ROLE OF GAMMA GLOBULINS IN PATHOGENESIS OF RENAL LESIONS IN SYSTEMIC LUPUS ERYTHEMATOSUS AND CHRONIC MEMBRANOUS GLOMERULONEPHRITIS, WITH AN OBSERVATION ON THE LUPUS ERYTHEMATOSUS CELL REACTION*‡

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PLATES 14 TO 23

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In a substantial per cent of cases of systemic lupus erythematosus there is a lesion of the glomeruli (1, 2), the so called "wire loop" lesion, the presence of which is pathognomonic of this disease with but few exceptions (3). This lesion is characterized by an irregular, rigid, and deeply eosinophilic thickening of one or more of the glomerular capillary loops. We have found that the thickened capillary walls, the wire loops, and the so called "hyaline thrombi" in glomeruli in systemic lupus erythematosus are the sites of characteristic deposition of serum γ-globulins. An illustration of this and related facts constitutes the principal purpose of this paper.

The analytic procedure used in the present work is the fluorescent antibody method of Coons (4), in theory applicable to the microscopic demonstration of almost any type of antigenically specific protein. With this method we have previously demonstrated (5) that γ-globulins are characteristically localized in the active glomerular lesions in glomerulonephritis and so called lipoid nephrosis (chronic membranous glomerulonephritis). We will illustrate in this paper also a further study of glomerulonephritis, of the membranous and the lobular types, in adults. Evidence has also been presented elsewhere (5) with the fluorescent antibody method that γ-globulins are localized in the glomerular lesions in amyloidosis (a finding confirmed and extended by others (6)) and in the glomerular and the arterial lesions in polyarteritis nodosa.

In a preliminary note dealing with studies of lupus erythematosus and other diseases, using the fluorescent antibody method, Vazquez and Dixon (7) have de-

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scribed the presence of a considerable concentration of γ-globulin in areas of fibrinoid change in renal arterioles and glomeruli. Very recently, Gitlin and his associates (8) have presented their work on fibrinoid in the collagen diseases, utilizing fluorescent antibody against human fibrin as a specific strain. Their illustrations and descriptions of fibrin deposition in the glomeruli in lupus erythematosus bear little if any resemblance to those which we present for localized γ-globulins.

Methods and Materials

A method of procedure comparable to that described elsewhere (5) for demonstrating tissue-localized γ-globulins has been followed in the present work, and only the necessary details will be given here. Tissues were obtained at autopsy, blocked in the unfixed state, frozen at −70°C., and stored at −20°C. Frozen sections were cut in a cryostat with a microtome setting of 4 μ, thawed, and dried on slides, and stored for short periods in the refrigerator until used. Tissue sections were washed with isotonic buffer–saline to remove soluble, unbound serum proteins prior to treatment with the specific fluors, henceforth referred to as the specific stains. The specific stains were flurescein conjugates, prepared by the method of Coons and Kaplan (4), of crude γ-globulin fractions of antisera obtained from rabbits and from a goat after immunization with human γ-globulin.[1] Analysis of the precipitin activity of these antisera by means of a gel diffusion technique (9) revealed the presence, in both, of heavy lines of reactivity with human γ-globulin and much weaker antibody components against α- and β-globulins. The major difference between the rabbit and goat antisera was the presence in the latter of much stronger antialbumin activity. The specific stain derived from the goat antiserum was used in the present work only for the study of chronic membranous glomerulonephritis. Non-specific staining was removed by absorption of the fluors with lyophilized tissue powders, prepared from the livers of the rat or the mouse.

The control reactions used to establish immunologic specificity were: (*a*) complete inhibition of specific staining by prior treatment of sections with unconjugated homologous antiserum, but no inhibition by prior treatment of companion sections with homologous normal serum (4); and (*b*) absence of specific staining in sections exposed to fluorescein-conjugated antibodies to heterologous γ-globulin.

When immunologic specificity was indicated by these reactions, further attempts were made to identify the stained tissue antigens by the use also of absorption procedures, in which aliquots of the conjugates were reacted, in antigen excess, with purified human γ-globulin (prepared by Dr. Leonhard Korngold by zone electrophoresis in starch) or with purified serum albumin. ***In this paper the term specific fluorescence, unless otherwise qualified, is used to designate the immunologically specific staining patterns that were completely inhibited by prior reaction of the conjugates with human γ-globulin, but which were not appreciably affected by prior reaction with human serum albumin.*** Trace amounts of β-globulin were present in both the purified γ-globulin and albumin, the latter containing also a small amount of α-globulin.

The anatomical material obtained for study by the fluorescent antibody method consisted of the kidneys obtained at autopsy on two cases (designated cases 1 and 2 below) with clinical and pathological diagnoses of systemic lupus erythematosus; and the kidneys obtained at autopsy on two cases (cases 3 and 4) with the clinical nephrotic syndrome and pathologic diagnoses of chronic membranous glomerulonephritis. The immediately relevant clinical, laboratory, and pathologic data were as follows:

Case 1 was a 45 year old female with a 4 years' history of clinically established systemic lupus erythematosus, a diagnosis supported by positive lupus erythematosus preparations,

[1] Human γ-globulin (Squibb) kindly provided through the courtesy of the American Red Cross.

For 1 year prior to death she had received treatment with corticosteroids and ACTH and had become hypertensive. Blood urea nitrogens ranging up to 100 mg./100 ml. had been present for 6 months prior to admission. On admission the patient was lethargic and had diffuse enlargement of lymph nodes. Blood pressure, 180/110. Urinanalysis: 24 hour protein excretion in excess of 6 gm.; sediment contained red cells and all forms of casts. Blood urea nitrogen, 150 mg./100 ml. Total serum protein, 5.0 gm./100 ml., albumin, 2.1, γ-globulin, 0.35. Serum lupus erythematosus preparation positive. Despite intensive therapy the patient remained uremic, developed congestive failure, and hyperpotassemia and died. Final anatomic diagnoses included: systemic lupus erythematosus; hypertensive and arteriosclerotic cardiovascular disease; pulmonary edema and uremic pneumonitis; and serous effusions. Organ weights: heart, 400 gm.; kidneys, 300 gm. together.

Case 2 was a 13 year old girl who had developed alopecia, skin rash, migratory polyarthritis, pleuritic chest pain, and peripheral edema 15 months prior to death. Lupus erythematous preparation was equivocal. The symptoms responded to meticorten, and the patient was asymptomatic for approximately 1 year, except for occasional bouts of cystitis without evidence of ascending urinary tract infection. Blood pressure, normal. Urinanalysis, 4—plus proteinuria but no hematuria. Blood urea nitrogen and urea clearance were normal. 3 months prior to death the patient became massively edematous and had smoky urine containing red cells and casts. Blood urea nitrogen, 26 mg./100 ml. Total serum proteins, 3.5 gm./100 ml., albumin, 2.1, γ-globulin, 0.35. The patient also had fever, tachycardia, joint pains, and pleural effusions, these symptoms responding to meticorten. The patient then developed grand mal seizures and for the first time became hypertensive, blood pressure 148/100. The seizures were prevented by increased doses of meticorten, but the blood urea nitrogen rose to 163 mg./100 ml., and the edema became more extensive. Acidosis developed along with severe epistaxis, the patient became more lethargic and expired. During the last 3 months of life the lupus erythematous preparation became strongly positive. Direct Coombs test was positive throughout the disease. Final anatomic diagnoses included: systemic lupus erythematosus; peripheral edema and ascites. Organ weights: heart, 280 gm.; kidneys together, 460 gm.

Case 3 was a 64 year old white female with an 8 months' history of peripheral edema and proteinuria, and with a clinical diagnosis of the nephrotic syndrome and hypertension. Blood pressure, 165/95. Urinanalysis, 4—plus albuminuria with a few casts, red cells, and leukocytes. Blood urea nitrogen, 23 mg./100 ml., rising to 58 terminally. Total serum protein, 4.5 to 4.9 gm./100 ml., albumin 1.6 to 1.9. Final anatomic diagnoses included: chronic membranous and sclerosing glomerulonephritis; generalized edema and serous effusion; arteriosclerotic and hypertensive cardiovascular disease. Organ weights: heart, 420 gm.; kidneys, right, 230 gm., left 200 gm.

Case 4 was a 43 year old white male with a 14 year history of clinically established nephritis with proteinuria and hypertension. Blood pressure, 180/110. Urinanalysis, 3—plus albuminuria with numerous red cells and many granular and hyaline casts. Blood urea nitrogen, 82 mg./100 ml., rising to 158 terminally. Total serum protein, 5.8 gm./100 ml., albumin, 3.9. Final anatomic diagnoses included: chronic sclerosing, lobular, and membranous glomerulonephritis; arteriolar nephrosclerosis, uremic pericarditis; edema and serous effusion; hypertensive and arteriosclerotic cardiovascular disease. Organ weights: heart, 600 gm.; kidneys, right 140 gm., left 135 gm.

