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IMMUNOCYTOCHEMICAL OBSERVATIONS ON THE VASCULAR NECROSIS AND RENAL GLOMERULAR LESIONS OF MALIGNANT NEPHROSCLEROSIS

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Although the characteristic vascular lesions[1,2] which represent the final stage of malignant hypertension are clearly related to the elevation of blood pressure, their pathogenesis is still unknown.[3] The high blood pressure is not the only factor,[4,5] but lowering of it ameliorates the lesions.[6] Hypertension does not invariably accompany or precede fibrinoid necrosis of arterioles,[7,8] and a relationship between hypertension and periarteritis nodosa has not been clarified. Some recent observations on the presence of antivessel antibodies in man[9] and rats[10] with hypertension suggest the role of an immune mechanism in the pathogenesis of these arterial lesions. Therefore, the localization of immunoglobulins, complement and other plasma proteins in the renal vascular and glomerular lesions was investigated immunocytochemically, in order to contribute to the understanding of the immune processes in malignant hypertension.

MATERIAL AND METHODS

Necropsy specimens of kidneys and other organs from 14 patients with malignant nephrosclerosis, of 4 patients with long-standing hypertension, and of 2 with renal cortical necrosis were studied (Table I). Formalin-fixed paraffin sections were stained with hematoxylin and eosin, with Gomori's elastica stain and with Weigert's fibrin stain, and were subjected to the periodic acid-Schiff (PAS) reaction after diastase digestion.

Tissue blocks were also quick frozen in dry ice-isopentane and stored at −20° C. Techniques described previously[11] were used for the fixation and staining of sections and for the preparation of fluorescein-labeled antisera. As suggested by Nairn,[12]

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antisera were also conjugated with lissamine-rhodamine B200, and adsorbed with activated charcoal (British Drug Houses, Ltd., The Ealing Corporation, Cambridge, Mass.). In this double-staining procedure, three sections were used: the first was stained with equal parts of rhodaminated antiserum and fluoresceinated antiserum for 30 minutes; the second was stained with rhodaminated antiserum for 30 minutes, washed quickly, and then stained with fluoresceinated antiserum. This procedure was reversed for the third section. Fluorescence microscopy observations were made with a Leitz Ortholux microscope, using two BG12 exciter filters and one OG4 or OG5 barrier filter. Pictures were taken with Anscochrome T/100 tungsten film and then converted to black and white negatives.

Antisera were prepared in rabbits against the following human plasma antigens: a) gamma globulin prepared using a DEAE cellulose column [13]; b) beta 2M globulin (19S macroglobulin) isolated by water precipitation of the serum from a patient with Waldenström's macroglobulinemia.[14] This antiserum was adsorbed with pure

TABLE I

CLINICAL DATA IN PATIENTS WITH MALIGNANT NEPHROSCLEROSIS

Necropsy number	Age	Sex	Race	Duration of hypertension (years)	Blood pressure (mm Hg)	Blood urea nitrogen (mm/100 ml)
18759	58	M	W	15	240–150	200
19083	6	F	N	3 months	220–150	90
19147	31	F	N	10	240–152	200
19389	69	F	W	3	210–105	108
19689	38	F	N	17	230–150	115
19738	39	M	N	10	250–150	240
19927	35	F	N	3	280–160	50
20296	56	F	N	7	300–200	77
20302	32	M	N	6 months	240–180	106
20326	39	M	W	4	250–140	265
20434	37	M	N	2	220–150	296
20659	67	M	N	2	240–156	182
L885	32	F	N	3	250–150	90
L886	54	M	W	7 months	210–130	160

gamma globulin; c) beta 1C globulin (complement) prepared by the method of Müller-Eberhard, Nilsson and Aronsson [15]; d) fibrinogen (Fraction I, Pentex, Kankakee, Ill.). This antiserum was purified by adsorption with human serum until only one precipitin line was seen with the agar double diffusion technique [16] against human plasma (since fibrinogen and fibrin cannot be differentiated immunologically, the term "fibrinogen" is used to indicate both); and e) albumin isolated by ammonium precipitation.[17] The specificity of antisera was checked by immunoelectrophoresis.[18]

Sections were treated with acid buffers, as previously described,[11] in an attempt to elute antibody gamma globulin from immune complexes. For identification, the fluorescent structures were encircled with a diamond pencil on the back of the slides. Then they were photographed, and their relationship to the scratched circles was mapped. The cover slips were removed, the slides were washed, then stained with hematoxylin and eosin, and rephotographed.

