosporine treatment increased cell proliferation in all three areas of the kidney. Interstitial cell proliferation accounted for most of this increase (table 2).

Discussion

Cyclosporine nephrotoxicity continues to be a major problem with its widespread use as an immunosuppressant. Both functional and structural changes in the kidneys of transplant patients and experimental animals treated with cyclosporine have been reported. In order to better elucidate the structural and functional alterations occurring in the kidney during cyclosporine administration, we have developed an *in vivo* model of cyclosporine nephrotoxicity in rats. Rats have been shown to be extraordinarily resistant to the toxic side effects of cyclosporine, and, thus, the dosage used in these studies was about 10 times the normal immunosuppressive dosages for patients. Because cyclosporine is primarily metabolized by the hepatic cytochrome P-450 system (Cunningham *et al.*, 1985), differences in rates of metabolism between rat and human may account for the differences in toxic potency. Because volume depletion has been shown to potentiate most forms of nephrotoxic injury, we placed the animals on a sodium-deficient diet to maximize any potential nephrotoxicity produced by this agent. Cyclosporine-treated rats lost weight during the first 4 to 6 days of dosing, after which the loss plateaued. This weight loss may be due to decreased food consumption.

A number of findings in these studies suggested that cyclosporine did not have a direct toxic effect on renal tubular epithelial cells. Histologically, the kidneys from these cyclosporine-treated rats displayed only minor changes, confined to focal areas of tubular atrophy and interstitial fibrosis in the outer cortex and a proliferation of interstitial cells in the inner stripe of the outer medulla. No evidence of widespread toxic tubular cell injury was found. Other investigators have also reported nonspecific vacuolization in the kidneys of cyclosporine-treated animals (Kirwan *et al.*, 1981; Mihatsch *et al.*, 1983; Whiting *et al.*, 1982). However, as initially suggested by Dieperink *et al.* (1983), these findings are probably vehicle or fixation artifacts because no increased vacuolization compared with controls was observed in our studies in which sesame oil was used as a vehicle and kidneys were fixed by perfusion. In addition, we found no increase in renal cellular or mitochondrial calcium after cyclosporine treatment. In a number of studies, an increase in cellular renal cortex calcium has been found to coincide with the appearance of morphologically evident renal