A large variety of control material, both normal and pathologic, representing several organs and tissues in man and in experimental animals has been studied in our laboratory with the fluorescent antibody method. Of relevance here were the kidneys in the aggregate from 20 cases, including "normal" kidneys as well as those the site of a variety of inflammatory (both infectious and non-infectious), degenerative, circulatory, and other common disorders. The renal pathologic diagnoses in the control material included, among others, acute and chronic

pyelonephritis, arterial and arteriolar nephrosclerosis, cortical necrosis, lower nephron nephrosis, and capillary hyaline thrombosis. Kidneys in cases of benign hypertension, shock, and proteinuria without notable renal morphologic change were also studied.

A 1000 watt mercury arc (General Electric) or a carbon arc (Bausch and Lomb), suitably filtered, provided the sources of illumination for fluorescence microscopy which was carried out, with the photographic recording of observations in black and white and color, at various magnifications including those provided by the use of oil-immersion objectives.

Paraffin sections prepared from formalin-fixed material and stained with hematoxylin and eosin; periodic acid-Schiff (PAS) reaction; alcian blue-PAS with orange G in phosphotungstic acid as counterstain; and phosphotungstic acid and hematoxylin were also studied as routine in this work. Frozen sections, companions to those stained with fluorescent antibody method, were stained with hematoxylin and eosin. Where indicated, Sudan IV was used as a stain for lipides and Congo red and crystal violet for amyloid.

RESULTS

Systemic lupus erythematosus.—

In the two examples of this disease studied, our observations on kidneys stained with fluorescent antibody for localized γ-globulin will be correlated with those morphologic features, present in either of our cases but impressively evident in one of them, which have been reported for the kidney by Klemperer and his associates (2) in their classical description of the pathology of systemic lupus erythematosus. The most characteristic finding was the specific apple-green fluorescence in the glomerular wire-loop lesions (Fig. 1) which in hematoxylin and eosin sections (Fig. 2) appeared as focal, irregular, rigid, and eosinophilic thickenings of the capillary wall, apparently involving the basement membranes and appertaining space that lies between capillary endothelium and visceral epithelium. Not all of the glomeruli exhibited localization of γ-globulins in keeping with the histologic observation that a few of the glomeruli displayed no structural alteration at all, while many were partially or completely obliterated by fibrosis, appearing blue-violet in the fluorescence microscope only by virtue of intrinsic fluorescence. Various distributions and intensities of specific fluorescence were present in the thickened glomerular capillary walls and in the wire-loop lesions (Figs. 3 and 5) to a considerable degree capable of correlation with variations in the pattern of deep eosinophilia observed in sections stained with hematoxylin and eosin (Figs. 4 and 6). The so called "hyaline thrombi" (2) were also the site of vivid specific fluorescence signifying the deposition of γ-globulins (Figs. 7 and 8). In some glomeruli specific fluorescence was seen to occupy two, more or less parallel, circumferential sites in the glomerular capillary walls (Fig. 9), consisting of an inner, granular, discontinuous deposition of material and an outer, denser, continuous deposition of material, the latter apparently bearing a relation to the basement membrane.

While in hematoxylin and eosin sections there were some foci in which the eosinophilia of the glomerular lesions was accentuated to that degree found in so called fibrinoid, these foci failed to stain like fibrin with phosphotungstic acid-hematoxylin or with orange G in phosphotungstic acid and with rare exception were negative with these stains. The sites of localization of γ-globulins in the glomerular lesions corresponded, however, to PAS-positive, violet and pink areas in sections stained

with the periodic acid–Schiff reaction. Some glomerular capsular crescents were the site of specific fluorescence (Fig. 10).

An exceptional finding was the presence of specific apple-green fluorescence in large cytoplasmic granules in tubular epithelium (Fig. 11). While the structure of these fluorescent granules was similar to that which one expects to find in hematoxylin and eosin sections at sites of severe hyaline droplet degeneration in tubules, this correlation was not established in this material although it was suggested by the rarity of occurrence of both specific tubular fluorescence and hyaline droplet degeneration. Among the numerous tubular protein casts there were a number of small, round casts that had specific fluorescence. In some interstitial foci of inflammatory cells, particularly in the cytoplasm of cells identified as immature and mature plasma cells, there was also specific fluorescence (Fig. 12). Renal arteries and arterioles were not remarkable except for the rare occurrence of small streaks of specific fluorescence in the intima and the media of some of the small arteries in one case. In companion hematoxylin and eosin sections, however, corresponding specific arterial and arteriolar lesions could not be demonstrated.

Each of the foregoing examples of specific fluorescence was completely inhibited by prior treatment of sections with unconjugated antiserum against human γ-globulins and by treatment of sections with conjugate from which specific antibodies had been precipitated and removed by combination with purified human γ-globulins (compare Figs. 13 and 14). Prior treatment of sections with homologous normal serum or absorption of the fluor with human serum albumin did not inhibit the specific staining reaction.

Chronic Membranous Glomerulonephritis.—

The kidneys were studied by the fluorescent antibody method for γ-globulins in two examples of this disease, characterized clinically by the prominence of the nephrotic syndrome. Aside from the membranous change (Fig. 16), many glomeruli in each case were the site also of sclerosis and in one case a few of the glomeruli had an accentuated lobular appearance (Fig. 21). As in previous observations (5), specific fluorescence indicating the localization of γ-globulins in glomerular basement membranes was a notable feature (Fig. 15). Specific apple-green fluorescence, readily differentiated from the blue-white intrinsic fluorescence of sclerotic glomeruli, was also seen in some of the glomeruli having a lobular configuration (Fig. 20), γ-globulins being apparently deposited in the altered mesangium (Fig. 21). In each case of membranous glomerulonephritis some of the dense protein casts in the tubules had specific fluorescence. However, when the antibodies against γ-globulin in the fluor were removed by precipitation with purfied γ-globulin, the residual fluor—now containing antibodies against serum albumin—continued to stain the tubular casts (Figs. 18 and 19), which presumably contained albumin, but failed to stain the glomeruli (Fig. 17).

Control Tissues and Organs.—

Normal kidneys as well as those the site of a variety of inflammatory, degenerative circulatory and other disturbances have been studied since the inception of our pathologic work with the fluorescene antibody method. The renal pathologic diagnoses included, among others, acute and chronic pyelonephritis, arterial and arteriolar

nephrosclerosis, cortical necrosis, capillary hyaline thrombosis, and lower nephron nephrosis. Kidneys in cases of benign hypertension, peripheral circulatory collapse, and benign proteinuria without notable renal morphologic change were also investigated. The presence of localized γ-globulins in the glomeruli and the tubules of these control kidneys has not been observed in our entire experience. While we have not had the opportunity to study with fluorescent antibody all of the lesions that are to be considered in the morphologic differential diagnosis of renal lupus erythematosus, excepting renal amyloidosis in whch γ-globulins are localized (5, 6), one example worthy of comment in this respect is hyaline thrombosis of glomerular capillaries occurring in shock. We have studied one such case with numerous capillary thromboses reminiscent of the hyaline thrombi in renal lupus, although we recognize, as stated by Klemperer and his associates (2), that the latter are in reality, or at least in part, intrusions into the lumens by intramural hyaline deposits. There was no glomerular localization of γ-globulins detectable in this case. The capillary thrombi contained fibrin stainable with phosphotungstic acid and hematoxylin.

DISCUSSION

One may now consider what anatomic, and conceivably pathogenetic, features common to the kidney in such diverse diseases as nephrotic glomerulonephritis, systemic lupus erythematosus, and secondary amyloidosis, might account for the glomerular localization of γ-globulins, as described in the present and earlier work. Eosinophilic thickening and alteration of the glomerular capillary wall, involving the basement membrane and the appertaining space that lies between the capillary endothelium and the visceral epithelium, are one such common morphologic feature, although in the several diseases there are distinctive structural attributes of the lesions, special tinctorial properties, and so on. Functionally, increased permeability of glomerular capillaries in the foregoing diseases is manifested by proteinuria and by the clinical prominence of the nephrotic syndrome.

