IMMUNOHISTOCHEMICAL ANALYSIS OF AMYLOID BY THE FLUORESCENCE TECHNIQUE*· ‡· §

By JACINTO J. VAZQUEZ, M.D., and FRANK J. DIXON, M.D.

(*Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh*)

Plates 50 to 55

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The pathogenesis of amyloidosis, usually considered a disease of disturbed metabolism or a degenerative process, remains unknown. Among several theories, that of an immunologic mechanism involving an antigen-antibody reaction has been postulated as a causative factor in the deposition of amyloid (1, 2). Although it has been rarely possible to test this postulation directly, the so called "fluorescent antibody" technique of Coons *et al.* (3, 4) does allow an approach to this problem since it makes possible an immunologic analysis of histological sections. Recently, observations of increased gamma globulin in the glomeruli of a case of amyloidosis have been reported, using this technique (5). However the specific localization of gamma globulin in the amyloid deposits was not described.

In the present study we have analyzed the amyloid deposits of secondary human and experimental amyloidosis in the rabbit for their homologous albumin and gamma globulin content, on the assumption that if an antigen-antibody reaction was responsible for the formation of amyloid, one might expect to find a specific concentration of gamma globulin within the deposits.

Materials and Methods

Purified human plasma fraction II;[1] human plasma fraction V;[2] bovine plasma fraction II;[3] crystallized bovine plasma fraction V[4] and rabbit plasma fractions II and V[5] were used for animal immunizations. Anti human gamma globulin and albumin (anti HGG and anti HSA) and anti bovine gamma globulin and albumin (anti BGG and anti BSA) were obtained after injecting albino rabbits averaging 3½ kilos according to the following schedule: On

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[1] Polymyelitis immune globulin. Lederle Laboratories C-420, Pearl River, New York.

[2] Armour and Co., lot 31796. Chicago, Illinois.

[3] Armour and Co., lot C-904.

[4] Pentex Inc., lot B-1205. Kankakee, Illinois.

[5] Pentex Inc.

days 1 and 2 the animals received 10 mg. of antigen subcutaneously. On days 3 and 4 the dose was increased to 20 mg. and on day 5 each animal received 30 mg/kilo intravenously. After 2 weeks the same course was given and repeated thereafter as needed. Anti rabbit gamma globulin and albumin (anti RGG and anti RSA) were obtained by immunization of a sheep with antigen in Freund adjuvant containing tubercle bacilli, according to the following schedule: One initial dose of 37.5 mg. was given intramuscularly. One month later the animal received 50 mg. and this dose was repeated thereafter at 1 month intervals as needed, always by the same route. The anti HGG, anti BGG, and anti RGG contained approximately 700 μg. of antibody N/ml. and the anti HSA, anti BSA, and anti RSA contained above 1 mg. of antibody N/ml. The globulins to be labelled were obtained by fractionation of antisera at 5°C. with equal volumes of saturated ammonium sulfate (pH adjusted to 7.0). This fractionation was repeated 3 times to remove hemoglobin from the precipitate. The precipitate was then reconstituted to ⅓ of the volume of the original serum and dialyzed against non-buffered saline until $(NH_4)_2SO_4$-free. Merthiolate (1/10,000) was added and the protein solutions were stored frozen until ready for conjugation with fluorescein isocyanate.

Characterization of Antisera.—Protein solutions before and after conjugation with fluorescein were immunologically characterized by the precipitin reaction both in fluid and gel media, the latter according to the "agar diffusion" technique of Ouchterlony (6). It was found that the precipitin reaction of fluorescein-labelled antisera in saline was inconsistent and sometimes only evident when complement was added to the system (7). However, specific precipitation in agar was obtained with conjugates, giving qualitative evidence of immunologic activity in the protein solutions employed. It has been previously found (4, 8) that the fluorescein label does not alter the immunologic and physical properties of the antibody molecule. With the agar diffusion technique it was found that at the single concentration employed, no cross-reaction occurred between HGG and anti RGG, or anti BGG, or anti HSA. The anti HGG serum contained 95 per cent anti HGG and 5 per cent anti beta globulin. No anti alpha globulin or anti albumin were detectable. Anti RGG serum was less pure, after routine absorption with RSA prior to conjugation it contained approximately 91 per cent anti RGG and 9 per cent anti beta globulin. Again, no anti alpha globulin was detectable.

Preparation of Tissue Material and "Fluorescent Antibody" Technique.—Fresh tissues were obtained promptly at autopsy, cut in small thin pieces (up to 1 $cm.^2$) and placed against the walls of stoppered test tubes. Tubes were plunged in a mixture of dry ice and butanol to obtain a quick freezing of the tissues in order to avoid histologic distortion. The material was then stored from a few days to several months in a deep freeze compartment (minus 20°C.) until ready for sectioning.

Sections, 5 to 7 μ in thickness, were cut in a refrigerated cabinet (minus 20°C.) with a rotary Minot microtome. They were mounted on clean slides and the following fixation to prevent inactivation of tissue antigens was employed: Slides were immersed in absolute alcohol (ethanol)-ether solution (50/50) for 10 minutes. Then they were transferred to 95 per cent ethanol at 37°C. for 20 minutes. After this they were washed in buffered saline for 10 minutes (two changes—5 minutes each). The slides were then ready for immediate staining with the fluorescent conjugates.

Tissues of human cases to be reported included liver, spleen, adrenals, and kidneys from two cases of amyloidosis secondary to chronic active pulmonary tuberculosis (cases 1 and 2), and liver and spleen from an additional case of amyloidosis secondary to chronic pyelonephritis and extensively infected decubitus ulcers (case 3). Experimental amyloidosis was produced in rabbits by injections of either casein or ribonucleic acid. Two animals received daily 5 cc. subcutaneous injections of casein (10 per cent casein-0.25 per cent NaOH, pH 7.0) for 56 days. Two animals received daily 10 cc. subcutaneous injections of ribonucleic acid (8 per cent RNA-0.05 M phosphate, pH 7.2) for 60 days. At the end of this period they were sacrificed and most of the viscera were obtained for study.

