Nephrotoxic Nephritis in Rabbits

The Role of the Sympathetic Nervous System

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The sympathetic nervous system and catecholamines play a major role in fibrin deposition in organs in rabbits after endotoxin administration. Glomerular fibrin deposition is also a key factor in the pathogenesis of nephrotoxic nephritis in rabbits, but the role of the sympathetic nervous system in this type of fibrin deposition has not been defined. We investigated sympathetic nervous system involvement in nephrotoxic nephritis using a model of isolated chemical sympathectomy with 6-hydroxydopamine. Different quantities of pooled nephrotoxic serum were injected intravenously into control and sympathectomized rabbits to produce a known spectrum of pathology in normal rabbits. Animals were killed and their organs were analyzed to ascertain that sympathectomy had been accomplished. Biochemical, immunohistologic, and histopathologic evaluation of the animals, comparing controls and sympathectomized rabbits, revealed no differences in the degree of renal damage for a given quantity of nephrotoxic serum. We conclude that, in the rabbit model, the sympathetic nervous system plays no significant role in the pathogenesis of fibrin deposition and glomerular damage in nephrotoxic nephritis. (Am J Pathol 90:689–700, 1978)

NEPHROTOXIC NEPHRITIS is produced experimentally by the intravenous injection of heterologous antibody directed against glomerular basement membrane (GBM) antigens of the recipient species.[1-6] After the heterologous antiserum is injected, localization occurs along the GBM, with fixation of autologous complement. Transient proteinuria develops during this heterologous phase. The autologous phase begins with the formation of antibody against the foreign anti-GBM antibody and is characterized by deposition of autologous antibody on the GBM and accumulation of fibrin in the mesangium, Bowman's space, and crescents. Swelling and proliferation of endothelial and mesangial cells is noted at the end of the first week. Fibrin deposition appears to be a critical pathogenetic factor in the glomerular pathology, not only as evidenced by its presence in large quantities but also by virtue of the beneficial effects of anticoagulation, defibrination, and polymorphonuclear leukocyte depletion.[6-10] However, the mechanism precipitating fibrin deposition is not well understood. We previously showed that chemical sympathectomy

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provides significant protection from renal fibrin deposition induced by endotoxin in rabbits.[11] The present study assesses the role of the sympathetic nervous system in the pathogenesis of fibrin deposition in nephrotoxic nephritis in rabbits.

Materials and Methods

Preparation of Nephrotoxic Serum and Induction of Nephrotoxic Nephritis

Normal rabbit kidneys were cut into slices and the cortex was separated from the medulla. After mincing and washing cortices in cold phosphate-buffered saline (PBS), pH 7.3, glomeruli were isolated by the method of Krakower and Greenspon,[12] lyophilized, and used to immunize a sheep. Twenty milligrams of glomeruli were suspended in PBS and mixed with an equal volume of complete Freund's adjuvant. The animal was serially immunized with a total of 80 mg antigen and was exsanguinated after 10 weeks. The sheep serum was heated at 56 C for 30 minutes, then absorbed with rabbit red cells and serum prior to injection. Samples from trial bleedings and the final bleeding were tested in normal rabbits. The most potent lots were pooled and used in the reported experiments conducted with normal control rabbits and with rabbits chemically sympathectomized with 6-hydroxydopamine (6-OHDA) as previously described. Rabbits received 6-OHDA, 50 mg/kg intravenously, followed by a second injection of 50 mg kg 20 hours later. One week later, the animal received 100 mg/kg twice after a 20-hour interval. The animals were entered into the study 1 week later. To produce a spectrum of disease, three different injection schedules were followed. The animals were divided into groups to receive 1.0, 1.5, and 2.0 ml of nephrotoxic serum intravenously. One milliliter of serum produced mild proteinuria, 100 to 500 mg/24 hr; 2 ml caused heavy proteinuria up to 5 g 24 hr. A severe lesion was present by 10 days in normal rabbits which received the higher dose of nephrotoxic serum and, therefore, all rabbits were killed on the tenth day.