One may ask whether the localized γ-globulins which occupy the aforementioned sites of eosinophilic alteration may simply represent protein molecules that have been partially or completely held back by the capillary walls, while the albumins and other proteins of smaller molecular size more readily pass through. That this is affirmed or denied is not proved by the present work but several observations have bearing upon the question. Glomerular-localized γ-globulins have not been detected in kidneys obtained at autopsy in patients with benign proteinuria, although to be sure the degree of proteinuria was in no instance equal to that present in the cases of membranous glomerulonephritis and systemic lupus erythematosus. Our study (10) of nephrotoxic nephritis, which is a form of membranous glomerulonephritis in experimental animals produced by the injection of nephrotoxic (antikidney) antiserum (11), also is pertinent to the discussion. Proteinuria occurred promptly after the injection of nephrotoxic serum and reached very high levels within 1 to 6 days,

thereafter receding; but localized autogenous globulins were barely detectable in the renal glomeruli during most of this early period, probably occurring then in such amounts as were to be found in the inflammatory edema fluid that was microscopically present. However, from 6 and 9 days to 3 months after injection, autogenous globulins were impressively localized in the basement membranes of glomeruli with a pattern of distribution, when stained with fluorescent antibody, comparable to that observed in chronic membranous glomerulonephritis in man.

Yet despite the preceding observations, the physical model of deposition of larger protein molecules on the basement membrane is supported by a recent study (12) of the ultrastructure of glomerular capillaries in renal amyloidosis in the mouse. The existence of three layers in the basement membrane was observed in this as in preceding work. The wider, osmiophilic middle layer (lamina densa (13–16)) contained no electronoptically detectable pores and was considered to function as the true ultrafilter of the glomerulus. The thickening of the basement membrane in experimental amyloidosis involved this layer exclusively. Of additional pertinent interest is the fact that the recently described pattern of localization of fibrin in the kidneys in systemic lupus erythematosus (8) bears little if any similarity to that for γ-globulin. From the point of view of physical attributes, fibrinogen has a greater molecular weight and longer and narrower molecular axes than does γ-globulin.

As pointed out by Klemperer and his associates (2), the greatest similarity to the wire-loop change in renal lupus erythematosus is seen in hematoxylin-eosin sections of early renal amyloidosis. Moreover, it is recognized that renal amyloidosis may coexist with systemic lupus. In the present study, staining procedures of proved efficacy in detecting amyloid in tissue sections from known cases of secondary amyloidosis were applied to the kidneys, but amyloid was not found in either case of systemic lupus erythematosus.

That the simple accumulation of inflammatory exudate in the capillary wall affords adequate explanation for the glomerular localization of γ-globulins in these several diseases seems unlikely—unless the inflammatory exudate be a distinctive one with an extremely high concentration of protein—for such a pattern of localization has not in our experience been observed in control kidneys, not, for example, in kidneys the site of non-suppurative and suppurative pyelonephritis nor in those the site of a variety of degenerative (arteriolosclerosis), circulatory (hypertension, capillary fibrin thrombosis, cortical necrosis), and other pathologic disturbances in which inflammation is also present. It is possible to state, therefore, that the presence of glomerular-localized γ-globulin, in concentrations as demonstrably high as illustrated in our work on glomerulonephritis and systemic lupus erythematosus, is not dependent upon the coexistence of hyalin, fibrin, hemorrhage, necrosis, inflammatory edema, or suppuration. We have shown, moreover, in an example of

chronic membranous glomerulonephritis that the glomeruli were positively stained for localized γ-globulin, whereas tubular protein casts in the same sections were positively stained for γ-globulins as well as for other serum proteins including albumin. The conclusion appears justified that the glomerular, localized proteins in this case of glomerulonephritis consisted of a greater concentration of γ-globulin and a lesser concentration of other serum proteins than was present in tubular protein casts, which presumably represent concentrated abnormal filtrates of the glomeruli. Of course, the fluorescent antibody method, in the views of its originator (17) and of those broadly experienced in its use, cannot be used for quantitative measurements, as for example, the determination of the concentration ratios of two or more different proteins by the expedient of comparing fluorescent intensities.

A further possibility to be considered in systemic lupus erythematosus is that the glomerular-localized γ-globulins include, as least in part, the lupus erythematosus factor. The lupus erythematosus factor is known to be a γ-globulin (18, 20), and the ability of the fluorescent antibody for human γ-globulin to react with the lupus erythematosus factor is strongly suggested by the accompanying illustrations pertaining to the lupus erythematosus cell reaction, Figs. 22, 24, and 25. A study of the lupus erythematosus cell reaction by the fluorescent antibody method is currently underway in our laboratories, and the results will be presented more fully elsewhere. However, we wish to draw attention to the fact that in a lupus erythematosus preparation prepared by the method of Snapper and Nathan using highly positive lupus erythematosus serum, the nuclei of leukocytes, while undergoing transformation and subsequent phagocytosis to form lupus erythematosus cells, developed specific apple-green fluorescence when stained with fluorescent antibody for human γ-globulin, Figs. 22 and 24. This specific nuclear fluorescence is completely inhibited by prior absorption of the fluorescent antibody stain with human γ-globulin. In the control (negative) lupus erythematosus preparation, moreover, the nuclei of the leukocytes did not display specific fluorescence when stained with fluorescent antibody for human γ-globulin, Fig. 23. Strong evidence that the material on the nuclei which is reacting with the fluorescent antibody is the lupus erythematosus factor comes from the demonstration that this factor can be absorbed on cell nuclei from various sources and on nucleoprotein isolated from nuclei (19, 20). However, whether the lupus erythematosus factor actually does become localized in glomeruli remains to be demonstrated.

Finally one may ask whether the localized γ-globulins are, at least in part, antibodies against renal localized antigens (of extrinsic or conceivably intrinsic origin), in keeping with the concept of hypersensitivity pathogenesis of these several diseases (21). Our work with nephrotoxic nephritis (10) has demonstrated that the antibody fraction of antikidney serum was localized in the glomerular basement membranes in a pattern very similar to that of autogenous

globulins in the nephritic rat and similar to that of γ-globulins in nephrotic glomerulonephritis in man. By virtue then of their protein composition and membranous location the localized γ-globulins fill an apparently necessary, *although not alone sufficient*, condition for establishment as antibodies. Proof as to whether localized γ-globulins are specific antibodies can be afforded by the fluorescent antibody method (22), provided that the antigen be known and that it be available in reasonable purity and in a soluble form suitable for laboratory usage. Efforts directed toward this end with special reference to the role of streptococcal antigens in acute glomerulonephritis and acute rheumatic carditis are currently underway in our laboratory.

SUMMARY AND CONCLUSIONS

Utilizing the fluorescent antibody method for the histologic demonstration of localized γ-globulins, we have made the following observations (in contradistinction to the lack of such findings in a variety of normal and pathologic, control kidneys).

In systemic lupus erythematosus (*a*) γ-globulins were localized in the thickened capillary walls, the "wire-loop" lesions, and the so called "hyaline thrombi" in glomeruli; (*b*) these sites of localization of γ-globulins were correlated to a considerable degree with the pattern of accentuated eosinophilia of the glomeruli, as seen in hematoxylin-eosin sections, or with the pattern of PAS-positive areas in the glomeruli in sections stained with the periodic acid–Schiff reaction; (*c*) and γ-globulins were localized rarely in large cytoplasmic granules in tubular epithelium and occasionally in glomerular capsular crescents, tubular protein casts, and inflammatory cells, particularly in the cytoplasm of cells identified as immature and mature plasma cells.

In nephrotic glomerulonephritis (*a*) γ-globulins were localized in the glomerular basement membrane and appertaining structures in chronic membranous glomerulonephritis; (*b*) γ-globulins were apparently localized in the altered mesangium in chronic lobular glomerulonephritis; and (*c*) in the tubular protein casts, presumably representing abnormal glomerular filtrates, γ-globulins were present in a lesser concentration and other serum proteins in a greater concentration than found in the glomeruli.

In positive lupus erythematosus preparations the nuclei of leukocytes, while undergoing transformation and subsequent phagocytosis to form lupus erythematosus cells, were the sites of localization of γ-globulin (presumably the lupus erythematosus factor) whereas in control preparations no nuclear localization of γ-globulin occurred.

These observations are discussed in relation to the pathogenesis of renal lesions in systemic lupus erythematosus, chronic membranous glomerulonephritis, and amyloidosis.

