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Glomerular Localization of Antigen and Antibody in Rabbits Following Intravenous Administration of Serum Cryoproteins from Homologous Animals with Acute Serum Sickness (37760)

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(Introduced by E. C. Curnen, Jr.)

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Serum cryoproteins have been described in a variety of diseases in man and laboratory animals. The detection of these cold insoluble proteins in the course of diseases thought to be attributable to complexes of antigen and antibody has prompted investigators to suggest that they represent immune complexes (1). In previous communications we have demonstrated that serum cryoproteins isolated from rabbits with acute serum sickness, the classical experimental model of immune complex disease, contain complexes of antigen and specific antibody (2–4). In this study the localization of cryoproteins in the renal glomerulus was determined by immunohistologic and morphologic examination of rabbit kidneys following intravenous injection of redissolved cryoproteins.

Materials and Methods. Serum sickness was produced in 20 New Zealand white rabbits, 3–3.5 kg, by administration of bovine serum albumin (BSA), 250 mg/kg intravenously and 10 mg/kg in Freund's complete adjuvant given simultaneously by subcutaneous injection. Because our previous studies indicated that cryoproteinemia was increased if animals were nephrectomized, bilateral nephrectomies were performed 9 days after BSA administration. Animals were bled by cardiac puncture on Days 10 and 11. Cryoproteins were isolated from the serum of each rabbit as previously described (5). Briefly blood was allowed to clot at 37° and serum removed after centrifugation at 2000 rpm for 20 min. The serum was recentrifuged to insure removal of cells and then placed at 2° for 72 hrs. Approximately 30–75 cc of serum was obtained from each animal. The refrigerated serum was then centrifuged at 5000 rpm at 4° for 60 min and the precipitate was washed four times with cold phosphate buffered saline — 0.01 *M* phosphate, 1 M saline (PBS) (pH 7.4) by centrifugation at 4°. A volume of PBS equal to twice the original volume of serum used was used for each wash. The precipitate was dissolved in 3–5 cc of 0.075 *M* barbital buffer (pH 8.6) at 37° for 4 hr. The redissolved precipitate was centrifuged at 10,000 rpm and the supernate was made isotonic by addition of 4.25% NaCl. The solution was passed through a 0.45 μm Millipore filter and protein determined by the Folin–Lowry method (5).

Individual samples were then pooled and aliquots of the solutions containing 8 to 20 mg of total protein were injected into the marginal ear vein of 7 male New Zealand white rabbits, 2–3 kg (Group I).

In order to fractionate the cryoprecipitates, portions were dissolved in 0.02 *M* citrate buffer (pH 3.2) for 4 hr at 37° and gel filtration chromatography on Sephadex G 200 was performed using 0.02 *M* acetate buffer (pH 5). The fractions obtained were dialyzed against phosphate buffered saline, concentrated by pressure dialysis and Ouchterlony immunodiffusion in agar gel and immunoelectrophoresis performed on each fraction using goat antisera to whole rabbit serum, rabbit IgG and BSA. The protein content of each solution was determined by the Folin-Lowry method and the fractions were tested against each other by Ouchterlony immunodiffusion in agar gel. In addition, each fraction was

TABLE I. Summary of Morphologic and Immunohistologic Findings in Animals Receiving Cryoproteins and Control Solutions.

