LOCALIZATION OF STREPTOCOCCAL ANTIGENS IN TISSUES

I. Histologic Distribution and Persistence of M Protein, Types 1, 5, 12, and 19 in the Tissues of the Mouse*

By MELVIN H. KAPLAN,‡,§ M.D.

With the Technical Assistance of Mary Meyeserian and Audrey Swift

(*From the House of the Good Samaritan, Children's Medical Center, and Department of Bacteriology and Immunology, Harvard Medical School, Boston*)

Plates 39 to 41

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In the present work the localization of streptococcal antigens in tissues has been reinvestigated with a more sensitive immunofluorescent technique than previously available (1).

Previous reports by Schmidt (2, 3) indicated that an immunofluorescent method could be applied to detect group A carbohydrate and a protein fraction extracted from streptococcal cells, but only in a few locations and after injection of relatively large doses. Following an intravenous injection of 10 mg. of carbohydrate in the mouse, immunofluorescent staining could be observed only in renal tubular cells, for 30 minutes or less after injection of antigen. The streptococcal protein antigen was detected in reticulo-endothelial cells of spleen and liver and in some of the glomerular loops and tubular epithelial cells of the kidney only for short periods of time. No antigen was detectable in any of these sites 60 hours after injection.

By contrast with these results, the distribution of other foreign proteins, such as bovine albumin, egg albumin, and human gamma globulin was found to be considerably more extensive (4). These antigens were found widely disseminated in reticulo-endothelial cells, connective tissue stroma, and vascular endothelium of all organs examined, and also within the hepatic cells, lymphocytes, and renal tubular cells. In many of these cell types they penetrated the cell nucleus.

It was conceivable that failure to demonstrate streptococcal antigen in tissue sites in which other foreign protein antigens became distributed may have resulted from a low sensitivity of the direct immunofluorescent technique to these particular antigens. This was suggested by the fact that the doses of streptococcal antigens had to be fairly high for detection; *e.g.*, 10

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§ Present address: Department of Medicine, Cleveland City Hospital, Cleveland.

mg. or more. The dose permitting the detection of other protein antigens, egg albumin, bovine albumin, and human gamma globulin (4) was of the order of 1 to 10 mg., while in the case of penumococcal polysaccharides, antigen could be readily traced after doses of approximately one-tenth this amount (5).

All these previous studies employed the so called direct immunofluorescent technique in which a foreign antigen in the tissue is revealed by fixation of fluorescent antibody. Subsequent to these studies, Weller and Coons (6) reported the use of an indirect method for the detection of virus in tissue culture. In this technique, considerably more sensitive than the direct method, anti-antibody labeled with fluorescein detects the site of interaction of antigen and specific antibody. Presumably the heightened sensitivity results from a higher ratio of fluorescent material to antigen in a double complex of antigen, antibody, and fluorescent anti-antibody.

In the present study, the indirect immunofluorescent method was utilized successfully for detection of streptococcal antigen in the tissues of the mouse. The streptococcal M protein antigen was well suited to the immunofluorescent method, since it could be extracted from streptococcal cells in a partially purified state and readily identified by its reaction with type-specific precipitating antiserum (7). Further, the biologic properties of this substance are of particular interest. It is the cellular component of the group A streptococcus which is related to virulence of this organism (8), and which permits division of group A strains into Lancefield types (9). As a component of the streptococcal cell, M protein is actively antigenic, inducing circulating antibodies to the specific M protein in patients with streptococcal disease (10, 11). Skin reaction to partially purified M protein shows the characteristics of delayed or tuberculin type hypersensitivity (12).

Types 1, 5, 12, and 19 M proteins were compared with respect to histologic distribution in the tissues of the mouse and relative rates of disappearance. The distribution of the type 12 M protein was of particular interest because of the recent evidence of Rammelkamp and coworkers (13, 14) that this type was associated frequently with outbreaks of acute glomerulonephritis.

Materials and Methods

Preparation of M Protein.—M proteins were prepared from types 1, 5, 12, and 19 streptococci according to the procedure outlined by Lancefield and Perlmann (15) for the preparation of type 1 M protein. The type 1 (Hib), 5 (Tri), and 19 (Has) streptococcal strains had been isolated from the throats of patients with acute rheumatic fever, at the House of the Good Samaritan, and had been preserved in sealed ampoules in the frozen state in a CO_2-box since their isolation. The type 19 Has strain was of particular interest in that it had been associated with multiple recurrent attacks of acute rheumatic fever with a high mortality rate in an epidemic at the House of the Good Samaritan in 1939. The type 12 strain (Nass) was made

available through the kindness of Dr. Charles H. Rammelkamp, and had been isolated in a focal outbreak of acute glomerulonephritis. All of these strains were found to be rabbit- and mouse-virulent following intraperitoneal injection of blood-broth cultures into these animals. For the purpose of preparing M protein the virulence of each of these strains was further enhanced by 6 serial passages through mice. Blood broth cultures of the resultant strains were found consistently mouse-virulent at dilutions between 10^{-4} and 10^{-6}.

