ANALYTICAL PATHOLOGY

II. Histopathologic Demonstration of Glomerular-Localizing Antibodies in Experimental Glomerulonephritis *

Robert C. Mellors, M.D.; Javier Arias-Stella, M.D.†; Malcolm Siegel, Ph.D., and David Pressman, Ph.D.‡

From the Divisions of Pathology (Drs. Mellors and Arias-Stella) and Experimental Pathology (Drs. Siegel and Pressman), Sloan-Kettering Institute for Cancer Research; the Pathology Laboratories (Drs. Mellors and Arias-Stella), Memorial Center for Cancer and Allied Diseases; and the Sloan-Kettering Division, Cornell University Medical College (Drs. Mellors and Pressman), New York, N.Y.

In a previous communication,[1] a method—in reality a new application of the technique of Coons and Kaplan[2] for the detection of antigens—was described for the histologic demonstration of the antigenic components of tissues and the sites of *in vivo* localization of antibodies. Aside from showing that the glomeruli and particularly their basement membranes were the principal renal antigens in the production of nephrotoxic serum, the method disclosed that antibodies localize in glomeruli during the acute stage of experimental glomerulonephritis produced in rabbits by the injection of purified foreign proteins, namely, bovine gamma globulins. The main purpose of the present paper is to describe in fuller detail the experimental conditions and observations pertaining to the rôle of glomerular-localizing antibodies in the pathogenesis of experimental glomerulonephritis. In addition, studies were made of three other histopathologic manifestations of allergy and hypersensitivity, namely, myocarditis, endocarditis, and angiitis. Since it is necessary to document the anatomical changes observed, a histopathologic description of the lesions occurring in the experimental animals is presented.

Materials and Methods

Experimental Procedure

Young, male, white rabbits with an average weight of 2,400 gm. were used. Fifteen animals received intravenous injections of bovine gamma globulins, while 4 served as normal control animals.

* This investigation was supported in part by research grant C-1345 from the National Cancer Institute of the National Institutes of Health, Public Health Service; by a grant from the Damon Runyon Memorial Fund; and by the United States Atomic Energy Commission under their contract A T (30-1) — 910.

Received for publication, November 5, 1954.

Presented at the Fifty-second Annual Meeting of the American Association of Pathologists and Bacteriologists, Houston, Texas, April 8, 1955.

† Kellogg Foundation Fellow in Pathology. Now at Faculty of Medicine, Lima, Peru, South America.

‡ Now at Roswell Park Memorial Institute, Buffalo, N.Y.

The bovine gamma globulins (Armour & Company, Fraction II) were neutralized, made up in physiologic sodium chloride solution, and sterilized, as proved with bacteriologic cultures, by pressure-filtration through a Seitz filter. The final concentration of protein was 8 gm. per 100 ml.; and this solution was given intravenously in a marginal ear vein at a dose of 10 ml. per kg. of body weight, administered once a week. As shown in Table I, 7 animals received one injection while the remainder received two to three injections. A small desensitizing dose (about 1 ml. per kg. of 1 per cent protein solution) was given intravenously about 6 to 12 hours prior to the second and third injections in order to prevent anaphylactic shock (which nevertheless occurred in rabbit S). In addition to receiving bovine gamma globulins, 4 animals (Table I) were subjected to unilateral nephrectomy with the intent of increasing the frequency or severity of the renal lesions.[3]

TABLE I

Number of Intravenous Doses of Bovine Gamma Globulins (BGG) and Other Treatment Received by Rabbits

Animals	Number of doses of BGG*	Other treatment
B, C, E, F, G	1	
I, J	1	Unilateral nephrectomy
A, H, S	2	
R, T	2½	
L	2½	Unilateral nephrectomy
Q	3	
N	3	Unilateral nephrectomy

* Each dose consisted of the intravenous injection of 10 ml. per kg. of body weight of 8 per cent bovine gamma globulins made up in sterile isotonic saline solution.

Animals were weighed daily, and they displayed in some cases a terminal weight loss. Twenty-four hour urine collections were made by placing the animals in metabolism cages on one or more occasions weekly. The urine was examined microscopically; and the protein concentration was estimated by the Kingsbury-Clark method.[4] When the quantity of protein was 20 mg. per 100 ml. of urine, or greater, on more than one occasion—and the finding was frequently verified by analysis of the bladder urine at necropsy—the result was recorded as positive for proteinuria.

The animals were sacrificed on or about the seventh day after the last injection of protein. They were anesthetized with sodium phenobarbital, given heparin to prevent blood coagulation, and perfused with isotonic saline solution under a hydrostatic pressure of 2.5 m. until the

abdominal and the thoracic viscera were grossly cleared of blood. The perfusion fluid entered the abdominal aorta in a cephalic direction and passed through the transected inferior vena cava. Bladder urine was taken for protein and microscopic analysis and on occasion for bacteriologic culture. The kidneys, liver, spleen, heart, and lungs were weighed (Tables II and III) and representative blocks were cut for

TABLE II

Weights of Perfused Organs in Normal Control Rabbits

Body weight	Organ weights as per cent of body weight				
	Kidney	Heart	Lung	Spleen	Liver
AV 2420	0.45	0.36	0.25	0.076	3.8
CI 2210-2630	0.39–0.51	0.31–0.41	0.19–0.31	0.046–0.106	2.4–5.2

AV = average; CI = confidence interval that statistically includes 99 per cent of the estimates of the average.

TABLE III

Weights of Perfused Organs in Rabbits Receiving Injections of Bovine Gamma Globulins

Animal	Body weight	Organ weights as per cent of body weight						
		Rt. kidney	Lt. kidney	Heart	Rt. lung	Lt. lung	Spleen	Liver
	gm.							
A	2690	0.36	0.38	0.28	0.23	0.17	0.074	4.1
B	2440	0.50	0.49	0.31	0.27	0.20	0.074	4.8
C	2100	0.49	0.53	0.45	0.26	0.20	0.11	4.5
E	2300	0.38	0.37	0.28	0.28	0.22	0.096	4.8
F	2640	0.44	0.49	0.38	0.23	0.20	0.076	5.7
G	3630	0.38	0.38	0.34	0.41	0.27	0.099	4.8
H	2440	0.42	0.52	0.37	0.27	0.19	0.11	3.8
I	1790	0.56	0.39*	0.42	0.25	0.18	0.10	5.3
J	2080	0.52	0.48*	0.41	0.26	0.19	0.13	3.8
L	2320	0.66	0.41*	0.50	0.21	0.18	0.13	2.8
N	1980	0.82	0.33*	0.41	0.45	0.31	0.14	6.3
Q	2830	0.43	0.51	0.25	0.27	0.18	0.10	4.8
R	2810	0.51	0.58	0.34	0.56	0.40	0.06	4.7
S	2270	0.50	0.52	0.31	0.34	0.28	0.18	4.5
T	2040	0.56	0.45	0.29	0.25	0.17	0.12	3.5

* Unperfused, normal kidney obtained by nephrectomy before injections of bovine gamma globulins were begun.

use in the following manner. Unfixed tissue for the study of antibody localization was frozen immediately on dry ice and stored at −10° C. Frozen sections, 3 to 5 μ thick, were subsequently prepared from this material by the method of Bush and Hewitt[5] and treated with the

fluorochroming procedure to be described. Blocks for routine histologic observation were fixed in 10 per cent formalin at pH 7.0. Paraffin sections were stained with Harris' hematoxylin and picric acid eosin and the periodic acid-Schiff reagent. When indicated, special stains such as scharlach R, Weigert's resorcin-fuchsin, Mallory's anilin blue, and colloidal iron[6] were used.

Demonstration of Localizing Antibodies

The method has been described in full elsewhere[1]; and similar procedures were used for the preparation of the globulin fraction from chicken antiserum to rabbit globulins, designated herein as Chi-anti-Rab, and for the labelling or coupling of this fraction with fluorescein isocyanate to obtain the fluorochroming reagent, designated herein as the fluor. When an unfixed, thin frozen section of rabbit tissue is washed with pH 8 isotonic borate buffer for 1 minute, incubated with a few drops of the fluor for 1 hour at room temperature, washed with borate buffer, rinsed and mounted in buffered glycerin, and examined with a fluorescence microscope, rabbit antibodies that are localized in the tissue become self-luminous because of their combination with the fluor. The immunochemical specificity of the fluorochroming procedure is verified[2] by demonstrating that either a decreased intensity or a total absence of fluorescence occurs when the tissue section is incubated with uncoupled Chi-anti-Rab prior to treatment with the fluor. All observations pertaining to antibody localization in the present study were verified by this demonstration. The selectivity of the procedure was enhanced also[2] by treatment of the fluorochroming reagent with lyophilized tissue protein. In some of the present work, the washed and sedimented fraction of homogenized rabbit liver was used for this purpose.

The method and the conditions of photographic photometry which permit a translation of the photographic density into the relative fluorescence intensity of histologic details are similar to those previously used.[1]

Results

Histopathologic Findings

The significant microscopic findings in the organs of rabbits receiving intravenous injections of bovine gamma globulins are given in Table IV. The classification and the incidence of lesions in 15 treated rabbits were as follows: glomerulonephritis, 12; myocarditis, 8; endocarditis, 3; interstitial pneumonia, 5; allergic angiitis, 4; lymphoid hyperplasia of the spleen, 15; allergic granuloma of the spleen, 2; and

parasites of the liver, 6. A brief description of the gross and the microscopic anatomy of the kidneys, heart, lungs, spleen, and liver in these animals follows.

