The Effect of Anticoagulation on Serum Sickness Nephritis in Rabbits[1] (36452)

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Coagulation processes have been implicated in the pathogenesis of some forms of human and experimental renal disease (1, 2). The demonstration of fibrinogen or its derivatives at the site of the glomerular lesion and modification of the clinical and pathologic events by means of anticoagulation have provided support for this hypothesis.

It has been demonstrated convincingly with nephrotoxic serum nephritis that anticoagulation has led to marked reduction in glomerular fibrinogen deposition and severity of the lesions (3–7). The present study was done to evaluate the effect of anticoagulation on another principal form of experimental immunologic renal disease, namely serum sickness nephritis, which results from glomerular deposition of antigen–antibody complexes induced by a single injection of foreign protein.

Materials and Methods. Twenty-eight New Zealand albino rabbits[3] weighing 2–3 kg and fed Purina rabbit chow, lettuce and carrots were used. This particular strain of rabbits has been used in our laboratory during the past 7 years and has not been observed to develop spontaneous renal disease.

A single iv injection of crystalline bovine serum albumin[5] (BSA), 250 mg/kg body weight, dissolved in isotonic saline was given to induce serum sickness nephritis. The animals were separated into 2 groups: 1. nonanticoagulated, 13 animals; 2. anticoagulated, 15 animals. Warfarin sodium[6] was given iv to the animals of Group 2 beginning on day 6 after injection of BSA, except in 4 animals where it was started on day 8. The dosage was 50 mg/kg body weight for the first 2 days of anticoagulation and a total of 100 mg on day 3. Subsequent doses were based on the prothrombin time which was done every 2–3 days. The dosage of warfarin was adjusted to maintain the prothrombin time between 25 and 35 sec. Blood was drawn from an ear vein or artery into a syringe containing 0.25 ml of a 0.1 *M* sodium oxalate solution; the prothrombin time was determined by a modification of the method of Quick (8). In our hands and as reported by others (9) the prothrombin time of normal rabbits is 6–8 sec.

The time of elimination of BSA from the plasma was determined by capillary precipitin test done every second day using high titered rabbit antiserum to BSA. When a precipitin reaction was no longer detectable antigen (BSA) elimination from the plasma was considered to have occurred. This usually took place around day 13 (12–21 days) following BSA injection. There was no difference in the time of antigen elimination between the 2 groups.

An open renal biopsy under pentobarbital sodium anesthesia was done in each animal prior to injection of BSA and within 3 days of elimination of antigen from the serum. This time was chosen for the second biopsy because it is known to be the time of maximum glomerular damage (10, 11). The kidney was exposed by a flank incision and a cortical specimen approximately 0.5 × 0.5 × 2.0 cm was obtained using a No. 15 scalpel

[1] Aided by Term Grant MT-1579 from The Medical Research Council of Canada.

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[3] Canadian Breeding Farm and Laboratories Ltd., St. Constant, Quebec, Canada.

[4] Ralston Purina Co. of Canada Ltd., Woodstock, Ontario, Canada.

[5] Pentex Inc., Kanakee, IL.

[6] Coumadin, Endo Laboratories, Garden City, NY.