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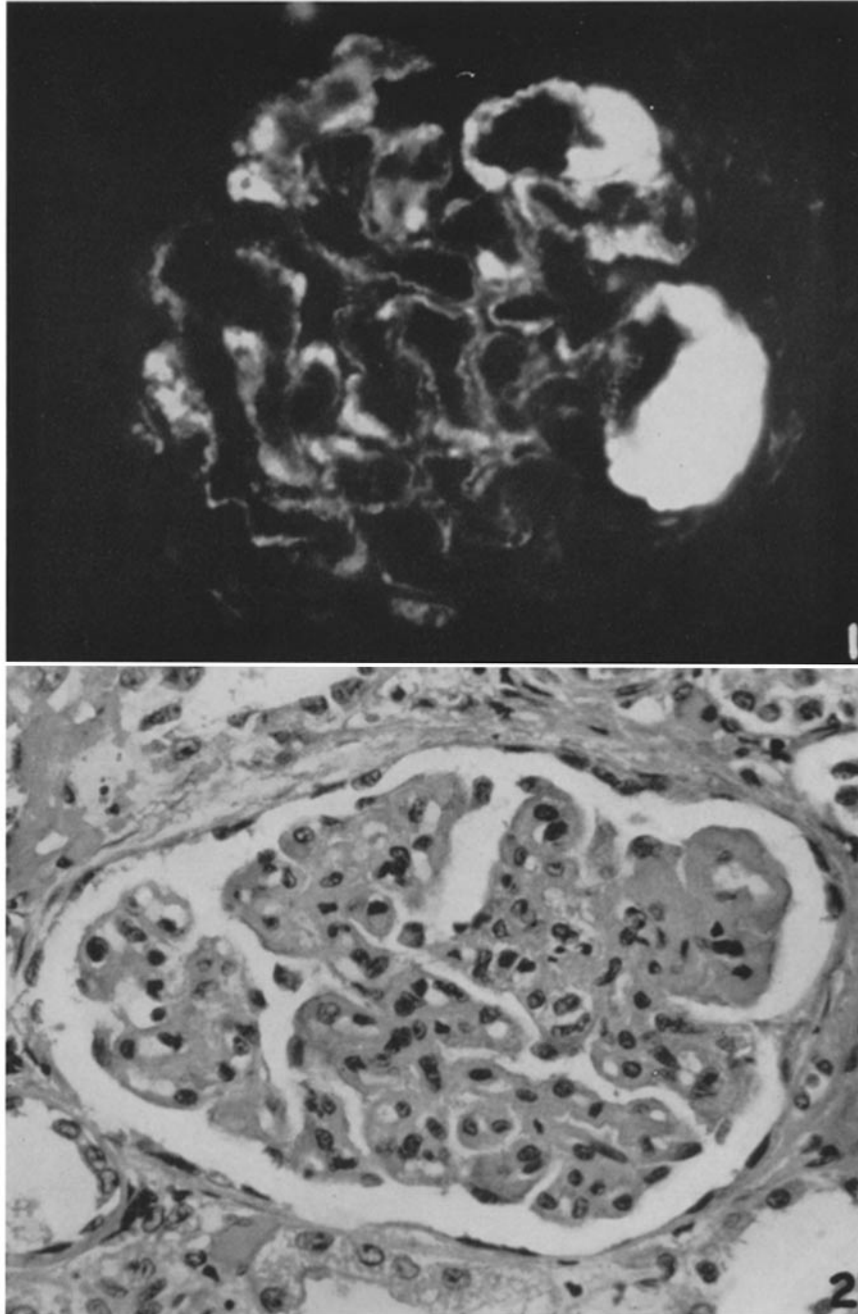
EXPLANATION OF PLATES

Plate 14

Fig. 1. Specific fluorescence of kidney section in systemic lupus erythematosus (case 1). White areas (apple-green in fluorescence microscope) indicate sites of γ-globulin localization in glomerular basement membranes and in two wire-loop lesions, present at 2 and at 4 o'clock. Frozen section, stained with fluorescent antibody for human γ-globulin. $\times$ 575.

Fig. 2. Hematoxylin-eosin stain of kidney section in systemic lupus erythematosus (case 1) showing thickened glomerular basement membranes, focally accentuated, with wire-loop lesion at 2 o'clock. Paraffin section. $\times$ 455.

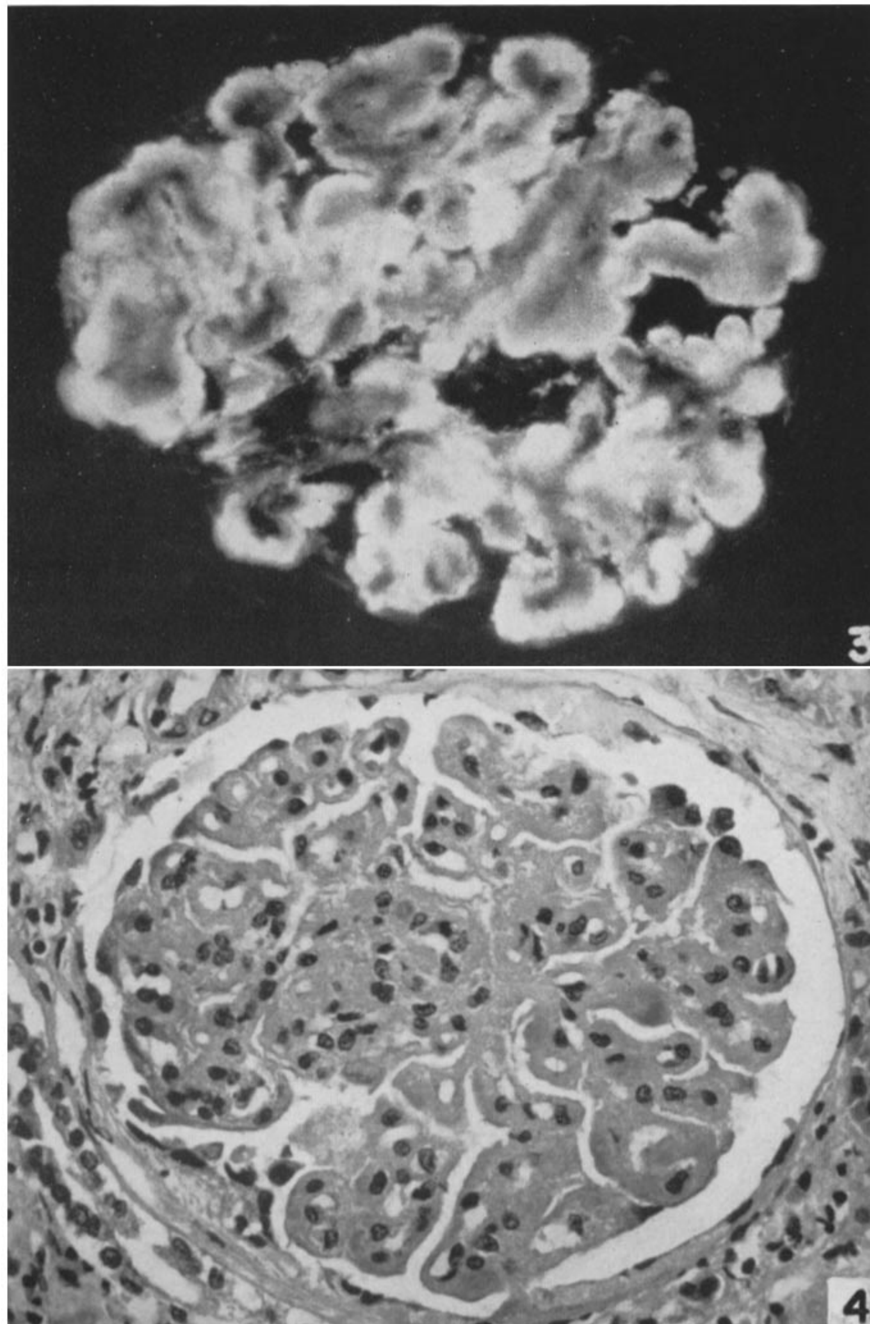


(Mellors *et al.*: Gamma globulins and pathogenesis of renal lesions)

PLATE 15

FIG. 3. Specific fluorescence of kidney section in systemic lupus erythematosus (case 1). γ-Globulin localization in diffusely thickened, glomerular basement membrane. Frozen section stained with fluorescent antibody for human γ-globulin. × 575.

FIG. 4. Hematoxylin-eosin stain of kidney section in systemic lupus erythematosus (case 1), showing diffuse thickening of glomerular basement membrane. Paraffin section, × 450.

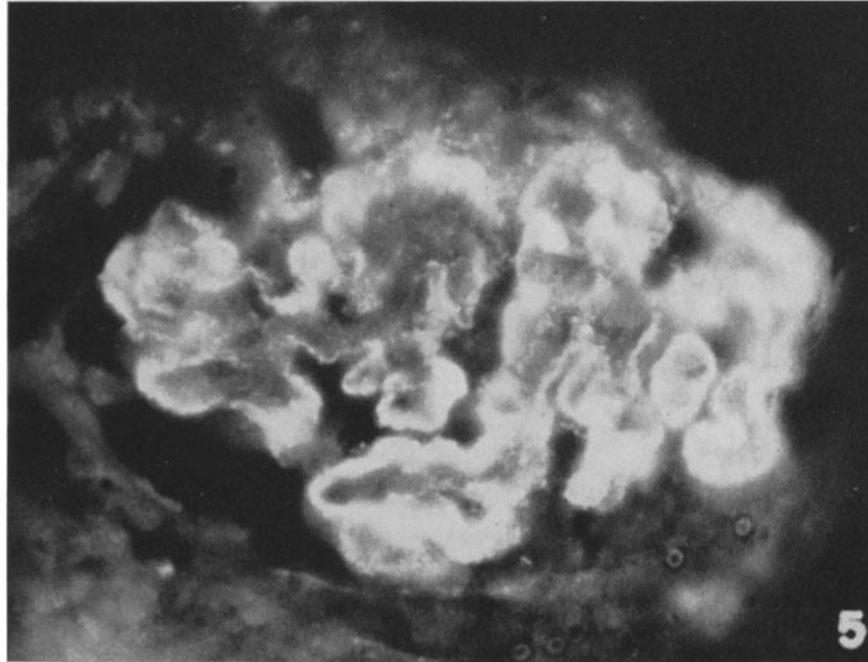


(Mellors *et al.*: Gamma globulins and pathogenesis of renal lesions)

PLATE 16

FIG. 5. Specific fluorescence of kidney section in systemic lupus erythematosus (case 2). γ-Globulin localization in thickened glomerular basement membrane. Frozen section stained with fluorescent antibody for human γ-globulin. $\times$ 525.

