VASCULAR LESIONS IN ANTI-KIDNEY SERUM NEPHRITIS OF THE RAT

JACOB CHURG, M.D., AND HIROSHI SAKAGUCHI, M.D.*

From the Departments of Pathology, Barnert Memorial Hospital, Paterson, N. J., and New Jersey College of Medicine and Dentistry, Jersey City, N. J.

Chronic glomerulonephritis induced in the rat by anti-kidney serum is often accompanied by hypertension and arterial lesions.[1] Among such lesions are calicific medial necrosis and acute necrotizing fibrinoid arteritis. During the current study of anti-kidney serum nephritis in the rat, both types of arterial necrosis were observed in animals who had developed severe chronic renal disease. These vascular lesions have been described and their relation to the renal disease discussed.

MATERIAL AND METHODS

Glomerulonephritis was induced in 155 rats by rabbit anti-rat-kidney serum as previously described.[2] The animals received 0.7 to 3 ml of serum intravenously, usually in a single injection, but sometimes the amount was divided into 2 or 3 daily doses. They were kept in metabolic cages and were allowed food (Purina® Chow) and water *ad libitum*. Urinalysis was performed at frequent intervals; non-protein nitrogen (NPN) was measured several times in the course of the study, the last examination being at the time of sacrifice.

Injection of the serum was followed within hours by increasing proteinuria, frequently reaching several grams per 100 ml of urine; by azotemia of over 100 mg per cent, occasionally over 200 mg per cent of NPN per 100 ml of blood, and often by edema. Recovery began after 1 week. Proteinuria lasting more than 6 weeks usually persisted throughout the remaining life of the animal, indicating the presence of chronic renal disease. In such animals, even if the NPN returned to normal, it usually increased again later.

Fifty-one animals with proteinuria persisting after 2 months were allowed to survive for an additional period of time. They were killed by exsanguination under ether anesthesia or died spontaneously 2½ to 11 months after induction of the disease. All tissues were fixed in formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin, the periodic acid-Schiff (PAS) reagent, Mallory trichrome, toluidine blue for metachromasia, Verhoeff's elastic tissue stain and the von Kóssa stain for calcium.

RESULTS

Renal Lesions. The changes of chronic glomerulonephritis were observed in all 51 animals and were severe in 40 of these. The severity of

Presented in part at the Sixtieth Annual Meeting of the American Association of Pathologists and Bacteriologists, Cincinnati, Ohio, April, 1963.

Supported by a grant from the Kidney Disease Foundation of Northern New Jersey, and by a Public Health Service Research Grant (AM-00918) from the National Institute of Arthritis and Metabolic Diseases.

Accepted for publication, June 27, 1965.

* Present address: Department of Pathology, Keio University Medical School, Tokyo, Japan.

the disease was roughly proportional to the dose of serum and to the duration of the disorder. The kidneys were at first enlarged, sometimes markedly so, being 2 to 3 times the normal size. They were pale and generally smooth. By the end of 3 to 4 months, they shrank to their normal or even to subnormal size and were firm and finely granular. Atrophy increased with progression of the disease. Microscopic changes corresponded to the gross appearance. At 2½ months, most of the glomeruli showed partial obliteration by intercapillary fibrosis and fibrous crescents, but many capillaries were still patent (Fig. 1). These glomerular changes progressed rapidly or slowly, terminating in diffuse fibrosis (Fig. 2). In addition to crescents, many glomeruli exhibited deposits of pink-staining homogeneous or somewhat fibrillar material, probably a protein, on the inside of the Bowman's capsule. This substance was similar in its staining reactions to the tubular casts. Later the deposits tended to calcify. At various stages one could observe glomeruli with dilated Bowman's space which probably corresponded to the tubules obstructed by casts. The tubules were at first rather well preserved, showing only focal atrophy (Fig. 1), but later became extensively atrophic (Fig. 2). In the terminal stages all the tubules were severely altered and were frequently surrounded by a calcified ring, probably calcified basement membrane. Large pink hyaline casts were observed in the tubules, mainly in the outer medulla, and later also in the cortex (Fig. 2) and in the inner medulla. With progression of the tubular atrophy, interstitial fibrosis increased as did the lymphocytic and plasma-cellular infiltration of the stroma. All animals with vascular lesions had severe renal disease, but the reverse was not always true since some animals with severe renal disease had no vascular lesions.