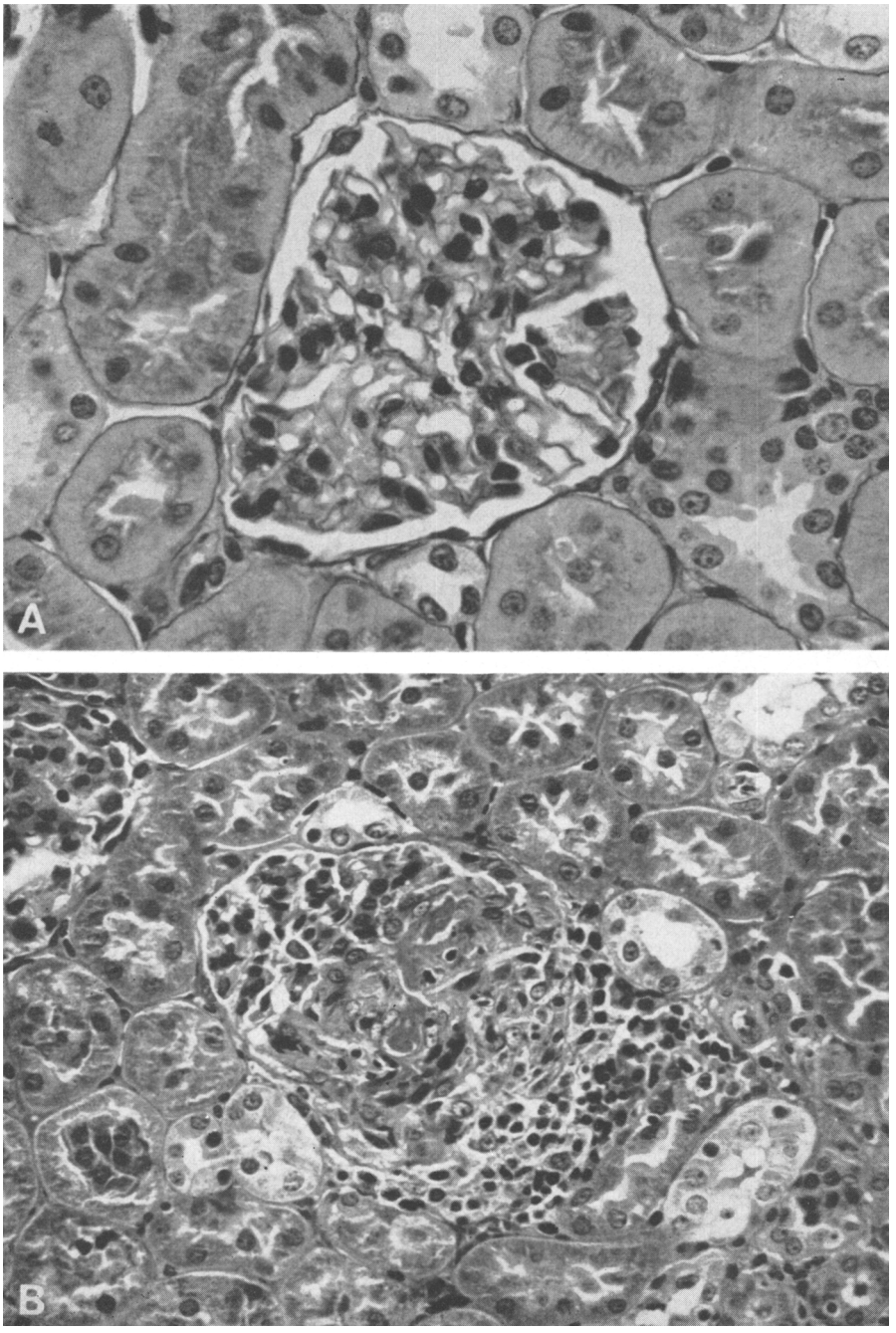

FIG. 1A. A normal glomerulus from a rabbit in the anticoagulated group prior to BSA injection (PAS stain, ×450). (B) A glomerulus from the same rabbit at time of BSA elimination showing mesangial proliferation, focal necrosis and pyknotic nuclear fragments, and hypercellularity. A focus of periglomerular mononuclear cell infiltration is seen (HPS, ×275).