TABLE IV

Incidence of Histopathologic Lesions and Alterations in Organs of Rabbits Receiving Bovine Gamma Globulins

Animal	Glomerulonephritis	Myocarditis	Endocarditis	Interstitial pneumonia	Angiitis	Lymphoid hyperplasia, spleen	Granuloma, spleen	Parasites liver
A	+	+	+	+		+		
B	+					+		
C	+	+	+			+		+
E	+					+	+	+
F	+	+				+		+
G	+		+	+	+	+	+	
H		+		+	+	+		
I						+		
J	+					+		
L	+	+				+		
N	+	+				+		
Q	+	+		+	+	+		+
R	+					+		+
S	+	+		+	+	+		
T						+		+
Incidence	12/15	8/15	3/15	5/15	4/15	15/15	2/15	6/15

The evidence for, and the incidence of, renal injury are given in Table V. Proteinuria occurred in 5 of 15 treated animals and by the criteria used was not present in the normal controls. Gross enlargement together with punctate hemorrhages in the superficial cortex occurred in the kidneys of one animal (R). Hypertrophy of the remaining kidney occurred in all animals having unilateral nephrectomy (data in Table III compared with those in Table II); and the average weight of the kidney (the sum of both divided by two) was increased in 2 nephrectomized animals (L and N). Microscopic findings of glomerular injury were present in 12 animals. The most frequent and diffuse lesion occurred in the glomerular capillaries and appertaining structures. The glomerular capillary change comprised three alterations which singly or collectively produced a partial or complete occlusion of the lumina of the capillary loops: (a) swelling or enlargement (and increased staining) of the cytoplasm of cells, a change which occurred principally in endothelial cells and to a lesser extent in epi-

thelial and mesenchymal cells; (b) widening of the intercapillary spaces and the presence therein of homogeneous and occasionally granular acidophilic material; and (c) alterations of the basement membranes consisting of thickening and the deposition of hyaline or fibrillar acidophilic material, a change which was more prominent in focal areas of the capillary tufts.

Cellular proliferation, mainly of endothelial origin, was the next most frequent evidence of glomerular injury. The lesion was focal in some

TABLE V

Evidence of Renal Injury in Rabbits Receiving Bovine Gamma Globulins

Animals	Proteinuria	Renal enlargement	Renal petechiae	Glomerulitis					Tubular casts
				Capillary change	Cellular proliferation	Cellular exudation	Focal necrosis	Crescent formation	
A				+					+
B				+	+			+	+
C				+					
E				+	+	+			+
F				+	+				+
G				+	+				
H									
I									
J				+					+
L	+	(+)		+	+				
N	+	(+)		+				+	+
Q	+			+	+		+	+	+
R	+	+	+	+	+			+	+
S	+			+	+				
T									

kidneys and diffuse in others, and in many glomeruli the proliferative change was confined principally or entirely to segmental or lobular portions of the capillary tufts. The endothelial proliferation completely occluded the capillary lumina in focal areas of some glomeruli. The increased number of polymorphonuclear leukocytes in the glomerular capillaries was noted in many cases and was clearly excessive in one animal (E). Foci of necrosis of glomeruli were present in one animal (Q), and the lesions were composed of fragmented nuclei of inflammatory and parenchymatous cells superimposed on hyalinized or structureless capillary walls. Crescent formation and adhesion between the capillary tuft and the glomerular capsule were present in 4 animals.

Protein casts in the glomerular space and in the convoluted tubules were of frequent occurrence, and red blood cells in various stages of degeneration were present in these locations on rare occasion. The walls of renal cortical arterioles and venules were thickened and hyalinized in several animals.

Interstitial pneumonia was present in 5 animals. The lesions were unevenly distributed within the lobe and among the lobes, were associated with allergic angiitis in all but one case, and were composed of large collections of mononuclear cells, including lymphocytes, monocytes, and plasma cells, together with a few neutrophils and eosinophils. The inflammatory exudate had an alveolar, septal, perivascular, and peribronchial distribution. Angiitis, when most extensive, was present in the arteries, arterioles, capillaries, venules, and veins of the lung and consisted of endarteritis, endophlebitis, and panvasculitis. The inflammatory exudate, which included mononuclear cells and serous fluid together with small numbers of neutrophils and eosinophils, was most prominent beneath the endothelium and in some cases was present in all coats of the vascular wall. In some blood vessels there was proliferation of endothelial lining cells with partial or total occlusion of the lumina. The cellular exudate of the interstitial pneumonitis appeared in some instances to arise by extension from the vascular inflammation.

Focal interstitial myocarditis was present in 8 of 15 treated animals. The lesions were composed of microscopic, nodular collections of mononuclear cells, principally Anitschkow myocytes but also lymphocytes and plasma cells, which had a predilection for distribution in the perivascular connective tissue of the myocardium. Necrosis of connective tissue as well as of muscle fibers frequently was present in the centers of the lesions.

Endocarditis, including mitral valvulitis, was present in 3 animals. The valvular lesions consisted of foci of edema and fibrinoid necrosis accompanied by exudation of mononuclear and polymorphonuclear cells and proliferation of cardiac histiocytes.

Hyperplasia of the lymphoid follicles of the spleen occurred in all of the treated animals. In two cases granulomas composed of nodular collections of epithelioid cells together with giant cells were present in the follicles of the spleen.

Coccidia were present in the livers, this being the only abnormal finding that was common to both the treated and the control animals.

Many of the histopathologic lesions are illustrated in Figures 1 to 17.

Localizing Antibodies

Representative photomicrographs of thin, unfixed, frozen sections of rabbit kidneys which were treated with the fluor (fluorescein-labelled Chi-anti-Rab) to demonstrate the localization of antibodies by fluorescence are shown in Figues 18 to 30; and the quantitative data are summarized in Table VI.

TABLE VI

Glomerular: Tubular Ratio of Fluorescence Intensity in Sections of Rabbit Kidneys Treated with the Fluorochroming Procedure for Demonstration of Localizing Antibodies

Group of animals	No. of animals	Glomerulitis	Ratio of fluorescence intensity		
			AV	CI	N
Control	2	Not present	1.4	1.3–1.5	52
Control+	6	Not present	1.4	1.3–1.5	94
Experimental	3	Not present	1.6	1.4–1.8	48
Experimental	3	Capillary change only	1.4	1.3–1.5	45
		Capillary change plus:			
Experimental	4	(a) cellular proliferation	2.0	1.9–2.2	69
Experimental	5	(b) proliferation, crescents, etc.	2.1*	2.0–2.3	101

AV = average.
CI = confidence interval which includes 99 per cent of the estimates of the average.
N = number of measurements.
Control = normal animals, perfused.
Control + = perfused and unperfused.
Experimental = injected with bovine gamma globulins.
* When the fluorochroming procedure is *blocked* by pretreatment of sections with Chi-anti-Rab, the values are: AV, 1.3; CI, 1.2-1.5, and N, 33, respectively.

The fluorescence in a section of normal kidney obtained from a control rabbit is due principally to non-specific combination between the histologic structures and the fluor. The fluorescence intensity of the glomerulus is slightly greater than that of many of the tubules, as judged by visual estimation (Figs. 18 and 19); and the glomerular: tubular ratio of fluorescence intensity, as measured photometrically, is 1.4 (Table VI).

In a kidney of a rabbit having glomerulitis with cellular proliferation, the fluorescence intensity of the glomeruli is much greater than that of the tubules (Figs. 20 to 23); and the analytical glomerular: tubular ratio of fluorescence intensity is 2.0 (Table VI). This is a statistically significant increase in the ratio relative to that for the normal control kidney, because, as shown in the fifth column of Table VI, the confidence intervals (lines 1 or 2 and 5) do not overlap. That the increased fluorescence intensity displayed in proliferative glomerulitis is due to the presence of glomerular-localizing antibodies is shown

as follows. If a section of kidney from a rabbit having proliferative glomerulitis is first treated with unlabelled globulin (Chi-anti-Rab) and then treated with the fluor, the fluorescence intensity of the glomerulus is then only slightly greater than that of the tubules (Figs. 24 and 25). The quantitative data (Table VI, last line and footnote) indicate that the glomerular:tubular ratio of fluorescence intensity is now 1.3, that is, essentially the same as the ratio in the control kidney (Table VI, lines 1 and 2). The explanation is that once the glomerular-localizing rabbit antibodies have combined with the unlabelled globulin, their further combination with the labelled globulin (the fluor) is prevented. The fluorescence which can be specifically blocked in this manner is due, therefore, to the presence of localizing antibodies which are concentrated in the glomerulus.

The glomerular:tubular ratio of fluorescence intensity is approximately 2.0 to 2.1 (Table VI, lines 5 and 6) in kidneys in which glomerulitis is accompanied by cellular proliferation (Figs. 20 to 23, and 28), cellular exudation (Fig. 27), or crescent formation (Fig. 26). However, if the glomerulitis consists only of those alterations in the glomerulus which were described as capillary changes in the discussion of histopathology, the glomerular:tubular ratio of fluorescence intensity is 1.4 (Table VI, line 4, and Fig. 29), which is indistinguishable from that in the normal control kidney.

If a rabbit, although receiving injections of foreign proteins, does not develop glomerulitis, the glomerular:tubular ratio of fluorescence intensity is 1.6 (Table VI, line 2, and Fig. 30), a value which is not statistically different from that in the normal control kidney.

In summary, antibodies, as demonstrated by the method of microfluorescence, are localized in the glomeruli of the kidneys of rabbits having glomerulitis of the proliferative, exudative, or crescentic type and induced by the injection of foreign proteins.

Preliminary studies have been made on other organs and tissues obtained from the animals in these experiments. The information at hand suggests that antibodies are localized in (a) the endothelium and subendothelium of pulmonary blood vessels that are the site of angiitis; (b) the endocardium and myocardium in carditis; and (c) the cytoplasm of plasma cells in the spleen of both normal and experimental animals. These findings will be the subject of a future report.

Discussion

The incidence, distribution, and types of glomerulitis and glomerulonephritis, as well as the associated allergic lesions, observed in the present work are similar to those described under comparable condi-

tions by other investigators (Table VII), notably, Rich and Gregory,[7] Hawn and Janeway,[8] Ehrich *et al.*,[9] More and Waugh,[3] Wissler *et al.*,[10] McLean *et al.*,[11] Hamilton-Paterson and Henderson,[12] Germuth,[13] and Hamilton and Fremes.[14]

The bearing that the present study has on the pathogenesis of glomerulonephritis must be viewed in historical perspective. The belief that human glomerulonephritis is of allergic origin has been expressed by clinicians, among them von Pirquet and Schick[15] and Volhard,[16] during the last half century. Fishberg,[17] in 1939, summarized the evidence in favor of the view that immuno-allergic mechanisms were in some way connected with the development of glomerulonephritis. The findings cited include: (a) the fact that glomerulonephritis complicating scarlatina or tonsillitis occurs during convalescence rather than at the height of the infection; (b) the not uncommon association of clinical glomerulonephritis with serum sickness; (c) the increased skin reactivity to filtrates of cultures of hemolytic streptococci shown by patients with glomerulonephritis; (d) an elevated titer of antibodies, including antistreptolysin, in the blood of patients with glomerulonephritis following scarlatina or pharyngitis; (e) the more frequent occurrence of glomerulonephritis in subacute bacterial endocarditis during the bacteria-free rather than the bacteremic stage of the disease; and (f) evidence from animal experiments which will be discussed.