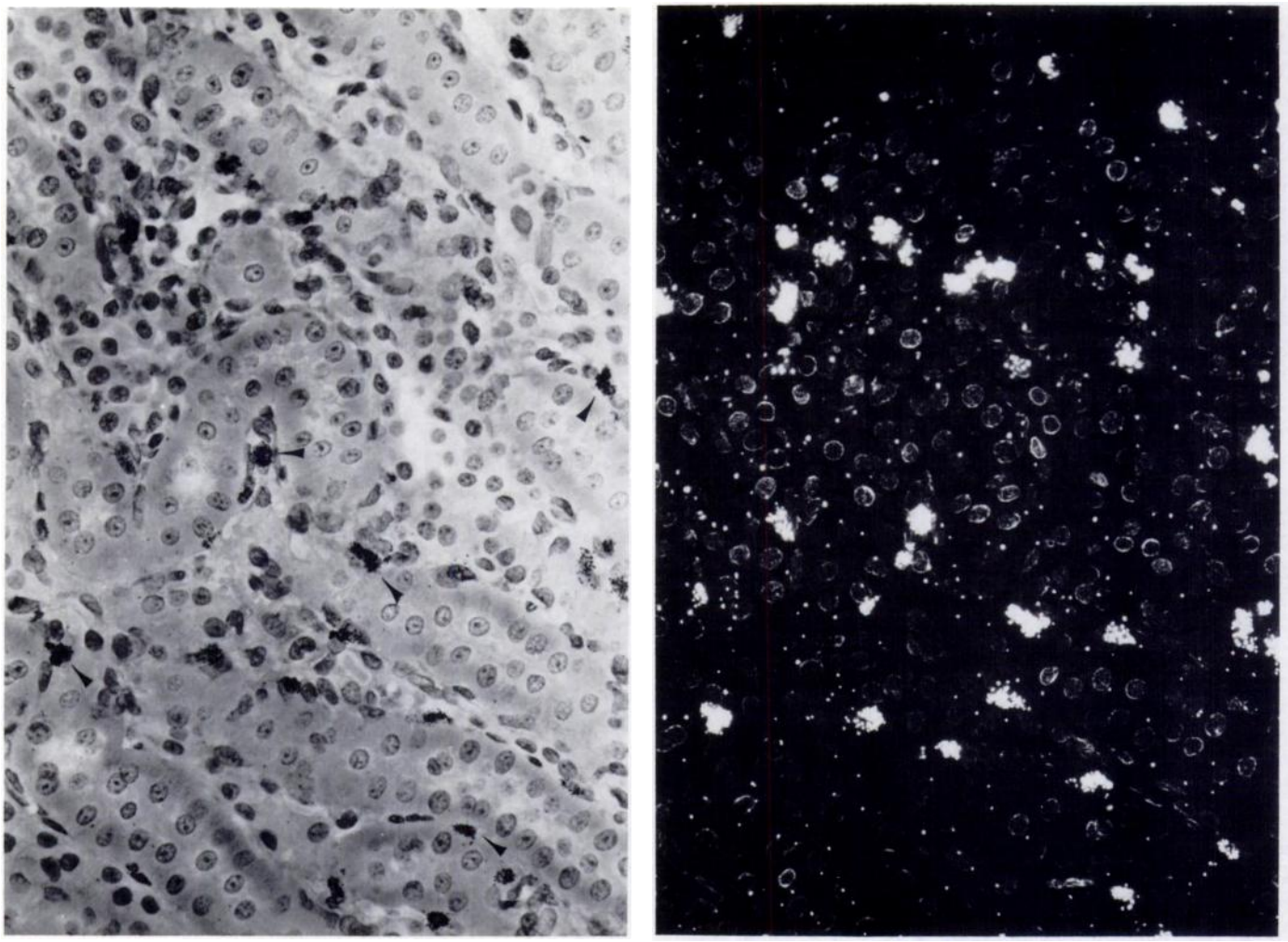

Fig. 8. Histoautoradiography of the corticomedullary region of the kidneys from rats treated for 4 days with 100 mg of cyclosporine per kg b.wt. In the left panel, [^{3}H]thymidine label is observed as black silver grains in interstitial cell nuclei (arrowheads). In the right panel, the dark-field exposure of the same area highlights the incorporation of [^{3}H]thymidine as light areas on a dark background.

TABLE 2

Histometric counts of [^{3}H]thymidine incorporation

Using a 63× objective, 25 fields/area were counted, for a total of 75 fields/kidney section. Values represent mean ± S.E. for n = 2 to 4. CS, cyclosporine.

	Total Cells		Tubule Cells		Interstitial Cells	
	Vehicle[a]	CS[b]	Vehicle	CS	Vehicle	CS
Whole kidney	10.0 ± 4.0	265.8 ± 57.0	2.5 ± 1.5	13.3 ± 3.3	7.5 ± 5.5	252.5 ± 59.2
Outer cortex	1.5 ± 0.5	72.3 ± 20.7	0.5 ± 0.5	3.3 ± 1.0	1.0 ± 0.0	69.0 ± 20.7
Inner cortex	6.5 ± 4.5	64.5 ± 14.2	0.5 ± 0.5	5.5 ± 2.4	6.0 ± 5.0	59.0 ± 16.1
Medulla-Papilla	2.0 ± 0.0	129.0 ± 35.3	1.5 ± 0.5	4.5 ± 1.6	0.5 ± 0.5	124.5 ± 35.4

[a] Rats were treated for 4 or 8 days with 1 ml/kg of 10% ethanol in sesame oil.
[b] Rats were treated for 4 or 8 days with 100 mg/kg of cyclosporine in vehicle.

tubular cell injury produced by nephrotoxins (Sastrasinh *et al.*, 1982; Weinberg *et al.*, 1983; Humes *et al.*, 1984). This observation supports our histopathologic findings that suggest that cyclosporine is at most only mildly toxic to renal tubule cells.