Controls in the fluorescent antibody studies included: a) use of unrelated fluorescent antisera; b) use of fluorescent sera on normal tissues; and c) blocking of the fluorescent antibody binding by prior application of non-fluorescent antibody.

RESULTS

The histologic findings in the 14 cases of malignant nephrosclerosis are listed in Table II. The vascular necrosis was eosinophilic, giving a positive stain for fibrin. Few erythrocytes, rare leukocytes and lipid were

TABLE II

PATHOLOGIC FEATURES IN PATIENTS WITH MALIGNANT NEPHROSCLEROSIS

Necropsy number	Kidneys			Other organs with arteriolar necrosis	Associated findings
	Combined weight (gm)	Arteriolar necrosis *	Glomerular necrosis *		
18759	270	+	+	Adrenal	
19083	96 (normal for age 135)	+	+	Adrenal, pancreas, spleen, g.i. tract	Chronic pyelonephritis
19147	210	++	+++	G.I. tract	
19389	225	++	+++	Liver, spleen	Rheumatic carditis
19689	190	+++	+++		Acute and chronic
19738	360	+	+		pyelonephritis
19927	170	+	+	G.I. tract	
20296	260	+	++		
20302	260	+++	++	Heart, spleen testis, g.i. tract	
20326	240	++	+++		Chronic pyelonephritis
20434	240	++	+++	Adrenal	
20659	260	+++	+++	Pancreas	
L885	270	+++	+++		
L886	260	+	+		

* Glomeruli or arterioles showing necrosis: +, 1–3%; ++, 4–6%; +++, 7% or more.

seen in the necrotic vessel walls. Marked periadventitial inflammatory reaction was seen in only one case (PM 19389). Intimal hyperplasia of intraparenchymal renal arteries was common. Necrosis of arterioles in organs other than kidneys was detected in 8 of the 14 cases. The renal glomeruli often showed involvement of the root of the glomerulus by fibrinoid necrosis and focal edema. In occasional glomeruli prominent exudate with inflammatory cells could be detected; diffuse hemorrhage was uncommon. Several glomeruli exhibited reparative changes: crescent-like proliferations, adhesions to Bowman's capsule and obliteration of capillaries.

The four cases of long-standing hypertension showed conspicuous hyaline deposition in the renal arterioles, and the two cases of renal cortical necrosis revealed areas of renal parenchymal necrosis with fibrin thrombi and necrosis in some of the arteries.

Immunocytochemical observations of the vascular lesions exhibited

gamma globulin in the wall of most of the arterioles with fibrinoid necrosis in 11 of the 14 cases (Table III). Occasionally, the specific fluores-

TABLE III

LOCALIZATION OF GAMMA GLOBULIN, COMPLEMENT AND FIBRINOGEN IN RENAL GLOMERULI AND VESSELS IN PATIENTS WITH MALIGNANT NEPHROSCLEROSIS

Necropsy number	Gamma globulin	Complement	Fibrinogen
18759	+	+	+
19083	+	+	+
19147	+	+	+
19389	+	+	+
19689	o	o	+
19738	+	+	+
19927	+	+	+
20296	+	+	+
20302	+	+	+
20326	o	o	+
20434	+	+	+
20659	+	+	+
L885	+	+	+
L886	o	o	+

cence was diffuse; often it was granular, and irregularly distributed in the vessel wall in greater prominence in the media and adventitia (Fig. 1). Attempts to elute gamma globulin with acid buffers were unsuccessful. Albumin and fibrinogen (Fig. 2) were usually detected in the region of arteriolar necrosis. Beta 2M globulin (19S macroglobulin) was absent from the arterial lesions. Complement (beta 1C globulin) was detected in some, but not all of the vessels with fibrinoid necrosis (Fig. 3). The distribution of complement was often irregular, granular and mainly in the media and adventitia (Figs. 4a,b).

Vessels with fibrinoid necrosis had an orange-green color when stained with fluorescein-labeled anticomplement antiserum and rhodamine-labeled antihuman gamma globulin antiserum. Similarly, the vessels usually assumed an orange-green color when they were stained with rhodamine-labeled antifibrinogen antiserum and fluorescein-labeled antigamma globulin or complement antiserum.

Not infrequently, however, areas of green fluorescence were noted in the media and adventitia of a vessel, while the intima and part of the media were stained orange by the antifibrinogen antiserum (Fig. 5). More rarely, only gamma globulin or complement could be found in the vessel without fibrinogen (Fig. 6). The hyperplastic proliferation of the renal artery intima was always devoid of the plasma proteins investigated (Figs. 7a,b). The vascular necrosis in organs other than kidneys revealed the presence of all plasma proteins.

