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Acute and Chronic Effects of Chemically Induced Unilateral Renal Disease in Rats[1] (38890)

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Turpentine has been reported to produce acute renal failure in man when used locally as an abortifacient (1). Morphologic lesions 6 wk after placement are predominantly tubular atrophy and dilatation, interstitial fibrosis, edema, and leukocytic infiltration. Although dysuria, hematuria, proteinuria, and glycosuria have been noted with turpentine toxicity, their significance has been overshadowed by signs and symptoms referable to other organ systems (2–5). Renal damage associated with turpentine has usually been transient and completely reversible (6). Local application of turpentine to one kidney in the rabbit followed by removal of the contralateral kidney has been associated with widely necrotizing arteritis (7–12). The turpentine-treated kidney usually develops unilateral perinephritis locally; however, acute and chronic effects on the contralateral kidney have not been investigated.

The purpose of this study was to examine the serologic and chemical alterations produced by turpentine applied to the kidney locally and to define the morphological and immunohistological alterations in the contralateral kidney. In addition the investigation was designed to develop a model for the study of unilateral renal disease and its relationship to bilateral nephropathy.

Materials and Methods. Fifty-three Sprague–Dawley rats 100–150 g body wt were anesthetized with ether after two baseline 24-hr urine collections. After flank incision one kidney was wrapped in turpentine soaked silk by a modification of the method of Campbell (7). Dual-shaped silk material about 6 cm in diameter perforated at the edges was backed with sterile plastic. A cylindrical aperture 1.2 x 2.0 cm was made and pursestring sutures placed both at the periphery of the apparatus and the central aperture. The central hole was placed over the exposed kidney and the pursestring tied loosely around the hilum. The kidney was then wrapped with the mildly saturated silk and the sutures at the periphery tied so as not to allow the escape of turpentine into the peritoneum. Three control groups of 15 animals each were used. In one group the kidney was wrapped in a similar manner but the silk was not treated with turpentine. In another a small amount of turpentine equivalent to the quantity used to soak the silk wrapping was injected intramuscularly. The third group was not treated.

The animals were placed in metabolic cages and fed Purina Rat Chow and water ad lib. Twenty-four-hour urine samples were collected daily for 2 wk and then every third day. Urine volume and quantitative urine protein excretion were recorded. The following studies were done at the time of sacrifice: serum creatinine, cholesterol, total protein, protein electrophoresis using cellulose acetate, and protein immunoelectrophoresis utilizing rabbit antisera to rat serum. Animals were sacrificed approximately 2, 5, and 8 wk after turpentine administration. Animals in the control group were sacrificed at the same times as the experimental animals.

At the time of sacrifice each kidney was weighed, examined grossly, and specimens

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were fixed in formalin for light microscopy. Four-micron sections were stained with periodic acid–Schiff (PAS) and hematoxylin and eosin (H and E). Immunohistological studies utilizing fluorescein isothiocyanate (FITC)-conjugated antisera to rat IgG, C3, and fibrinogen were performed on 4-μm frozen sections. Renal arteries and veins were examined grossly and by light microscopy.

On selected 24-hr urine protein samples, urine was concentrated to 10 mg protein per ml by negative-pressure dialysis (Amicon) and characterized by immunoelectrophoresis using antisera to normal rat serum and by cellulose acetate electrophoresis. Attempts to demonstrate antiglomerular basement membrane or antibody to renal proximal tubular epithelial (RTE) antigen from the serum of experimental and control animals were performed by double-layer immunofluorescence using frozen sections of normal rat kidney and FITC-conjugated rabbit antisera to RTE. Immunofluorescent techniques have been previously described (13). No attempt was made to elute and characterize glomerular fixed antibodies from the kidneys.

Results. Experimental animals were divided into three groups:

I. Animals dying or sacrificed 4–14 days after surgery (22) rats; A. Animals with significant proteinuria (13 animals); B. Animals without significant proteinuria (nine animals).

II. Animals sacrificed 4–5 wk after surgery (14 animals).

III. Animals sacrificed at 8 wk after surgery (six animals). Eleven animals found dead for an apparently long period of time were excluded from the study.