Group	Animal	Solution	Total protein (mg)	Time of sacrifice (hr)	Proliferation[a,b]	Immunohistology[b]		
						IgG	BSA	B_1C
I	22	Cryoprotein	20	48	Tr	1–2+	0	Tr
	29	Cryoprotein	15	48	Tr	Tr–1+	2+	0
	33	Cryoprotein	12	72	1+	2+	Tr	Tr
	67	Cryoprotein	15	96	1+	Tr–1+	1+	Tr
	68	Cryoprotein	8	96	1+	1+	0	0
	466	Cryoprotein	8	48	0	Tr–1+	0	0
	487	Cryoprotein	8	66	1+	1–2+	Tr	Tr
II	473	Cryoprotein fraction A	15	48	0	0	0	0
	475	Cryoprotein fraction A	15	72	0	0	0	0
	472	Cryoprotein fraction B	8	48	0	0	0	0
	476	Cryoprotein fraction B	8	72	0	0	0	0
III	485	Fraction A + B	8	48	0	Tr	Tr	Tr
	488	Fraction A + B	8	72	Tr	Tr	Tr	Tr
IV	471	IgG + BSA	20	48	0	0	0	0
	474	IgG + BSA	20	72	0	0	0	0
V[c]	477, 428, 481	Agg. IgG + BSA	40	48–96	0	0	0	0
VI[c]	426, 227	Agg. BSA + IgG	40	72, 96	0	0	0	0
VII[c]	408–411	IgG + BSA	40	48–96	0	0	0	0
VIII[c]	69– 73	Buffer	0	72	0	0	0	0

[a] Proliferation = increase in mesangial matrix and increase in epithelial or endothelial cells.

[b] Graded by severity or intensity 0–4+; Tr = trace.

[c] All rabbits in Group V–VII received 40 mg total protein and animals from each of these groups were sacrificed at 48, 72 and 96 hr. The five animals in Group VIII received buffer and sacrificed at 72 hr following injection. None of the animals in Groups V–VIII showed morphological or immunohistologic changes.

placed at 2° for 72 hr and studied for the presence of cryoprecipitates as described previously. Control animals were injected with 8–15 mg of each fraction (Group IIA and B). A solution of recombined fractions was also administered intravenously to rabbits (Group III).

Other groups of animals (Table I) received a mixture containing 10 mg of normal rabbit IgG + 10 mg of BSA in 0.075 *M* barbital buffer (pH 8.6) which had been kept at 2° for 5 days (Group IV); 20 mg of aggregated IgG + 20 mg BSA (Group V), 20 mg of aggregated BSA + 20 mg of normal rabbit IgG (Group VI), 20 mg of normal IgG + 20 mg BSA (Group VII), and a solution of barbital buffered saline (Group VIII). All solutions were centrifuged at 10, 000 rpm and passed through an 0.45 μm Millipore filter prior to intravenous administration. All animals received the same volume (3 ml) of solution.

Animals were sacrificed with pentobarbital 2–4 days following injection of the solutions. Immunohistologic studies were performed on frozen sections of kidneys as previously described (6). Fluorescein isothiocyanate labeled antisera to rabbit IgG (provided through the courtesy of Dr. Alfred Michael of the University of Minnesota), B_1C (provided through the courtesy of Dr. Hans Muller-Eberhard, Division of Experimental Pathology, Scripps Clinic and Research Institute, La Jolla, CA) and BSA (courtesy of Dr. Konrad Hsu) were used. Tissue was fixed in Bouin's solution for light microscopy. Four micrometer sections were stained with hematoxylin and eosin (H&E) and periodic acid Schiff (PAS).

Results. Two peaks were eluted from

Sephadex G 200 after cryoprecipitates were treated with citrate buffer. The first fraction eluted was designated (A) and the smaller molecular weight fraction referred to as (B).

Immunodiffusion and immunoelectrophoresis demonstrated the presence of rabbit IgG in Fraction A and revealed BSA in Fraction B. A precipitin band was formed between the two fractions by immunodiffusion in agar gel. Neither fraction by itself precipitated in the cold.