Some of the renal glomeruli, especially those with focal necrosis, contained gamma globulin (Figs. 8a,b), complement (Figs. 9a,b) and albumin. Fibrinogen was generally diffusely localized along the basement membranes and in the intercapillary space (Fig. 10). In areas of glomerular hemorrhage, fibrinogen was also observed in capillary lumens (Figs. 11a,b). When sections were stained with rhodamine-labeled antifibrinogen and fluorescein-labeled antihuman gamma globulin or complement, many glomeruli appeared orange-green. Few glomeruli, however, were stained diffusely orange or orange-green with some structures distinctively green (Fig. 12). Rare glomeruli contained only gamma globulin or complement; none showed beta 2M globulin.

The three cases of malignant nephrosclerosis in which the lesions were devoid of gamma globulin and complement, fibrinogen was detectable in the fibrinoid necrosis of the arterioles and glomeruli. In the two instances with renal cortical necrosis, only fibrinogen was found in the necrotic arteries. The four cases of long-standing hypertension and conspicuous renal arteriolar hyalinization failed to show plasma proteins in the kidney sections, except for occasional intimal staining with fibrinogen.

Discussion

The present investigation indicates that all plasma proteins, including complement, were found in vascular lesions in 11 of 14 cases with malignant nephrosclerosis. The presence of gamma globulin in the vascular lesions, together with albumin and fibrinogen, is in keeping with the thought that injury of any type to a blood vessel, particularly hypertension,[19] increases its permeability to plasma proteins. The presence of complement, however, suggests an immunologic component, since complement fixes on immune complexes which, as recently demonstrated experimentally,[20] may be trapped in the vessel wall during a state of increased permeability.

Complement was not detected in the vascular lesions in renal cortical necrosis, in the arterial alterations associated with long-standing hypertension, and in the renal tubular casts, indicating that complement is not a common finding in exudates or areas where proteins are deposited nonspecifically. The presence of complement in a tissue is suggestive of the aggregation of antibody with complement *in vivo*, with ensuing tissue-damaging action in the "sensitized tissue." Complement is also fixed by aggregated gamma globulin,[21] but immunohistochemical techniques cannot differentiate between complement bound to immune complexes and that bound to aggregated gamma globulin, which is also cytotoxic when injected into man or animal.[22] The failure of acid buffers to elute gamma globulin does not necessarily imply a non-antibody gamma globulin,

since gamma globulin can permeate the tissue so strongly that it cannot easily be eluted.[23]

Immunohistochemical analysis of arteries in the so-called collagen diseases have indicated the presence of complement in fibrinoid necrosis. In periarteritis nodosa, complement,[23,24] gamma globulin[25-27] and fibrinogen[25,27,28] have been detected. Gamma globulin[27] and fibrinogen[29] have also been found to be localized in scleroderma. In lupus erythematosus and rheumatic fever, however, gamma globulin[27,30-32] and complement,[24] but not fibrinogen, have been described. All plasma proteins, including complement, were found in the vascular lesions in a case of malignant nephrosclerosis which was briefly set out in a previous report.[24] In their study of three cases of malignant hypertension, Fennel, Reddy and Vazquez described a preferential localization of fibrin or fibrinogen in the arterial lesions.[29]

While it is possible that a variety of mechanisms might lead to lesions with similar plasma protein localization, the presence of complement in some vascular fibrinoid necrosis may indicate a common pathogenesis but a variable means of expression.

Several findings suggest that factors other than hypertension play a role in malignant nephrosclerosis. The arterial lesions have been noted to be: a) rare in patients over 65,[4,33,34] although the incidence of hypertension is high in this age group; b) present in only a small percentage of hypertensive patients[4,34-36] and not in all cases of malignant hypertension[4,33-36]; c) found also in patients having systolic blood pressure below 200 mm of Hg[4,34]; d) characteristically distal to the arterial narrowing area which would therefore be protected from the high pressure[4]; e) seldom found in patients with pheochromocytoma[37]; f) not infrequently associated with chronic glomerulonephritis, pyelonephritis and periarteritis nodosa,[4,34] diseases in which an immunologic component has been implicated. While fibrinoid necrosis can be produced experimentally by conspicuous elevation of the blood pressure,[38,39] high blood pressure seems to be only one of the factors involved in the mechanism of arteriolar necrosis. In animals arteriolar necrosis: a) is not seen in dogs with high blood pressure sustained for more than 5 years,[40] or up to 7 years after ligation of the moderator nerve[41]; and, b) develops after x-ray treatment which seems to "sensitize" the vessels.[42] Therefore, factors other than hypertension, such as arterial spasm,[43] anoxia, changes in blood volume, oxygen saturation, venous pressure,[44] altered metabolism,[45] infections,[46] toxic and allergic processes,[4] a combination of hypertension and humoral factors,[40] have been implicated in the pathogenesis of the arteriolar necrosis of malignant hypertension.