In all animals in Group I-A, 24-hr urine protein excretion increased from baseline values of 2.8–10 mg (Mean 6.06) to peak levels of 33–193 mg (Mean 104). The onset of significant proteinuria was between day 3 and 6. Proteinuria was accompanied by a marked increase in total volume and the electrophoretic pattern of protein excretion suggested tubular proteinuria (14). The clinical, morphological, serological, and urinary findings in this group are summarized in Table I. Morphological alterations in the wrapped kidney ranged from focal necrosis, interstitial inflammation, tubular dilatation, and renal capsular thickening to severe tubular and cortical necrosis. Vessels showed thickening and inflammation. Mild morphological changes were observed in the contralateral kidney and vessels. Fibrinogen was localized in the turpentine-treated kidney in 13 animals and in the contralateral kidney in five. Serum cholesterol was normal in this group as in all other groups. The total serum protein concentration and the albumin and globulin levels as well as the serum creatinine in this group are quite striking (Table I). Figure 1 compares the daily urine volume and protein excretion of a representative animal in this group with that of a control.

Of the nine animals in Group I-B, one died and three were sacrificed within 4 days of surgery. All showed subcapsular thickening or fibrosis of the turpentine-treated kidney. In only one was the serum creatinine elevated. The other six animals sacrificed between the 9th and 14th postoperatively day showed no alterations in urine protein excretion. Immunohistologic findings were negative in all members of this group and the contralateral kidney was morphologically normal. Vascular alterations observed on the turpentine-treated side were milder and less frequent than in animals in Group I.

The serologic, chemical, morphologic, and immunohistologic findings and the 24-hr urine protein excretion in Group II animals are summarized in Table II. Vessels uniformly showed thickening and inflammation. All but two animals (nos. 1 and 48) had transient proteinuria between 31 and 40 mg for 2–6 days between postoperative day 6 and 13. Eight animals had recurrent proteinuria >30 mg/24 hr at the time of sacrifice while control animals excreted 2.4–14.0 mg (Mean 8.06) protein. Three animals had hyperproteinemia. Elevations were reflected in the globulin fraction particularly in the alpha 2 and immunoglobulin fractions. The course of protein excretion of a typical animal from this group is shown in Fig. 2.

All animals in Group III except No. 24 had proteinuria usually of short duration

TABLE I. Group I-A.

Animal no.	No. days post-surgery	Serum creatinine (mg/100 ml)	Serum protein (g/100 ml)	Serum albumin (%)	Wrapped kidney					Nonwrapped kidney					Urine protein (mg)[b]
					Weight (g)	Morphology	Immunohistology[a] IgG	BiC	F	Weight (g)	Morphology	Immunohistology IgG	BiC	F	
17*	5				1	Severe interstitial fibrosis and inflammation tubular dilatation	0	0	1+	1.3	Normal	0	0	0	40
19	10	1.12	7.23	32	1	Severe interstitial inflammation tubular dilatation and atrophy	0	0	1+	1.4	Normal	0	0	Tr	40
22[c]	9				3	Occasional glomerular necrosis, interstitial inflammatory infiltrate	0	0	1+	1.3	Normal	0	0	0	40
30[c]	5				0.9	Severe tubular atrophy	0	0	1+	1.1	Normal	0	0	0	43.7
31	16	2.2	8.3	44	1.6	Severe tubular dilatation and atrophy, interstitial inflammatory infiltrate	0	0	1+	1.4	Focal mild centrolobular hyalinization and proliferation, focal glomerular basement membrane ↑	0	0	1+	135.8
32[c]	8				1	Cortical and tubular necrosis	0	0	2+	0.9	Glomeruli engorged with rbc, focal proliferation	0	0	0	82
35	15	0.9	9.4	43	1.1	Cortical necrosis	2+		1+	1.2	Normal	0	0	1+	37
38[c]	5				1.2	Tubular necrosis, interstitial inflammatory infiltrate	0	0	1+	1.0	Normal	0	0	0	193
39[c]	4				1.1	Occasional necrotic glomeruli	0	0	1+	0.8	Normal	0	0	0	37
59	9	2.93	7.3	29	0.8	Cortical necrosis	0	0	2+	1.05	Normal	0	0	1+	122
61	7	2.9	6.9	38	1.07	Acute tubular necrosis	0	0	1+	.85	Normal	0	0	1+	67
63	4	2.88	7.8	60	1.107	Moderate tubular dilatation and atrophy	0	0	1+	1.76	Normal	0	0	0	79
65[c]	4				1.131	Acute tubular necrosis	0	0	1+	1.08	Normal	0	0	0	135