Masugi,[18] in work reported during 1928 to 1934, produced experimental glomerulonephritis in rabbits by the injection of a specific antikidney serum. The clinical, chemical, and anatomical findings in the experimental animals corresponded closely to those in human diffuse glomerulonephritis; and the renal disease once established continued its progress and eventually produced death by renal insufficiency. Our recent findings with the microfluorescence method reported elsewhere,[1] together with the earlier work of others,[19-26] indicate that the glomeruli, and particularly their basement membranes, are the principal antigenic components that promote the formation of, and undergo combination with, the antibodies in antikidney serum.

Many investigators, since the early studies of Longcope,[27] have attempted to produce glomerulonephritis experimentally by sensitizing animals to foreign proteins, bacteria, and bacterial products. Some of this work has already been summarized in Table VII. Allen[28] stated that, of all the experimental procedures used, those employing injections of gamma globulins or antikidney antibodies produce histologic lesions which most closely simulate those of the spontaneous disease of human beings. To the foregoing evidence, all of which suggests an

TABLE VII

Glomerulitis, Glomerulonephritis, and Other Allergic Lesions Produced in Rabbits by Injections of Foreign Proteins

Reference	Material injected	Glomerulitis or glomerulonephritis			Other allergic lesions
		Incidence	Distribution	Principal lesion	
Rich & Gregory[7]	Horse serum	10/14	Diffuse	Proliferative	Periarteritis
Hawn & Janeway[8]	Bovine gamma globulin	11/19	Diffuse?	Proliferative and exudative	Carditis, arthritis, and arteritis
Ehrich *et al.*[9]	Horse serum	45/56	Diffuse	Proliferative, exudative, and necrotizing	Carditis, arteritis, and granulomas of spleen
More & Waugh[3]	Bovine gamma globulin	14/18	Diffuse	Proliferative	Carditis
Wissler *et al.*[10]	Horse serum	7/46	Focal	Necrotizing	Carditis and arteritis
McLean *et al.*[11]	Horse serum	20/31	Diffuse	Proliferative	None
Hamilton-Paterson & Henderson[12]	Horse serum	10/31	Diffuse	Membranous and proliferative	Periarteritis
Germuth[13]	Bovine albumin	28/34	Diffuse	Membranous and proliferative	Carditis and granulomas of spleen
Hamilton and Fremes[14]	Horse serum	28/51	Diffuse	Membranous and proliferative	Arteritis and carditis

underlying allergic pathogenesis of glomerulonephritis, is to be added the finding, first reported by Veil and Buchholz[29] and extended in the observations of others,[30,31] that the titer of serum complement is decreased in patients or animals with acute glomerulonephritis. This finding may have explanation in the work of Germuth,[18] who has shown in animals with induced hypersensitivity that the tissue lesions, among them glomerulonephritis, occur during the time when antigen is removed from the circulation following combination with circulating antibody.

The present study is the first to demonstrate that gamma globulin (presumably rabbit antibody to injected protein) is localized in the glomeruli in acute glomerulonephritis in the experimental animal. This localization of antibody, which is presumably due to a previous fixation in the glomeruli of some of the injected antigen, is an essential requisite for the allergic pathogenesis of glomerulonephritis. The analytical method used in this work should permit the study of the pathogenesis of other diseases, among them rheumatic fever and other so-called collagen diseases, rheumatoid arthritis, and certain types of purpura and encephalomyelitis. Gamma globulins have now been detected in the glomerular lesions in lipid nephrosis, acute glomerulonephritis, and secondary amyloidosis (Mellors and Ortega[32]).

Summary and Conclusions

The main purpose of this investigation was to study the rôle of tissue-localizing antibodies in the pathogenesis of experimental glomerulonephritis. The unique feature of the study was the histopathologic application of a microfluorescence method for demonstrating the histologic sites of localization of antibodies *in vivo*.

Of 15 rabbits receiving one or more intravenous injections of purified foreign proteins (bovine gamma globulins), 12 developed glomerulonephritis; 8, myocarditis; 3, endocarditis; 5, interstitial pneumonia; 4, allergic angiitis; 15, lymphoid hyperplasia of the spleen, and 2, allergic granulomas of the spleen.

The evidence of renal injury in 12 animals consisted variously of proteinuria, renal enlargement, renal petechiae, tubular casts, and glomerulitis with capillary change, cellular proliferation, cellular exudation, focal necrosis, and crescent formation.

Antibodies were localized in the glomeruli of the kidneys of rabbits having acute glomerulonephritis of the proliferative, exudative, or crescentic type. Such localization, not heretofore demonstrable, is clearly an essential requisite for the allergic pathogenesis of glomerulonephritis.

Preliminary studies of other organs in these sensitized animals suggested that antibodies were localized in the blood vessels in angiitis and in the heart in endocarditis and myocarditis.

The analytical method used should permit the study, already in progress, of the rôle of antigen-antibody interactions in the pathogenesis of a number of human diseases, among them, glomerulonephritis, rheumatic fever, disseminated lupus erythematosus, rheumatoid arthritis, thrombotic thrombocytopenic purpura, and certain types of encephalomyelitis.

We wish to thank Mr. John Hlinka and Miss Betty Corcoran for assistance.

REFERENCES

1. Mellors, R. C.; Siegel, M., and Pressman, D. Analytic pathology. I. Histochemical demonstration of antibody localization in tissues, with special reference to the antigenic components of kidney and lung. *Lab. Investigation,* 1955, **4**, 69–89.
2. Coons, A. H., and Kaplan, M. H. Localization of antigen in tissue cells. II. Improvements in a method for the detection of antigen by means of fluorescent antibody. *J. Exper. Med.,* 1950, **91**, 1–13.
3. More, R. H., and Waugh, D. Diffuse glomerulonephritis produced in rabbits by massive injections of bovine serum gamma globulin. *J. Exper. Med.,* 1949, **89**, 541–554.
4. Kolmer, J. A.; Spaulding, E. H., and Robinson, H. W. Approved Laboratory Technic. Appleton-Century-Crofts, Inc., New York, 1951, ed. 5, p. 142.
5. Bush, V., and Hewitt, R. E. Frozen sectioning. A new and rapid method. *Am. J. Path.,* 1952, **28**, 863–873.
6. Rinehart, J. F., and Abul-Haj, S. K. An improved method for histologic demonstration of acid mucopolysaccharides in tissues. *A. M. A. Arch. Path.,* 1951, **52**, 189–194.
7. Rich, A. R., and Gregory, J. E. The experimental demonstration that periarteritis nodosa is a manifestation of hypersensitivity. *Bull. Johns Hopkins Hosp.,* 1943, **72**, 65–88.
8. Hawn, C. V. Z., and Janeway, C. A. Histological and serological sequences in experimental hypersensitivity. *J. Exper. Med.,* 1947, **85**, 571–590.
9. Ehrich, W. E.; Forman, C. W., and Seifer, J. Diffuse glomerulonephritis and lipid nephrosis. Correlation of clinical, morphological, and experimental observations. *A. M. A. Arch. Path.,* 1952, **54**, 463–503.
10. Wissler, R. W.; Smull, K., and Lesh, J. B. The effects of various horse serum fractions in producing cardiovascular and renal lesions in rabbits. *J. Exper. Med.,* 1949, **90**, 577–594.
11. McLean, C. R.; Fitzgerald, J. D. L.; Younghusband, O. Z., and Hamilton, J. D. Diffuse glomerulonephritis induced in rabbits by small intravenous injections of horse serum. *A. M. A. Arch. Path.,* 1951, **51**, 1–11.
12. Hamilton-Paterson, J. L., and Henderson, W. G. Serum nephritis in rabbits. *Brit. J. Exper. Path.,* 1952, **33**, 67–75.
13. Germuth, F. G., Jr. A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. *J. Exper. Med.,* 1953, **97**, 257–282.

14. Hamilton, J. D., and Fremes, N. E. The natural history of experimental glomerulonephritis produced by foreign protein. *Am. J. Path.*, 1954, **30**, 127–145.

15. von Pirquet, C., and Schick, B. Die Serumkrankheiten. Franz Deuticke, Leipzig and Wien, 1905, pp. 60–68.

16. Volhard, F. Die doppelseitigen hämatogenen Nierenerkrankungen (Bright'sche Krankheit). Julius Springer, Berlin, 1918, pp. 358–472.

17. Fishberg, A. M. Hypertension and Nephritis. Lea & Febiger, Philadelphia, 1939, ed. 4, p. 448.

18. Masugi, M. Über die experimentelle Glomerulonephritis durch das spezifische Antinierenserum. Ein Beitrag zur Pathogenese der diffusen Glomerulonephritis. *Beitr. z. path. Anat. u. z. allg. Path.*, 1933–34, **92**, 429–466.

19. Greenspon, S. A., and Krakower, C. A. Direct evidence for the antigenicity of the glomeruli in the production of nephrotoxic serums. *Arch. Path.*, 1950, **49**, 291–297.

20. Heymann, W., and Lund, H. Z. Lipemic nephrosis in rats. *Science*, 1948, **108**, 448–449.

21. Krakower, C. A., and Greenspon, S. A. Localization of the nephrotoxic antigen within the isolated renal glomerulus. *A. M. A. Arch. Path.*, 1951, **51**, 629–639.

22. Pressman, D.; Eisen, H. N., and Fitzgerald, P. J. The zone of localization of antibodies. VI. The rate of localization of anti-mouse-kidney serum. *J. Immunol.*, 1950, **64**, 281–287.

23. Pressman, D.; Hill, R. F., and Foote, F. W. The zone of localization of anti-mouse-kidney serum as determined by radioautographs. *Science*, 1949, **109**, 65–66.

24. Pressman, D., and Keighley, G. The zone of activity of antibodies as determined by the use of radioactive tracers; the zone of activity of nephritoxic antikidney serum. *J. Immunol.*, 1948, **59**, 141–146.

25. Smadel, J. E. Experimental nephritis in rats induced by injection of anti-kidney serum. I. Preparation and immunological studies of nephrotoxin. *J. Exper. Med.*, 1936, **64**, 921–942.

26. Solomon, D. H.; Gardella, J. W.; Fanger, H.; Dethier, F. M., and Ferrebee, J. W. Nephrotoxic nephritis in rats. Evidence for the glomerular origin of the kidney antigen. *J. Exper. Med.*, 1949, **90**, 267–272.