Recently it has been suggested that immune processes which follow

or are concomitant with the hypertension contribute to the pathogenesis of this disease process. Complement-fixing and precipitating antibodies to human vascular antigen have been described in hypertensive patients,[9] and hypertension has been produced in animals by the injection of kidney extracts in Freund's adjuvant.[10] The high incidence of malignant nephrosclerosis in Negro patients also strengthens the possibility that immune reactions are implicated in this disease, since in the Negro race alterations of the gamma globulin-forming system[47–49] and high incidence of disease with an immunologic component, such as lupus erythematosus,[50] have been described.

The immunocytochemical investigations presented suggest the following hypothesis for the pathogenesis of at least some of the vascular necrotic lesions in malignant hypertension. Arteries with minimal damage and increased permeability, produced perhaps by hypertension or by toxins of renal failure, are the site of localization or formation of immune complexes. The possibility may also be entertained that increased pressure forcefully filters antigen-antibody complexes. Complement is deposited with ensuing liberation or formation of cytotoxic substances, an increase of damage to the vessel wall, and subsequent permeation of plasma proteins.

Summary

The deposition of plasma proteins has been investigated with immunofluorescent techniques in 14 cases of malignant nephrosclerosis. Gamma globulin, complement (beta 1C globulin), fibrinogen and albumin were identified in the arteriolar fibrinoid necrosis and in the glomerular lesions of 11 of the 14 cases. Occasionally only gamma globulin and complement were found, sometimes only fibrinogen was present. The hyperplastic proliferation of the intima of intraparenchymal renal arteries were devoid of the plasma proteins investigated. Complement and gamma globulin were not identified in the kidneys in cases of long-standing hypertension or in those with renal cortical necrosis.

The hypothesis is presented that one of the mechanisms of tissue damage in malignant nephrosclerosis is the deposition or formation of immune complexes in an area of minimal alteration, with subsequent fixation of complement, ensuing damage and increased permeability of all plasma proteins.

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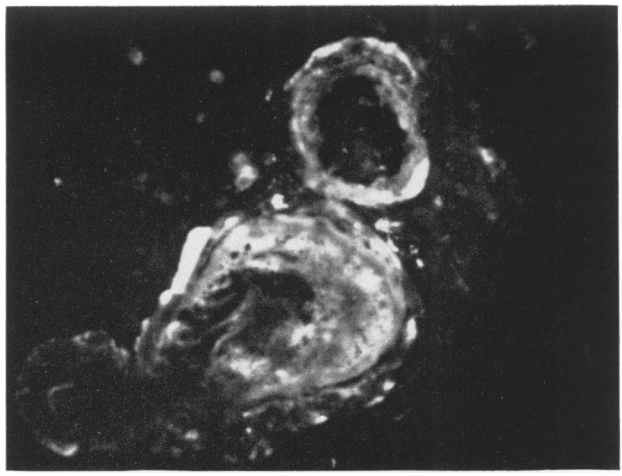
LEGENDS FOR FIGURES

FIG. 1. Kidney in a patient with malignant nephrosclerosis. Cryostat section treated with fluorescein-labelled antihuman gamma globulin. Two arteries contain gamma globulin. In the larger one it is localized in part of the vessel, mainly in the media and adventitia. × 250.

FIG. 2. Section adjacent to that shown in Figure 1 showing fibrinogen diffusely staining the wall of an artery. Fluoresceinated anti-fibrinogen antiserum. × 250.

FIG. 3. Section adjacent to that shown in Figure 2. One of the 3 arterioles contains fluorescein-labeled antihuman complement. × 250.



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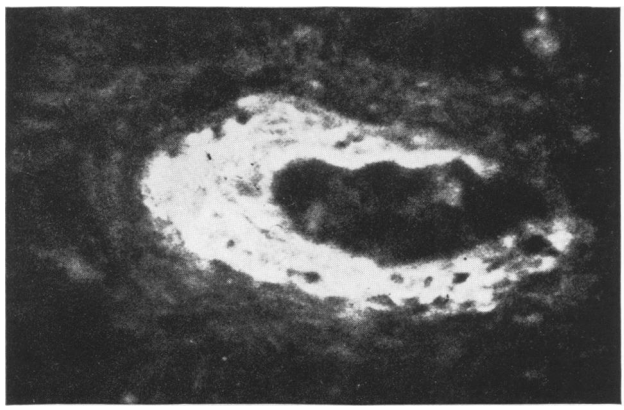