[a] Graded by intensity: IgG, BiC nodular; F (fibrinogen) intercapillary 0–4+.
[b] Twenty-four-hour urine protein in milligrams just prior to sacrifice or death.
[c] Animal died.

for 1–6 days (average 2 days). Protein excretion ranged from 30–150 mg/24 hr. Only one animal had significant proteinuria at sacrifice and this was suggestive of glomerular origin. Again those animals in this group with hyperproteinemia reflected increase in the globulin fraction. The clinical, morphological, and immunohistologic findings in this group are summarized in Table III. Renal inflammation of the major vessels was observed on the turpentine-wrapped side but not on the contralateral side.

Differences in weight of the treated and nontreated kidneys were small and were thought to reflect either hypertrophy of the nontreated kidney or atrophy of the treated.

None of the control groups showed renal morphologic or large-vessel alterations, proteinuria, polyuria, or serum chemical abnormalities. Figures 3 and 4 show representative morphological and immunohistological studies of the contralateral nontreated kidneys.

Attempts to identify anti-GBM or anti-RTE antibodies in the serum were unsuccessful.

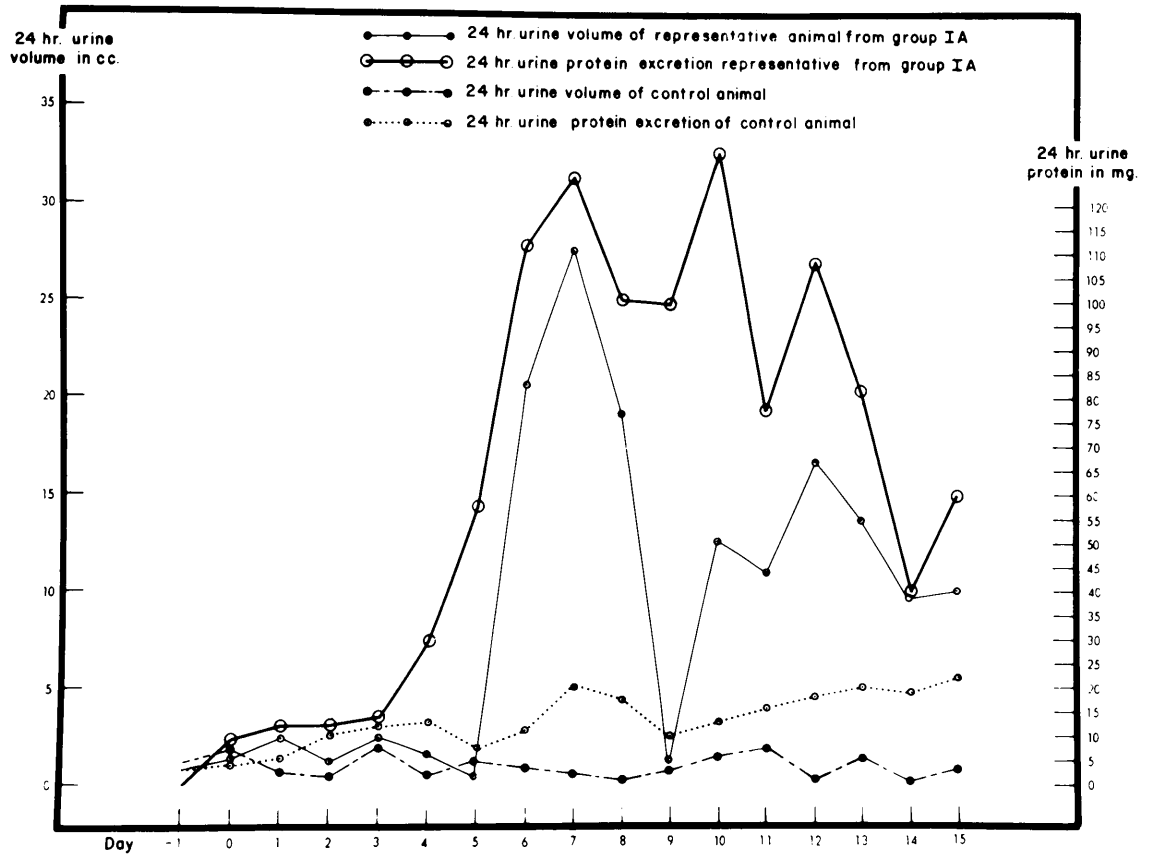

FIG. 1. Compares the daily urine volume and protein excretion of a representative animal in Group I-A with that of a control.

Discussion. The toxic effects of turpentine and the association of this chemical with renal disease are summarized by Cornell and Goldman (1). The local effects of this substance on the vasculature, blood pressure, and plasma after removal of the contralateral kidney have been carefully studied (7–12).

The morphologic alterations in the turpentine-wrapped kidney in this study are similar to those observed in acute cortical or tubular necrosis, hyperacute homograft rejection, or diseases which affect blood flow. The pattern of distribution of fibrinogen in the absence of IgG or β_1C in otherwise normal kidneys has been observed in hyperacute renal homograft rejection (15, 16).

Unilateral renal disease has been associated with the nephrotic syndrome and bilateral immune deposit disease (17). Although this relationship is unclear, it has been postulated that renal injury may lead to release of autologous kidney antigens giving rise to an autologous immune complex disease.