FIG. 6. Hematoxylin-eosin stain of kidney section in systemic lupus erythematosus (case 2) showing thickened glomerular basement membrane, focally accentuated. Paraffin section, $\times$ 425.



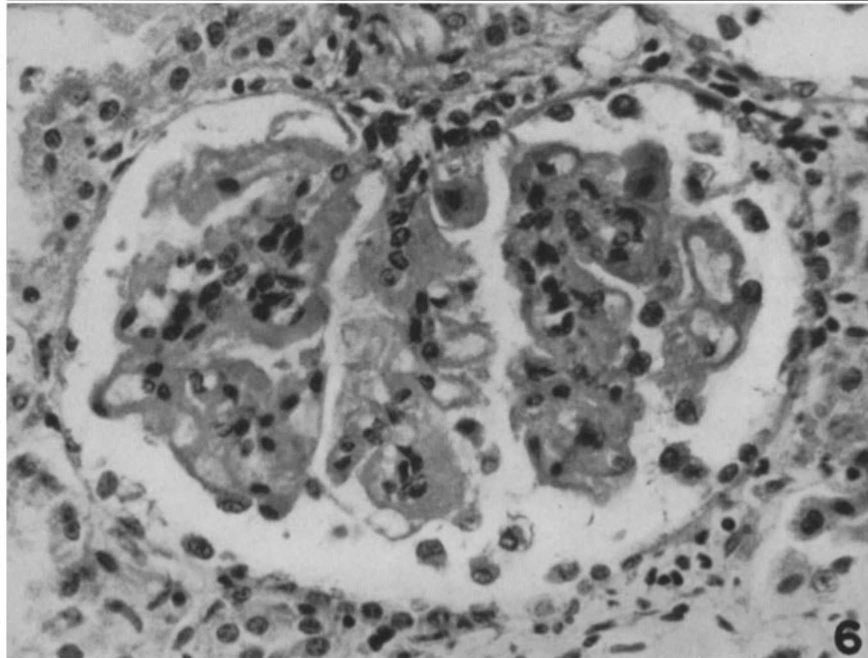


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Plate 17

Fig. 7. Specific fluorescence of kidney section in systemic lupus erythematosus (case 1). γ-Globulin localization in thickened glomerular basement membrane and also in large masses at 12 to 4 o'clock, the so called hyaline thrombi. Frozen section stained with fluorescent antibody for human γ-globulin. $\times$ 350.

Fig. 8. Periodic acid–Schiff (PAS) hematoxylin stain of kidney section in systemic lupus erythematosus (case 1) showing thickened (PAS-positive) glomerular basement membrane and so called hyaline thrombi, the latter at 2 to 4 o'clock. Paraffin section, $\times$ 500.

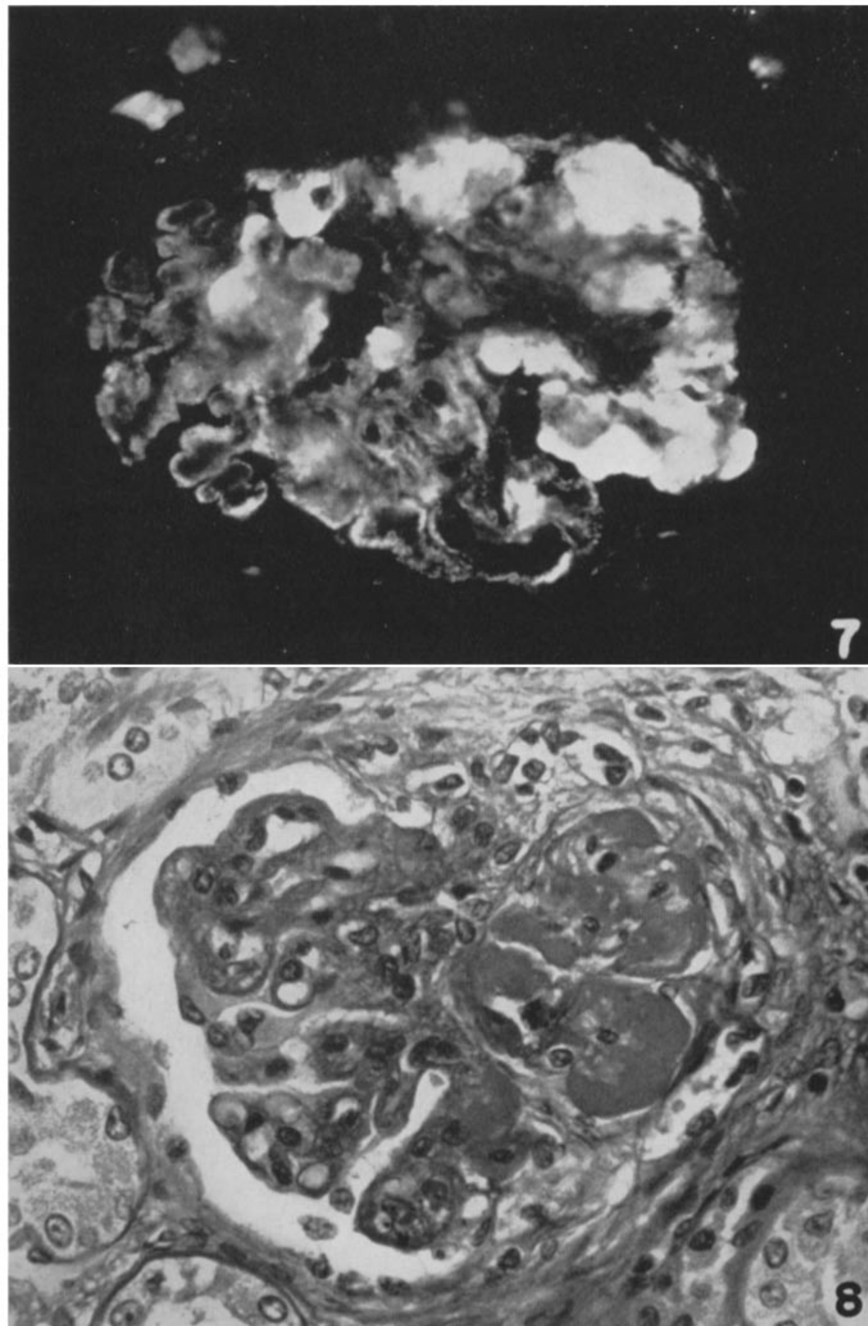

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PLATE 18

FIG. 9. Specific fluorescence of kidney section in systemic lupus erythematosus (case 1). γ-Globulin localized in thickened glomerular basement membrane and, as shown in transected capillary (arrow), occupying two, more or less parallel, circumferential sites consisting of an inner, granular discontinuous distribution and an outer, denser, membranous distribution. Frozen section stained with fluorescent antibody for human γ-globulin. $\times$ 450.

FIG. 10 A. Specific fluorescence of kidney section in systemic lupus erythematosus (case 1). γ-Globulin localization in capsular crescent at 8 to 11 o'clock as well as in glomerular foci, accentuated at 3 and 6 o'clock. Frozen section stained with fluorescent antibody for human γ-globulin. $\times$ 325.

FIG. 10 B. Hematoxylin-eosin stain of kidney section (case 1) showing cellular capsular crescent (with no evidence of fibrinoid) and thickened glomerular capillaries. Paraffin section, $\times$ 400.