Immunofluorescence and Histopathology

Kidney tissue was processed for immunofluorescence as described previously.[13] Sections were examined for rabbit IgG, C-3, fibrin, albumin, and sheep IgG. In addition, portions of kidney were fixed in buffered neutral formalin, embedded in paraffin, and serially sectioned at 3μ. Sections were stained with hematoxylin and eosin (H&E), periodic acid–Schiff reagent (PAS), and colloidal iron. Fluorescence sections were graded without knowledge of the animals' study group. All glomeruli in a section were counted and graded as negative, as showing segmental mesangial fibrin deposits, or as showing global involvement of the entire glomerulus. The intensity of staining for rabbit IgG, C-3, and sheep IgG was scored on a 0 to 4+ basis. Light microscopic sections were graded without knowledge of the animals' study group according to the following schema:

A. Glomerular cellularity: normal = 0; focal slight hypercellularity = 1+; moderately diffuse = 2+; diffuse and pronounced = 3+

B. Crescents: none = 0; 25% of all glomeruli = 1+; 25 to 50% = 2+; 50% involvement = 3+

C. Fibrin: same schema as crescents

D. Red cell casts: none = 0; occasional = 1+; 1 in every 10× field = 2+; >1 in each 10× field = 3+

E. Protein casts: same schema as red cell casts

Tissue Catecholamine Determinations

At death the heart and kidneys were sealed in plastic bags and frozen at −50 C until they were analyzed. For analysis of norepinephrine and epinephrine, the organs were

thawed, washed in saline, weighed, minced, and homogenized in cold 5% trichloroacetic acid. After filtering, the filtrate, pH 8.3, was adsorbed on aluminum oxide columns, washed with deionized water, and eluted with 0.25 N acetic acid; the catecholamine content was determined fluorometrically as previously described.[11]

Biochemical Studies

Blood urea nitrogen (BUN) was determined in the University of Virginia Hospital Renal Laboratory by standard methods. Animals were placed in metabolic cages and 24-hour urine samples were collected for total urinary protein determinations using 3% sulfosalycylic acid with bovine serum albumin as a standard.[14]

Statistical Analysis

Catecholamine levels and histologic scores are recorded as the mean ± standard error of the mean. Data were analyzed by the Student t and χ^2 tests.

Results

Catecholamine Levels

Chemical sympathectomy induced a significant depletion of norepinephrine in heart and kidney (Table 1). Nephrotoxic serum produced a further slight decrement in kidney in chemically sympathectomized rabbits compared with 6-OHDA controls, but there was little additional depletion in cardiac levels. Nephrotoxic serum injected into normal animals had no effect on cardiac levels of norepinephrine; it did produce a slight, but not statistically significant, decrement in renal norepinephrine levels when given in the larger doses of 1.5 and 2.0 ml. Both 6-OHDA and nephrotoxic serum lowered the epinephrine content of heart and kidney; the range of levels was quite wide.

Urinary Protein and BUN

The urine protein excretion and BUN are summarized in Table 2. The quantity of protein excreted was quite variable but usually corresponded to the dose of nephrotoxic serum injected. Chemical sympathectomy did not alter proteinuria in any predictable fashion. Mild BUN elevation was present in animals receiving larger amounts of nephrotoxic serum, but there were no differences between normal and 6-OHDA–pretreated rabbits.

Immunofluorescence

All animals receiving nephrotoxic serum demonstrated bright linear staining for sheep IgG (Figure 1A) along the GBM. Rabbit IgG was also noted in a linear pattern of deposition of 3+ intensity in all nephrotoxic serum recipients. Rabbit C-3 was present in trace amounts in a similar

Table 1--Catecholamine Levels (μg/g tissue)

Group	Milliliters of NTS	Norepinephrine*		Epinephrine*	
		Heart	Kidney	Heart	Kidney
Normal rabbits		0.969 ± 0.059 (13)	0.138 ± 0.10 (15)	0.033 ± 0.009 (13)	0.005 ± 0.001 (13)
NTN (4)	1		0.133 ± 0.016† (4)		
(16)		1.022 ± 0.084 (16)		0.118 ± 0.008‡ (16)	0.000 ± 0.000‡ (16)
NTN (12)	1.5 & 2.0		0.107 ± 0.016 (12)		
6-OHDA (9)		0.118 ± 0.013‡	0.023 ± 0.003‡	0.005 ± 0.002‡	0.006 ± 0.005
6-OHDA-NTN (14)	1.0,1.5, & 2.0	0.090 ± 0.08‡ (14)	0.014 ± 0.001‡ (13)	0.006 ± 0.001‡ (14)	0.002 ± 0.001‡ (14)

* Values expressed as mean ± SEM.
† $0.05 < P < 0.10$ vs normal.
‡ $P < 0.05$ vs normal.
No. of animals in each group is indicated in parentheses.
NTS = nephrotoxic serum, NTN = nephrotoxic nephritis, 6-OHDA = chemically sympathectomized.