Attempts to produce bilateral immune complex nephritis in the experimental animal by unilateral renal vein constriction on arterial occlusion have been unsuccessful and the above hypothesis is pure speculation. Although the turpentine model cannot be construed as accomplishing this goal, there are several related observations which may contribute to understanding human renal disease. The hypergammaglobulinemia and the immune deposit disease in the noninvolved kidney suggests that unilateral disease and unilateral renal vessel inflammation produced by a nonimmunologic mechanism may lead to the production of a bilateral

TABLE II. Group II.

Animal no.	No. days post-surgery	Serum creatinine (mg/100 ml)	Serum protein (g/100 ml)	Serum albumin (%)	Wrapped kidney					Nonwrapped kidney					Urine protein (mg)[b]
					Weight (g)	Morphology	Immunohistology[a] IgG	B1C	F	Weight (g)	Morphology	Immunohistology IgG	B1C	F	
1	34	1.8	7.7	63	—	Not done necrotic	0	0	0	1.2	Normal glomeruli, large	0	0	0	0
14	35	0.5	8.3	40	1	Focal tubular dilatation and atrophy	0	0	1+	1	Minimal glomerular basement membrane, thickening	Tr	Tr	Tr	34
16	35	2.21	8.25	42	1	Marked tubular atrophy and dilatation	Tr	Tr	Tr	1.1	Same as above and focal centrolobular hyalinization	1+	Tr	2+	25
20	35	1.7	7.73	60	0.8	Many obsolescent glomeruli, moderate tubular atrophy	0	0	Tr	1.2	Normal	0	0	Tr	25
23	35	0.86	7.73	58	1	Capsular thickening	0	0	0	1.2	Normal	0	0	0	2.3
25	35	1.9	7.8	60	0.9	Normal	0	0	0	1.1	Normal	0	0	0	0.7
33	35	1.8	7.23	74	0.35	Normal	0	0	0	0.9	Normal	0	0	0	0.7
34	36	1.8	7.79	63	0.8	Cortical necrosis, arterial medial thickening	0	0	0	1	Normal	0	0	0	36.5
44	28	2.13	9.9	45	1.06	Focal tubular dilatation and atrophy	0	0	0	1.05	Normal	Tr	Tr	1+	31
45	34	1.4	7.5	65	.93	Focal tubular dilatation and atrophy	0	0	0	1.2	Mild focal glomerular basement membrane thickening	1+	1+	1+	16.25
46	34	2.5	7.9	61	.89	Focal tubular dilatation and atrophy	0	0	0	0.93	Mild focal glomerular basement membrane thickening	1+	1+	1+	62
47	34	1.6	7.3	60	.9	Focal tubular dilatation and atrophy	0	0	0	0.93	Normal	Tr	Tr	Tr	35
48	34	1.6	7.9	61	.74	Normal	0	0	0	0.83	Normal	0	0	0	0
49	30	1.5	9.3	45	.76	Glomeruli obsolescent	0	0	0	1.01	Mild focal glomerular basement membrane thickening	1+	1+	1+	140