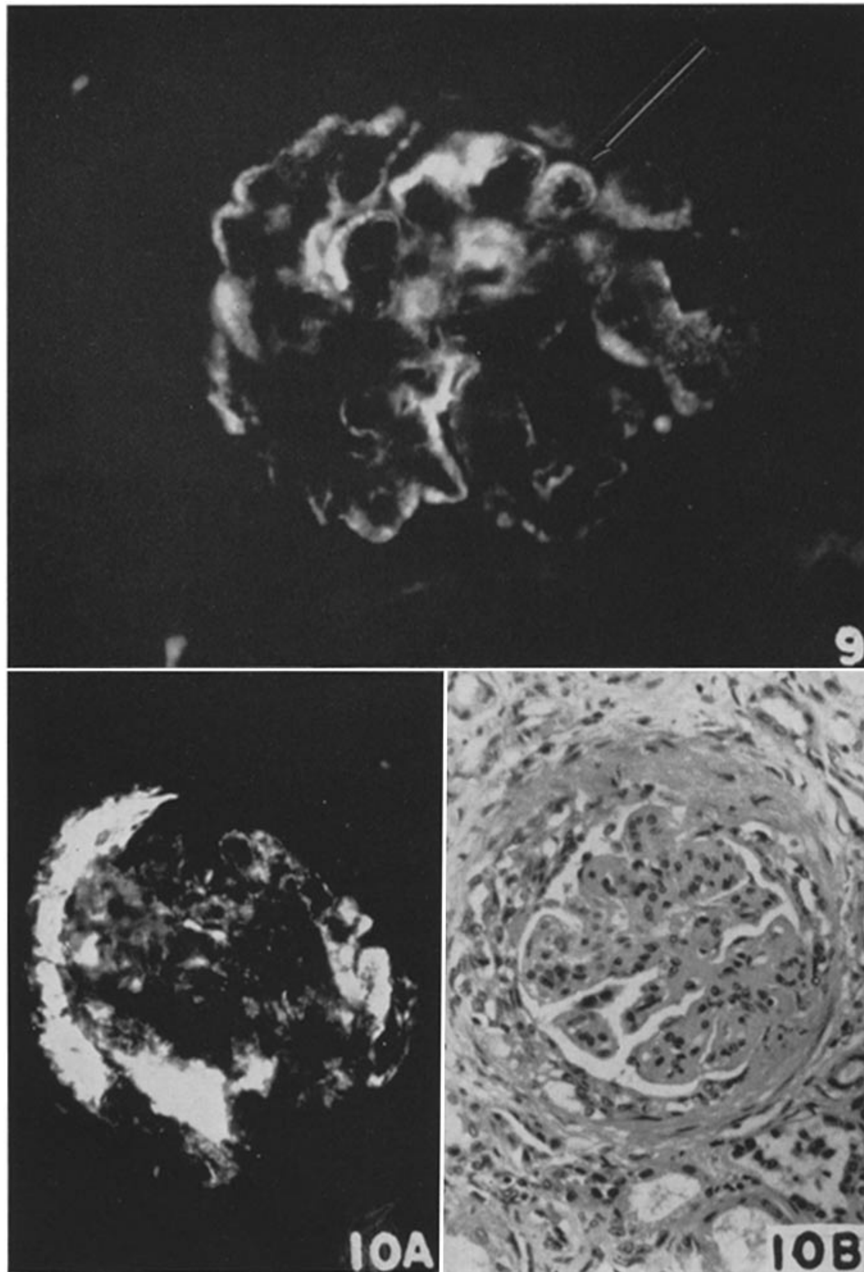

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Plate 19

Fig. 11. Specific fluorescence of kidney section in systemic lupus erythematosus (case 1) showing localization of γ-globulins in large cytoplasmic granules in tubular epithelium. Oval tubular lumen, large central black space; nuclei of tubular epithelium, small ovoid black impressions at outer circumference. Frozen section stained with fluorescent antibody for human γ-globulin. $\times$ 625.

Fig. 12. Specific fluorescence of kidney section in systemic lupus erythematosus (case 1) showing localization of γ-globulins in cytoplasm of interstitial inflammatory cells including immature and mature plasma cells. Frozen section stained with fluorescent antibody for human γ-globulin. $\times$ 750.

Fig. 13. Specific fluorescence of kidney section in systemic lupus erythematosus (case 1) showing localization of γ-globulins in four renal glomeruli. The fluorescence observed elsewhere in particles and in a tubular cast (right lower corner) is of the intrinsic type with a color readily distinguished in the fluorescence microscope from the specific apple-green. Frozen section stained with fluorescent antibody for human γ-globulin. $\times$ 80.

Fig. 14. The absorption of the specific stain (that used in Fig. 13) with purified human γ-globulin, in antigen excess, completely inhibits all the specific fluorescence of a kidney section in systemic lupus erythematosus (case 1). Several glomeruli were present in this field of illustration but, lacking fluorescence, they are invisible. Only the intrinsic fluorescence of a tubular protein cast and of the wavy internal elastic lamellae of a blood vessel is seen. Frozen section, $\times$ 125.