The isomer II of aminofluorescein was prepared by Dr. P. Maurer according to the method of Coons *et al.* (4). The procedure for conversion to fluorescein isocyanate and conjugation with antibody was identical to that described by Coons. A slight modification of the technique was followed in the purification of fluorescein conjugates. Dialysis of the conjugates, 4 to 10 days, against phosphate buffered saline (pH 7.0) at 5°C. was employed to remove the non-conjugated fluorescein from the solution. However, it was found that in spite of prolonged dialysis, non-conjugated fluorescein was still present. In order to eliminate the non-conjugated fluorescein more completely and to save time, conjugates were precipitated at 0°C. to minus 2°C. with absolute ethanol at a final concentration of 35 per cent and centrifuged in the cold. Usually three resolutions and reprecipitations were needed to obtain a supernatant free of fluorescein. After final resolution the conjugates were dialyzed for 24 hours to remove the ethanol. Further purification of the conjugates was accomplished by absorption with acetone-dried liver powder, mainly bovine, except when anti bovine antisera were used, although no significant differences were noted when preparations from the livers of other species were employed. The conjugates were absorbed twice, for ½ hour at room temperature with mechanical shaking and cleared by high speed centrifugation.

One or two drops of the conjugate were then placed on the prepared section and the slide was covered with a Petri dish containing wet filter paper to prevent evaporation. After 30 minutes, the conjugate was removed from the section by tilting the slide and the section washed in 4 changes of buffered saline with gentle agitation for 10 minutes. The section was immediately mounted with a clean coverslip and the slides were stored at 5°C. until microscopic observation. When non-fluorescent antiserum was used prior to the specific staining (see staining scheme) the non-fluorescent protein solution was applied for 30 minutes, then washed in 4 changes of buffered saline with gentle agitation. The fluorescent conjugate was then applied for 10 minutes, according to the staining procedure of Coons *et al.* (4).

Microscopy and Photography.—The source of light for ultraviolet microscopy was a water-cooled, high pressure mercury lamp[6] with a high light emission in the near ultraviolet and blue bands and a light intensity of 64,000 lumens. A standard binocular Zeiss-Winkel microscope with bright field condensers was used for visual observation with an interchangeable monocular tube for photographic recording. The cross-filter technique for ultraviolet microscopy was employed with several filter combinations for observation of different fluorescence in tissues. One filter combination consisted of BG 12 and UG 2 Schott exciter filters[7] with the addition of a blue heat-resisting filter[7] between the source of ultraviolet light and the condensers, and BG 23 and GG4 Schott barrier filters.[7] This combination made possible the observation of the blue autofluorescence of tissues as well as the specific bright green fluorescence given by the fluorescent stain. This system was used mainly for visual observation and color photography. The other combination consisted of a blue filter of standard thickness (Corning CS5-58) at the source of light and a yellow protective filter (Corning CS3-69) at the ocular. This system permitted a greater amount of exciting ultraviolet light to pass to the specimen and was used mainly for photographic recording. With this latter filter system, tissue autofluorescence was yellow in contrast with specific green-yellow fluorescence of the stain.

Exact duplicate photographic exposure, developing and printing were done on experimental and control sections.

Control Studies for the Specificity of Results.—The interpretation of findings of serum protein concentrations in tissue lesions is difficult since specific localization of a given protein must be differentiated from any non-specific outpouring or accumulation of serum proteins in zones of inflammation or degeneration. For this reason great emphasis has been placed on control

[6] Scopicon, Inc. Chauncey, New York.

[7] Zeiss-Winkel equipment for fluoroscence microscopy.

observations and all lesions revealing concentrations of gamma globulin have also been analyzed for their albumin content in an attempt to rule out non-specific localization of serum proteins. The following staining scheme was employed in each case.

1. One section was examined unstained for the presence of autofluorescence.

2. One section was stained with specific fluorescent antiserum for the antigen under study, either gamma globulin or albumin.

3. Additional sections were stained with heterologous fluorescent antisera (anti HGG, anti HSA, anti RGG, anti RSA, anti BGG, and anti BSA, according to the tissue antigen under study).

4. One section was flooded with non-fluorescent antiserum prior to the staining with fluorescent antiserum, to saturate the antigen in the tissue.

TABLE I

Staining Scheme and Typical Results with Specific Fluorescence Due to Human Gamma Globulin in Tissues

	Treatment of tissue sections							
	1	2	3			4	5	6
	Unstained	Fluorescent anti HGG	Fluorescent anti RGG	Fluorescent anti BGG	Fluorescent anti HSA	Non-fluorescent anti HGG fluorescent anti HGG	Complex HGG and Fluorescent anti HGG	Pretreated 40 per cent formal
Gamma globulin containing lesion	0*	1 + 2	0	0	3	0	0	4
Comparable normal tissue	0	1	0	0	3	0	0	4

* 0, Normal tissue autofluorescence; 1, Specific fluorescence for human gamma globulin normally present in tissues; 2, Specific fluorescence for increased human gamma globulin in lesions; 3, Specific fluorescence for albumin normally present in tissue; 4, Increased tissue autofluorescence.

5. One section was stained with complexes of antigen-fluorescent antibody (labelled antiserum absorbed with small increments of homologous antigen).

6. One section was treated with 40 per cent formalin for 30 minutes prior to the specific staining to modify the immunologic properties of the protein antigen.

7. Sections of comparable normal tissues were stained identically to those under study.

The expected results obtained with this staining scheme are illustrated in Table I.

Quantitative Studies.—Attempts to quantitate the tissue antigen-fluorescent antibody reaction were undertaken using I^{131} as a second label for the antibody which could be quantitatively determined throughout the reaction. For this purpose, fluorescent anti-HGG was lightly labelled with I^{131} (1 atom of iodine or less per molecule of protein) according to a modification of the method employed by Talmage *et al.* (9). During iodination, about $\frac{1}{5}$ of the globulin became insoluble at a neutral pH and was discarded. This did not impair subsequent observations of stained sections under ultraviolet microscopy.

Once the antibody solution was doubly labelled (fluorescein plus I^{131}) it was used as a specific immunologic stain in the same manner as described before. Sections thus stained were washed and allowed to dry and then placed in the beta counter for radioactivity measurements. All sections, including those of normal tissue control were of approximately equal area

and cut at the same thickness in order to permit a comparable estimate of the amount of radioactivity attached to normal and amyloid sections.

Radioautographs were also made in order to compare areas of high radioactivity with those of amyloid deposits. Using medium contrast lantern slides, optimum exposures were made when the product of micromicrocuries contained in the tissue section multiplied by the number of hours of exposure equaled 250.

RESULTS

In all three cases of human amyloidosis as well as in experimental amyloidosis, the diagnosis was made on paraffin sections stained with hematoxylin-eosin (Figs. 1, 5, 7, 11, 14, 16). Congo red stains were positive in sections of human amyloidosis but negative in sections of experimental animals. The extent of involvement of various organs in these cases is given in table II.