[a] Graded by intensity.
[b] Twenty-four-hour urine protein in milligrams just prior to sacrifice or death.

immune complex nephritis. However, turpentine itself by entry into the blood stream may alter serum or nonrenal tissue proteins, an hypothesis which we have suggested as the mechanisms of glomerular injury in some states (13). This hypothesis seems an unlikely explanation for all of the current findings, in view of the absence of disease in control groups. Hydrocarbons have been associated with autoimmune (Anti-GBM) nephritis probably by toxicity to lung or to other basement membrane structures which

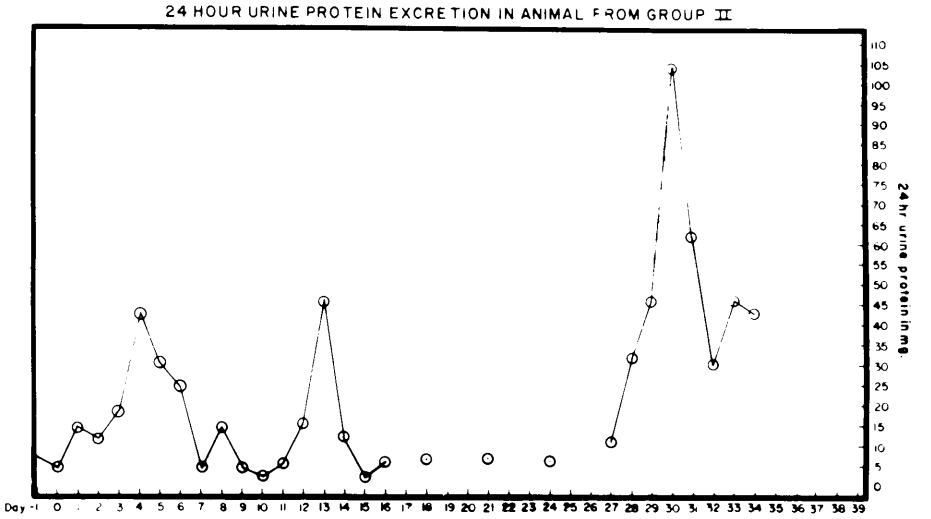

FIG. 2. Shows serial daily urine excretion from an animal from Group II.

TABLE III. Group III,

Animal no.	No. days post-surgery	Serum creatinine (g/100 ml)	Serum protein (mg/100 ml)	Serum albumin (%)	Wrapped kidney						Nonwrapped kidney					Urine protein (mg)[b]
					Weight (g)	Morphology	Immunohistology[a] IgG	B1C	F	Weight ((g)	Morphology	Immunohistology IgG	B1C	F		
2	54	1.05	8.9	49.3	0.7	Marked interstitial fibrous chronic inflammatory infiltrate	0	0	Tr	1.1	Minimal focal glomerular basement membrane thickening and focal	1+	1+	2+		10.9
13	58	1.67	7.73	60	1.2	Focal interstitial fibrosis, scattered areas of tubular atrophy and chronic inflammation	0	0	Tr	1.2	Normal	0	0	+		6.8
15	58	1.99	7.1	66.6	1	Focal interstitial fibrosis; scattered areas of tubular atrophy and chronic inflammation	0	0	0	1.1	Normal	0	0	0		1.56
21	58	2.99	7.3	55	1.1	Marked interstitial fibrosis, tubular atrophy, chronic inflammatory infiltrate	0	0	Tr	1.1	Moderate focal segmental glomerular basement membrane thickening epithelial proliferative	+	+	+		40
24	58	1.23	7.7	52	1	Normal	0	0	Tr	1.2	Minimal focal centrolobular scarring	0	0	2+		2.39
27	58	2.46	6.86	41	1	Focal interstitial fibrosis	0	0	2+	1	Minimal focal centrolobular scarring	0	0	2+		9.35

[a] Graded by intensity.