The lack of significant tubule cell necrosis suggests that decreased renal function is primarily due to hemodynamic alterations. We performed RBF and GFR measurements on conscious, restrained rats that had been treated with cyclosporine (100 mg/kg/day) for 4 or 8 days. Initially there was a decline in RBF and GFR to 70% of control values. These declines were followed by a further decline in GFR to 34% of control after eight doses, whereas no further reduction in RBF was observed. The decline in RBF was primarily due to a significant increase in renal vascular resistance. After eight doses of cyclosporine, a significant drop in blood pressure occurred; however, blood pressure was still within the autoregulatory range. Blood pressure will decrease in response to either a decline in cardiac output or volume contraction. Because cyclosporine is not known to be toxic to the myocardium, a fall in cardiac output most likely did not occur. Thus, the combination of a low sodium diet and cyclosporine treatment probably caused volume contraction, which led to the decrease in blood pressure. A cyclosporine-induced decline in GFR has been well documented in both animal (Whiting *et al.*, 1982;

Dieperink *et al.*, 1983; Tonnesen *et al.*, 1983) and clinical studies (Myers *et al.*, 1984). The mechanism of cyclosporine-induced declines in RBF is presently unclear. Moss *et al.* (1985) have shown that i.v. infusion of cyclosporine has a generalized sympathoexcitatory action, apparently through a direct effect on afferent nerve endings, so that the decline in RBF may be due to a direct effect of cyclosporine on renal nerve inputs to produce an increase in renal vascular resistance. In addition, rats with expanded extracellular fluid volume receiving an acute infusion of cyclosporine exhibit reduced salt and water excretion from innervated kidneys only (Moss *et al.*, 1985). Murray *et al.* (1985) have shown that the decline in RBF in rats given 20 mg/kg of cyclosporine i.v. is not blocked by the angiotensin II-converting enzyme inhibitor, captopril (50 mg/kg), but is blocked by the *alpha* adrenergic antagonist, phenoxybenzamine, or surgical denervation of the kidney. In contrast, Jao *et al.* (1986), also using the rat model, demonstrated that 10 mg/kg of captopril blocked the renal vasoconstriction caused by i.v. administration of 5 mg/kg of cyclosporine. Obviously, further study is needed to determine the mechanism of the cyclosporine-induced decline in RBF.