TABLE II

Localization of Amyloid Deposits

	Spleen	Liver	Kidney	Adrenal
Human (case 1)	+++	+++	+++	++
Human (case 2)	+++	+	+++	++
Human (case 3)	+++	+++	*	*
Rabbit (RNA)	++	0	0	0
Rabbit (casein)	+	0	0	0

Degree of amyloid deposition graded from +++ extensive to + slight and 0 absent.
* Not available.

Hyperglobulinemia was present in all human cases but no electrophoretic studies were available. Paper strip electrophoresis was done on serum of experimental animals. Those receiving RNA showed an average of 10 gm/100 ml. total serum protein with 30 per cent albumin; 18 per cent alpha globulin; 19 per cent beta globulin; and 33 per cent gamma globulin. Those receiving casein showed an average of 13 gm./100 ml. total serum protein with 21 per cent albumin; 16 per cent alpha globulin; 14 per cent beta globulin; and 49 per cent gamma globulin. In comparison, normal rabbit serum controls showed an average of 6.2 gm./100 ml. total serum protein with 60 per cent albumin; 10 per cent alpha globulin; 15 per cent beta globulin; and 15 per cent gamma globulin.

Observations of Fluorescence.—When sections of human cases were examined unstained with ultraviolet light, it was seen that the amyloid deposits showed a slight degree of autofluorescence, most evident in glomerular deposits in the kidneys (Fig. 3). No autofluorescence of amyloid was found in the rabbits. The amyloid deposits in all human tissues studied when stained with fluorescent anti human gamma globulin showed a bright green fluorescence specific for human gamma globulin. This fluorescence was sharply limited to the amyloid

deposits. Cell nuclei found within the amyloid stood out clearly by virtue of their failure to fluoresce (Figs. 2, 6, 8, 9, 12, 15). In all three human cases the amyloid in spleen stained brighter than that in liver, kidney, or adrenal. When similar sections were stained with fluorescent anti human albumin, they showed only a faint green specific fluorescence, indicative of small amounts of albumin in the amyloid deposits (Figs. 4, 10). No specific fluorescence was found when sections were stained with heterologous anti gamma globulins. The specific fluorescence was greatly diminished and in some instances abolished completely

TABLE III

Radioactivity Measurements in Sections of Spleen and Kidney from Amyloid Case 2; Liver from Amyloid Case 1 and Normal Control Tissues after Stain with I^{131}- Labelled Fluorescent anti HGG

Tissue	Stain	Beta counts per min.	Per cent increase over normal	Per cent inhibition
Amyloid liver (case 1)	I^{131} fluorescent anti HGG	8230	55	—
Amyloid liver (case 1)	Inhibited*	1974	—	76
Normal liver	I^{131} fluorescent anti HGG	5284	—	—
Normal liver	Inhibited*	1940	—	63
Amyloid spleen (case 2)	I^{131} fluorescent anti HGG	7655	64	—
Amyloid spleen (case 2)	Inhibited*	2756	—	64
Normal spleen	I^{131} fluorescent anti HGG	4660	—	—
Normal spleen	Inhibited*	2445	—	48
Amyloid kidney (case 2)	I^{131} fluorescent anti HGG	12659‡	105	—
Amyloid kidney (case 2)	Inhibited*	3198	—	74
Normal kidney	I^{131} fluorescent anti HGG	6170	—	—
Normal kidney	Inhibited*	2632	—	58

* Non-fluorescent anti HGG prior staining with I^{131} fluorescent anti HGG.

‡ The presence of tubular casts containing gamma globulin might have accounted in part for the high radioactivity measurements in this instance.

when non fluorescent anti HGG was used prior to specific staining with fluorescent anti HGG (Fig. 13). Similar results were obtained when sections were treated with 40 per cent formalin prior to the specific staining or were stained with complexes of antibody and corresponding antigen.

The same results as those in human cases were obtained when sections of amyloid containing spleens of rabbits were stained with the corresponding fluorescent antibody (anti RGG). In these cases a bright green fluorescence was noted in a perifollicular arrangement (Figs. 17, 18) corresponding to the amyloid deposits seen in hematoxylin-eosin sections, indicating a concentration of gamma globulin in these areas. All control stains in these cases showed practically complete inhibition of specific fluorescence (Fig. 19).

Results of Quantitative Studies.—A representative example of a preliminary study on the quantitation of I^{131}-labelled fluorescent anti HGG fixed in tissue sections is shown in Table III.

According to radioactivity determinations liver sections containing amyloid took up slightly more than one and a half times the amount of anti HGG that was taken up by comparable sized sections of normal liver; sections of spleen containing amyloid took up about one and two-thirds times as much anti HGG as did control sections and kidney sections containing amyloid a little more than twice as much as was taken up by control sections. This corresponded approximately to the histologic estimation of amyloid in these organs. When sections containing amyloid were inhibited with non-fluorescent anti-HGG prior to the specific stain, there was a two-thirds to three-fourths reduction in fixation of radioactivity. With control sections the use of non-fluorescent antisera resulted in a reduction of about one-half to two-thirds.

DISCUSSION

The protein nature of "amyloid" has long been recognized although its exact chemical composition has not been established.

Hass *et al.* (10, 10*a*, 10*b*) showed that amyloid is composed of a matrix of protein plus a polysaccharide similar to chondroitin sulfate. Carnes and Forker (11) and Wagner (12) are of the opinion that the mucopolysaccharide component of amyloid differs from chondroitin sulfate. Wagner (12) in a chemical and electrophoretic study of the protein composition of amyloid in two human cases, showed that in one case it was composed of alpha globulin and in the other of alpha and beta globulins, both with an associated mucopolysaccharide component. Although we cannot rule out the possibility of the presence of globulins other than gamma globulin in amyloid, we feel that the method employed in the present work is more direct and sensitive in regards to histochemical analysis of amyloid than that in which extraction from tissues and chemical and electrophoretic analysis are employed. Giles and Calkins (13) studied the chemical composition of a relatively pure amyloid deposit in the liver of a patient with secondary amyloidosis, concluding that it was probably composed of a mixture of predominantly a hydrophilic protein, glycoprotein, and polysaccharide. They stated that the carbohydrate fraction could not represent more than 4 per cent of the solid material.

Our findings are in agreement with those citing a protein character of amyloid, particularly those demonstrating its globulin nature, and suggest that gamma globulin might represent one of the major components of amyloid.