Mass cultures were made in Todd-Hewitt broth prepared with fresh beef heart and neopeptone and sterilized by filtration through porcelain filters. The cultures were grown in four 8 liter lots of medium for 18 hours and collected either in a Sharples or bucket-type centrifuge. Each extraction procedure was carried out with the cells collected from 16 liters of culture. The cells were washed twice with sterile saline in a 250 ml. centrifuge bottle and then suspended in 80 ml. saline. The pH was adjusted to 2.0 with 6 N HCl, and the cell suspension then heated in a boiling water bath with constant stirring and kept at 95°C. for 10 minutes. After cooling in running water, it was adjusted to pH 7.5 with 2 N NaOH, centrifuged, and the supernatant removed. The sediment was washed with 20 ml. of saline, and the washing pooled with the supernatant. Second and third extractions were carried out in 40 ml. volumes of saline in a similar manner. The pool of combined supernatant fluids and washings from these 3 extractions was filtered through a Buchner funnel with the aid of supercel, and then through a fine sintered glass filter. The filtrate was acidified to pH 2.0 with 6 N HCl, and the mixture allowed to stand overnight in the cold. The resulting precipitate was dissolved in 30 ml saline buffered at pH 8.0 with 0.01 M phosphate, and the pH adjusted to 8.0 with 1 N NaOH if necessary. Crystalline ribonuclease[1] (protease-free) was added to a concentration of 0.001 mg./ml., and the mixture placed in a dialyzing sac, and digestion thus carried out at 37°C. for 5 hours with concurrent dialysis against 0.01 M phosphate-buffered saline, pH 8.0. Dialysis was continued in the cold overnight against fresh changes of buffer.

M protein was precipitated from the digested extract with $(NH_4)_2SO_4$ between 0.33 and 0.6 saturation. The precipitate from this first fractionation was redissolved in 20 ml. distilled water, dialyzed overnight in the cold against buffered saline pH 7.0, and this solution again precipitated between 0.33 and 0.6 saturation ammonium sulfate. Following this reprecipitation, the solution containing M protein was dialyzed against frequent changes of physiologic saline for 3 days in the cold. This dialyzed solution was centrifuged at high speed to sediment traces of insoluble material and was then dried from the frozen state by lyophilization.

The resulting powder was thus a mixture of M protein fraction and salt. The protein content of the powder was determined by nitrogen analysis of a weighed aliquot. The per cent of the total solid representing protein was calculated for each preparation using 6.25 as the protein conversion factor. The protein content of these dried preparations varied in the range from 6 to 11 per cent. For the purpose of this study, the amount of M protein injected into mice was measured by the total protein content of the injected solution, and was expressed as milligrams M protein fraction.

No attempt was made to purify M protein further. Complete purification of M protein has not as yet been accomplished. The best preparation of type 1 M protein by Lancefield and Perlmann (15) was free of nucleic acid, contained 2.46 per cent sulfur, and had an isoelectric point of 5.3. It gave only one peak by electrophoresis but with sufficient boundary spreading to suggest inhomogeneity. Immunization of rabbits with such preparations indicated that purification was not complete and that antigenic impurities were still present. Studies by Schmidt (16) of types 19 and 14 M proteins prepared according to the procedure of Lancefield and Perlmann, differed from the type 1 M protein, in that the former substances were insoluble in distilled water, but soluble in salt solution of low ionic strength. Partial purification

[1] Supplied by Worthington Biochemical Corporation, Freehold, New Jersey.

of these M proteins was achieved by selective precipitation following prolonged dialysis against distilled water. On the basis of the electrophoretic pattern of the type 19 M protein preparation, which was not homogeneous, Schmidt estimated its M protein content as approximating 70 per cent.

It will be noted that in the present study, the M protein preparations were dialyzed against saline and dried as a mixture with salt. For injection of mice, solutions were prepared by dissolving sufficient quantity of the dried powder in distilled water to give a solution containing 1.0 mg. protein per ml.

Serologic tests with these preparations showed no precipitin reaction with specific anti-group A carbohydrate serum or with anti-M sera of heterologous types. With homologous typing sera, precipitin reactions were obtained with all four preparations at a protein concentration of 0.01 mg. per ml. (1:100,000). This compared favorably with the reactivity of the type 1 M protein preparation of Lancefield and Perlmann, assuming that the antisera were of similar reactivity.

Anti-M Sera.—Type- and group-specific anti-streptococcal sera were kindly made available to us for this study through the courtesy of Dr. Elaine Updyke, Communicable Disease Center, United States Public Health Service, Chamblee, Georgia.

Mice and Injection of Antigen.—Male albino Swiss mice of the Schwentker strain, weighing approximately 20 gm., were injected intravenously in a tail vein with 0.5 ml. of a solution containing 1.0 mg. M protein per ml. Paired mice were injected for each time interval studied and were sacrificed after varied time intervals by decapitation while under light ether anesthesia. These time intervals ranged from 30 minutes to 8 days. The organs to be examined were removed, quick-frozen at −70°C. within 10 minutes after death, and stored at −20°C. until frozen sections were prepared. As a routine, sections were taken from heart, kidney, lung, spleen, liver, inguinal lymph node, skeletal muscle, and skin. Sections were also taken frequently from stomach, duodenum, colon, and adrenal gland. The central nervous system was not examined.

Immunofluorescent Method.—Anti-rabbit globulin was prepared in the goat as previously described (17) and labelled with fluorescein isocyanate (1). Non-specific staining for mouse tissues using the indirect immunofluorescent method was found to be an especially complex problem, since such staining resulted from reactions of both the labelled anti-rabbit globulin and the specific rabbit antistreptococcal serum with normal mouse tissues. In particular, this varied markedly with the specific organ under study: Non-specific staining was most marked in the case of spleen, skin, and kidney, and distinctly less in the case of liver, lung, and heart.

These non-specific staining reactions usually involved vascular endothelium, reticulin, and connective tissue sites. Such non-specific reactions could not be abolished by absorbing both serum and fluorescent conjugate with acetone-dried mouse liver powder or packed liver suspensions. Absorption with mouse liver preparations removed non-specific staining for liver, but was not effective in abolishing staining of heart, spleen, kidney, or skin. As a general rule, it was felt that optimal absorption was obtained when the tissue absorbent employed was derived from the same organ as that under study.