27. Longcope, W. T. The production of experimental nephritis by repeated proteid intoxication. *J. Exper. Med.*, 1913, **18**, 678–703.

28. Allen, A. C. The Kidney. Grune & Stratton, New York, 1951, p. 114.

29. Veil, W. H., and Buchholz, B. Der Komplementschwund im Blute. Eine bedeutsame immunobiologische Reaktion beim rheumatischen Infekt und ihre Beziehung zur allgemeinen Pathologie des Rheumatismus. *Klin. Wchnschr.*, 1932, **11**, 2019–2021. (Cited by Masugi.[18])

30. Lange, K.; Slobody, L.; Graig, F.; Ogur, G.; Oberman, J., and LoCasto, F. Serum complement in acute glomerulonephritis and the nephrotic syndrome. *Pediatrics*, 1951, **8**, 814–820.

31. Schwab, L.; Moll, F. C.; Hall, T.; Brean, H.; Kirk, M.; Hawn, C. V. Z., and Janeway, C. A. Experimental hypersensitivity in the rabbit. Effect of inhibition of antibody formation by x-radiation and nitrogen mustards on the histologic and serologic sequences, and on the behavior of serum complement, following single large injections of foreign proteins. *J. Exper. Med.*, 1950, **91**, 505–526.

32. Mellors, R. C., and Ortega, L. G. In preparation.

[*Illustrations follow*]

LEGENDS FOR FIGURES

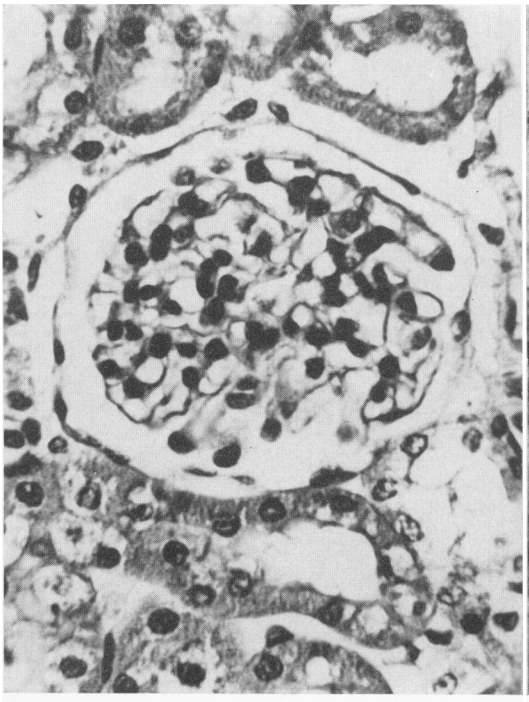
Figs. 1 to 17. Perfused rabbit organs. Sections stained with hematoxylin and eosin unless otherwise specified.

FIG. 1. Control rabbit. Normal glomerulus displaying thin-walled patent capillaries. × 475.

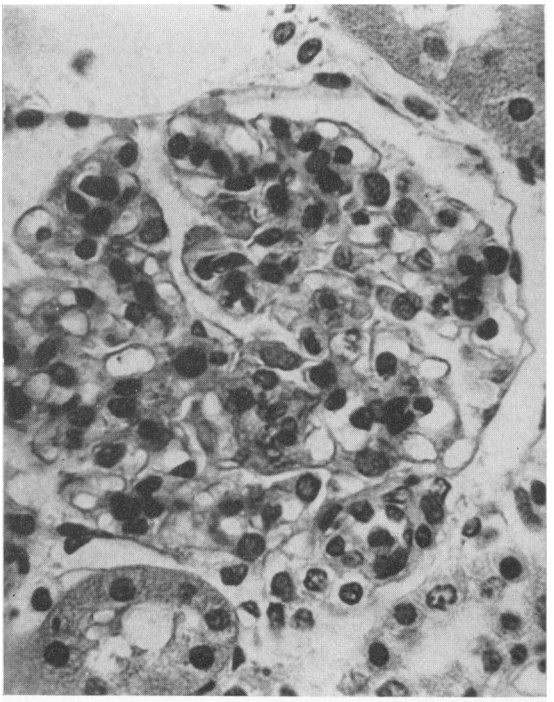
FIG. 2. Rabbit B. Glomerulitis with swelling of cells, widening of basement membrane, and reduction of the lumen of the capillaries in the glomerulus. × 475.

FIG. 3. Rabbit Q. Glomerulitis with deposition of large masses of PAS-staining granular material in the glomerulus. Periodic acid-Schiff (PAS) stain. × 475.

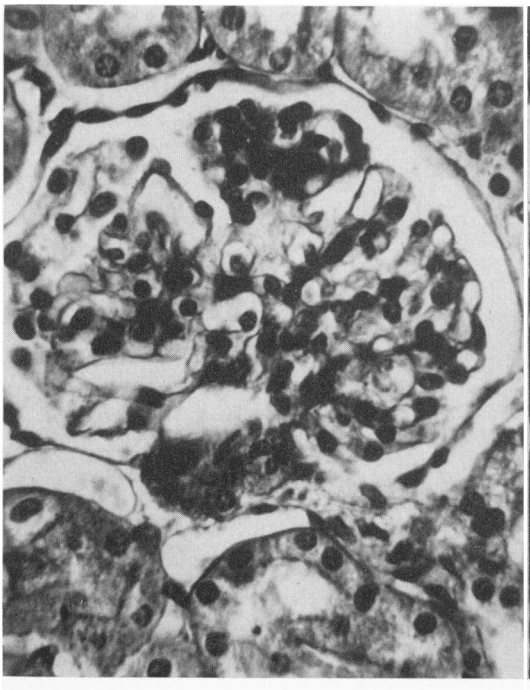
FIG. 4. Rabbit Q. Glomerulitis with thickening of the basement membrane and widening of the intercapillary spaces, the change being more prominent in one half of the glomerulus. × 475.



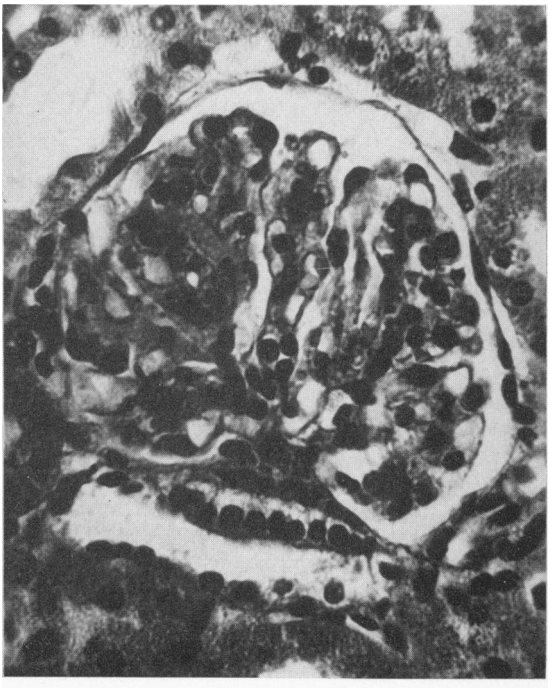
1



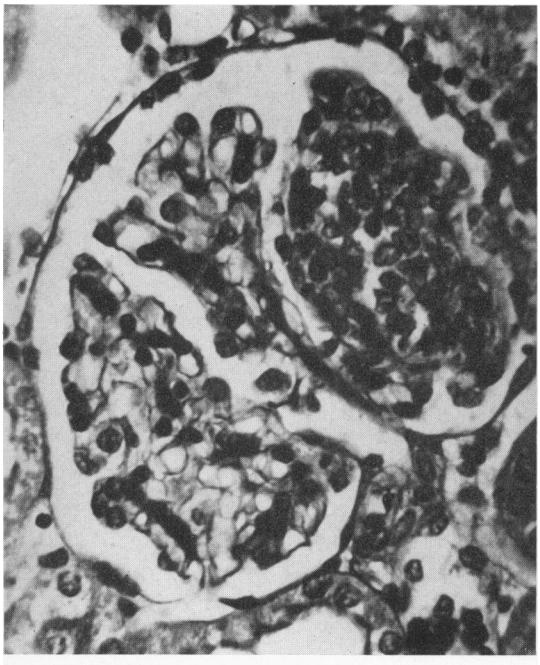
2



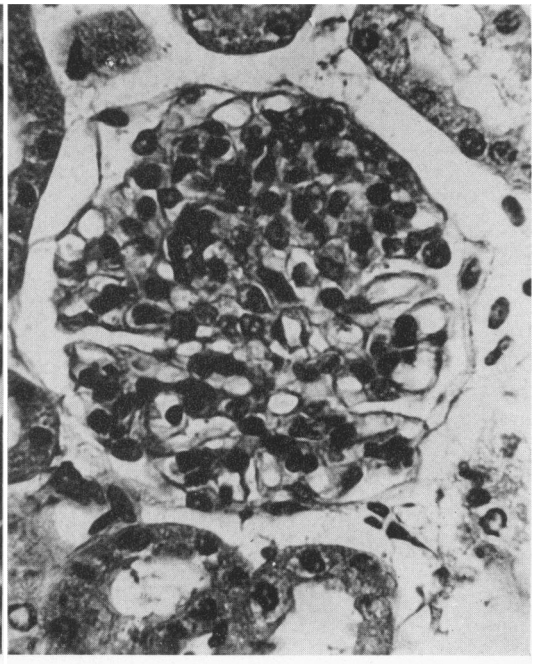
3



4



5



6

FIG. 5. Rabbit Q. Focal proliferative glomerulitis. PAS stain. × 475.

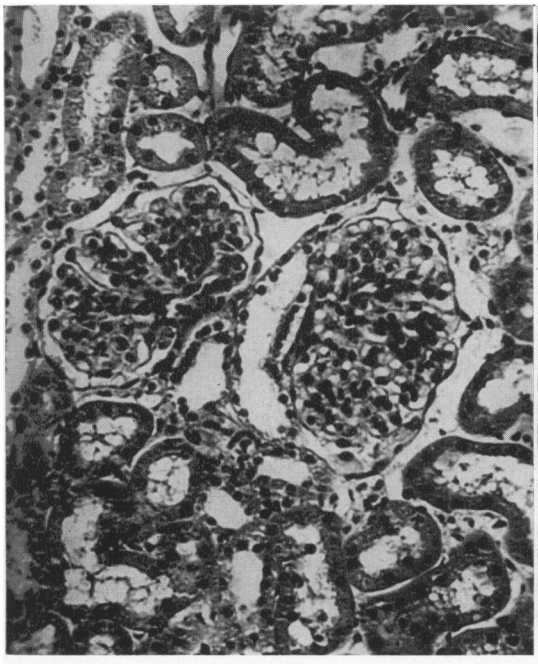
FIG. 6. Rabbit G. Proliferative glomerulitis, more accentuated in one half of the glomerulus. × 475.