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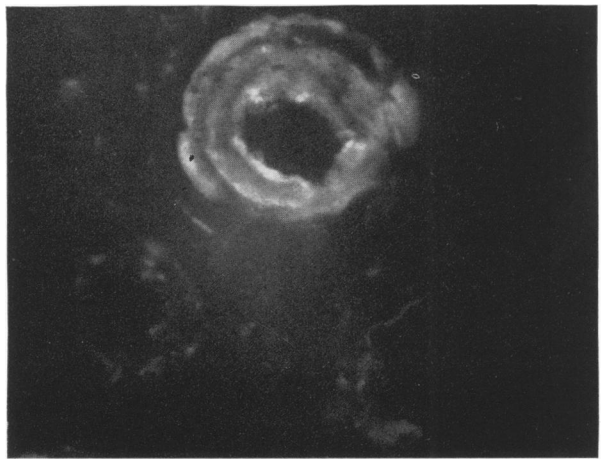

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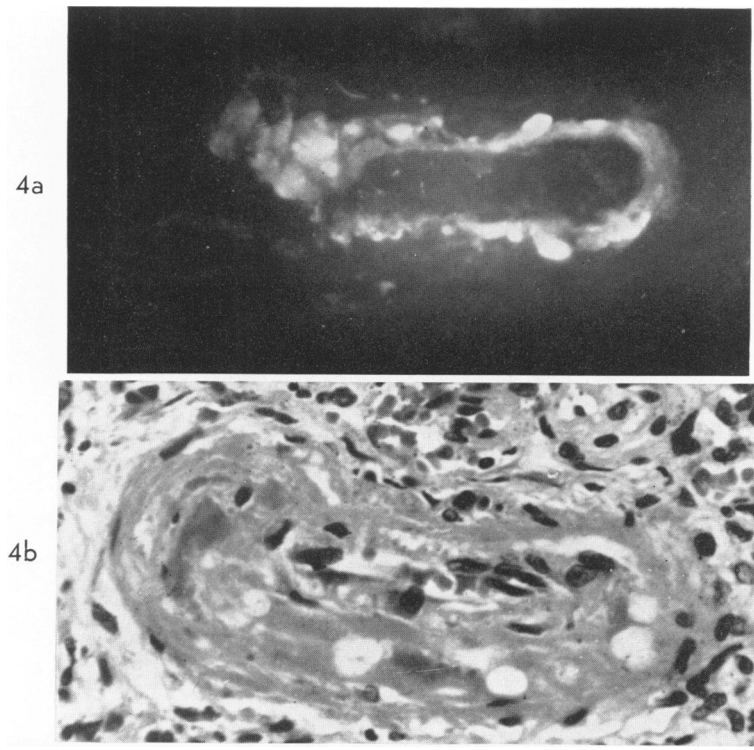


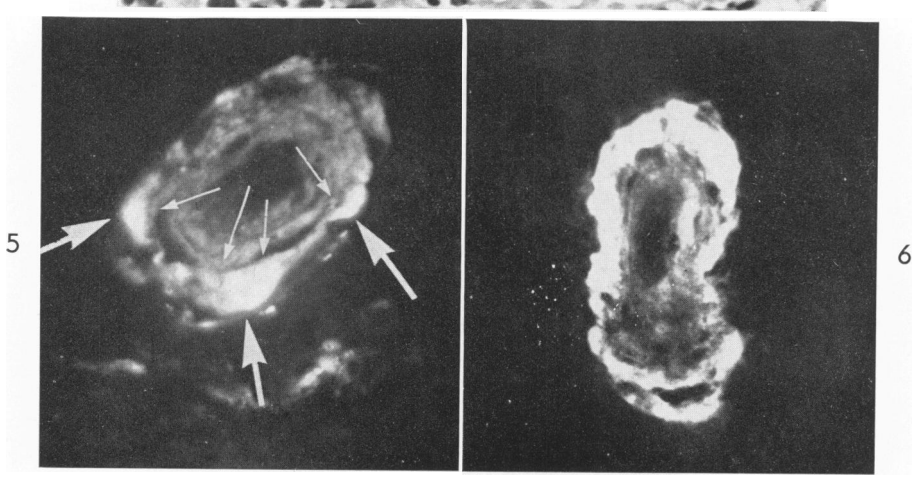


FIG. 4a. A section treated as that shown in Figure 3. Complement is present in aggregates and droplets in the wall of an artery with eosinophilic fibrinoid necrosis shown in Figure 4b (stained with hematoxylin and eosin). × 250.