Vascular Lesions. Arterial lesions were observed in 32 animals. Fourteen showed calcific medial necrosis of the aorta and its major branches; 11 revealed acute necrotizing arteritis of small arteries, mainly in the splanchnic region but also in the testis, heart and thyroid and in 7 animals, both lesions were present simultaneously. Of the 51 animals, 24 lived 2½ to 4 months. Eleven of these exhibited arteritis, and 3, arteritis and medial necrosis. The remaining 27 animals lived 4 to 11 months; 14 of these had calcific medial necrosis and 4, both medial necrosis and arteritis. Moreover, in animals that had both lesions, arteritis was in the healing stage.

The lesions of arterisis were very similar to those that have been produced in rats by perirenal cellophane or silk encapsulation or by constriction of renal arteries.[3-5] The arteritis consisted of fibrinoid necrosis, mainly in the media, with proliferation of mononuclear cells in the adventitia and to a lesser degree in the intima (Figs. 3 and 4). A variable

number of polymorphonuclear leukocytes and lymphocytes were present. In more advanced lesions, fibrinoid necrosis appeared also in the intima (Fig. 4). Healing was usually by fibrosis of the wall, though regeneration occurred if the damage was slight.

Medial necrosis was seen predominantly in the aorta but also in the main coronary arteries, in the carotids and the iliacs and sometimes in the main renal and mesenteric arteries, but not in the smaller renal or splanchnic branches. A similar type of medial necrosis has been described after total or subtotal nephrectomy and after administration of poorly soluble sulfonamides.[6-8] Necrosis and calcification usually began by dissolution of muscle fibers in the middle third of the media followed by calcification, distortion, and sometimes fragmentation of the elastic fibers (Figs. 5 and 6). In severely affected vessels, damage to the wall was often followed by aneurysmal dilatation. As a rule, there was no inflammatory reaction. Healing occurred by calcification followed in more severe instances by cartilaginous and bony metaplasia. Appreciable amounts of metachromatic material were often seen near the areas of necrosis and occasionally were more diffusely distributed in the media.

Other Organs — Stomach. In 10 animals with medial necrosis, the muscularis of the stomach showed extensive changes, especially at the junction of the cardiac and fundic parts. These changes consisted of necrosis and lysis of smooth muscle fibers, occasionally accompanied by calcification of the stroma (perimysium) (Figs. 7 and 8). As a rule, there was no inflammatory response, though occasional foci of neutrophils were seen. The alterations in the muscularis were identical with those seen after bilateral nephrectomy or sulfonamide adminstration.[8,9]

Heart. Focal collections of mononuclear cells were observed in the myocardium of many animals. They were apparently unrelated to the vascular lesions.

Cecum. Severe inflammation and ulceration of the mucosa were present in a number of animals with advanced renal changes. These lesions appeared to be analogous to the uremic colitis in man.[9]

Comment

Hypertension and vascular disease are often encountered in experimental glomerulonephritis. The cause of each is poorly understood. Inflammation and subsequent scarring lead to the narrowing of the glomerular vascular bed and to a decrease in renal perfusion. It has been suggested that this decrease activates the Goldblatt mechanism, stimulates release of renin by the kidneys, and increases formation of angiotensin in the blood. These events may explain the hypertension of acute glomerulonephritis. In chronic glomerulonephritis, an alternate explana-