It was found possible to resolve this problem effectively by using as absorbent, a homogenate of mixed mouse organs. Following at least two absorptions with such a mixed organ homogenate, both normal and anti-M sera could generally be applied to normal mouse tissue sections of different organs with freedom from non-specific reactions. In isolated cases, a third absorption was required.

A sample procedure used in the preparation of such a mixed organ homogenate was as follows:—

Twenty mice weighing 30 gm. each were sacrificed by etherizing, and the following organs excised and pooled separately: liver, kidney, spleen, heart, lung, and skeletal muscle. A ho-

mogenate was prepared from each kind of organ, as follows: Each pool of organs was minced in 20 ml. of saline and homogenized in the cold in a Waring blendor until a fine suspension was obtained. The resulting homogenate was next poured into a 50 ml. wide-mouthed tube and frozen and thawed once to effect lysis of the red cells in the tissue suspension. Following thawing of the emulsion, it was washed three times with 30 ml. volumes of buffered saline to remove all of the hemoglobin from the suspension. (Removal of hemoglobin from the tissue was important, since admixture of hemoglobin with the conjugate during absorption reduced its fluorescence and inhibited its immunofluorescent staining properties). After washing 3 times, the tissue suspension was poured into 15 ml. graduated tubes and packed by centrifuging at 3,000 R.P.M. for 30 minutes. Packed homogenates were thus prepared for each of the mouse organs. Equal volumes of tissue suspension from each of the 6 organ homogenates were mixed, and this mixed homogenate packed in 1.0 ml. volumes in small test tubes. Such tubes of packed homogenate could be stored effectively at −20°C. in the deep-freeze for several months.

Absorption of serum and conjugate was carried out as follows: To 1.0 ml. of packed homogenate, 2.0 ml. of serum or conjugate was added, the resultant viscous mixture stirred thoroughly, and then shaken gently on a shaking machine at room temperature for 2 hours. The tubes were centrifuged at high speed, and the supernatants removed and mixed with fresh tissue homogenate for a second absorption. Merthiolate was added to a final concentration of 1:20,000 to the sera or conjugates thus absorbed.

Fixation and Staining of Frozen Sections.—Unfixed frozen sections of mouse tissues were cut at a thickness of 4 micra in a cryostat (4). "Fixation" of sections in ethanol or methanol resulted in complete loss of antigen from the tissue, presumably because of the solubility of M protein in these solvents (7). Acetone was quite effective as fixative and preserved the precise histologic localization of this antigen. Fixation and staining were then carried out as follows: The sections were immersed in acetone at room temperature for 30 minutes, and dried in an incubator at 37°C. for 30 minutes. To a dry section, 1 drop of absorbed type-specific antistreptococcal serum was added, and reaction allowed to occur for 45 minutes in a moist chamber. The sections were then washed in 2 changes of buffered saline, pH 7.0, for 10 minutes, with gentle agitation on a shaking machine. Next, absorbed anti-rabbit globulin conjugate was applied for 45 minutes, and the sections washed as before, and mounted in glycerol buffer.

The ultraviolet microscope, equipped with a carbon arc as the ultraviolet source, and used with a darkfield condenser, has been previously described (1, 17).

Tests of Specificity of Staining.—Controls of specificity of staining consisted of the following tests:—

(*a*) Failure of anti-rabbit globulin conjugate to stain sections previously allowed to react with normal rabbit serum or heterologous anti-M sera.

(*b*) Failure of staining of sections by specific antiserum absorbed with the homologous M protein preparation.

(*c*) Failure of staining of sections with anti-rabbit globulin conjugate absorbed with rabbit globulin.

(*d*) Failure of staining of sections following fixation in ethanol.

Only the first control was performed on a routine basis for each organ examined. The remaining controls were carried out during the course of study of each M protein with a given antiserum. It will be noted that specific inhibition tests (1) were not carried out: In the indirect immunofluorescent method such an inhibition test requires antiserum from a species of animal other than rabbit. Theoretically, prior treatment of the section with this antiserum would inhibit subsequent reaction with the rabbit antiserum, and thus prevent visualization by the anti-rabbit globulin conjugate. Such a serum was not available in the present study.

RESULTS

Histologic Distribution of Streptococcal M Protein.—The distribution of the four different M proteins studied, 1, 5, 12, and 19, was similar; and in the following description, the term M protein will be used in a general sense to refer to all four substances. The following description is that of the histologic localization of M protein 3 hours after the intravenous injection of 0.5 mg. M protein fraction.

Heart.—Localization of M protein in the heart was quite sparse and limited almost exclusively to endocardial lining cells and the adjacent subendocardium. In these sites, antigen was usually present as discrete focal deposits (Figs. 1–3). Auricular endocardium was more active in this respect than ventricular endocardium, since M protein deposits could be found regularly in auricular sections and relatively infrequently in the ventricles. No appreciable difference with respect to such localization was noted between the left and right sides of the heart. Endocardial deposits of antigen were noted also in the mitral and tricuspid valves; however, this was a relatively infrequent observation despite routine examination of sections through these valves. Aortic and pulmonary valves were examined less frequently, and were not observed to contain antigen in the specimens examined. Within the myocardium itself, traces of antigen could be detected occasionally in scattered sites in the interstitial connective tissue between muscle fibers. No antigen was noted within myofibers. Endothelium of blood vessels including capillaries contained antigen occasionally. Within the epicardium, occasional small foci of deposited antigen were noted in the connective tissue.

Liver.—In the liver, M protein was taken up in considerable concentration by the Kupffer cells lining the sinusoids (Fig. 4). Antigen was not detected within hepatic cells or within bile canaliculi. In scattered hepatic cells, staining of nuclei could be observed, although antigen could not be specifically detected in the cytoplasm of these cells (Fig. 4).