FIG. 7. Rabbit B. Diffuse proliferative glomerulitis of moderate severity accompanied by alterations in the capillaries. × 195.

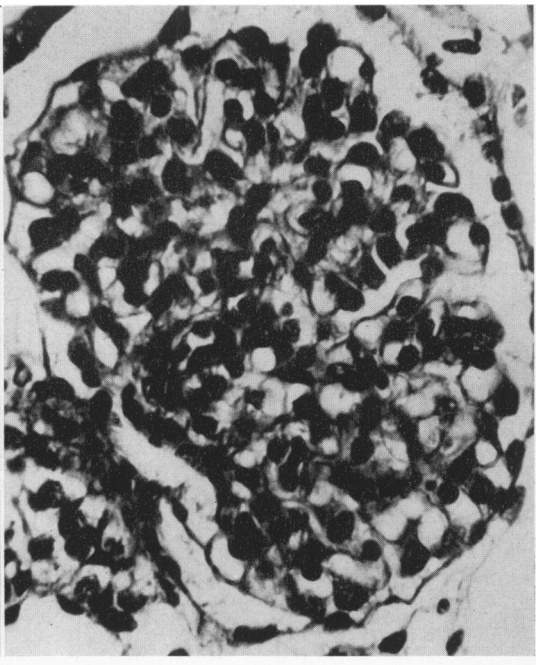
FIG. 8. Rabbit Q. Diffuse proliferative glomerulitis with uniform involvement of the glomerulus. × 470.

FIG. 9. Rabbit E. Diffuse exudative and proliferative glomerulitis. × 470.

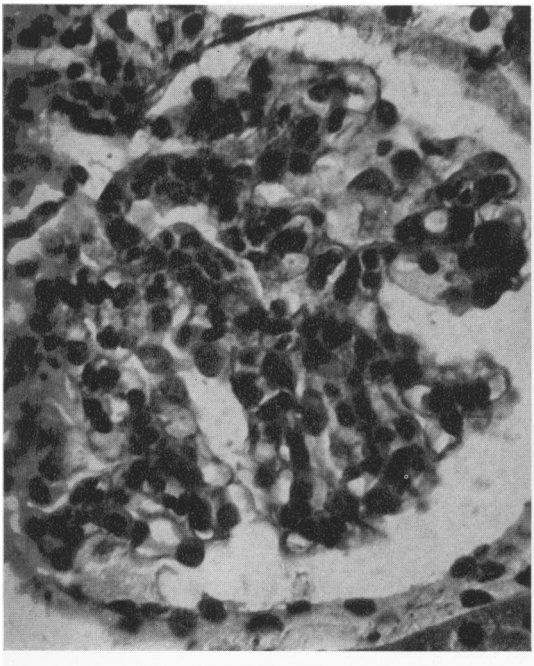
FIG. 10. Rabbit Q. Glomerulitis with focal area of early necrosis in which nuclear fragmentation is conspicuous. × 470.



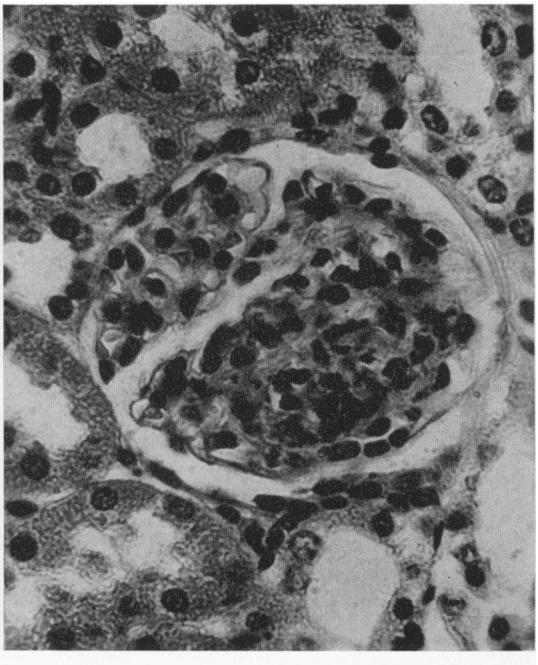
7



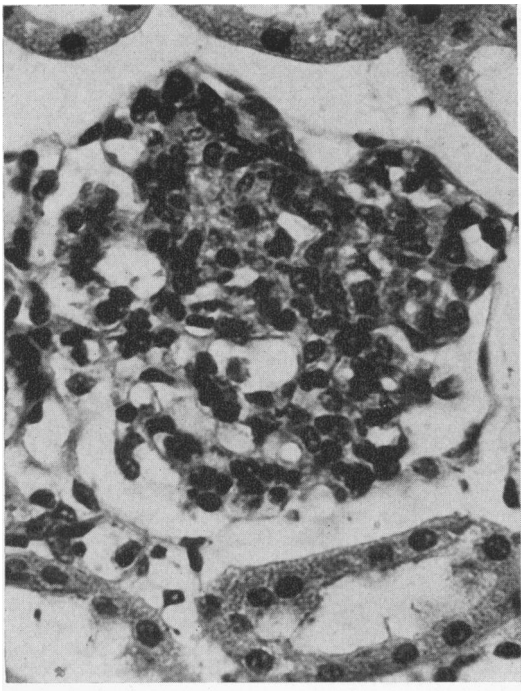
8



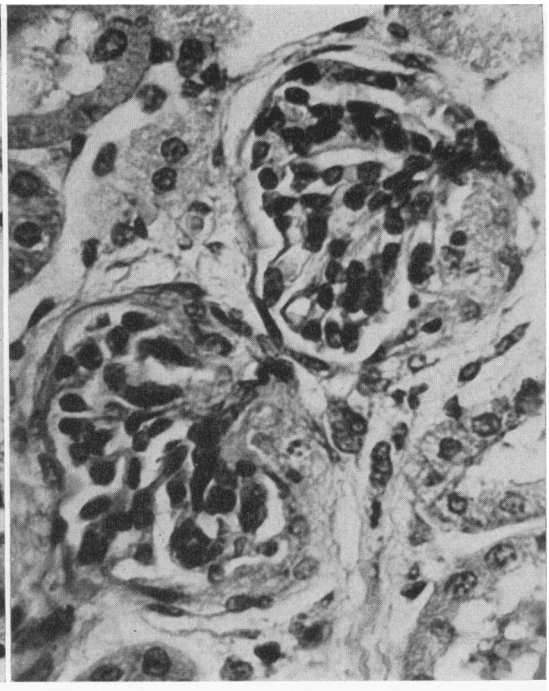
9



10



11



12

FIG. 11. Rabbit G. Proliferative glomerulitis with early fibrosis and retraction. × 480.

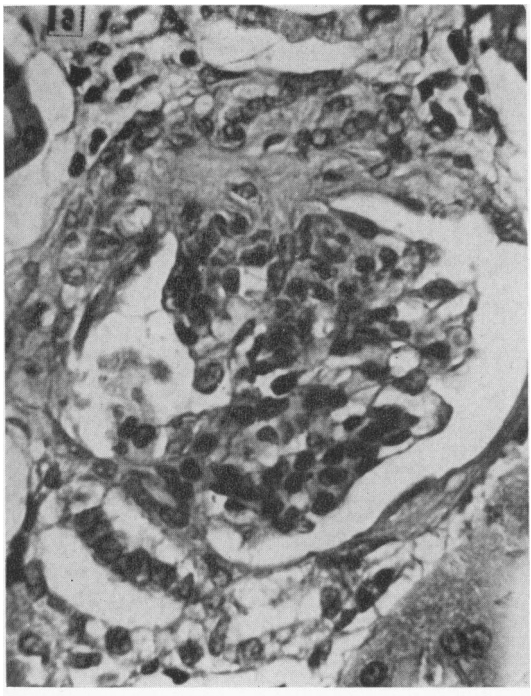
FIG. 12. Rabbit F. Proliferative glomerulitis with more advanced intracapillary and extraglomerular fibrosis. × 480.

FIG. 13. Rabbit R. Glomerular crescent with adhesion between the capillary tuft and Bowman's capsule. × 480.

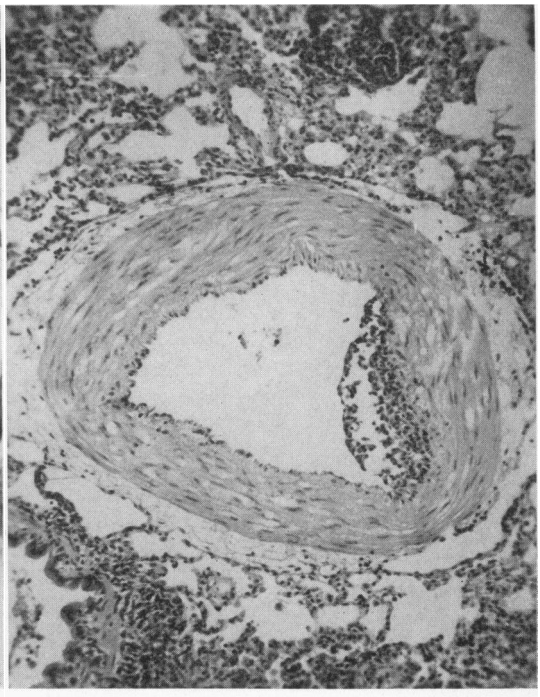
FIG. 14. Rabbit G. Endarteritis in a small pulmonary artery. × 95.

FIG. 15. Rabbit G. Panvasculitis and interstitial pneumonitis. × 95.