No attempt has been made to review the different theories on the etiology and pathogenesis of amyloidosis. Extensive reviews on this subject have been made elsewhere (2–14). The finding of gamma gobulin in amyloid deposits raises the following possibilities: (a) This finding might be due to an artefact in which there is a non-specific deposition of fluorescent antiserum in altered

tissue. (*b*) It might represent a non-specific exudation of plasma proteins in affected tissues similar to that occuring in inflammation. (*c*) It might represent a local non-immunologic accumulation of circulating gamma globulin. (*d*) It might represent a specific combination of tissue-fixed antibody with circulating antigen, or the combination of antibody with antigen fixed in the tissues.

(*a*) The first possibility has been ruled out in these studies by the control observations. However, conditions in which there were tissue alterations morphologically similar to amyloid were also studied. It was found that the hyaline "corpora amylacea" in prostate did not contain demonstrable gamma globulin. Likewise, necrotic tissue from cases of acute myocardial infarction and cortical renal necrosis failed to reveal the presence of gamma globulin. Hyaline in myocardial fibrosis and arteriolonephrosclerosis, etc. also failed to reveal the presence of gamma globulin. On the other hand, the so called "paramyloid" in a lymph node from a case of rheumatoid arthritis showed concentrations of gamma globulin.

(*b*) The possibility that the globulin concentration in amyloid represents a non-specific exudation of serum protein due to increased capillary permeability was ruled out by a comparison of the observations in amyloid with the relative concentrations of gamma globulin and albumin in acute inflammatory lesions, using the fluorescent antibody technique. In contrast to the high globulin–low albumin concentrations found in amyloid the acute inflammatory lesions showed a greater concentration of albumin than globulin as might have been expected with increased vascular permeability in view of the smaller molecular size of the albumin.

(*c*) That the globulin localization might represent a metabolic deposition of circulating gamma globulin is a possibility since the circulating globulin levels were elevated in these cases. In this connection it should be mentioned that an increase of plasma gamma globulins has been found at the very beginning of amyloid deposition; however during the course of the disease the gamma globulin levels may decrease (15). Eisen (16) also called attention to the fact that in several cases of myeloma with amyloidosis there was hyperglobulinemia early in the disease with a fall in the globulins later, in contrast to uncomplicated myeloma cases in which the hyperglobulinemia persisted throughout the disease. In line with these observations, we have found a higher level of serum gamma globulin in rabbits receiving casein than in those receiving RNA while the degree of amyloidosis was more pronounced in those animals given RNA. It might be postulated that the relative decrease of plasma gamma globulin in the presence of deposition of amyloid results from sequestration of circulating gamma globulin into the amyloid deposits. This is in agreement with our observations on the fate of the injected radiolabelled gamma globulin in rabbits with experimental amyloidosis in which a significant decrease in the half-life of the injected globulin occurs (17). In this study control rabbits had a gamma globu-

lin half-life of 4.7 days while rabbits which received RNA and developed amyloid showed gamma globulin half-lives averaging 3.9 days.

(*d*) It is also possible that the concentration of gamma globulin in amyloid is the result of an immunologic mechanism, resulting from a specific combination of circulating antibody with antigen in the tissues (1) or to an antigen-antibody reaction occurring at the site of antibody formation, mainly plasma cells and cells of the reticulo-endothelial system, as suggested by Latvalahti (18). Teilum (19, 20) also considers amyloid as an expression of an immunologic reaction. The present study does not establish an antigen-antibody reaction as the cause of formation of amyloid but is consistent with this hypothesis. Further studies are needed to demonstrate specifically that the localized globulin is indeed antibody.

CONCLUSION AND SUMMARY

The immunohistochemical composition of amyloid deposits in secondary human amyloidosis and experimental amyloidosis in rabbits was studied by means of the "fluorescent antibody" technique of Coons *et al.* Quantitative studies of the relative amounts of gamma globulin present in the amyloid deposits by the use of radioiodinated fluorescent antibody are reported. It is concluded that amyloid deposits in several organs from cases of secondary human amyloidosis and experimental amyloidosis in rabbits contain considerable concentrations of gamma globulin. The presence of gamma globulin in amyloid might be interpreted as either a metabolic deposition of circulating globulin present in high concentrations in the plasma or as a result of an immunologic reaction involving antigen and antibody.