Lung.—Bright amorphous deposits of material could be detected in the walls of capillaries in scattered areas throughout the lung. In certain of these sites, antigen was deposited as collections of particles or small rounded masses, which appeared to lie within capillary and alveolar walls (Figs. 5 and 6). No antigen was detected in the bronchioles or their adventitia. Within the lung parenchyma, antigen was also noted within scattered macrophages or "dust cells."

Kidney.—In the kidney, M protein was deposited largely within endothelial cells of the glomeruli, where it was observed most frequently as collections of droplets or globular masses (Figs. 7 through 12). Careful study of the sites of these rounded masses suggested that they may have been formed as droplets extruded by glomerular endothelial cells (Fig. 10). These masses gave a positive stain for glycoprotein by the Schiff-periodic acid method.

Occasionally the distribution of M protein within a glomerular tuft followed an irregular course (Fig. 9); and it was presumed that in such instances the antigen may have become adsorbed to the basement membrane. These characteristics of the glomerular localization of M protein were not peculiar to any particular M protein type, but were exhibited by all four preparations. Antigen was also detected as scattered particles within capillary walls and in the connective tissue stroma surrounding the tubules; however, its concentration in these sites was comparatively small. It was not noted within epithelial cells of any of the convoluted or collecting tubules or within the lumens of these tubules. However, tests of the urine 24 hours after the injection of 0.5 mg. of M protein, showed the presence of antigen by precipitin reaction with specific antiserum, indicating that it was being excreted by the kidney. No nuclear staining was noted in cells of either glomeruli or tubules.

Spleen.—M protein was limited almost entirely to the red pulp of the spleen, with only occasional penetration of the antigen into lymphoid nodules (Figs. 13 through 16). In the red pulp, it was observed within sinusoidal endothelial cells, reticular cells, and in cells surrounding the sinuses. The reticular cells were brilliantly stained, and occasionally faint stippling of nuclei could be seen; but this was distinctly an infrequent observation. M protein was not observed within the interior of lymphoid nodules except within scattered reticular cells, and within cells at the periphery of the nodule which were thought by their stellate appearance to be reticular, in nature. The antigen was also absorbed to collagenous stroma and reticulin, and was taken up by connective tissue cells. Such concentration of antigen was particularly prominent around the trabeculae and in the adventitia of the larger vessels (Figs. 14 and 15).

Lymph Node.—In the lymph node, M protein was present chiefly in reticulo-endothelial cells in the medullary and subcapsular sinuses and in cells immediately adjacent to these sinuses (Figs. 17 and 18). Antigen was not observed in the cortical lymphoid nodules.

Adrenal Gland.—In the adrenal gland, M protein was present in high concentration in the cells lining the cortical sinusoids and in macrophages and capillary endothelial cells in the medulla. To a lesser extent, discrete particles of antigen were observed within scattered cortical cells of all three zones of the adrenal cortex, particularly within cells of the zona reticularis, at the juxta-medullary region.

Gastrointestinal Tract.—Sections of stomach, duodenum, appendix, and colon were examined. Traces of antigen could be observed in the endothelium of the capillaries extending into the submucosae, and occasionally within cells of the serous coat. No material was noted in the mucosal epithelium, or in smooth muscle walls.

Skin.—Traces of M protein were present in scattered capillaries in the dermis, in the adjacent connective tissue cells and also adsorbed to adjacent collagen fibers.

Skeletal Muscle.—Rare traces appeared in capillaries surrounding the muscle fibers. No antigen was noted within the connective tissue stroma or within the muscle fibers.

TABLE I

*Persistence of Streptococcal M Proteins in the Tissues of the Mouse**

Organ	M protein type	Interval after injection of 0.5 mg. intravenously				
		3 hrs.	1 day	2 days	4 days	8 days
Heart	1	+	0	0	0	0
	5	+	+	Trace	0	0
	12	+	Trace	0	0	0
	19	+	+	Trace	Trace	0
Kidney	1	+++	++	+	+	Trace
	5	+++	+	Trace	0	0
	12	++	++	+	Trace	Trace
	19	++	++	+	+	Trace
Spleen	1	+	Trace	0	0	0
	5	++	Trace	0	0	0
	12	++	+	Trace	0	0
	19	++	+	Trace	0	0
Liver	1	+++	++	Trace	Trace	0
	5	+++	0	0	0	0
	12	++	+	0	0	0
	19	+++	+	Trace	0	0
Lung	1	+	+	0	0	0
	5	++	+	Trace	0	0
	12	+++	++	+	0	0
	19	++	+	Trace	Trace	0

* The dose was 0.5 mg. M protein given intravenously. Results are taken from at least 2 mice sacrificed at each interval. The relative concentration of antigen within a given organ was estimated from 0 to +++.

Comparative Persistence of M Proteins in Tissues.—The relative persistence of M proteins in various organs, with respect to the different serologic types, is given in Table I. It may be observed that the relative amount of M protein taken up by a given organ soon after injection, as for example, at 3 hours, was approximately the same for each of the M protein types. The subsequent rate of disappearance of each type of antigen was similar,with due allowance for the variability of the method. There is, perhaps, some suggestion from the data that the type 5 M protein may have been somewhat more rapidly

removed. In general, at the end of 48 hours, the amount of M protein in all the organs studied was remarkably reduced. By 4 days, antigen had practically disappeared below detectable levels in most tissues, with the possible exception of the kidney, where, except for the type 5 material, traces persisted for 8 days (*cf.* Figs. 7 to 12).