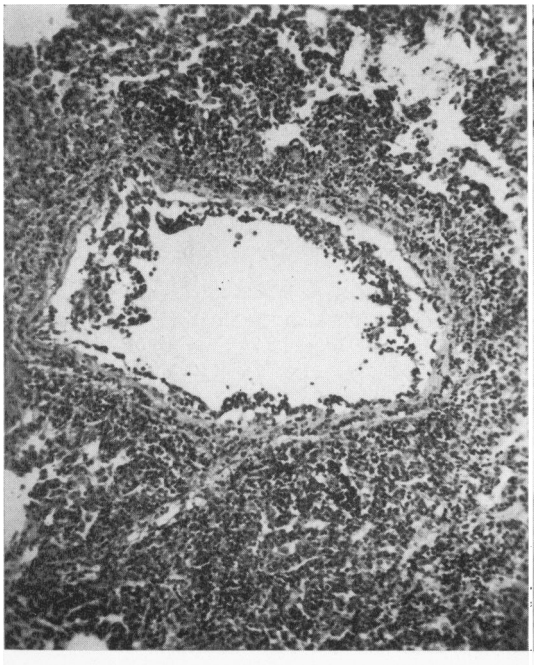
FIG. 16. Rabbit C. Mitral valve with acute valvulitis and a large focus of fibrinoid necrosis. × 95.



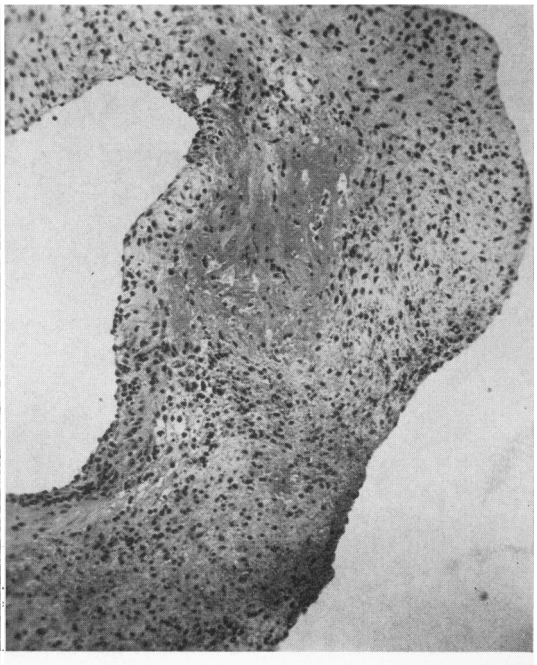
13



14



15



16

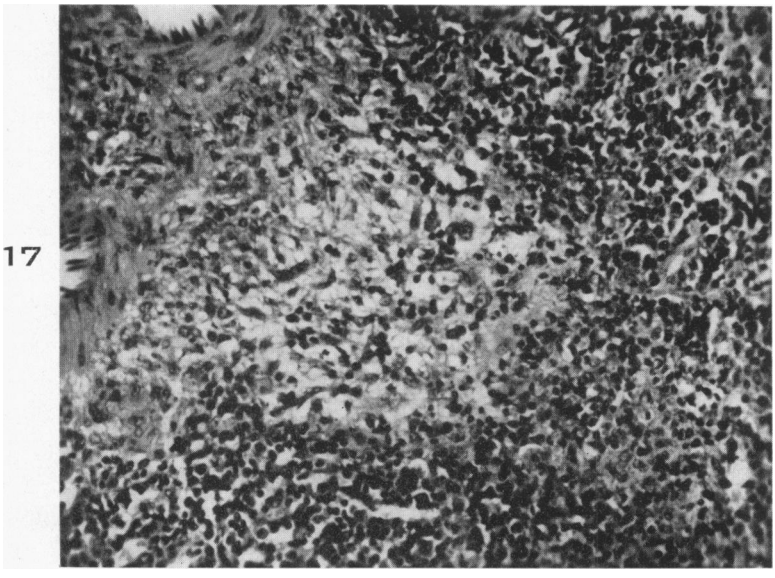
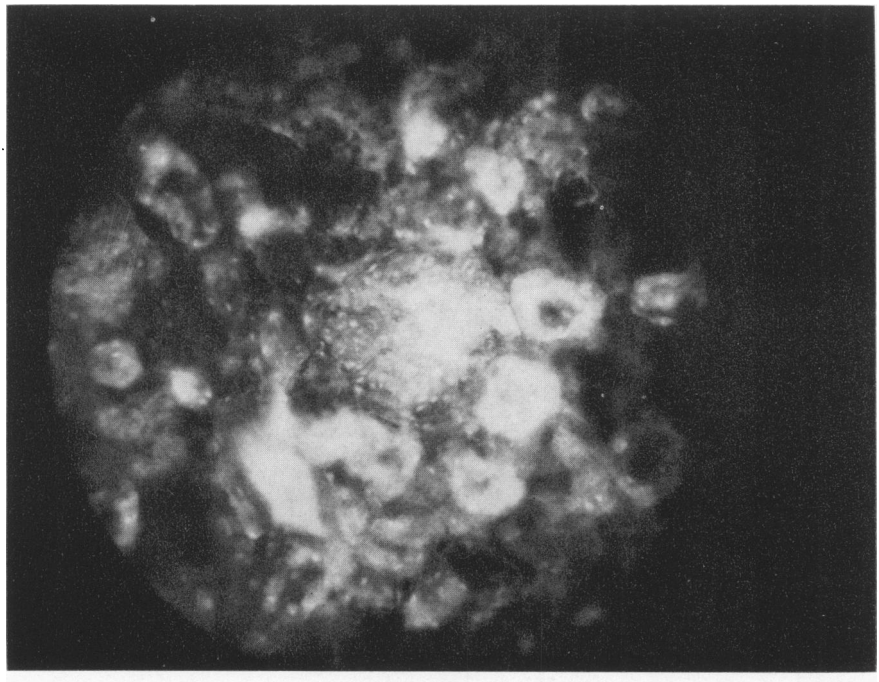


FIG. 17. Rabbit G. Spleen with perivascular allergic granuloma. × 175.

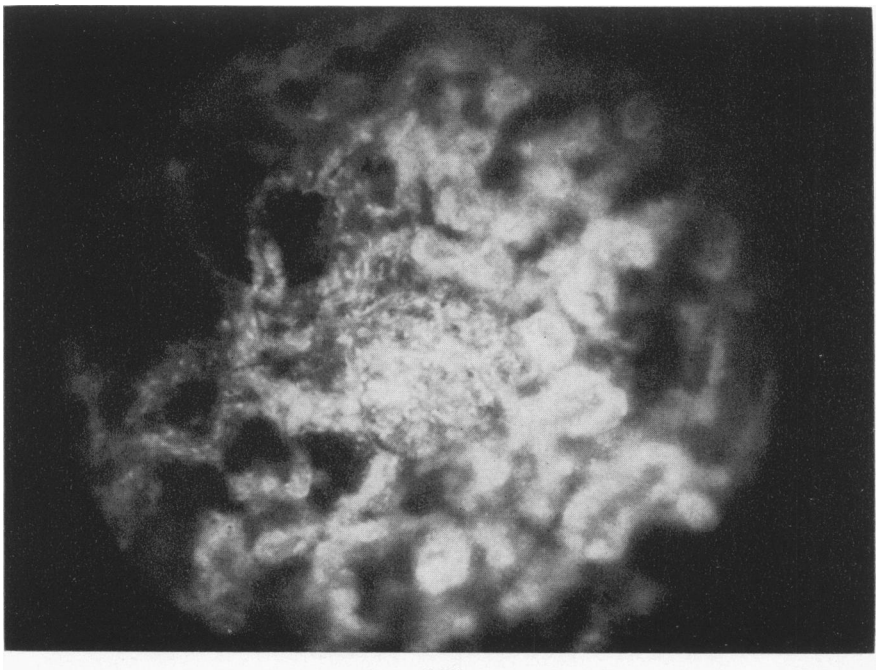
Figs. 18 to 30. Rabbit kidneys. Unfixed, frozen sections treated with the fluor (fluorescein-labelled Chi-anti-Rab) to demonstrate the localization of antibodies by fluorescence. Fluorescence photomicrographs except where otherwise stated. × 200.

FIG. 18. Normal control rabbit. Normal glomerulus and tubules. The fluorescence in the section is due principally to non-specific combination with the fluorochrome. The fluorescence intensity of the glomerulus is slightly greater than that of many of the tubules.

FIG. 19. Another normal control rabbit. Normal glomerulus and tubules.



18



19

FIG. 20. Kidney of a rabbit (Q) having proliferative glomerulitis. The fluorescence intensity of the glomeruli is much greater than that of the tubules.

FIG. 21. Phase photomicrograph of the same field seen in Figure 20. × 200.