A decline in RBF may potentiate cyclosporine-induced toxic injury to the tubules. Devineni *et al.* (1983) has shown that partial renal ischemia potentiates cyclosporine-induced nephrotoxicity in dogs. This finding substantiates the observations from two transplant centers that prolonged periods of ischemia increased the deleterious effects of cyclosporine on the initial function of renal allographs (Flechner *et al.*, 1983; Canadian Multicentre Transplant Study Group, 1983). To further explore this possibility, we utilized a more sensitive test for the assessment of renal tubule cell injury. Laurent *et al.* (1983) have shown that the incorporation of [^{3}H]thymidine into DNA is a sensitive index of acute renal tubule cell injury after administration of very low doses of gentamicin in the rat. Although the label was found both in tubule cells and interstitial cells after gentamicin administration, incorporation of radiolabeled thymidine was predominantly within tubules. In similar studies, we found that incorporation of [^{3}H]thymidine into renal DNA was significantly increased by cyclosporine treatment. However, unlike the studies with gentamicin, histoautoradiographic sections of these tissues revealed that interstitial cells, and not tubule cells, were the predominant site of [^{3}H]thymidine uptake. This evidence supports our histopathologic conclusion that cyclosporine is not a major tubule cell toxin but, rather, causes at most only a mild tubule cell injury associated with a dramatic cellular proliferation within the renal interstitium. We have shown a 3-fold increase in [^{3}H]thymidine incorporation into the outer cortex of cyclosporine-treated rats and that 95% of this is due to interstitial cell proliferation. Similarly, in the inner cortex and medullary-papillary regions, there is an 8- and 11-fold increase, respectively, in [^{3}H]thymidine incorporation, with the large majority due to interstitial cell proliferation. This increase in renal interstitial cell proliferation is the first description of a substantial renal interstitial effect of this nephrotoxic agent. Morphologic characteristics of these cells indicate that they are mononuclear cells, but further study is needed before these cells can be identified as either fibroblasts or activated inflammatory cells. In addition, the relationship between cyclosporine dosage and interstitial cell proliferation needs to be established. Whether this cellular proliferation is responsible for the mild interstitial nephritis observed in rats in these studies and in those of Simonton *et al.* (1983) has not yet been established. The possibility that a similar cyclosporine-induced interstitial cell proliferation is responsible for the interstitial fibrosis observed after human renal (Bohman *et al.*, 1985) and heart (Myers *et al.*, 1984) transplant deserves further study. The relationship between cyclosporine-induced renal perfusion alteration and this proliferative process also needs to be explored.

Acknowledgments

The excellent technical assistance of Pai-Sung Chen, Batul Hamid, M. Christine Holden and Sanjeevkumar Patel is appreciated.

References

Bohman, S. D., Klintmalm, G., Ringden, O., Sundelin, B. and Wilczek, H.: Interstitial fibrosis in human kidney grafts after 12 to 46 months of cyclosporine therapy. Transplant. Proc. **17:** 1168-1171, 1985.

Burton, K.: A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. **62:** 315-322, 1956.

Canadian Multicentre Transplant Study Group: A randomized clinical trial of cyclosporine in cadaveric renal transplantation. N. Engl. J. Med. **309:** 809-815, 1983.

Cunningham, C., Burke, M. D., Wheatley, D. N., Thomson, A. W., Simpson, J. G. and Whiting, P. H.: Amelioration of cyclosporine-induced nephrotoxicity in rats by induction of hepatic drug metabolism. Biochem. Pharmacol. **34:** 573-578, 1985.

Devineni, R., McKenzie, N., Duplan, J., Keown, P., Stiller, C. and Wallace, A. C.: Renal effects of cyclosporine: Clinical and experimental observations. Transplant. Proc. **15:** 2695-2698, 1983.

Dieperink, H., Starklint, H. and Leyssac, P. P.: Nephrotoxicity of cyclosporine—an animal model: Study of the nephrotoxic effect of cyclosporine on overall renal and tubular function in conscious rats. Transplant. Proc. **15:** 2736-2741, 1983.

Flechner, S. M., Van Buren, C., Korman, R. A. and Kahan, B. D.: The nephrotoxicity of cyclosporine in renal transplant recipients. Transplant. Proc. **15:** 2689-2694, 1983.

Hsu, C. H., Kurtz, T. W., Preuss, H. G. and Weller, J. M.: Measurement of renal blood flow in the rat. Proc. Soc. Exp. Biol. Med. **149:** 470-472, 1975.

Humes, H. D., Sastrasinh, S. and Weinberg, J. M.: Identification of a competitive inhibitor of the gentamicin-renal membrane binding interaction which ameliorates gentamicin nephrotoxicity. J. Clin. Invest. **73:** 134-147, 1984.

Jao, S., Waltzer, W. and Arbeit, L. A.: Acute cyclosporin induced decrease in GFR is mediated by changes in renal blood flow and renal vascular resistance. Kidney Int. **29:** 431, 1986.

Kirwan, P. D., Baxter, C. R., Duggin, G. G., Hall, B. M., Horvath, J. S., Sheil, A. G. R. and Tiller, D. J.: Giant mitochondria, renal transplant biopsy and cyclosporine. Lancet **2:** 146, 1981.