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EXPLANATION OF PLATES

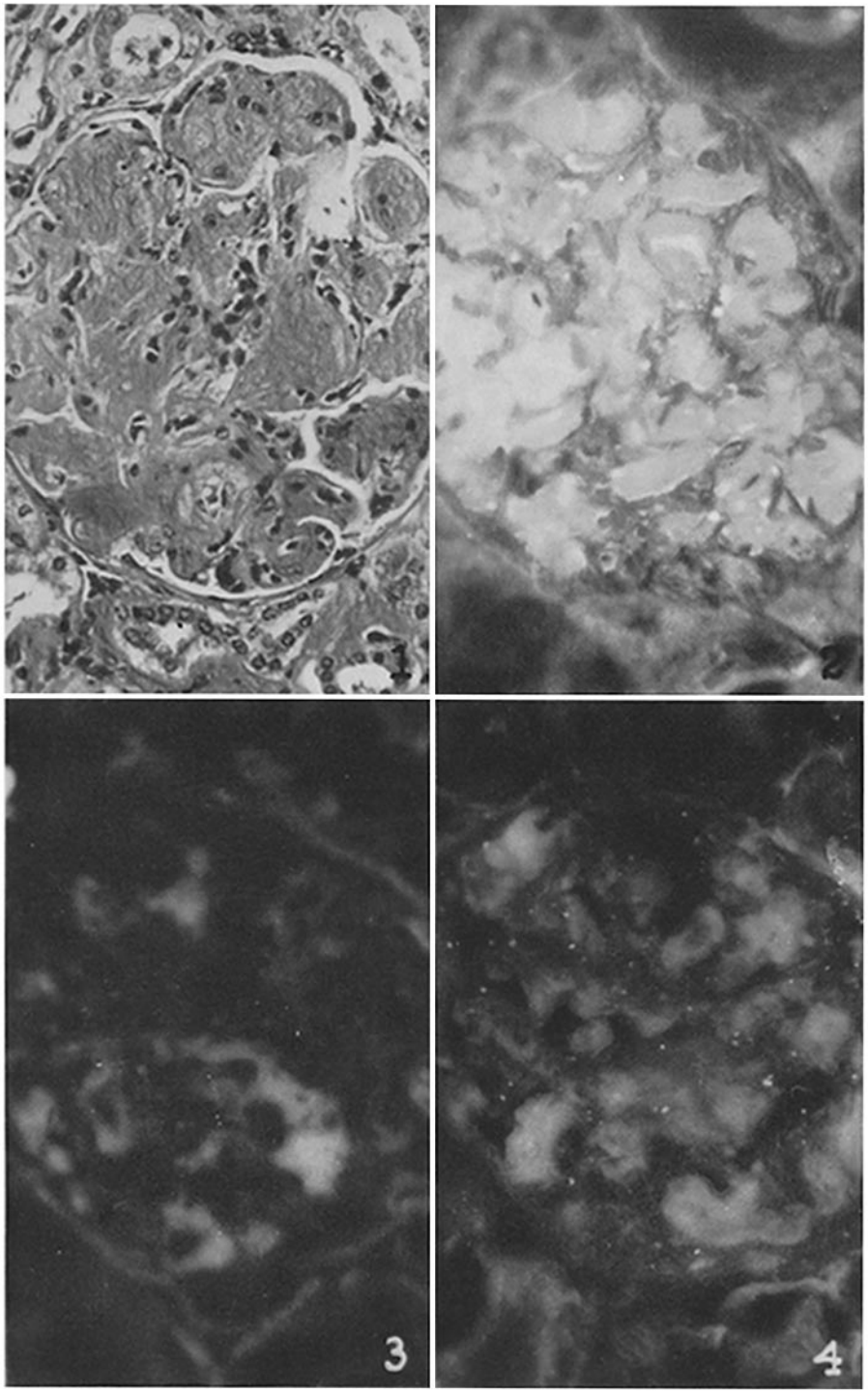
PLATE 50

FIG. 1. Section of kidney of human case 1. One glomerulus showing abundant deposition of amyloid in the capillary tuft. Hematoxylin-eosin. × 205.

FIG. 2. Similar section of kidney from human case 1 stained with fluorescent anti HGG. Note intense specific fluorescence in aggregate form corresponding to the amyloid deposits seen in Fig. 1 indicating the presence of gamma globulin within the deposits. Fluorescent micrograph. × 216.

FIG. 3. Section of kidney similar to that of Fig. 2, unstained, to show autofluorescence of some of the amyloid deposits. Fluorescent micrograph. × 216.

FIG. 4. Similar section of kidney to that of Fig. 2 stained with fluorescent anti HSA. Note some specific fluorescence of amyloid deposits indicating the presence of small amounts of albumin. Compare the degree of fluorescence with that in Fig. 2 for estimation of relative amounts of gamma globulin and albumin within the amyloid deposits. Fluorescent micrograph. × 216.

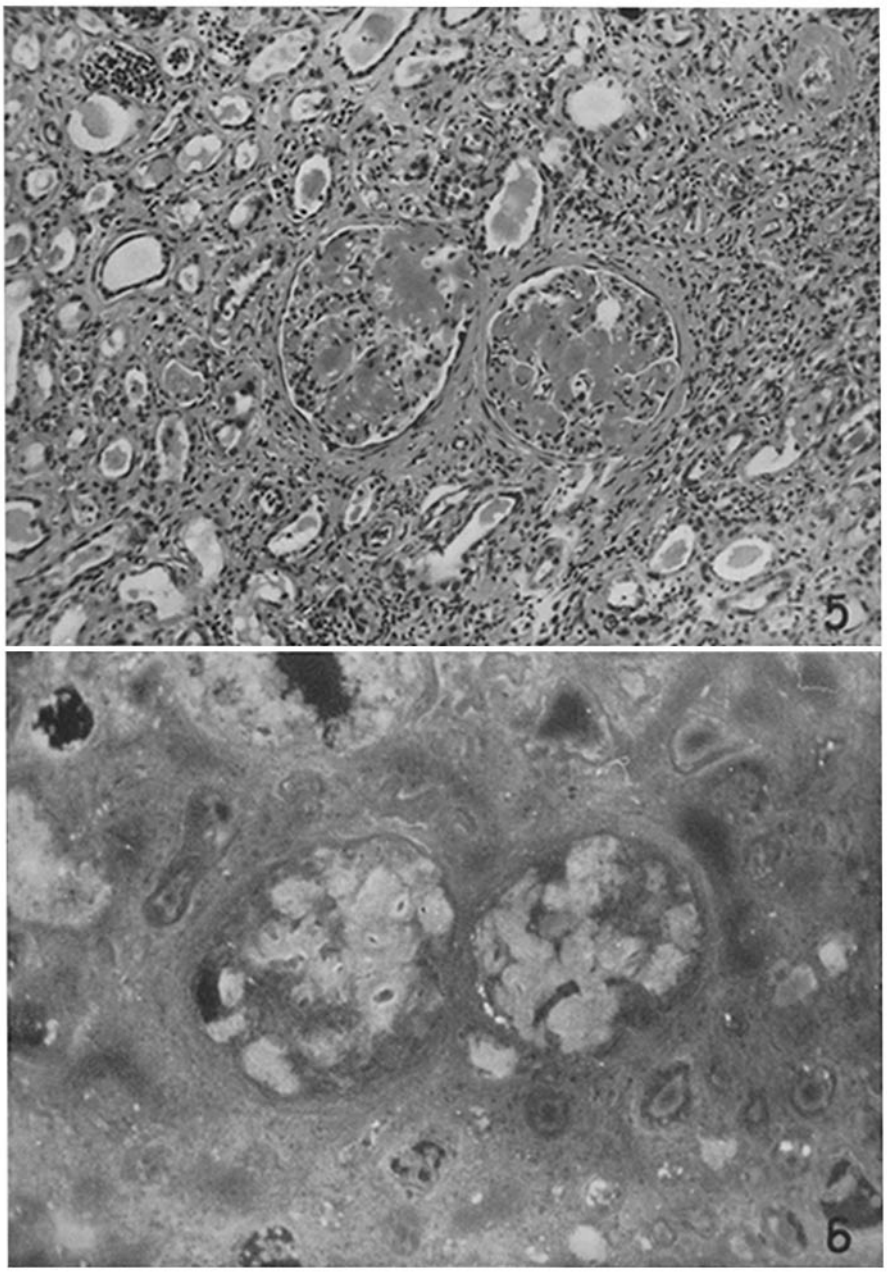


(Vazquez and Dixon: Immunohistochemical analysis of amyloid)

PLATE 51

FIG. 5. Section of kidney from human case 2. Two glomeruli showing abundant deposition of amyloid in the capillary tuft. Note amorphous casts in adjacent tubules. Hematoxylin-eosin. × 108.