Table I summarizes the morphological and immunohistological findings in rabbits receiving redissolved cryoproteins as well as in

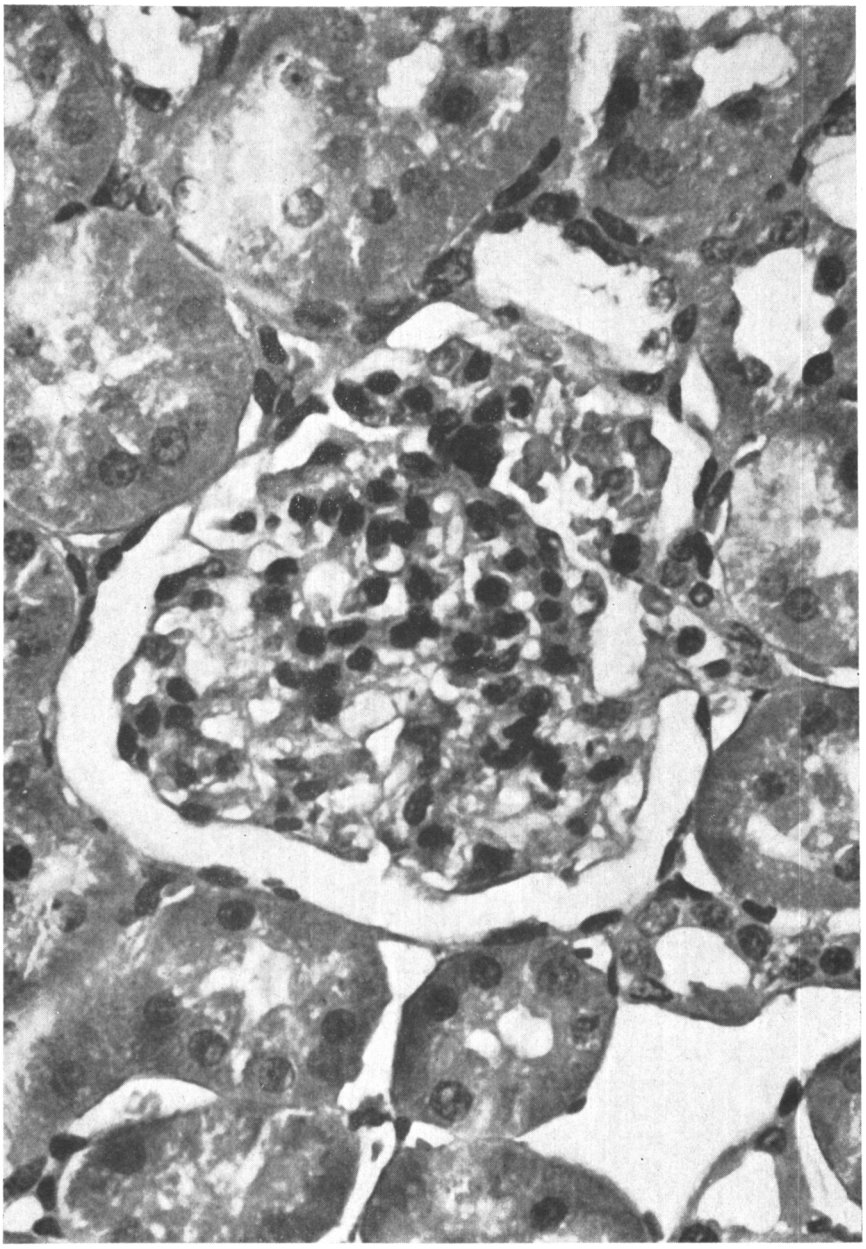

FIG. 1. A representative glomerulus from rabbit No. 22 sacrificed 2 days after receving 20 mg cryoprotein. Polymorphonuclear leucocyte infiltration and mild segmental proliferation are seen.

the control animals. The total protein administered and the interval between administration and sacrifice are included.