FIG. 5. A section, adjacent to that shown in Figure 3, was stained with rhodamine-labeled antifibrinogen and fluorescein-labeled antihuman complement. Orange-green staining appeared in the intima and media while some areas in the media and adventitia (between arrows) were green. × 250.

FIG. 6. The same section shown in Figure 5. This artery stained green only, indicating the presence of complement alone. × 250.

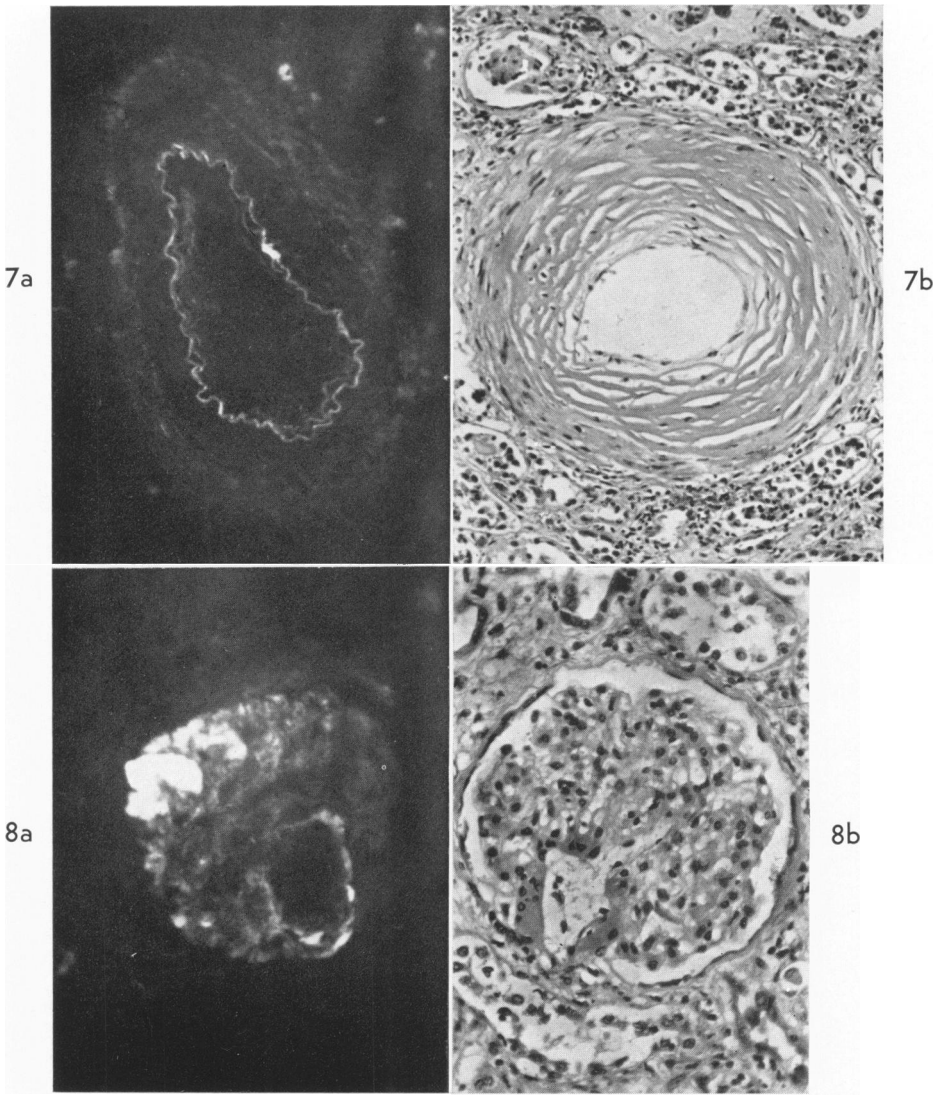


FIG. 7a. Kidney in a patient with malignant nephrosclerosis. Section treated with fluorescein-labeled antihuman fibrinogen antiserum. No fibrinogen is seen. The wavy line indicates the autofluorescent elastica. × 100.

FIG. 7b. A section adjacent to that shown in 7a. Intimal hyperplastic proliferation is evident. Hematoxylin and eosin stain. × 100.

FIG. 8a. A glomerulus in a patient with malignant nephrosclerosis. Section stained by fluorescein-labeled antihuman gamma globulin antiserum. Gamma globulin is localized in the fibrinoid necrosis in the afferent arteriole in the root of the glomerulus and in the glomerular tuft. × 250.