FIG. 6. Similar section of kidney from human case 2 stained with fluorescent anti HGG. Note the intense specific fluorescence within the glomeruli corresponding to amyloid deposits as seen in Fig. 5. The circumscribed extra glomerular fluorescent areas correspond to tubular casts containing gamma globulin. Fluorescent micrograph. × 138.



(Vazquez and Dixon: Immunohistochemical analysis of amyloid)

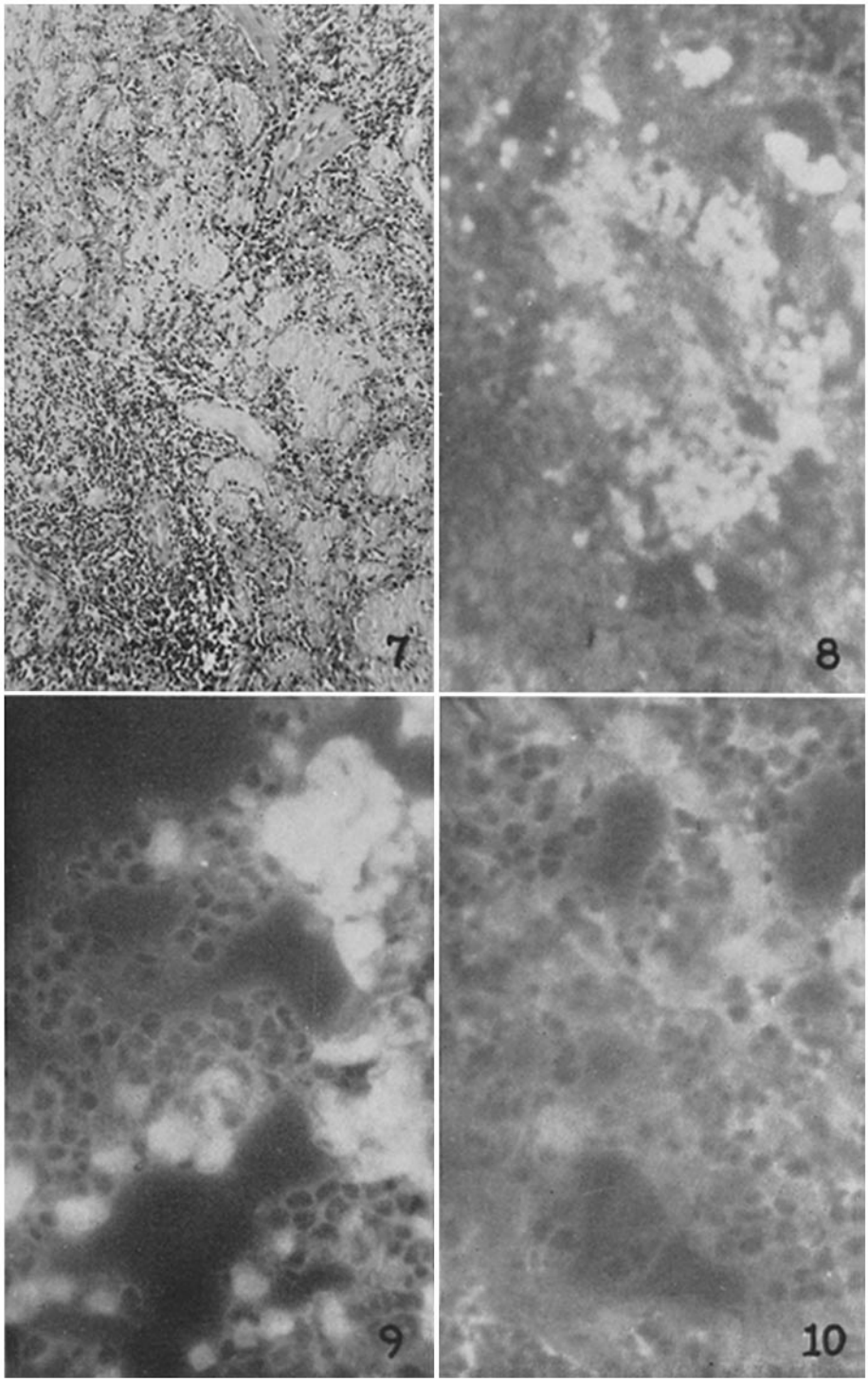
PLATE 52

FIG. 7. Section of spleen from human case 3. Note appreciable deposition of amyloid within splenic pulp. At lower left corner some lymphoid tissue around a small arteriole is seen. Hematoxylin-eosin. × 72.

FIG. 8. Similar section of spleen from human case 3 stained with fluorescent anti HGG showing appreciable specific fluorescence of amyloid deposits, indicating the presence of gamma-globulin. Fluorescent micrograph. × 92.

FIG. 9. A higher magnification of Fig. 8 to show in detail the bright specific fluorescence of amyloid deposits. Note by contrast the outline of non-fluorescent cells probably lymphocytes of the splenic pulp. Fluorescent micrograph. × 364.

FIG. 10. Section of spleen adjacent to that seen in Fig. 9 stained with fluorescent anti HSA. Note some fluorescence indicating the presence of small amounts of albumin within the amyloid deposits but much less appreciable when compared to that seen in Fig. 9. Fluorescent micrograph. × 364.