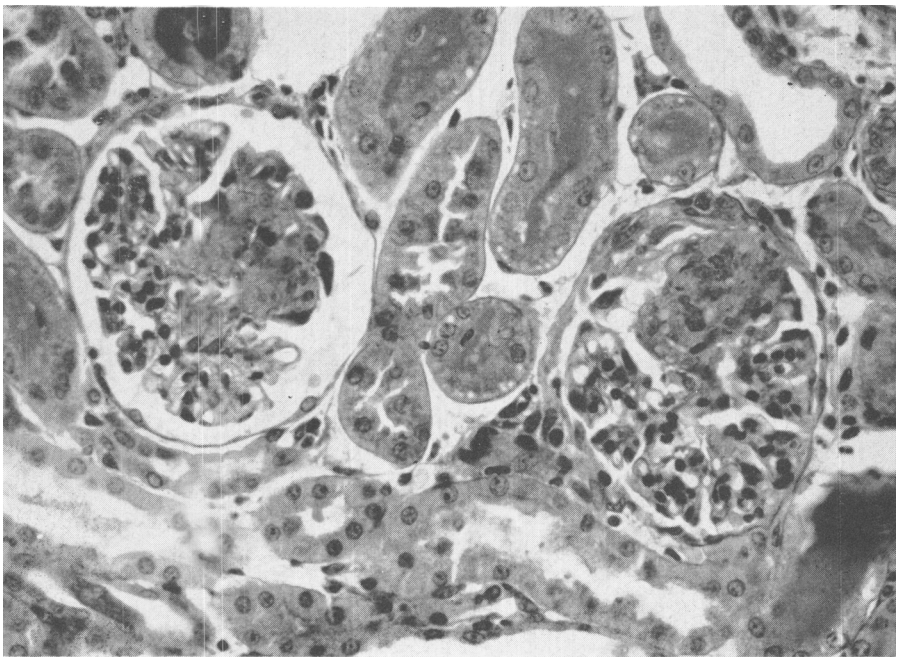

FIG. 2. These 2 glomeruli are from a rabbit in the nonanticoagulated group at the time of antigen elimination. In each there is a lobule with marked mesangial proliferation which has led to obliteration of patent capillary loops. The remaining lobules show mild hypercellularity. Some of the tubules contain proteinaceous casts (PAS, ×275).

blade. Hemostasis was achieved with several 3-0 cat gut sutures and topical pressure. In some animals Gelform[7] was applied to the kidney incision. A single dose of penicillin G (400,000 units) and streptomycin (0.25 g) was given im following the biopsy.

The tissue was divided into 2 parts. One was frozen in isopentane precooled to —70° in dry ice and stored at —100° for immunopathologic study. This tissue was sectioned at —20° and stained for fluorescent microscopy as described previously (12, 13) using fluorescein isothiocyanate labeled antisera to rabbit gamma globulin, beta$_{1C}$ globulin (B_{1C}) and fibrinogen[8] and to BSA. Antiserum to fibrinogen reacts with fibrinogen, fibrin, and intermediate derivatives (1, 14). Inhibition of fluorescence occurs when antifibrinogen antiserum is absorbed prior to staining with a particulate suspension of washed fibrin (15). Thus immunofluorescent studies using antifibrinogen antiserum may detect fibrinogen, its intermediate derivatives, or fibrin. For convenience we will refer to material detected using antifibrinogen antiserum as fibrinogen. The other piece was fixed in 10% cobalt formalin for light microscopy; the stains used were hemalum phloxine saffron (HPS), periodic acid–Schiff (PAS), and periodic acid silver methenamine (PASM).

Antisera for immunofluorescent microscopy were prepared in the following manner. Guinea pig antirabbit B_{1C} was made according to the method of Mardiney and Muller-Eberhard (16). Rabbit anti-BSA was prepared by repeated iv injection of a saline solution of the antigen following initial intradermal and sc immunization using an emulsion of BSA in complete Freund's adjuvant. Sheep antisera to rabbit gamma globulin[9] was prepared by repeated iv injections of alum precipitated antigen.

[7] The Upjohn Co., Kalamazoo, MI.

[8] Guinea pig antirabbit fibrinogen was obtained from Dr. Roger Herdman of the University of Minnesota, Minneapolis, MN.

[9] Rabbit gamma globulin, Cohn fraction II, Pentex Inc., Kanakee, IL.