Light microscopic examination of the kidneys of animals receiving redissolved cryoproteins revealed mild mesangial proliferation with minimal focal segmental endothelial proliferation. Occasional polymorphonuclear leucocytes were observed in the glomeruli (Fig. 1). The glomerular basement membranes were normal, tubules were intact and no proteinaceous material was observed in the tubular lumina. Interstitial changes were not noted. No lesions were seen in the control

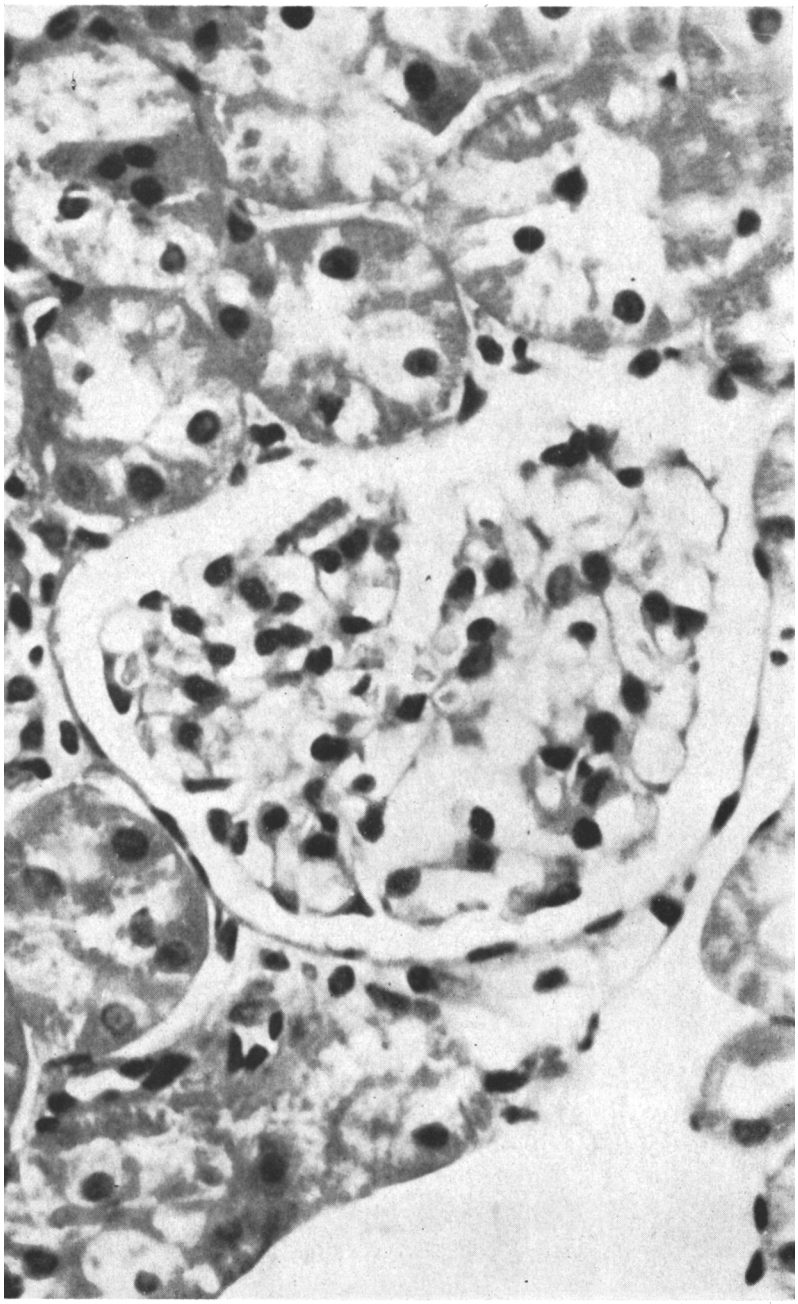

FIG. 2. A representative glomerulus from a control animal.

groups (Fig. 2).

Rabbit IgG was localized along the glomerular capillary walls and in the mesangial region in a granular pattern in all of the animals receiving cryoproteins (Fig. 3). Similarly granular deposits of BSA were noted in glomeruli of 4 of these animals (Fig. 4). Trace amounts of B_1C were observed in a few animals. Immunohistologic studies on control animals revealed no abnormalities.