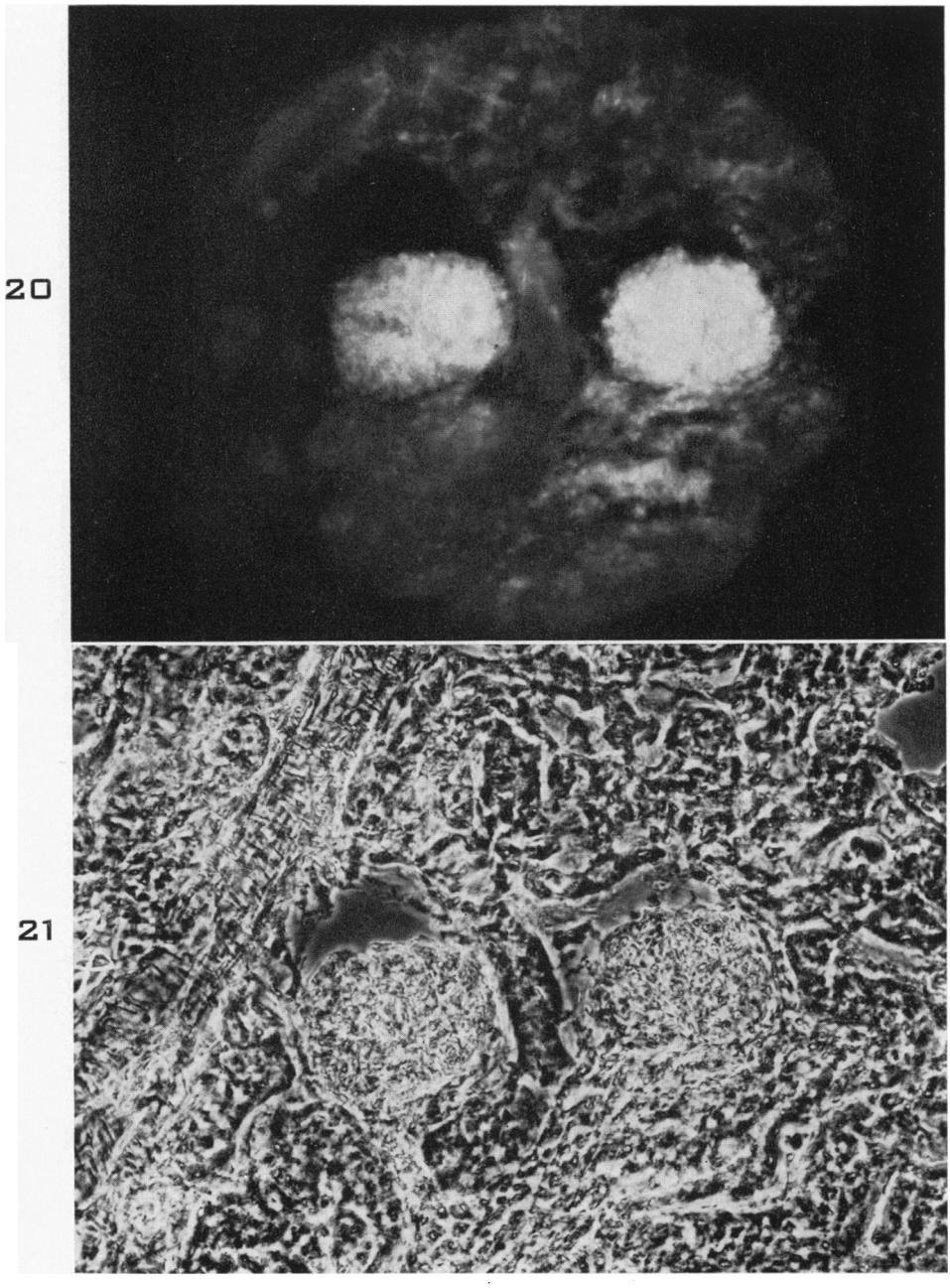
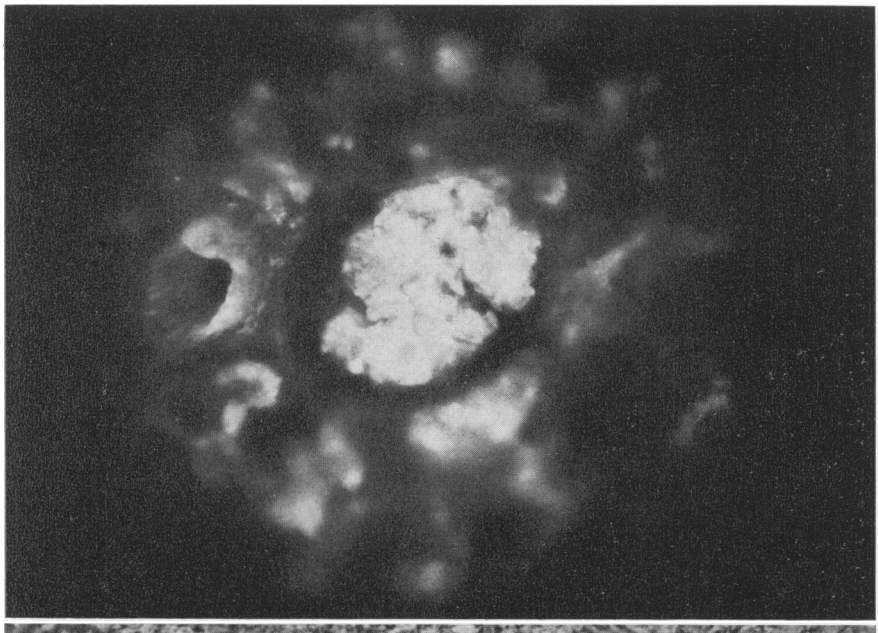
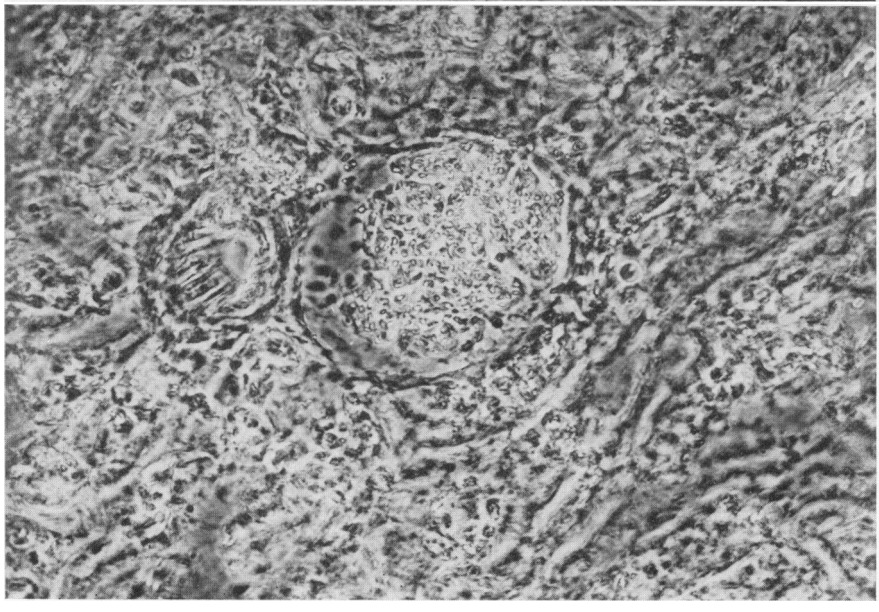


FIG. 22. Kidney of a rabbit (R) having proliferative glomerulitis. The fluorescence intensity of the glomerulus is much greater than that of tubules.

FIG. 23. Phase photomicrograph of same field seen in Figure 22. × 200.



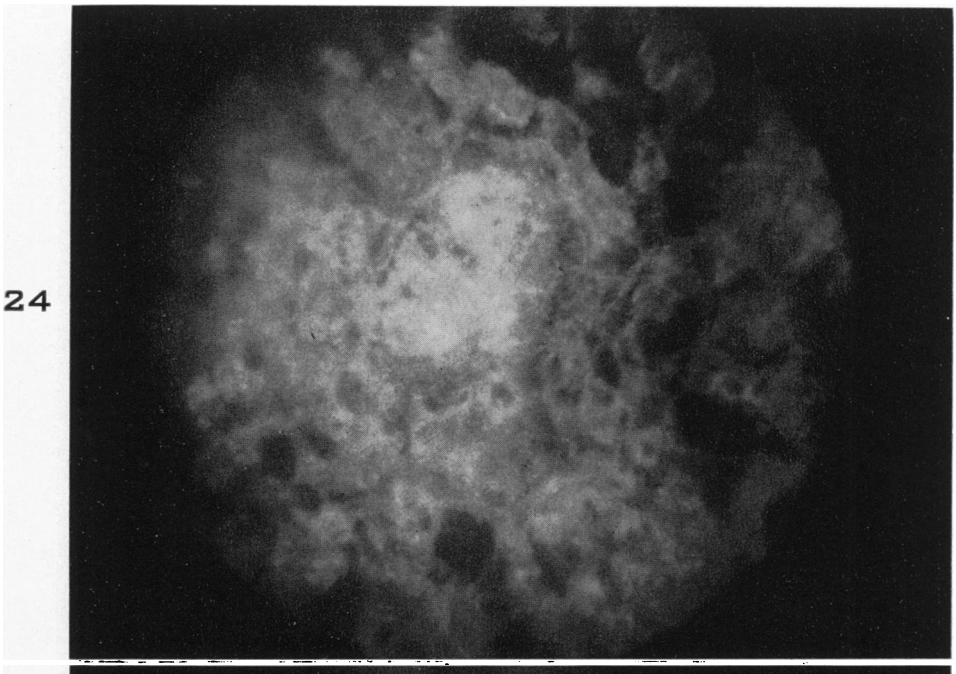
22



23

FIG. 24. Kidney of a rabbit (R) having proliferative glomerulitis. The specific fluorescence has been blocked, however, by treatment of the section with unlabelled globulin (Chi-anti-Rab) prior to using the fluor. The fluorescence intensity of the glomerulus is then only slightly greater than that of the tubules, indicating that much of the glomerular fluorescence illustrated in Figures 22 and 23 is inhibited by this immunochemical procedure. The fluorescence which can be specifically blocked in this manner is therefore due to the presence of localizing antibodies which are concentrated in the glomerulus.

FIG. 25. Another microscopic field with conditions identical to those in Figure 24.