Table 2—Protein Excretion and BUN*

Dose of NTS (ml)	Proteinuria (mg/24 hr)		BUN (mg/dl)	
	NTN	6-OHDA-NTN	NTN	6-OHDA-NTN
1.0	98 ± 55	362 ± 272	15 ± 1	23 ± 2
1.5	3655 ± 1867	810 ± 391	38 ± 25	20 ± 4
2.0	5400 ± 829	5900 ± 1307	34 ± 0.7	38 ± 17

* Values expressed as mean ± SEM.
NTS = nephrotoxic serum, NTN = nephrotoxic nephritis, BUN = blood urea nitrogen.

pattern. There were no detectable differences in the intensity of staining between normal and 6-OHDA rabbits for sheep IgG, rabbit IgG, or rabbit C-3. Rabbit fibrin in a segmental (Figure 1B) or global depositional pattern (Figures C and D) was detected with a frequency increasing in direct proportion to the amount of injected nephrotoxic serum. These data are presented in Table 3. The number of glomeruli and severity of involvement increased in both 6-OHDA–pretreated animals and control nephrotoxic recipients. The number of animals and quantity of involvement was slightly less with regard to 6-OHDA rabbits receiving 2 ml of nephrotoxic serum than with regard to controls, but this difference was not significant.

Histopathology

Histologic changes were characterized chiefly by proliferation of Bowman's capsular epithelium to form extracapillary crescents, usually in association with fibrin deposition (Figure 2). Some glomerular tufts were slightly hypercellular. There was only minimal interstitial cellular infiltrate, but glomeruli were occasionally surrounded by a cuff of lymphocytes. Scattered tubules contained red blood cell casts. The results of

Table 3—Fibrin Score*

Group	Dose of NTS (ml)	(n)	Percent trace-negative	Percent segmental	Percent global
6-OHDA-NTN	1	(4)	80 ± 16	10 ± 6 (2)	10 ± 10 (1)
NTN	1	(4)	96 ± 4	4 ± 4 (2)	1(1)
6-OHDA-NTN	1.5	(4)	62 ± 12	32 ± 10(4)	5 ± 2 (3)
NTN	1.5	(4)	59 ± 20	24 ± 10(4)	18 ± 11(2)
6-OHDA-NTN	2.0	(6)	76 ± 12	14 ± 6 (4)	10 ± 8 (3)
NTN	2.0	(8)	44 ± 10	24 ± 5 (8)	31 ± 10(7)

* Values expressed as mean score ± SEM for all rabbits in the group.
Actual number of animals in the group whose kidneys had the particular type of involvement is indicated in parentheses.
NTS = nephrotoxic serum, NTN = nephrotoxic nephritis.

histologic scoring of serial PAS and H&E sections are presented in Table 4. The severity of the lesions tended to increase with administration of larger quantities of nephrotoxic serum, although there was less difference between rabbits receiving 1.5 ml nephrotoxic serum and those receiving 20 ml nephrotoxic serum. As noted by immunofluorescence, there was a slight decrease in the number of 6-OHDA animals with various lesions as compared with controls, but this was not significant. Representative lesions from a 6-OHDA and control animal are shown in Figures 2A and B. In both groups of rabbits, the histologic lesion varied from minimal glomerular hypercellularity and few other findings to a severe proliferative glomerulonephritis with fibrin, crescents, red cell casts, and protein casts. Neither of two observers was able to differentiate between control and 6-OHDA–treated animals in the double blind studies of sections.