FIG. 8b. The same glomerulus shown in Figure 8a. Hematoxylin and eosin stain. × 250.

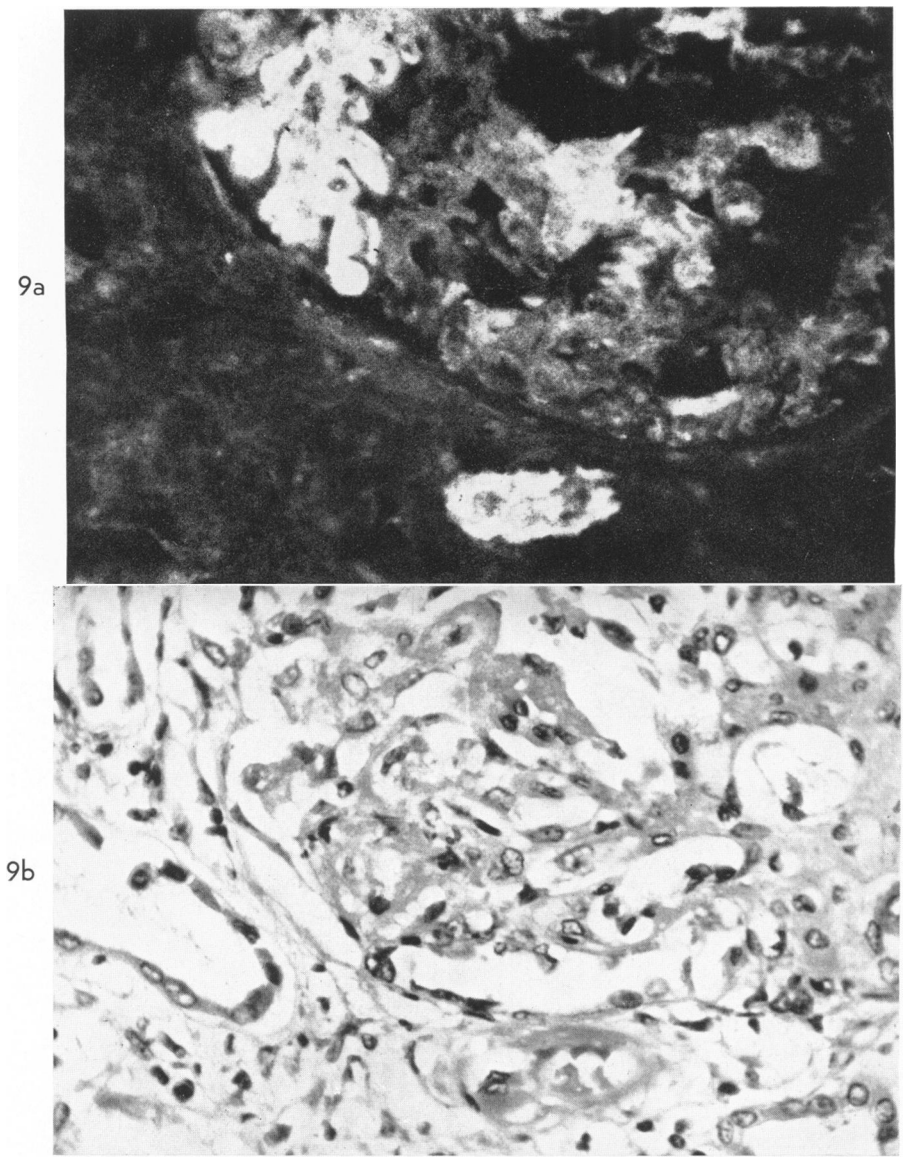
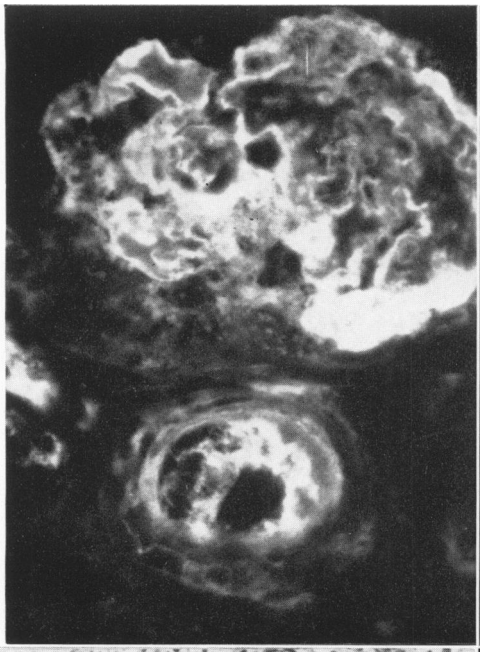