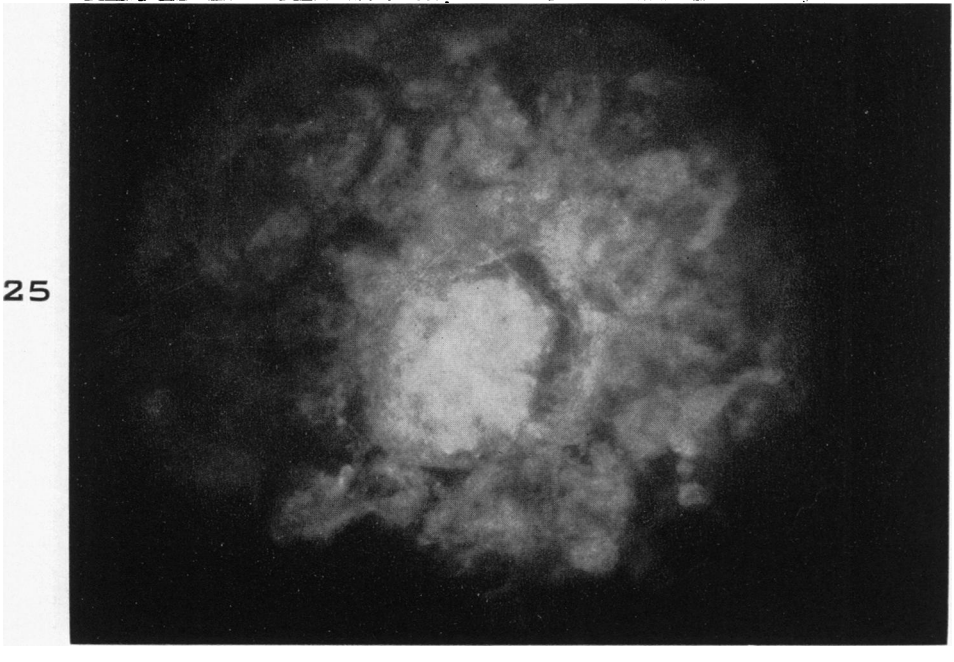
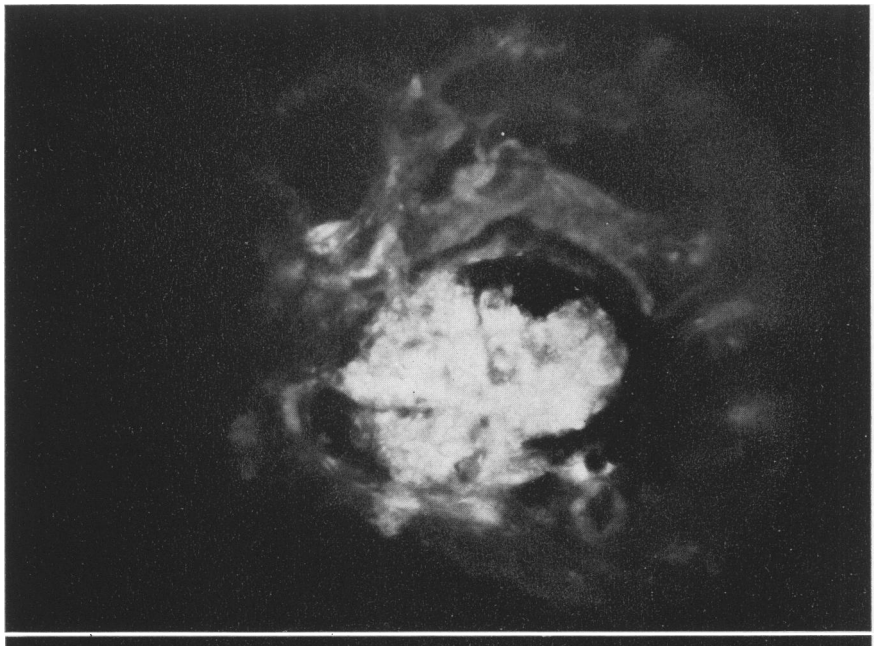
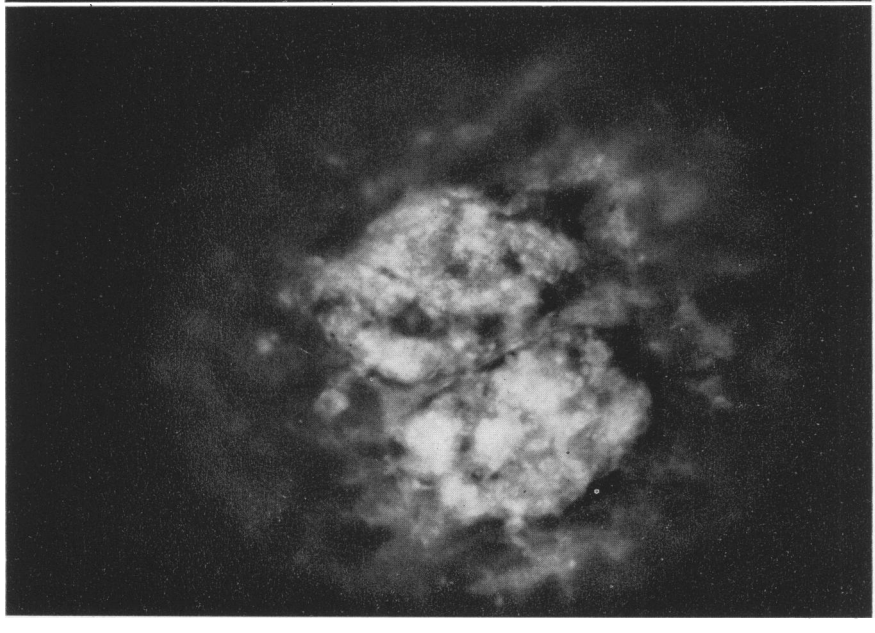


FIG. 26. Kidney of a rabbit (N) having glomerulitis and crescent formation. The relative fluorescence intensity of the glomerulus is increased.

FIG. 27. Kidney of a rabbit (E) having proliferative and exudative glomerulitis. The relative fluorescence intensity of the glomeruli is increased.





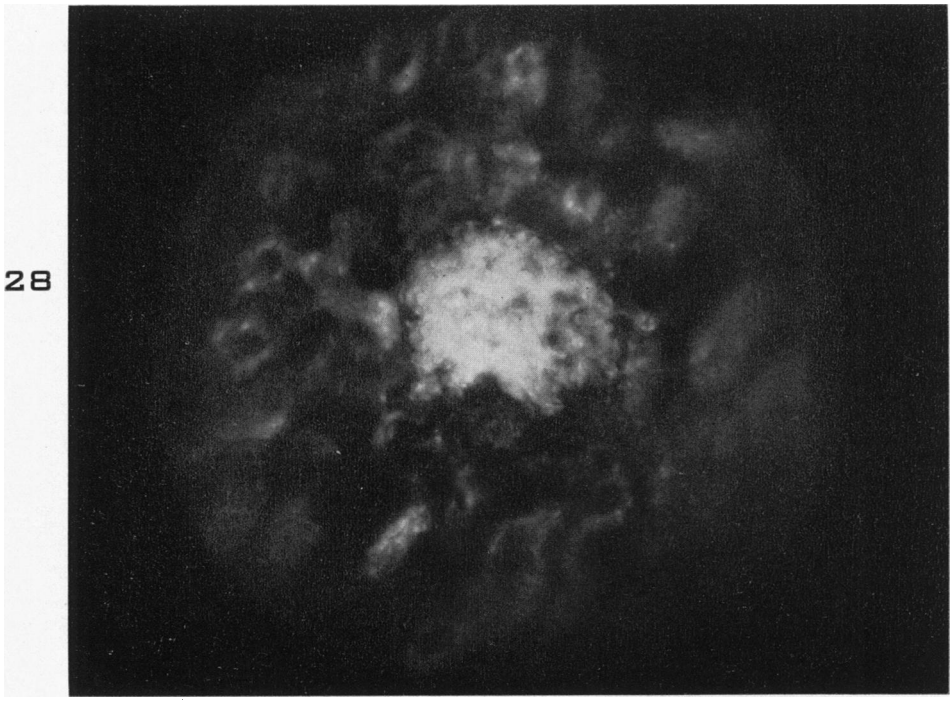
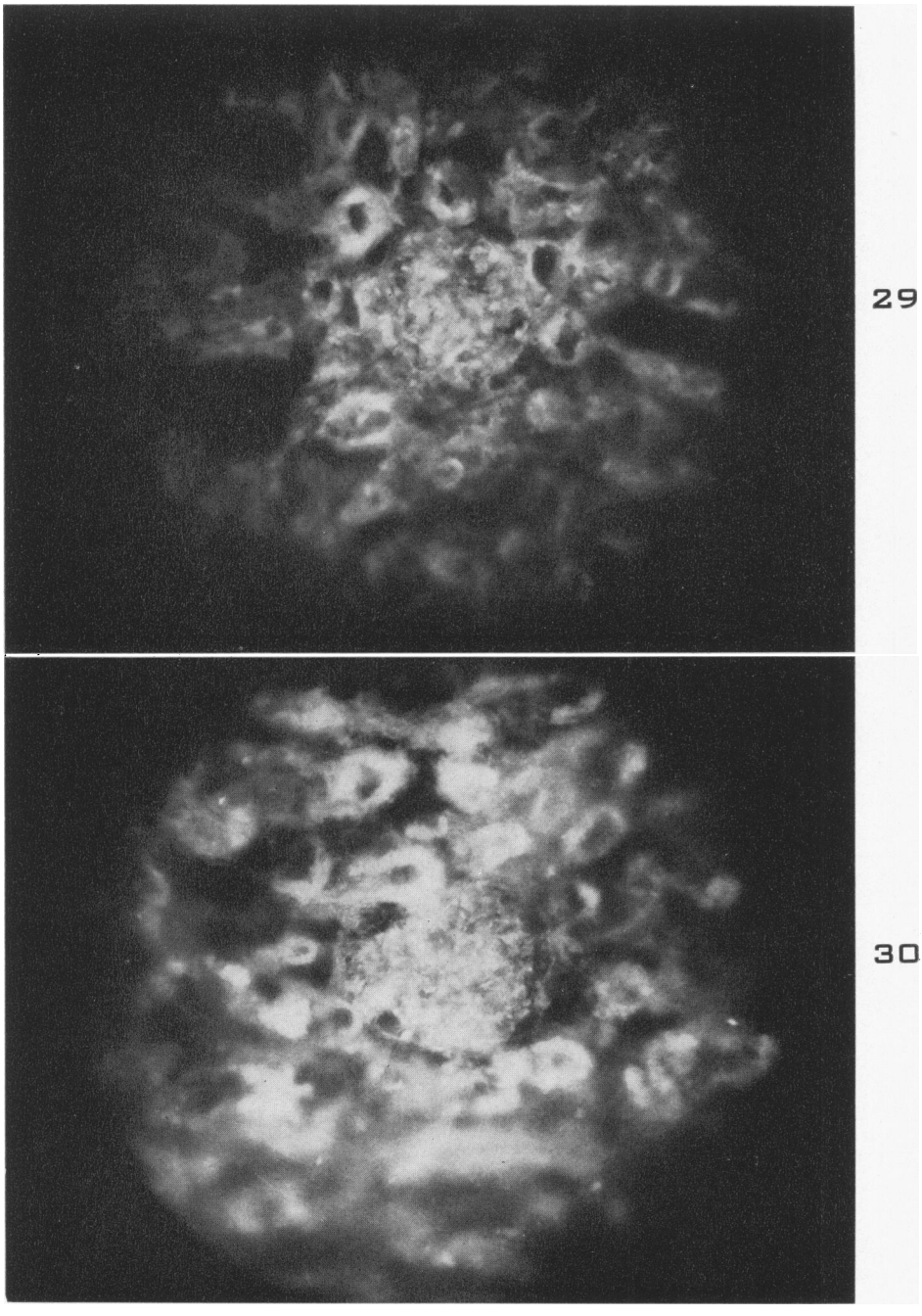


FIG. 28. Kidney of a rabbit (G) having proliferative glomerulitis. The relative fluorescence intensity of the glomerulus is increased.

FIG. 29. Kidney of a rabbit (C) having glomerulitis with capillary change only. The fluorescence intensity of the glomerulus is only slightly greater than that of the tubules.

FIG. 30. Kidney of a rabbit (T) which received injections of bovine gamma globulins but did not develop glomerulitis. The fluorescence intensity of the glomerulus is only slightly greater than that of the tubules.



29

30