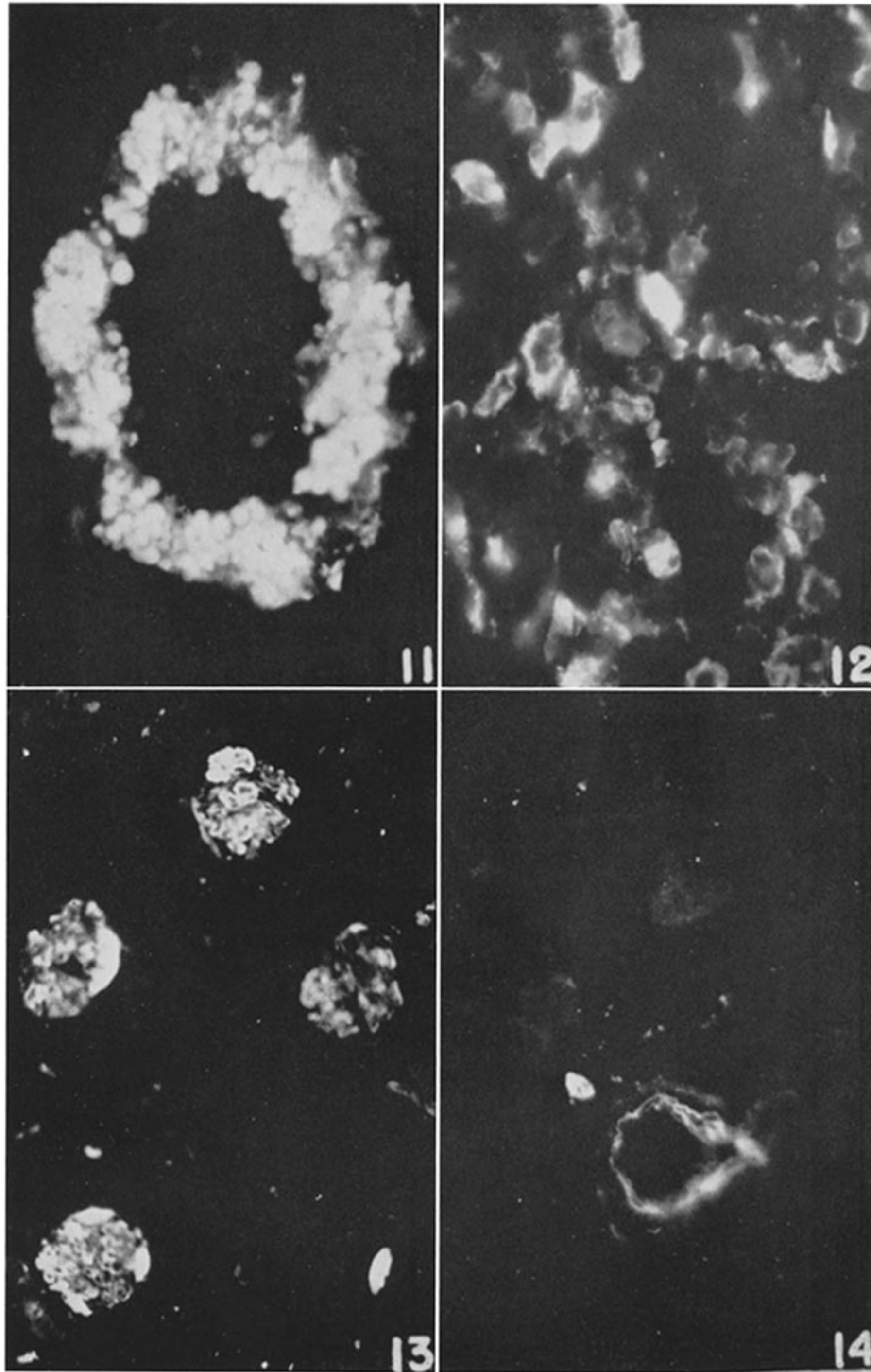

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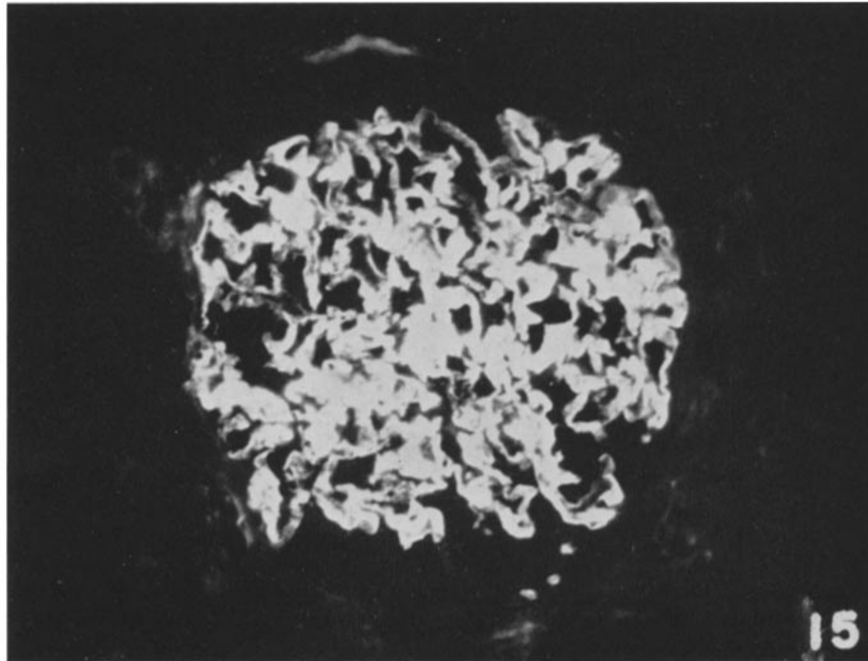
PLATE 20

FIG. 15. Specific fluorescence of kidney section in chronic membranous glomerulonephritis (case 3). γ-Globulin localization in diffusely thickened glomerular basement membrane. Frozen section stained with fluorescent antibody for human γ-globulin. $\times$ 340.

FIG. 16. Hematoxylin-eosin stain of kidney section in chronic membranous glomerulonephritis (case 3) showing diffusely thickened glomerular basement membrane. Paraffin section. $\times$ 390.



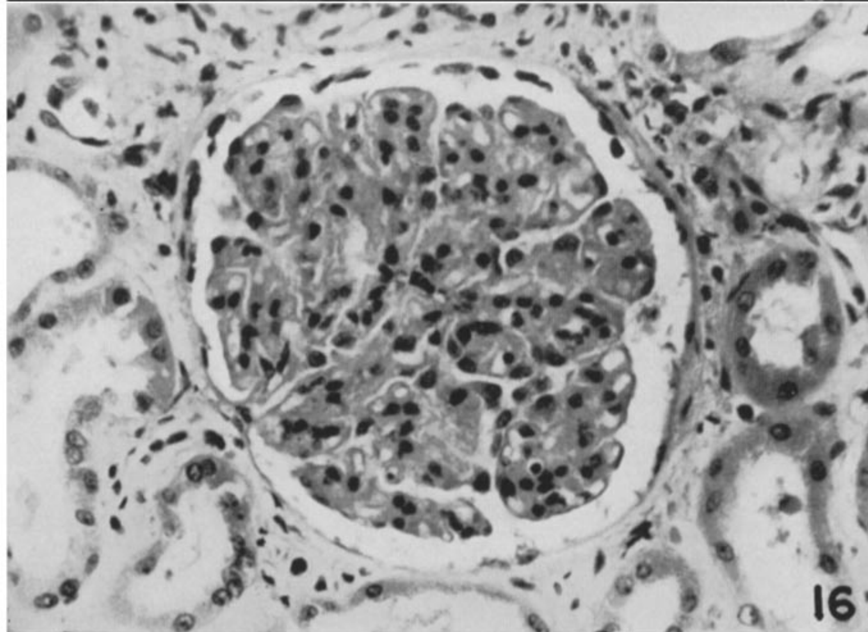


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PLATE 21

FIG. 17. The absorption of the specific stain (that used in Fig. 15) with purified human γ-globulin, in antigen excess, completely inhibits the specific fluorescence of a glomerulus (arrows at Bowman's capsule), representative of all glomeruli present, in a kidney section in chronic membranous glomerulonephritis (case 3), but only partially inhibits the specific fluorescence of some tubular protein casts (see Figs. 18 and 19). Frozen section. $\times$ 340.

FIG. 18 and 19. The absorption of the specific stain (that used in Fig. 15) with purified human γ-globulin, in antigen excess, only partially inhibits the apple-green fluorescence of some tubular protein casts (arrow) in chronic membranous glomerulonephritis (case 3). The protein casts presumably contain not only γ-globulin but also other serum proteins, these latter, including serum albumin, reacting with the residual stain. Frozen section. $\times$ 190.