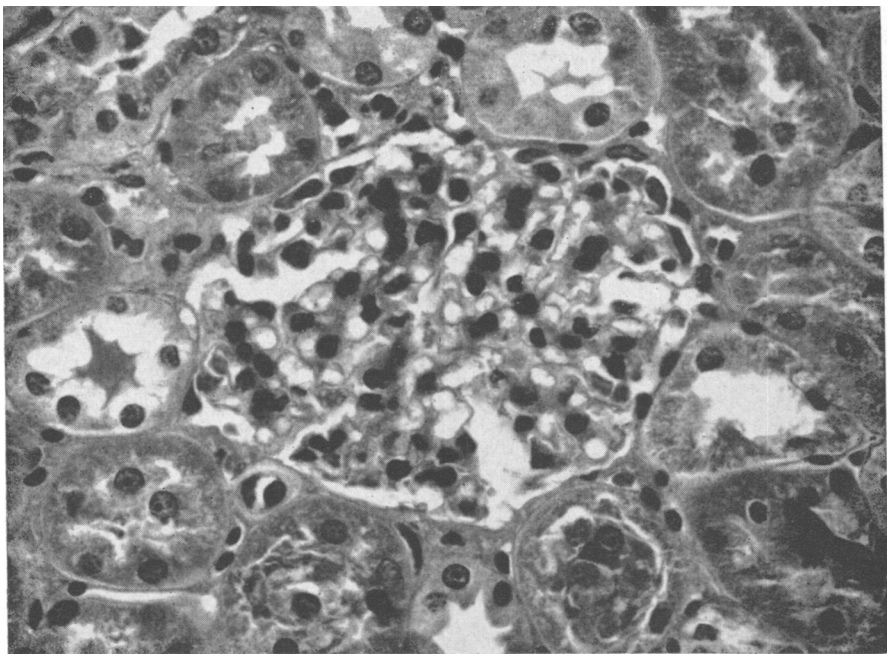

FIG. 3. This glomerulus is from an animal in the anticoagulated group at time of BSA elimination from the serum. Although there is minimal mesangial hypercellularity the glomerulus is essentially normal. Of interest however is the fact that an adjacent glomerulus showed extensive involvement manifested by glomerular hypercellularity and pronounced mesangial proliferation. This focal nature of the distribution of lesions was seen in animals of both groups (HPS, ×450).

Sections were examined independently by both authors without knowledge of the animal's group or time of biopsy. In assessing changes seen by light microscopy the following particular features were studied and graded on a scale from 0 (normal) to 4+ (severe): number of glomeruli involved, presence of inflammatory cells, reduction in open capillary loops, mesangial cellular and/or fibrillar proliferation, capillary basement membrane changes, glomerular epithelial crescents and adhesions to Bowman's capsule, tubular casts, and interstitial inflammation. For fluorescent microscopy the presence of glomerular or interstitial fluorescence for each of the specific antigens was assessed and graded from 0–4+. If fluorescence was seen the extent and pattern of deposition was noted.

TABLE I. Light Microscopy.[a]

	0	1	2	3	4+
Group 1, nonanticoagulated		1	8	4	
Group 2, anticoagulated	1	3	6	4	1

[a] Specific abnormalities were graded on a scale from 0 to 4+ as described in the text. This table is a composite based on the overall assessment of the individual abnormalities found. The values refer to the number of animals.

Results. Light microscopy (Figs. 1A and B, 2 and 3; Table I). No lesions were seen in the kidney biopsies obtained prior to injection of BSA. At the time of antigen elimination lesions were observed in all animals except one from Group 2. There were no differences in the severity or type of lesion between the animals of either group. The number of glomeruli involved varied between animals. In animals with less severe glomerular lesions only about 50% of the glomeruli were affected whereas in animals showing severe glomerular lesions virtually all glomeruli were involved to some extent. In less severely involved glomeruli the lesions were segmental, leaving other lobules normal. The main lesions consisted of proliferation of the