[b] Twenty-four-hour urine protein in milligrams just prior to sacrifice or death.

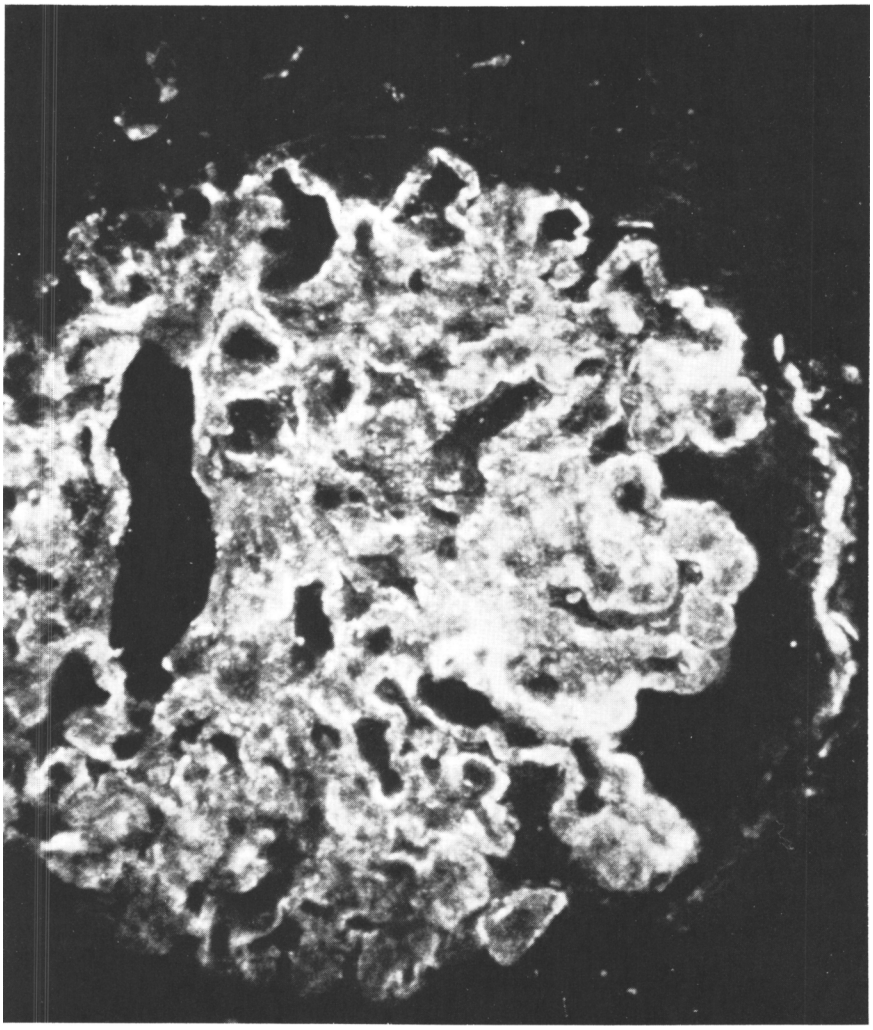

FIG. 3. Immunofluorescent localization of IgG in the glomerulus of the contralateral kidney of animal no. 2 (Group III) at 58 days after onset of the experiment.

cross react with lung, releasing these antigens into the circulation (18). This disease, however, is characterized by linear deposition of host IgG and C3 on the GBM and by anti-GBM antibodies in the serum. An alternative explanation of the current findings is that turpentine, by altering renal tissue or renal vasculature, leads to a release of renal or vascular antigens resulting in an autologous immune complex disease.