tion is possible, namely, that extensive atrophy of tubules gives rise to renoprival hypertension. Unfortunately, a high blood pressure reading provides no clue to its origin. Vascular lesions seen in glomerulonephritis are also non-specific. As is well known, necrotizing arteritis can be induced in animals not only by renal damage but also by injection of foreign protein[10,11]; calcific medial necrosis (smooth muscle necrosis) is seen after administration of sympathicomimetic amines,[11] parathyroid hormone,[12] vitamin D,[13] etc. When these lesions are seen in the rat following renal manipulations, however, the type of manipulation is definitely correlated with the vascular alterations; interference with renal hemodynamics by means of an arterial clamp or by wrapping the kidney in silk is followed by hypertension and by acute necrotizing arteritis. Total or subtotal nephrectomy or severe damage to the tubules, particularly those in the medulla, are associated with hypertension and with calcific medial necrosis ("cytolytic" necrosis of smooth muscle).[9,14] Consequently the type of vascular alteration may be used to indicate whether hypertension accompanying a renal disease is "renal" or "renoprival" in origin. Since hypertension, on occasion, occurs without any vascular lesions and vascular changes appear in the absence of hypertension, it has been suggested that arterial necrosis is caused by a different factor or by a different constellation of factors from those causing elevation of blood pressure.[15]

Until this point has been clarified, it may be better to speak in more general terms of a "renal state" and a "renoprival state," to denote two different sets of alterations each of which includes hypertension and vascular changes. The principal mechanism of the "renal state" is probably release by the kidney into the circulation of a pressor, and perhaps also of an angiotoxic, substance. The cause or causes of the "renoprival state" are not actually known. The normal kidney may possibly inactivate pressor and angiotoxic substances produced elsewhere in the body, or the kidney may play an important role in the metabolism of the smooth muscle cells. Particular suspicion has been attached to the disturbance in the metabolism of calcium and phosphorus which occurs in severe renal insufficiency.[1] It has been shown, however, that "renoprival" hypertension and vascular lesions can appear in the absence of renal excretory failure.[16,17] Some of our animals with medial necrosis (smooth muscle necrosis) had only mild or moderate renal insufficiency as measured by the NPN level in the blood. The relation of the kidney to hypertension has been reviewed by Peart.[18]

The results of this study suggest that both the "renal" and the "renoprival" mechanisms operate in chronic glomerulonephritis in the rat and that they operate in sequence. In the early stages of the disease, the main

vascular lesion is acute necrotizing arteritis—in the late stages, calcific medial necrosis. These findings agree well with the concept that the Goldblatt mechanism is activated by the narrowing of the renal vascular bed, but becomes de-activated when the blood flow decreases toward zero. The role of the tubules in the development of the "renoprival" state is presumptive, but it is supported by the experience with sulfonamide nephropathy.[8,9]

Though the pathogenesis of human glomerulonephritis is not necessarily the same as that of anti-kidney serum nephritis, the evolution of lesions is quite similar leading to eventual obliteration of glomeruli and atrophy of tubules. The morphologic analogy suggests also that in man the hypertension of glomerulonephritis may be in part "renal" and in part "renoprival." Unfortunately, in man, vascular lesions, when they occur, cannot be used to support this premise. The principal necrotizing lesion is fibrinoid degeneration of arterioles which is probably related to the severity and duration of the hypertension rather than to its origin and to the concomitant renal insufficiency. It is well known that the "renal" (Goldblatt) mechanism can operate in man, but doubts have been expressed about the occurrence of the "renoprival state," because man can live without kidneys for a long time and fail to show elevation of blood pressure.[19] This argument is not necessarily valid, because people without kidneys are kept alive by means of an artificial kidney or peritoneal dialysis which may remove the extra-renal pressor substances from the body or may help to counteract the metabolic disturbances. Recently Sommers, Robbins, Babin and Knaack, using a different approach ("mapping technique"), came to the conclusion that hypertension of chronic pyelonephritis was most likely "renoprival" in origin.[20] Hypertension in advanced chronic glomerulonephritis may prove, at least in part, to be of similar nature.