Discussion. Serum sickness induced by BSA antigen is the classical model of immune complex mediated inflammatory tissue disease. The role of immune complexes, plasma mediators and leukocytes in the pathogenesis of injury has been well defined (7–9). These investigators have demonstrated that renal deposition of immune complexes depends on their concentration, size and ratio of antigen to antibody in the complexes as well as other factors. They have suggested that the reticuloendothelial system clears large complexes in antigen excess and these complexes as well as those in antibody excess are not pathogenic for the kidney.

The serum cryoproteins which appear in BSA-induced serum sickness have been characterized previously (2–4). Cryoproteinemia and antigen cryoprecipitation reach peak values when immune catabolism is occurring (2). The cryoprotein contains BSA and IgG antibody with specificity for this antigen. Recent work with radiolabeled BSA antigen has shown that a large portion of antibody bound antigen may cryoprecipitate from the serum of rabbits with serum sickness (3).

In this study the glomerular fixation of redissolved cryoproteins was demonstrated. The presence of IgG and BAS in a granular pattern in the renal glomerulus suggests that the immune complexes in the cryoprotein are deposited in the glomerulus and remain these for at least 4 days. The presence of IgG and BSA in glomeruli of the animals inject-

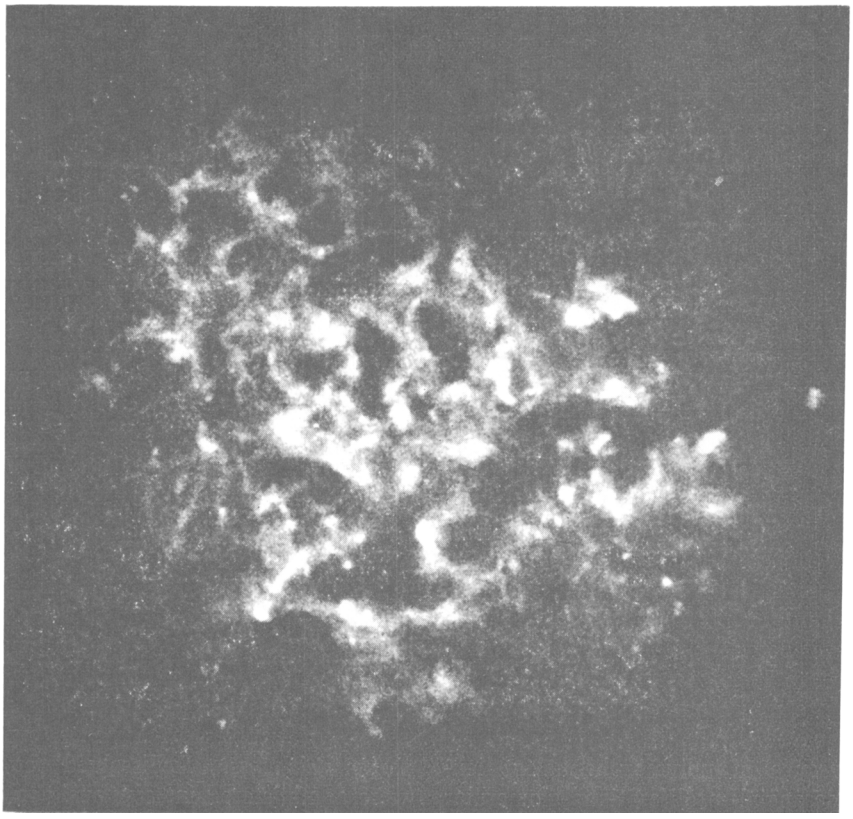

Fig. 3. Immunofluorescent localization of IgG in animal No. 33 sacrificed 3 days after receiving 12 mg of cryoprotein.