Localization of M Protein in the Tissues of Infected Mice.—Distribution of M protein was examined in mice inoculated with types 5 and 12 mouse-virulent strains. In each case, groups of 4 mice were inoculated intraperitoneally with 0.1 ml. of a 10^{-4} dilution of a 12 hour blood-broth culture. Two mice were sacrificed from each group when moribund. The interval between inoculation and sacrifice varied from 24 to 36 hours. In these mice, M protein could be detected regularly in the spleen, and liver, and in particular concentration on the serous surfaces of these organs; however, antigen could not be detected in heart, lung, or kidney. Heart blood cultures obtained from these mice at the time of sacrifice were positive for the inoculated organism.

In the spleen, clumps of single cells or short chains of cocci were brightly fluorescent within localized abscesses. Adjacent reticulo-endothelial cells and infiltrating polymorphonuclear cells were brightly and diffusely stained. Antigen remained localized around these focal abscesses and did not become distributed throughout the organ. Similarly in the liver, antigen could be demonstrated within focal abscesses containing clumps of cocci and within the surrounding inflammatory cells and Kupffer cells. As in the spleen, distribution of antigen was limited to the vicinity of focal abscesses.

DISCUSSION

The sensitivity of this immunofluorescent technique is difficult to estimate in view of the undefined degree of purification of M protein; however, assuming that the preparations employed contained 50 per cent of M protein, an approximation which seems reasonable in the light of the estimated 70 per cent purification achieved by Schmidt using similar methods for preparation of type 19 M protein (16), it would follow that the actual dose injected in these experiments was approximately 250 micrograms. Since this amount was 10 times the minimal detectable dose (as defined by the minimal dose permitting demonstration of antigen in the spleen 3 hours after injection) it will be seen that the method is sensitive to a dose of approximately 25 micrograms of antigen.

Although the application of the method was limited to M protein in the present study, the technique appears feasible for other streptococcal antigens as well. The most serious difficulties in its use arise from non-specific staining, but this can be eliminated by exhaustive absorption of serum and conjugate with pooled tissue homogenates.

However, this procedure does not answer a question which has been seriously considered in connection with the poststreptococcal diseases: Is there an antigen which is shared by the group A streptococcus in common with con-

nective and vascular tissue? While the immunofluorescent technique might be regarded as an ideal tool for the investigation of this problem, non-specific reactions between tissue elements and either normal or immune serum make interpretation extremely difficult. Furthermore, streptococi grown in infusions of beef heart with added neopeptone, *e.g.* Todd-Hewitt medium, absorb antigenic constituents from the medium. In our studies, constituents for which antigenicity has been observed include a myocardial sarcoplasmic antigen (17) and a substance of blood group A specificity present in the neopeptone (18, 19). Thus, proof that the group A streptococcus contains an antigen cross-reacting with connective and vascular tissue would require more precise technical and experimental controls than those employed in the present study.

The localization of streptococcal M protein in mouse tissues differed in several respects from that of animal foreign proteins and bacterial polysaccharides (4, 5, 20). M protein did not become as widely disseminated throughout the extra-vascular compartment as these other antigens, but was largely confined within the reticulo-endothelial system. In the case of the heart, for example, antigen was limited essentially to the auricular or ventricular endocardium or subendocardium, with rare traces in the myocardial connective tissue. This was in marked contrast with the extensive dissemination throughout the myocardial vascular and connective tissues of polysaccharide and animal protein antigen. This difference in distribution could not be attributed to the smaller dosage of M protein injected, since the same results were obtained when the dose was increased by 10 times.

In the kidney, M protein was concentrated almost entirely within the glomerular tufts and appeared only in traces in the interstitial stroma and capillary walls. In the liver, spleen, lymph node, and lungs, it was present in marked concentration in reticuloendothelial cells, but did not enter appreciably the adjacent tissue cells; *i.e.*, the hepatic cords, lymphoid nodules, or lung parenchyma. Extensive penetration of all of these sites had been observed with the other antigens.

The finding of a streptococcal antigen in endocardial and subendocardial sites in these studies is consistent with the concept that immunologic reactions to deposited streptococcal antigen may be involved in the pathogenesis of rheumatic fever, but the non-specific nature of the localization must be emphasized. Pneumococcal polysaccharides and animal protein antigens have also been observed in these sites, and such localization may perhaps represent only a general characteristic of the distribution of foreign antigens.

While the localization of streptococcal M protein in the kidney, particularly within renal glomeruli, and its tendency to persist within the glomerular tufts are of interest, the more significant finding would seem to be the similar localization and persistence in the kidney of the type 12 M protein as compared with the other types. This observation suggests that the localization and persistence of M protein in the mouse kidney is not necessarily related to the "nephritogenic" properties of the type 12 streptococcus.

SUMMARY

A method has been described for the detection of streptococcal antigens in tissues using the indirect immunofluorescent technique. This method has been applied to the histologic distribution in the mouse of M protein of types 1, 5, 12, and 19. Histologic localization of these M proteins was similar, and their rates of disappearance from the tissues were comparable.

The major sites of deposition were the endocardium and adjacent subendocardium of the heart, alveolar walls of the lung, glomerular tufts of the kidney, and reticulo-endothelial cells of liver, spleen, lymph nodes, and adrenal gland. M protein was distributed in considerably lesser concentration in capillary endothelium and connective tissue sites in myocardium, kidney, skin, and gastrointestinal tract. Traces were also present in adrenal cortical cells. It was observed only rarely in cell nuclei.

After injection of 0.5 mg. M protein fraction, the concentration of antigen diminished to undetectable levels in all organ sites by 4 days, except in the renal glomerulus, where traces were visible at 8 days. In mice injected with streptococcal culture intraperitoneally, M protein was detected at sites of focal abscesses in liver and spleen, and on the serous surfaces of these organs.