Summary

Among 51 rats with chronic anti-kidney serum nephritis, 32 had necrotizing arterial lesions. Eleven animals showed acute inflammation of small arteries; 14 exhibited calcific medial necrosis ("cytolytic smooth muscle necrosis") of large elastic arteries, and in 7 there were both lesions.

Arteritis was most common 2½ to 4 months after induction of nephritis while medial necrosis was seen mainly after the fourth month.

Animals with arteritis showed moderately advanced glomerular disease and comparatively little tubular atrophy. Animals with calcific medial necrosis had advanced glomerular lesions and extensive tubular atrophy. The relation of both types of arterial lesions to the "renal" and "renoprival" state has been discussed.

It is suggested that in experimental animals, as well as in man, vascular lesions and hypertension in the early stages of glomerulonephritis are of "renal" origin and are probably caused by the secretion of angiotoxic or pressor substances by the kidneys. In advanced chronic glomerulonephritis, on the other hand, vascular lesions and hypertension may be related to the loss of detoxifying or metabolic functions of the tubules ("renoprival" state).

References

1. Hackel, D. B., and Heymann, W. Vascular lesions in rats with nephrotoxic renal disease. *Arch. Path. (Chicago)*, 1959, **68**, 431–437.
2. Churg, J.; Grishman, E., and Mautner, W. Nephrotoxic serum nephritis in the rat. Electron and light microscopic studies. *Amer. J. Path.*, 1960, **37**, 729–749.
3. Friedman, B.; Jarman, J., and Klemperer, P. Sustained hypertension following experimental unilateral renal injuries. Effects of nephrectomy. *Amer. J. Med. Sci.*, 1941, **202**, 20–29.
4. Smith, C. C.; Zeek, P. M., and McGuire, J. Periarteritis nodosa in experimental hypertensive rats and dogs. *Amer. J. Path.*, 1944, **20**, 721–735.
5. Wilson, C., and Byron, F. B. The vicious circle in chronic Bright's disease. Experimental evidence from the hypertensive rat. *Quart. J. Med.*, 1941, **10**, 65–93.
6. Kolff, W. J., and Fisher, E. R. Pathologic changes after bilateral nephrectomy in dogs and rats. *Lab. Invest.*, 1952, **1**, 351–363.
7. Morrison, A. B. Experimentally induced chronic renal insufficiency in the rat. *Lab. Invest.*, 1962, **11**, 321–332.
8. Churg, J., and Lehr, D. Cardiovascular and smooth muscle lesions in the course of experimental nephropathy. (Abstract) *Amer. J. Path.*, 1954, **30**, 638–639.
9. Churg, J. Renal and renoprival vascular disease in the rat. *Arch. Path. (Chicago)*, 1963, **75**, 547–557.
10. Rich, A., and Gregory, J. E. The experimental demonstration that periarteritis nodosa is a manifestation of hypersensitivity. *Bull. Hopkins Hosp.*, 1943, **72**, 65–88.
11. Kellaway, T. D.; Herbertson, B. M., and Mortimer, T. F. Arterial lesions induced in rabbits by soluble foreign protein and by methoxamine. *J. Path. Bact.*, 1962, **84**, 45–51.
12. Hueper, W. C. Arteriosclerosis. *Arch. Path. (Chicago)*, 1944, **38**, 162–181, 245–285, 350–364; 1945, **39**, 117–131, 187–216.
13. Ham, A. W. Coronary and aortic sclerosis, periarteritis nodosa, chronic nephritis and hypertension as sequelae to a single experimentally produced widespread calcium precipitation in the rat. (Abstract) *Arch. Path. (Chicago)*, 1940, **29**, 731.
14. Churg, J.; Strauss, L., and Paronetto, F. Some Aspects of the Pathology of Hypertension. Vascular Lesions in Experimental and Human Hypertension. In: Hypertension. Mendlowitz, M. (ed.). Grune & Stratton, New York and London, 1961, pp. 15–32.
15. Campbell, W. G., Jr., and Santos-Buch, C. A. Widely distributed necrotizing arteritis induced in rabbits by experimental renal alterations. I. Comparison

with the vascular lesions induced by injections of foreign serum. *Amer. J. Path.*, 1959, 35, 439–465.