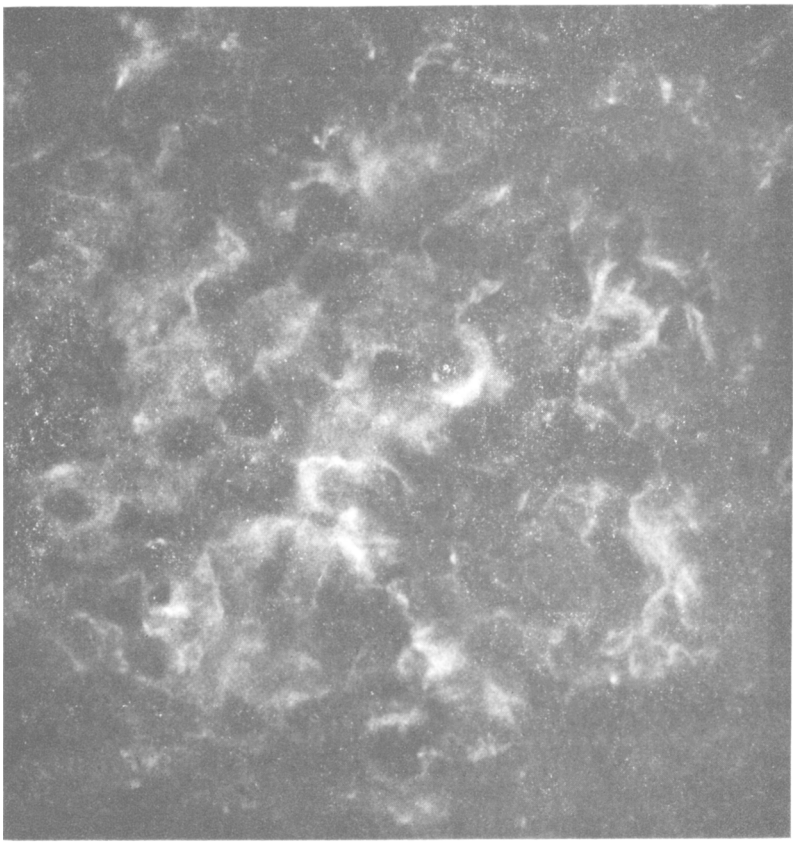

Fig. 4. Immunofluorescent localization of BSA in animal No. 29 sacrificed 2 days after receiving 15 mg cryoprotein.

ed with cryoproteins, the failure to localize these in the glomeruli after administration of fractions following dissociation of the precipitate at acid pH and gel filtration and the absence of the proteins in the several animal controls suggest that this phenomenon is not due to denaturation of aggregation. The paucity of glomerular B_1C is surprising since cryoproteins can fix complement *in vitro*. This however may be due to the small amount of cryoprotein injected. Similarly the mildness of the morphological changes may be related to the amount of protein injected and the short interval between injection and sacrifice. The failure to localize BSA in all animals may be due to saturation of antigenic sites by high affinity antibody.

The presence of polymorphonuclear leukocytes, mesangial proliferation, glomerular deposition of IgG and BSA suggests that the cryoprecipitates contain antigen–antibody complexes which fulfill the criteria for production of vascular membrane injury. The dissociation of the cryoprotein by acid pH, and the demonstration of antigen and antibody which recombine at neutral pH demonstrate that these cold insoluble serum precipitates probably contain antigen–antibody complexes. The observation that individual fractions isolated by gel chromatography after acid treatment of the cryoglobulins fail to precipitate in the cold further indicates that cryoproteins may represent immune complexes of antigen and specific antibody.

Summary. To study the kidney fixing properties of serum cryoproteins on the renal glomerulus, cryoproteins were isolated from serum of rabbits with acute experimental serum sickness. Redissolved cryoproteins were administered intravenously to untreated rabbits in doses of 8–20 mg total protein. Animals were sacrificed 2–4 days following

administration and morphological and immunohistological studies were performed on the kidneys. Localization of antibody, antigen and to a lesser extent B_1C was observed. No alterations were noted in control animals. These studies support our hypothesis that cryoproteins in serum sickness contain antigen–antibody complexes which are of immunopathologic significance.

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