The histologic distribution of M protein is compared with that described previously for pneumococcal polysaccharide and animal protein. Differences in the extent of distribution and in the characteristics of antigen deposition are pointed out.

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EXPLANATION OF PLATES

PLATE 39

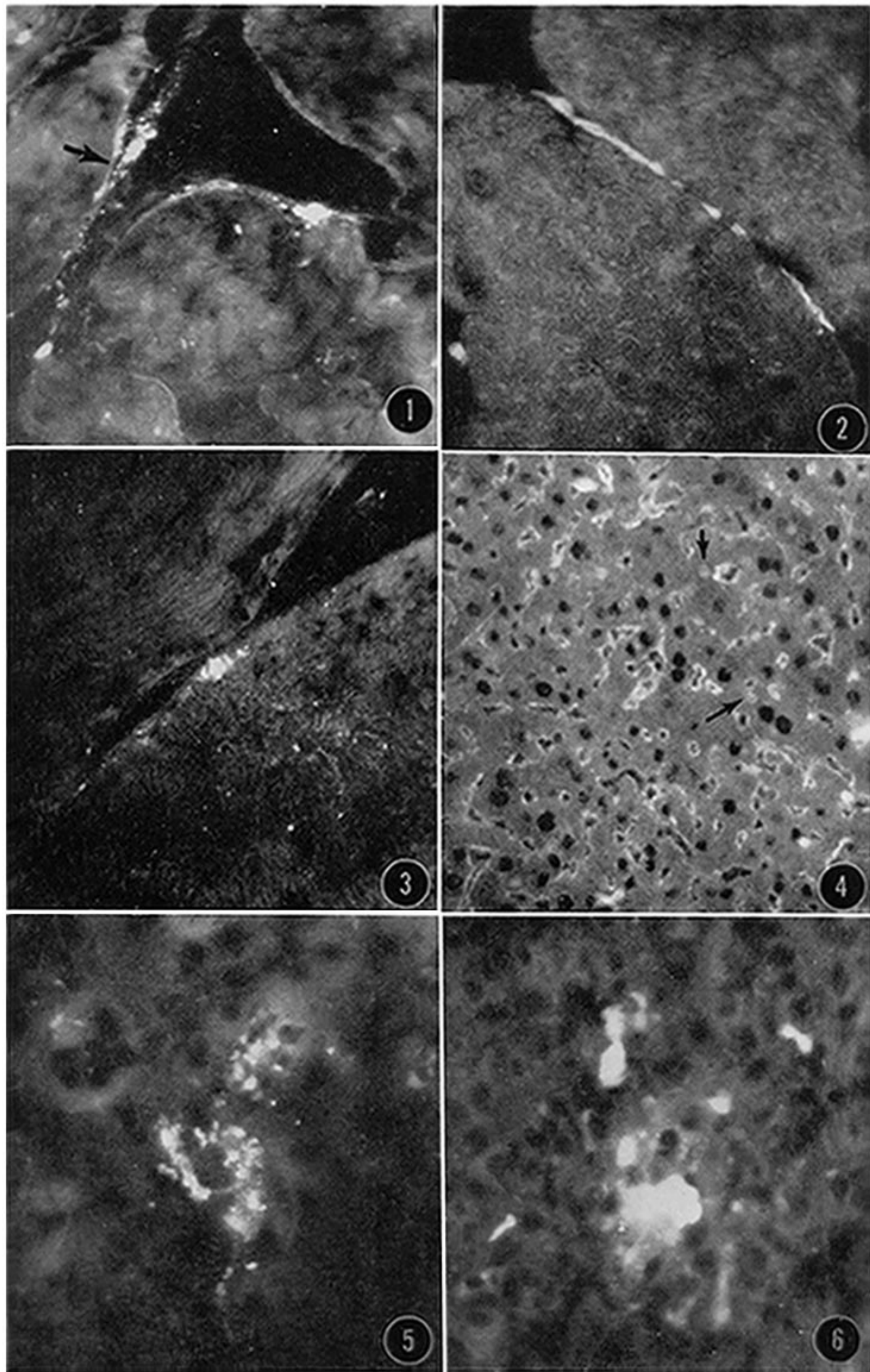
FIG. 1. Left auricle, mouse 150, injected with 0.5 mg. M protein fraction, type 5; 24 hours after injection. Bright fluorescent deposits of antigen are concentrated focally in the endocardial lining cells and in the adjacent subendocardium. Antigen has been taken up by a portion of endocardium, which has been partially stripped away in the preparation of the section (arrow). Traces of deposited antigen may be observed in the connective tissue stroma between myofibers at the upper left. × 400.

FIG. 2. Left ventricle, mouse 111, injected with 0.5 mg. M protein fraction, type 12; 24 hours after injection. The M protein is taken up in the endocardial lining cells. The grey mottling of the myocardium is due to the natural blue-grey autofluorescence; no green fluorescence was observed in the myocardium. × 400.

FIG. 3. Left ventricle, mouse 123, given 0.5 mg. M protein fraction, type 19; 24 hours after injection. A deposit of antigen may be observed within the endocardium and extending into the subendocardium. × 400.

FIG. 4. Liver, mouse 141, injected with 0.5 mg. M protein fraction, type 19; 3 hours after injection. Antigen may be observed in the Kupffer cells lining the sinusoids, but not in the hepatic cell cytoplasm. Occasional hepatic cell nuclei show penetration by antigen (arrows). × 200.

FIGS. 5 and 6. Lung, mouse 119, injected with 0.5 mg. M protein fraction, type 12; 24 hours after injection. Aggregate of antigen deposited within the substance of the lung and which appears to lie within capillary and alveolar walls. The lung sections are compressed in such unfixed frozen sections, making precise histologic localization difficult. × 400.