Discussion

Sympathectomy, which provided dramatic protection from endotoxin-induced glomerular fibrin deposition, afforded no protection from nephrotoxic nephritis. The difference in patterns of renal fibrin deposition clearly suggests action on different pathogenetic processes. After endotoxic injection, intravascular coagulation ensues, combined with stasis in capillaries and intraluminal fibrin deposition in glomerular capillaries.[15-17] In nephrotoxic nephritis, fibrin deposition appears in the expanded mesangium, Bowman's space, and crescents. Catecholamines could play a role in local fibrin deposition in nephrotoxic nephritis. Vasospasm probably occurs as an initial event after the injection of nephrotoxic serum, as suggested by transient oliguria.[18,19] Periarteritis and malignant nephrosclerosis in humans, both entities causing local ischemia, can produce a histologic lesion similar to that seen in rabbits with nephrotoxic nephritis.[20,21] In addition, epinephrine infusion into the renal arteries in dogs results in crescentic nephritis.[22] Thus, the initial deposition of heterologous anti-GBM antibody and complement fixation might be associated with local norepinephrine release and its consequences, ie, increased ischemia, endothelial damage, and a setting for perpetuation of the initially induced nephropathy. On the other hand, the autologous phase of nephrotoxic nephritis might be associated with glomerular proliferation, release of norepinephrine, and local fibrin deposition from the same sequence. Our failure to find any significant differences in renal catecholamine levels with control rabbits receiving nephrotoxic serum suggests that the degree of damage of the pathologic process involved was not associated in control animals with lowering of tissue norepinephrine levels. This in no way proves that norepinephrine was or was not released, since the synthetic capability for

Table 4—Histology*

Group	Dose of NTS (ml)	No. of animals	Glomerular cellularity	Percent crescents	Percent fibrin	Casts	
						RBC	Protein
6-OHDA-NTN	1	4	0.4 ± 0.2 (2)	7.0 ± 7 (1)	3.0 ± 3 (1)	0.2 ± 0.2 (1)	0.8 ± 0.5 (2)
NTN	1	4	0.9 ± 0.4 (3)	0.7 ± 0.7 (1)	0.7 ± 0.7 (1)	0.5 ± 0.5 (1)	0.5 ± 0.5 (1)
6-OHDA-NTN	1.5	4	2.0 ± 0.5 (4)	5.0 ± 3 (3)	5.0 ± 2 (3)	2.0 ± 0.5 (4)	2.0 ± 0.6 (4)
NTN	1.5	4	2.0 ± 0.5 (4)	7.0 ± 4 (2)	17.0 ± 10 (2)	2.0 ± 0.4 (4)	1.0 ± 0.5 (3)
6-OHDA-NTN	2	6	1.0 ± 0.3 (5)	9.0 ± 6 (3)	7.0 ± 6 (2)	0.8 ± 0.4 (3)	2.0 ± 0.6 (4)
NTN	2	8	2.0 ± 0.4 (6)	5.0 ± 3 (5)	12.0 ± 4 (7)	1.0 ± 0.3 (6)	1.0 ± 0.3 (7)

* Values expressed as mean score ± SEM for all rabbits in the group.
Actual number of animals in the group whose kidneys had the particular type of involvement is indicated in parentheses.
NTS = nephrotoxic serum, NTN = nephrotoxic nephritis.

norepinephrine is very great; there could be a marked increase in turnover, but the studies with 6-OHDA indicate that this possibility is unlikely. Despite the marked decrease in norepinephrine levels in tissues of sympathectomized animals, we were unable to detect any differences in the disease process. If norepinephrine were involved in the pathogenesis of the ischemia and proliferation occurring with nephrotoxic nephritis, then one might expect some alteration in either the clinical course or histopathologic findings in sympathectomized rabbits.

Experimental nephrotoxic nephritis in rabbits has been studied extensively. The role of complement as well as the type and quantity of heterologous antiserum in the production of the lesion have been well delineated [23-28] and will not be reviewed here. Local fibrin deposition in the kidney in the course of nephrotoxic nephritis appears to be a key factor in consequent glomerular damage, as judged by a beneficial protective effect of anticoagulation, fibrin depletion by ancroid, and depletion of polymorphonuclear leukocytes.[2,10,23-27] Diminution of glomerular damage and crescent formation with anticoagulants is probably associated with an initially milder form of nephrotoxic nephritis, since studies with rapidly progressive severe nephrotoxic nephritis have shown no benefit of anticoagulation.[29-31] We selected a dosage schedule which would provide a spectrum of pathology from mild proliferative changes to moderate proliferation and crescent formation because we were interested in detecting any protective effect of chemical sympathectomy on nephrotoxic nephritis. A beneficial effect might then warrant studies of sympathectomy in severe rapidly progressive disease. However, we were unable to detect any differences in 6-OHDA–pretreated rabbits and controls. The degrees of proteinuria and azotemia were comparable for each of the groups, the immunofluorescence findings of immunoglobulin and fibrin deposition were similar, and we were repeatedly unable to distinguish between control and sympathectomized rabbits on a histopathologic basis.