Kurtz, T. W., Maletz, R. M. and Hsu, C. H.: Renal cortical blood flow in glycerol-induced acute renal failure in the rat. Circ. Res. **38:** 30-35, 1976.

Laurent, G., Maldague, P., Carlier, M. and Tulkens, P. M.: Increased renal DNA synthesis in vivo after administration of low doses of gentamicin to rats. Antimicrob. Agents Chemother. **24:** 586-593, 1983.

Mihatsch, M. J., Thiel, G., Spichtin, H. P., Oberholzer, M., Brunner, F.P., Harder, F., Olivieri, V., Bremer, R., Ryffel, B., Stocklin, E., Torhorst, J., Gudat, F., Zollinger, H. U. and Loertscher, R.: Morphological findings in kidney transplants after treatment with cyclosporine. Transplant. Proc. **15:** suppl. 1, 2804-2835, 1983.

Moss, N. G., Powell, S. L. and Falk, R. J.: Intravenous cyclosporine activates afferent and efferent renal nerves and causes sodium retention in innervated kidneys in rats. Proc. Natl. Acad. Sci. U.S.A. **82:** 8222-8226, 1985.

Munro, H. N. and Fleck, A.: The determination of nucleic acids. Methods Biochem. Anal. **14:** 113-176, 1966.

Murray, B. M., Paller, M. S. and Ferris, T. F.: Effect of cyclosporine administration on renal hemodynamics in conscious rats. Kidney Int. **28:** 767-774, 1985.

Myers, B. D., Ros, J., Newton, L., Luetschor, J. and Perlroth, M.: Cyclosporine-associated chronic nephropathy. N. Engl. J. Med. **311:** 699-705, 1984.

Rocher, L. L., Milford, E. L., Kirkman, R. L., Carpenter, C. B., Strom, T. B. and Tilney, N. L.: Conversion from cyclosporine to azathioprine in renal allograph patients. Transplantation (Baltimore) **38:** 669-674, 1984.

Sastrasinh, M., Weinberg, J. M. and Humes, H. D.: Effects of gentamicin on calcium uptake by renal mitochondria. Life Sci. **30:** 2309-2315, 1982.

Shell, A. G., Hall, B. M. and Tiller, D. J.: Australian trial of cyclosporine in cadaveric donor renal transplantation. Transplant. Proc. **15:** 2485-2489, 1983.

Simonton, S. C., Rynasiewicz, J. and Sibley, R. K.: Light microscopic and electron microscopic features of experimental cyclosporine A nephrotoxicity. Lab. Invest. **48:** 78A, 1983.

TIETZ, N. W. Fundamentals of Clinical Chemistry, 2nd ed., pp. 991-999, WB Saunders Co., Philadelphia, 1976.

TONNESEN, A. S., HAMNER, R. W. AND WEINMANN, E. J.: Cyclosporine and sodium and potassium excretion in the rat. Transplant. Proc. **15:** 2730–2735, 1983.

VISSCHER, G. E., ROBISON, R. L. AND ARGENTIERI, G. J.: Quantitiative morphological evaluation of experimentally induced atheroma. Artery **12:** 95–103, 1983.

WEINBERG, J. M., HARDING, P. G. AND HUMES, H. D.: Alterations in renal cortex cation homeostasis during mercuric chloride and gentamicin nephrotoxicity. Exp. Mol. Pathol. **39:** 43–60, 1983.

WHITING, P. H., THOMSON, A. W., BLAIR, J. T. AND SIMPSON, J. G.: Experimental cyclosporine A nephrotoxicity. Br. J. Exp. Pathol. **63:** 88–94, 1982.

Send reprint requests to: H. David Humes, M.D., Chief, Medical Service, VA Medical Center, 2215 Fuller Road, Ann Arbor, MI 48105.