16. FLOYER, M. A. The effect of nephrectomy and adrenalectomy upon the blood pressure in hypertensive and normotensive rats. *Clin. Sci.*, 1951, 10, 405–421.
17. MUIRHEAD, E. E.; GROLLMAN, A., and VANATTA, J. Hypertensive cardiovascular disease ("Malignant hypertension"). *Arch. Path.* (*Chicago*), 1950, 50, 137–150.
18. PEART, W. S. Hypertension and the Kidney. In: Renal Disease. BLACK, D.A.K. (ed.). Blackwell Scientific Publications, Oxford, 1962.
19. MERRILL, J. P., and GIORDANO, C. The role of the kidney in human hypertension. I. Failure of hypertension to develop in the renoprival subject. *Amer. J. Med.*, 1961, 31, 931–940.
20. SOMMERS, S. C.; ROBBINS, G. B.; BABIN, D. S., and KNAACK, C. T. Chronic pyelonephritis, renal tubular atrophy, and hypertension. *Arch. Intern. Med.*, 1962, 110, 505–510.

Appreciation is expressed to Mr. A. Prado for technical assistance and to Mr. J. Tanczos for the microphotographs.

[*Illustrations follow*]

LEGENDS FOR FIGURES

Photomicrographs were prepared from sections stained with hematoxylin and eosin.

FIG. 1. Kidney, 11 weeks after injection of anti-kidney serum. There is moderate fibrosis of glomeruli and slight tubular atrophy. × 90.

FIG. 2. Kidney, 6 months after injection of anti-kidney serum. Advanced fibrosis of glomeruli is encountered with extensive tubular atrophy and large casts in some of the tubules. × 90.

FIG. 3. Section from the same animal shown in Figure 1. Severe arteritis and periarteritis appear in the pancreas. × 90.

FIG. 4. Twelve weeks after injection of anti-kidney serum. Fibrinoid necrosis and marked inflammation are manifest in a small mesenteric artery. × 400.