Although tissue norepinephrine depletion was not "complete" in the present study, the levels were reduced 90% compared with those of normals and were similar to those attained in the studies of endotoxin and intramuscular coagulation previously reported.[11] Therefore, we conclude that catecholamines and the sympathetic nervous system either play an insignificant role or are not involved in the pathogenesis of nephrotoxic nephritis in rabbits.

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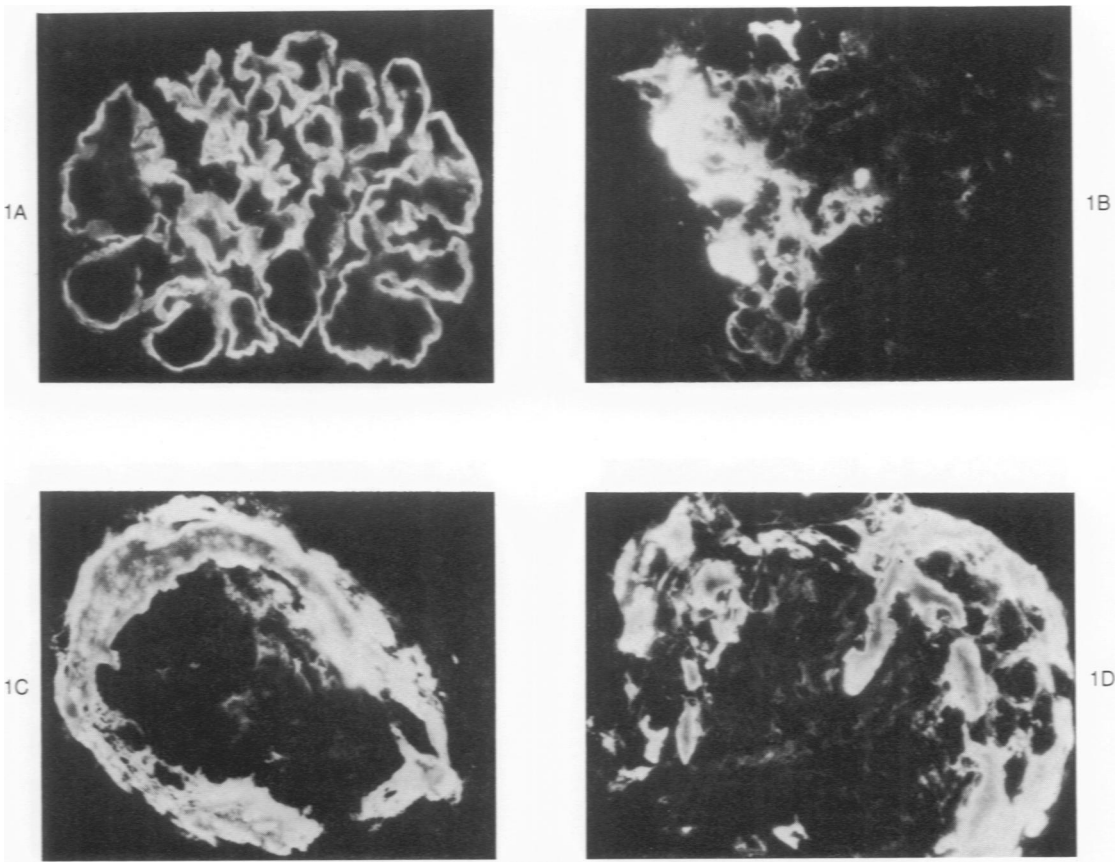

Figure 1A—Glomerulus illustrating linear sheep IgG along the GBM by immunofluorescence using goat antisheep IgG. (× 460) **B**—Glomerulus demonstrating segmental fibrin deposition. From a rabbit that received 2 ml nephrotoxic serum. (Sheep antirabbit fibrinogen, × 400) **C**—Glomerulus from a nephrotoxic nephritis rabbit, demonstrating global involvement. (Sheep antirabbit fibrinogen, × 340) **D**—Glomerulus from a rabbit sympathectomized with 6-OHDA showing global fibrin deposition. (× 400)