(Kaplan: Distribution of M protein in mouse tissues)

Plate 40

Fig. 7. Kidney, mouse 109, injected with 0.5 mg. M protein fraction, type 12; 1 hour after injection. All glomeruli show remarkable concentration of antigen. Within the glomerular tufts, antigen is present within endothelial cells, as discrete deposits varying considerably in size from small particles to large globular masses. Traces may also be observed in the peritubular capillaries. Antigen is entirely absent from the convoluted tubules. × 400.

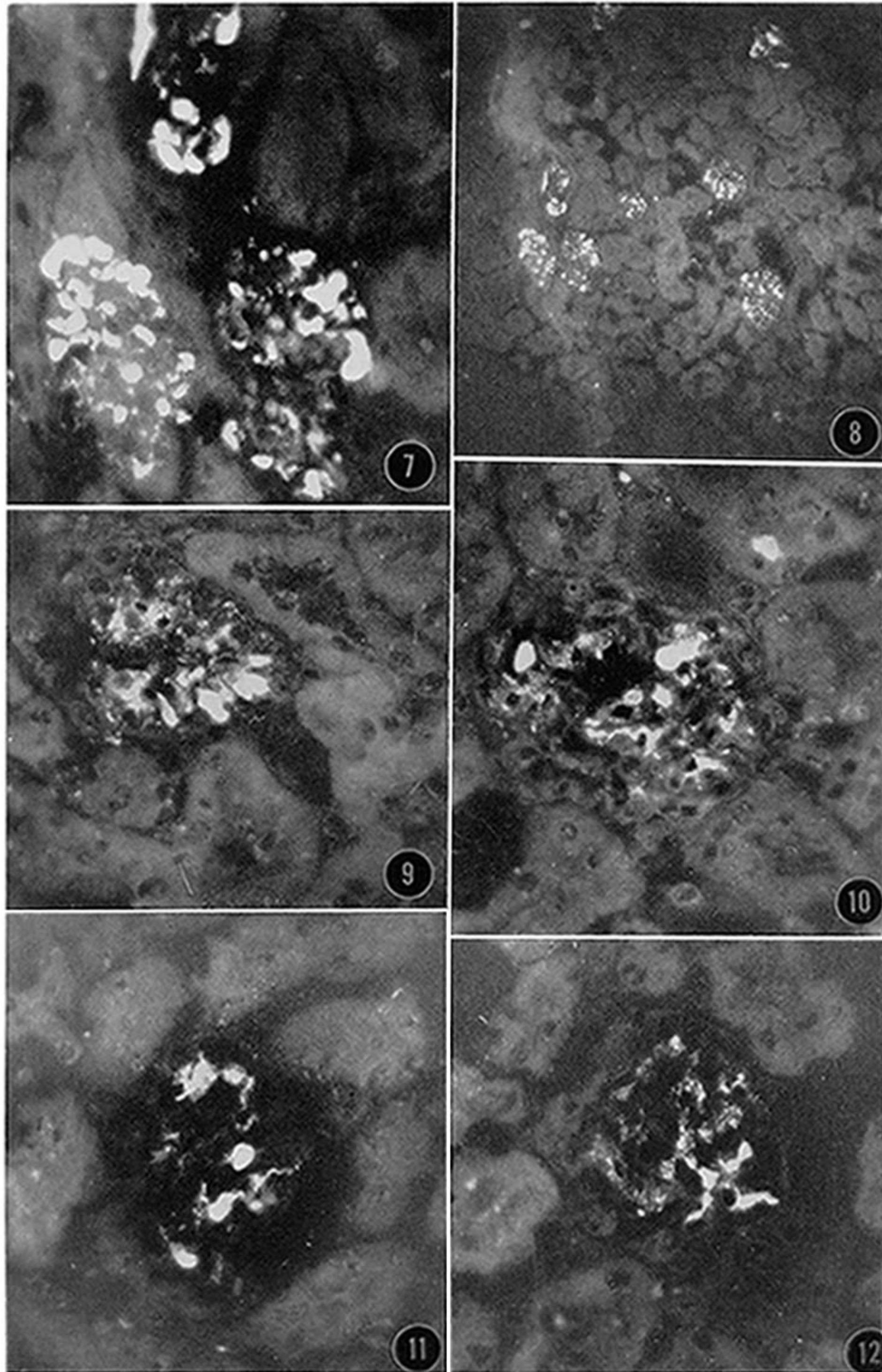
Fig. 8. Kidney, from same mouse as in Fig. 7; low power view showing the focal concentrations of antigen in the glomerular tufts. Traces are present in peritubular capillaries. The tubules are autofluorescent and do not contain antigen. × 100.

Fig. 9. Kidney, mouse 153, given 0.5 mg. M protein fraction, type 1; 3 hours after injection. Antigen may be observed within the cytoplasm of endothelial cells of the glomerulus, as well as in droplet-like condensations within the glomerular tuft. Traces are also present in peritubular capillaries. × 400.

Fig. 10. Kidney, from same mouse as in Fig. 9. The globular condensations of antigen are contiguous to endothelial cells. In the center of this tuft, a droplet which contains antigen appears to have been extruded from the cell. × 400.

Fig. 11. Kidney, mouse 154, injected with 0.5 mg. M protein fraction, type 1; 24 hours after injection. Within the glomerulus, bright discrete droplets of antigen are seen connected by a thin irregularly coursing condensation of material which is presumed to represent antigen localized adjacent to or in the glomerular basement membrane. × 400.