Summary. Chemically induced unilateral renal disease was associated with a high incidence of proteinuria, diuresis, a morphological spectrum ranging from perinephritis to acute tubular or cortical necrosis, and unilateral or bilateral glomerular fibrinogen deposition during the first 2 wk after induction. Later, a decrease in proteinuria and return to normal urine output was not infrequently followed by recurrent proteinuria, hypergammaglobulinemia, morphological alterations, and deposition of IgG and β_1C on the glomerular basement membranes and mesangium of the contralateral kidney and the treated kidney. Intercapillary deposition of fibrinogen in association with IgG and β_1C was occasionally observed in one or both kidneys. The mor-

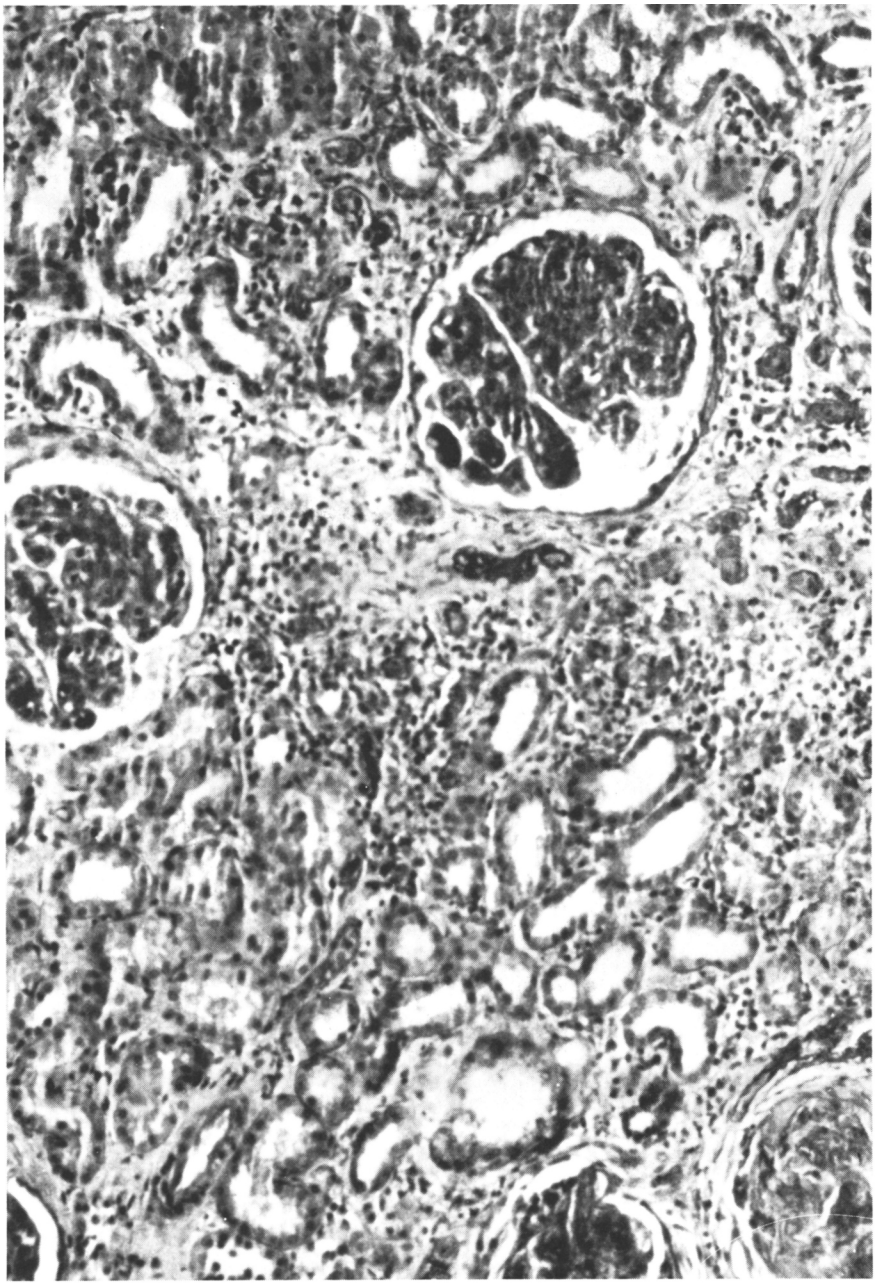

FIG. 4. PAS staining of a field of the contralateral kidney at 8 wk.

phologic, immunohistologic, serologic, and chemical findings suggest that this model may be useful for further defining the course and prognosis of unilateral renal disease produced by vascular insufficiency.

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