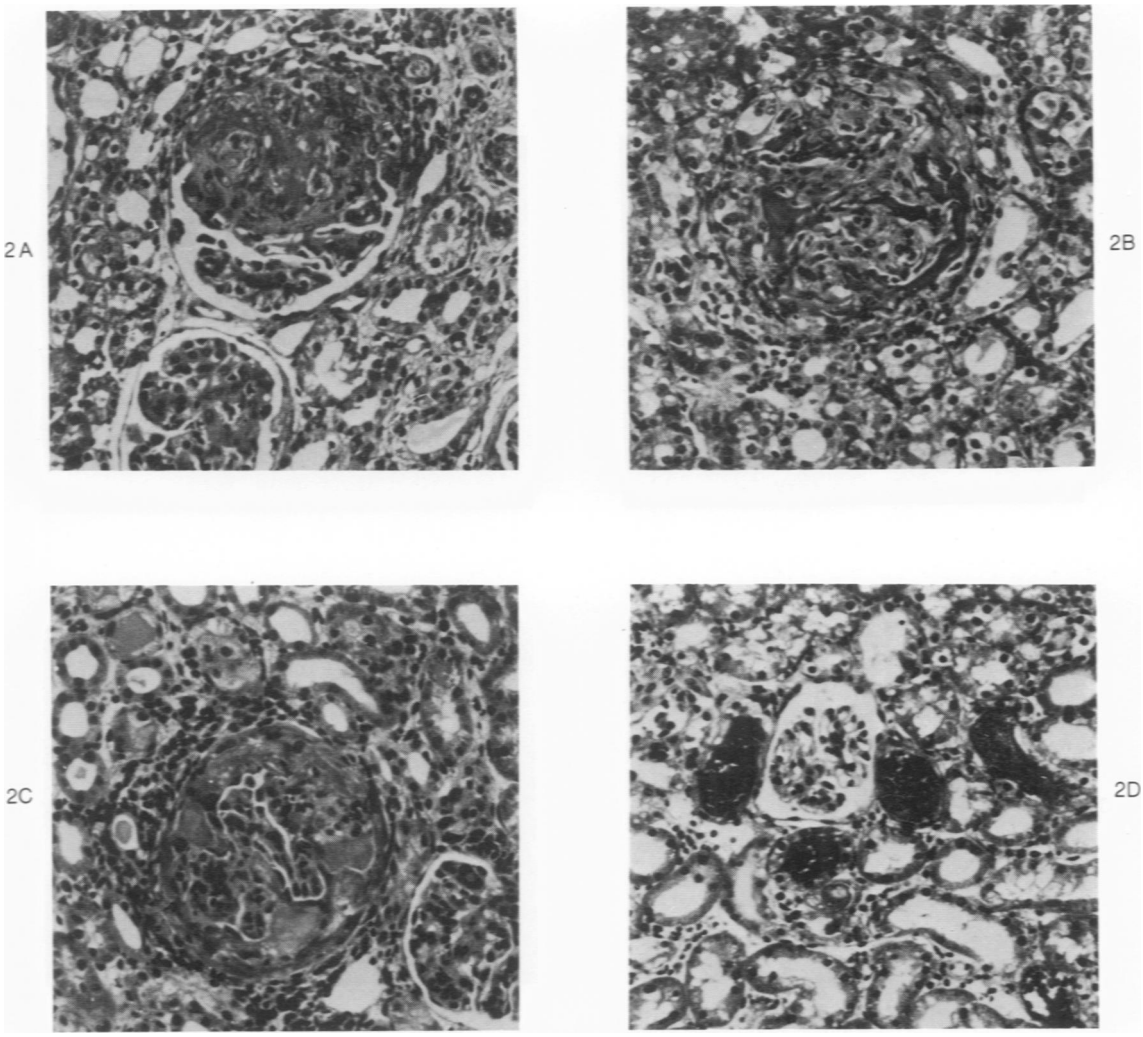

Figure 2—Histologic sections of representative glomeruli. **A**—Pronounced proliferation of Bowman's capsular epithelium with compression of glomerular capillary tuft in an animal sympathectomized with 6-OHDA and receiving 2 ml nephrotoxic serum. (H&E, × 200) **B**—Obliteration of tuft by proliferation of Bowman's capsular epithelium in association with fibrin deposits in an animal receiving 2 ml nephrotoxic serum alone. (H&E, × 200) **C**—Periglomerular infiltrate of lymphocytes in a 6-OHDA animal receiving 1.5 ml nephrotoxic serum. (H&E, × 200) **D**—Tubular casts of red cells and a normal glomerulus from a rabbit with nephrotoxic nephritis receiving 2 ml nephrotoxic serum. (H&E, ×200)