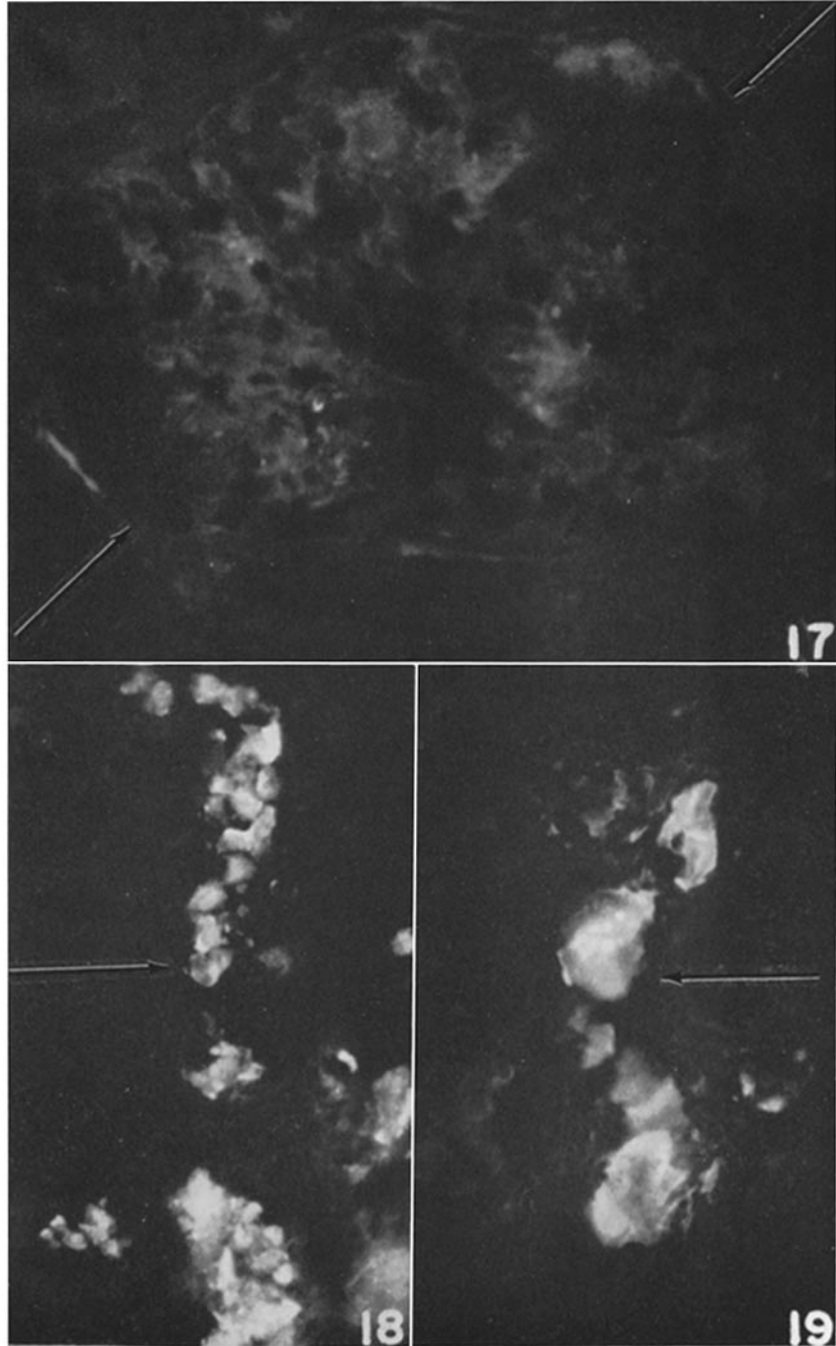

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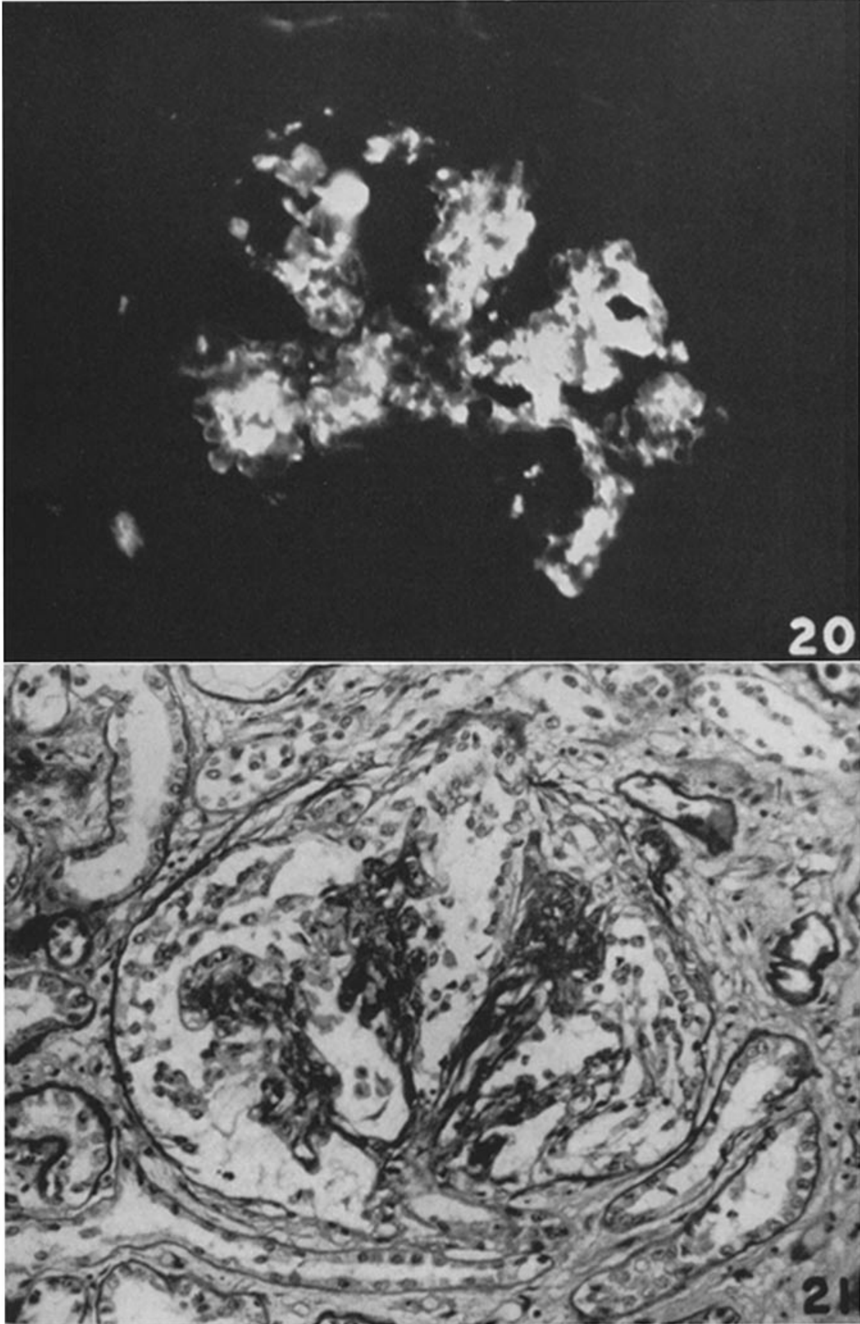
PLATE 22

FIG. 20. The specific fluorescence of a kidney section in chronic lobular glomerulonephritis (case 4). γ-Globulin localization in the central portions, presumably the mesangium, of several lobulated tufts of a glomerulus. Frozen section stained with fluorescent antibody for human γ-globulin. $\times$ 275.

FIG. 21. Periodic acid–Schiff stain of a kidney section in chronic lobular glomerulonephritis (case 4) showing accentuated lobular configuration of glomerulus and prominent PAS-staining mesangium. Paraffin section. $\times$ 230.



(Mellors *et al.*: Gamma globulins and pathogenesis of renal lesions)

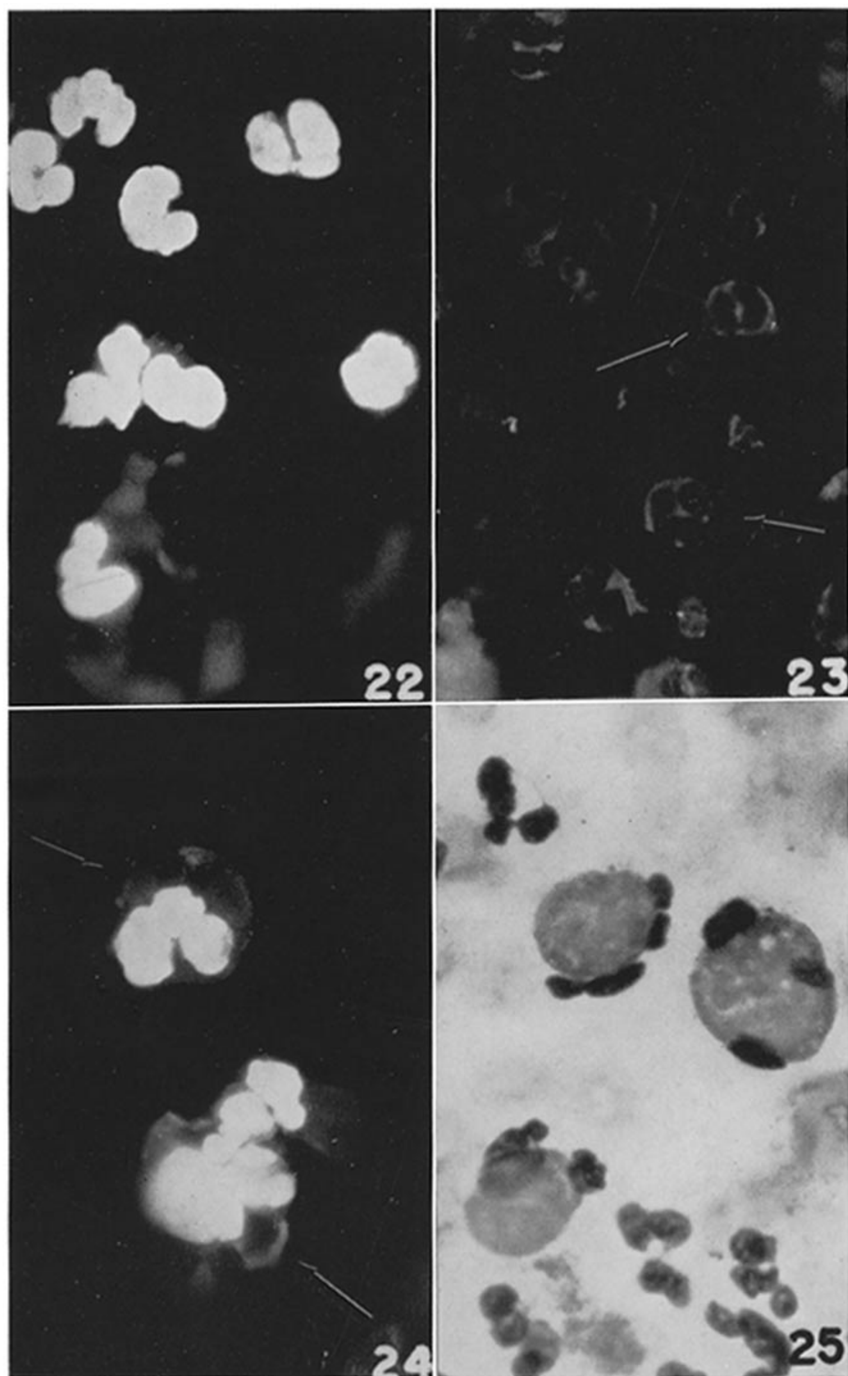
Plate 23

Fig. 22. Specific fluorescence of leukocytes in a positive lupus erythematosus preparation. γ-Globulin localization in the nuclei of leukocytes which are undergoing transformation prior to phagocytosis and formation of lupus erythematosus cells. Unfixed lupus erythematosus preparation treated with acetone for 10 minutes and stained with fluorescent antibody for human γ-globulin. $\times$ 500.

Fig. 23. Total absence of specific fluorescence in nuclei (arrows) of leukocytes in a negative lupus erythematosus preparation. Fluorescent antibody procedure identical with that used in Fig. 22. $\times$ 500.

Fig. 24. Specific fluorescence of leukocytes in a positive lupus erythematosus preparation. γ-Globulin localization in nuclei of leukocytes which are undergoing transformation and phagocytosis (arrows point to *non-fluorescent* nuclei of phagocytic cells) to form lupus erythematosus cells. Procedures identical with those used in Fig. 22. $\times$ 650.

Fig. 25. Wright's stain of a positive lupus erythematosus preparation (a companion to that shown in Figs. 22 and 24) showing lupus erythematosus cells, $\times$ 1100.



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