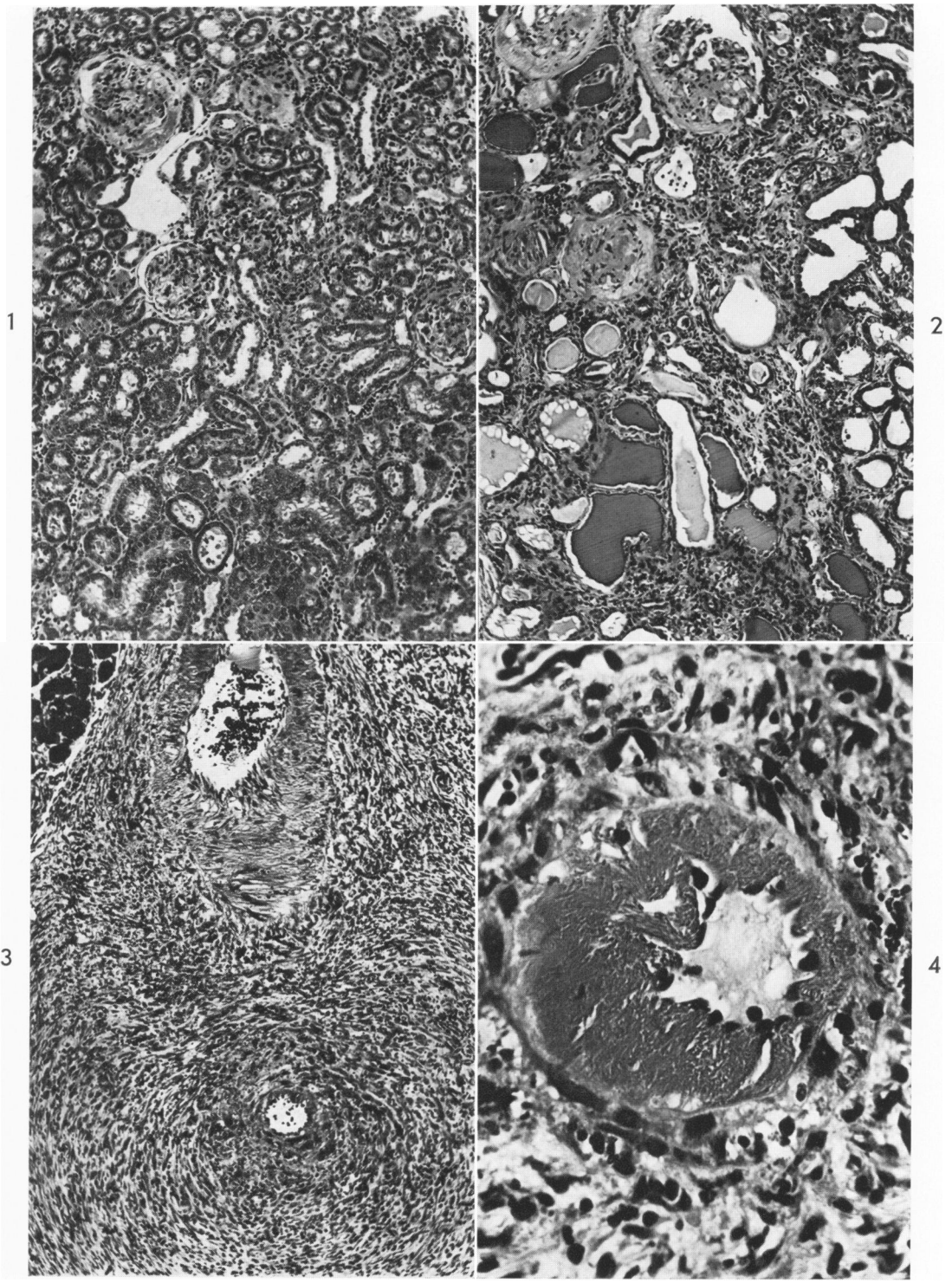
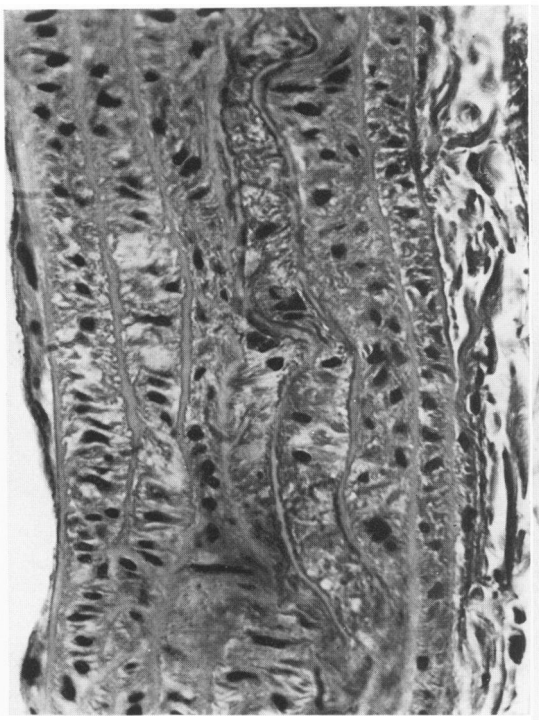


FIG. 5. Fourteen weeks after injection of anti-kidney serum. The aorta exhibits a streak of smooth muscle necrosis in the mid-third of the media. × 400.