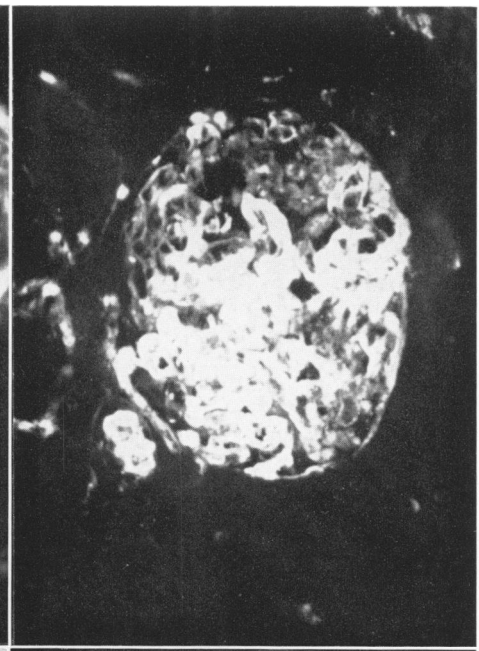
FIG. 9a. A section adjacent to that shown in Figures 8, stained with antihuman complement. Complement is localized in an arteriole and in part of the glomerulus. × 400.

FIG. 9b. Same section shown in Figure 9a stained with hematoxylin and eosin. Even after some distortion caused by processing, necrosis of the arteriole and of the glomerular tuft is apparent. × 400.

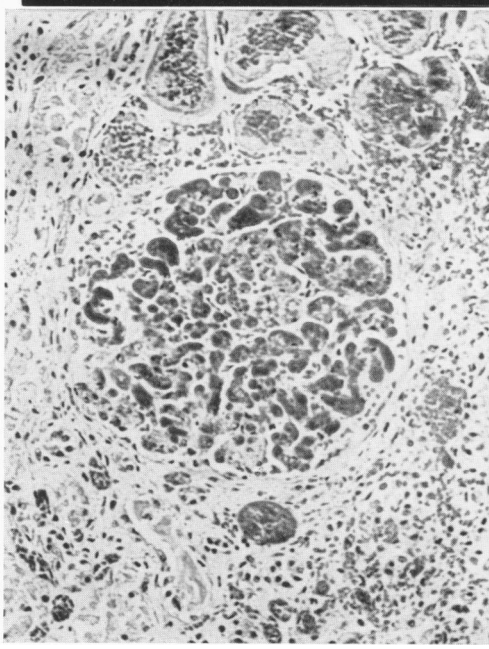
FIG. 10. A section adjacent to that shown in Figure 9. Fibrinogen appears in an afferent arteriole in the glomerulus along the basement membranes and in the intercapillary space. Fluorescein-labeled antifibrinogen antiserum. × 250.



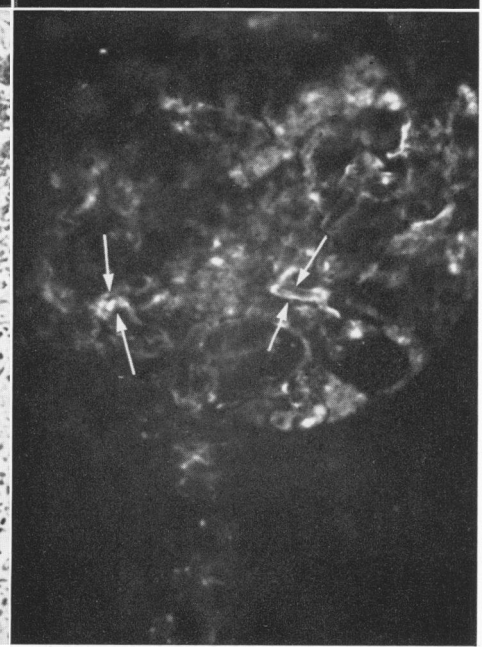
10



11a



11b



12

FIG. 11a. A section parallel to that shown in Figure 10. Fibrinogen may be seen in a small arteriole and is diffusely distributed in the glomerulus in areas of hemorrhagic necrosis. × 250.

FIG. 11b. A section adjacent to that shown in Figure 11a, stained with hematoxylin and eosin. × 250.

FIG. 12. A section adjacent to that shown in Figures 8 stained with rhodamine-labeled antihuman fibrinogen and fluorescein-labeled antihuman complement. The glomerulus was orange-green with some areas (arrows) more distinctly green. × 250.