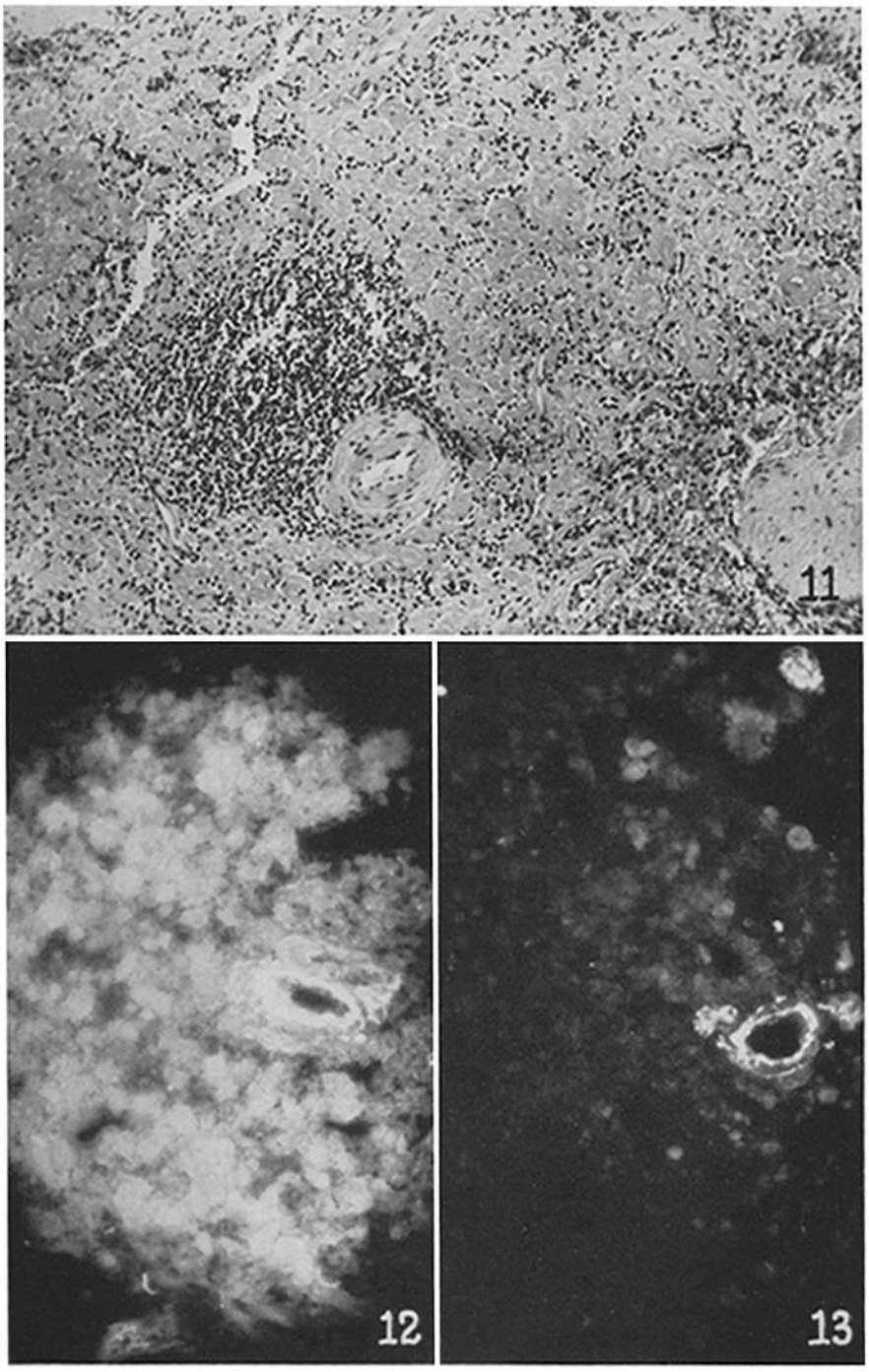
(Vazquez and Dixon: Immunohistochemical analysis of amyloid)

PLATE 53

FIG. 11. Section of spleen from human case 2 showing marked deposits of amyloid in splenic pulp. A central arteriole with some lymphoid tissue are seen as remnants of a Malpighian follicle. Hematoxylin-eosin. × 108.

FIG. 12. Similar section of spleen from human case 2 stained with fluorescent anti HGG. Note the specific (bright green) fluorescence of amyloid deposits around a central arteriole showing marked autofluorescence of the lamina elastica. Fluorescent micrograph. × 92.

FIG. 13. Section of spleen similar to that in Fig. 12 in which non-fluorescent anti HGG was applied prior to the specific staining with fluorescent anti HGG to saturate the antigen in the tissue. Note the markedly diminished specific fluorescence of amyloid deposits when compared to that in Fig. 12. The bright autofluorescence of the arteriole stands out by comparison. A bright circumscribed area of fluorescence in the upper right corner of the picture represents autofluorescence of connective tissue. Fluorescent micrograph. × 92.

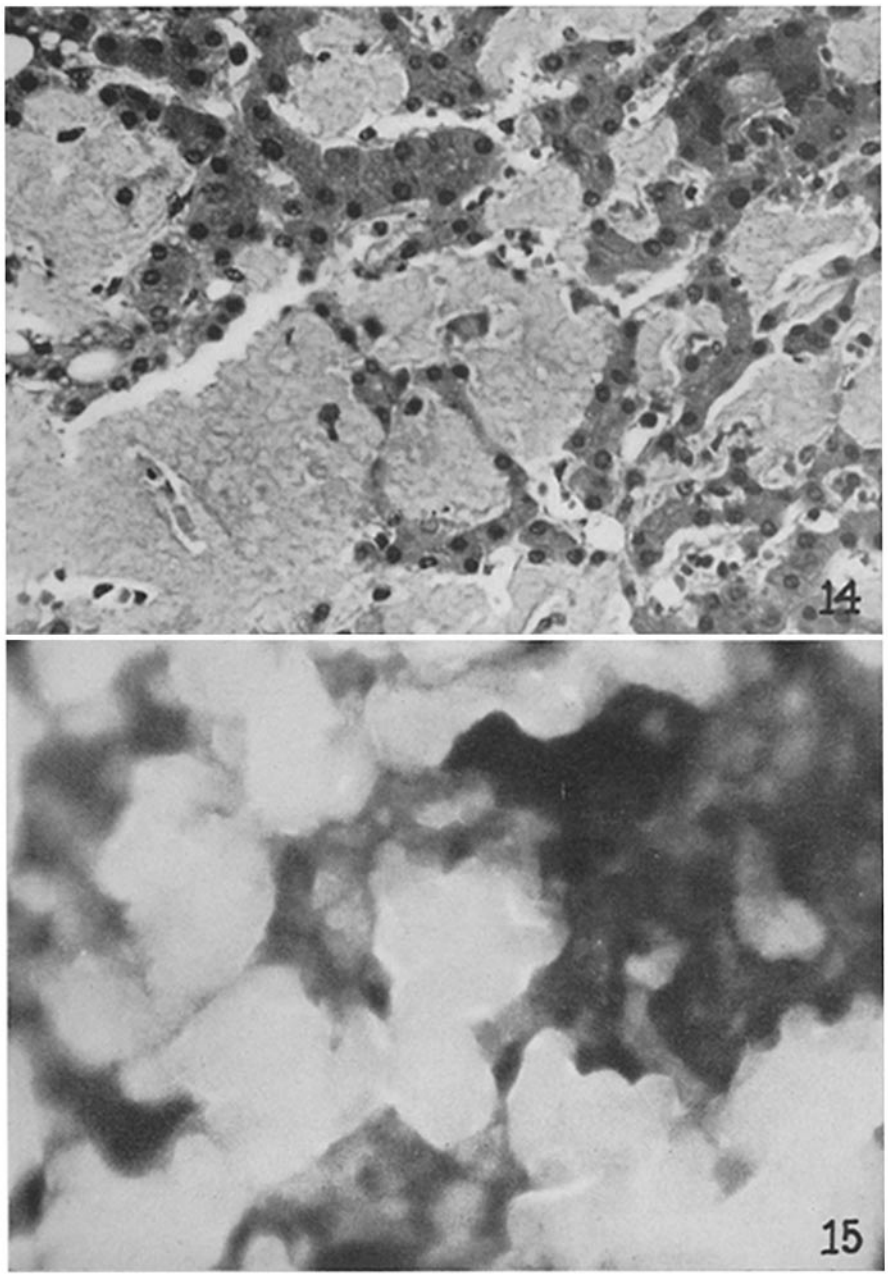


(Vazquez and Dixon: Immunohistochemical analysis of amyloid)