Fig. 12. Kidney, mouse 156, given 0.5 mg. M protein fraction, type 1; 4 days after injection. Antigen is present within glomerular endothelial cells as small granular or amorphous deposits. × 400.



(Kaplan: Distribution of M protein in mouse tissues)

Plate 41

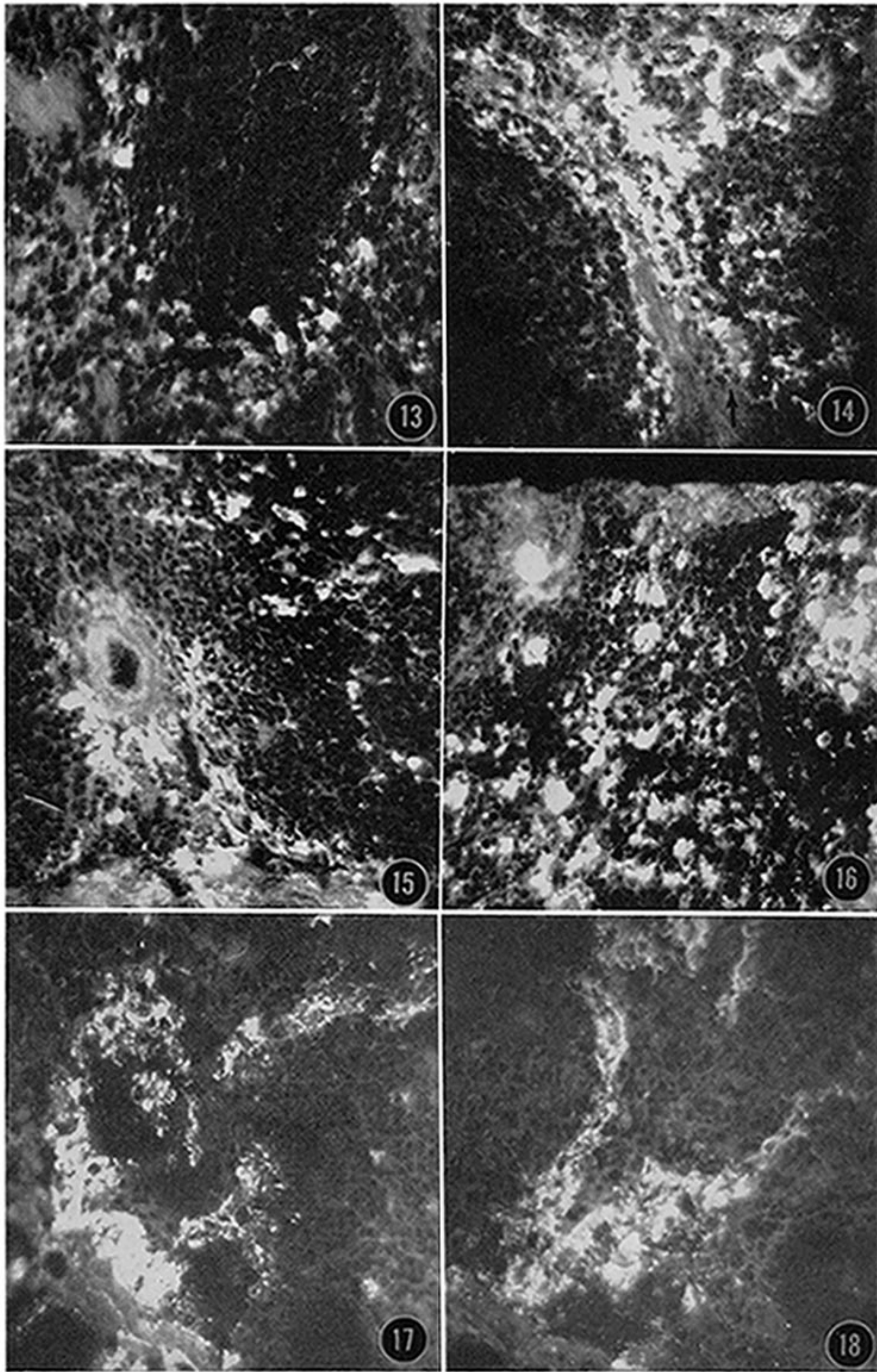
Fig. 13. Spleen, mouse 122, injected with 0.5 mg. M protein fraction, type 19; 1 hour after injection. M protein is present in the red pulp surrounding a lymphoid nodule. Within the red pulp the reticulo-endothelial elements are brightly laden with antigen, whereas in the white pulp, only scattered reticular cells, particularly at the periphery of the nodule contain antigen. × 200.

Fig. 14. Spleen, from mouse 122, as described in Fig. 13. M protein is adsorbed to collagenous bundles of a trabecula, and is present within cells between the collagen bundles (arrow). × 200.

Fig. 15. Spleen, mouse 109, injected with 0.5 mg. M protein, type 12; 1 hour after injection. Antigen is present in the adventitial connective tissue of an artery, and in adjacent phagocytic cells. Green fluorescent staining was not observed in the endothelium of this vessel; the internal elastic membrane shows bright blue autofluorescence. × 200.

Fig. 16. Spleen, mouse 109, as described in Fig. 15. Antigen is present in considerable concentration within the cytoplasm of cells surrounding the sinusoids of the red pulp. The sinusoidal lining cells also contain antigen but are less brightly stained in this section. × 200.

Figs. 17 and 18. Inguinal lymph node, mouse 122, injected with 0.5 mg. M protein fraction, type 19; 1 hour after injection. Antigen is concentrated within the reticulo-endothelial cells within and adjacent to the medullary sinuses, and does not penetrate adjacent lymphocytic cells. × 200.



(Kaplan: Distribution of M protein in mouse tissues)