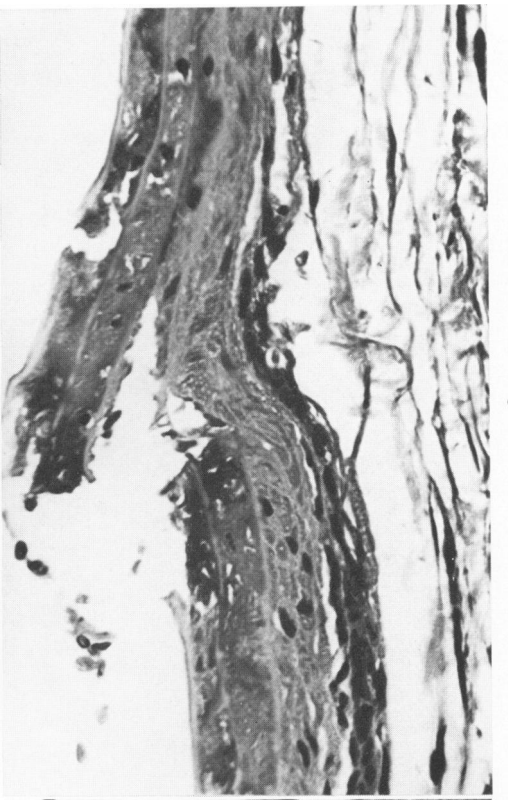
FIG. 6. Section from the same animal shown in Figure 2. Marked thinning of the aorta and extensive calcifying necrosis are shown. The "fracture" is probably an artifact. × 550.

FIG. 7. Section from the same animal shown in Figure 6. "Cytolytic" necrosis of smooth muscle appears in the wall of the first part of the stomach. × 100.

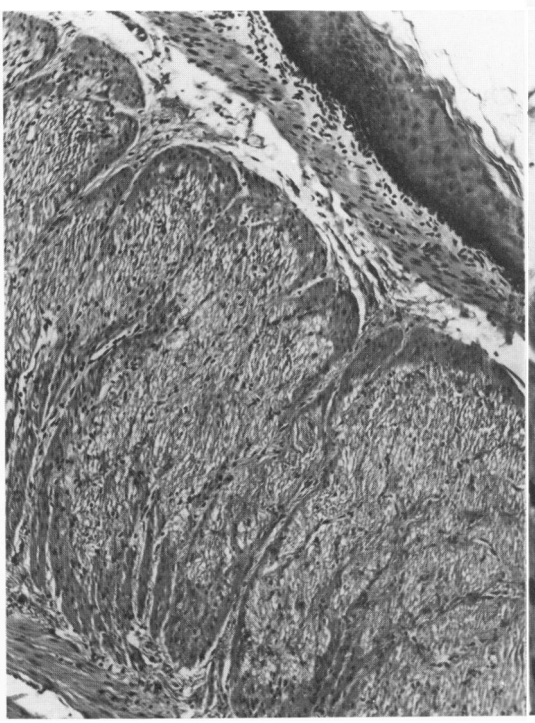
FIG. 8. A higher magnification of the area shown in Figure 7. Complete disappearance of the muscle fibers and preservation of the stroma are shown. × 400.



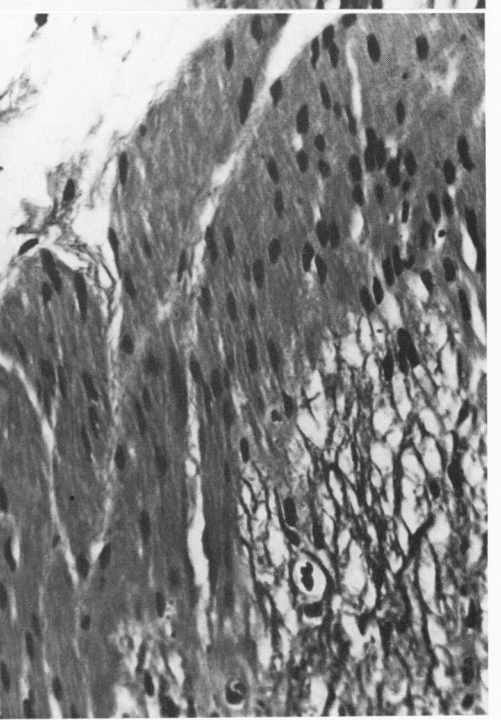
5



6



7



8