PLATE 54

FIG. 14. Section of liver from human case 1 showing abundant deposition of amyloid. Note some liver cell cords flattened by amyloid deposits. Hematoxylin-eosin. × 308.

FIG. 15. Similar section of liver from human case 1 stained with fluorescent anti HGG in which areas of marked specific fluorescence indicate the presence of gamma globulin in amyloid deposits. Fluorescent micrograph. × 324.



(Vazquez and Dixon: Immunohistochemical analysis of amyloid)

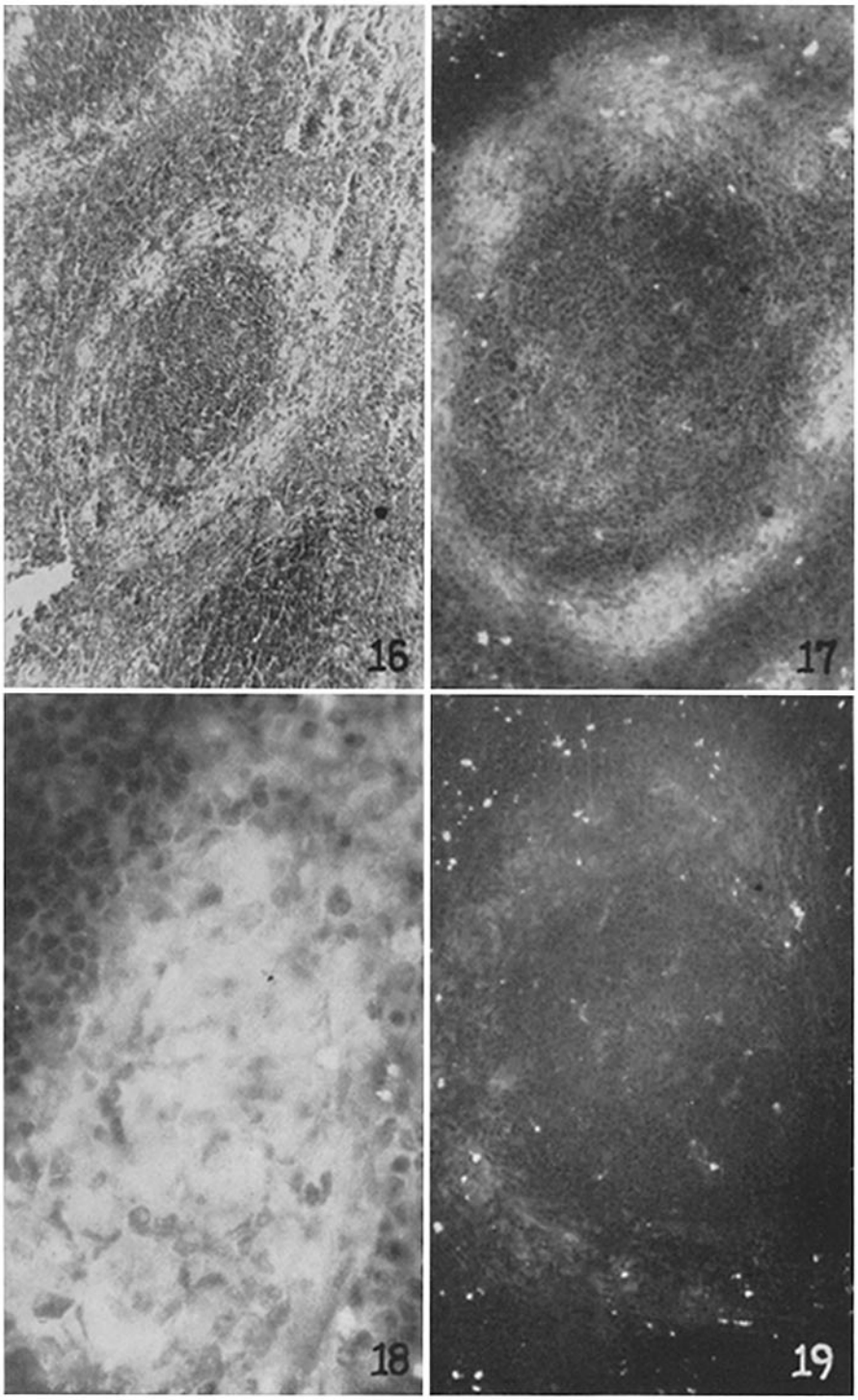
PLATE 55

FIG. 16. Section of spleen of rabbit injected with RNA showing deposition of amyloid in a perifollicular location. Hematoxylin-eosin. × 72.

FIG. 17. Similar section of spleen of rabbit injected with RNA and stained with fluorescent anti RGG. Note the appreciable specific fluorescence in a circular fashion corresponding to the amyloid deposits in a perifollicular location seen in Fig. 16. Fluorescent micrograph. × 92.

FIG. 18. A higher magnification of Fig. 17 to show in more detail the bright specific fluorescence of amyloid deposits indicating the presence of homologous gamma globulin. Note the round outline of negative fluorescence within the deposits probably representing the nuclei of cells. Likewise, outside the amyloid deposits, the outlines of non-fluorescent cells, probably lymphocytes, are seen. Fluorescent micrograph. × 364.

FIG. 19. Section of spleen adjacent to that seen in Fig. 17 in which non-fluorescent anti RGG was applied prior to the specific staining with fluorescent anti RGG to saturate the antigen in the tissue. Note the almost complete absence of specific fluorescence of amyloid deposits when compared to Fig. 17. The scattered bright dots represent non-specific fluorescence probably of altered leukocytes. Fluorescent micrograph. × 92.



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