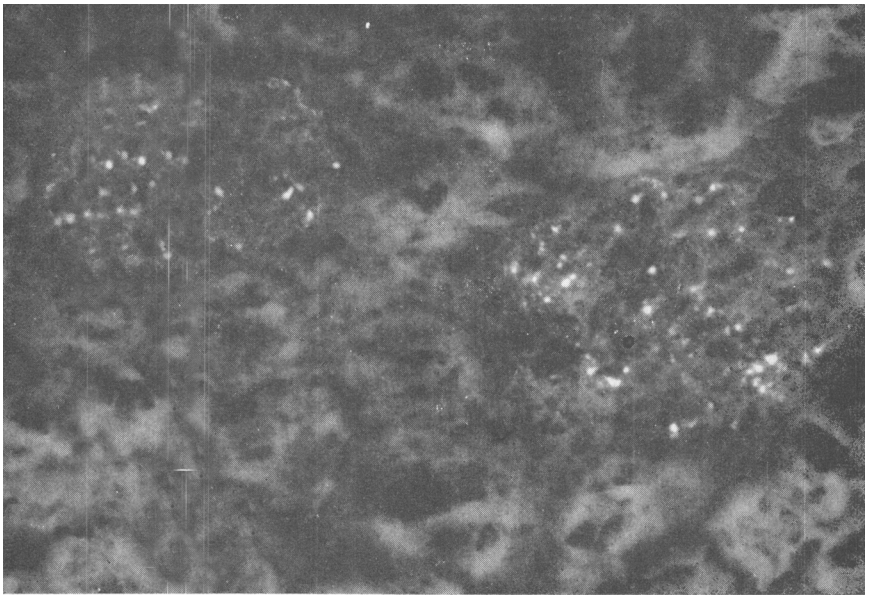

FIG. 4. Two glomeruli stained for rabbit gamma globulin from an anticoagulated animal at time of antigen elimination are shown. Scattered discrete nodules of fluorescence are seen which occasionally appear to follow the course of the capillary loops. Severe lesions were seen by light microscopy (original magnification ×312).

mesangium with a variable increase in the number of mesangial nuclei, reduction in the number of open capillary loops in affected lobules, polymorphonuclear leukocyte infiltration, and in the more severely involved glomeruli focal necrosis and epithelial crescents. Some glomeruli showed adhesions to Bowman's capsule. The glomerular capillary basement membranes were usually of normal thickness, however occasional localized thickening was observed. Foci of interstitial mononuclear cells and tubular casts were seen in about half of the biopsies and were more common in those with severe glomerular damage.

Fluorescence microscopy (Figs. 4 and 5; Table II). No fluorescence was seen for rabbit gamma globulin, B_{1C}, fibrinogen or BSA in any of the biopsies prior to BSA injection. At the period of antigen elimination a minority of the animals in each group showed positive fluorescence for these antigens. The number of animals showing positive fluorescence for B_{1C} and gamma globulin was higher in Group 1 than in Group 2. In only one animal was BSA detected. The B_{1C} and gamma globulin deposits were distributed in a discrete nodular pattern along capillary basement membranes or within the mesangium and involved about one eighth to one third of the glomerular surface area. Fluorescence for fibrinogen was not common and even when detected was only of 1–2+ intensity. It appeared to be within the capillary lumina or the mesangium and, in several instances, was seen in Bowman's space.

Discussion. Fibrinogen deposition has been noted in the glomerular lesions of a variety of human renal diseases, (1, 17–20) and in nephrotoxic serum nephritis in the experimental animal (5–7). The value of anticoagulation in patients with these diseases has been explored (21–24) and a favorable response has been observed in some cases. There is, furthermore, convincing evidence that in nephrotoxic serum nephritis both the clinical course and pathologic changes can be modified by anticoagulation (3–7). The relation between improvement following anticoagulation and underlying pathogenetic mechanisms is not clear. In some of these disorders intravascular coagulation is proba-

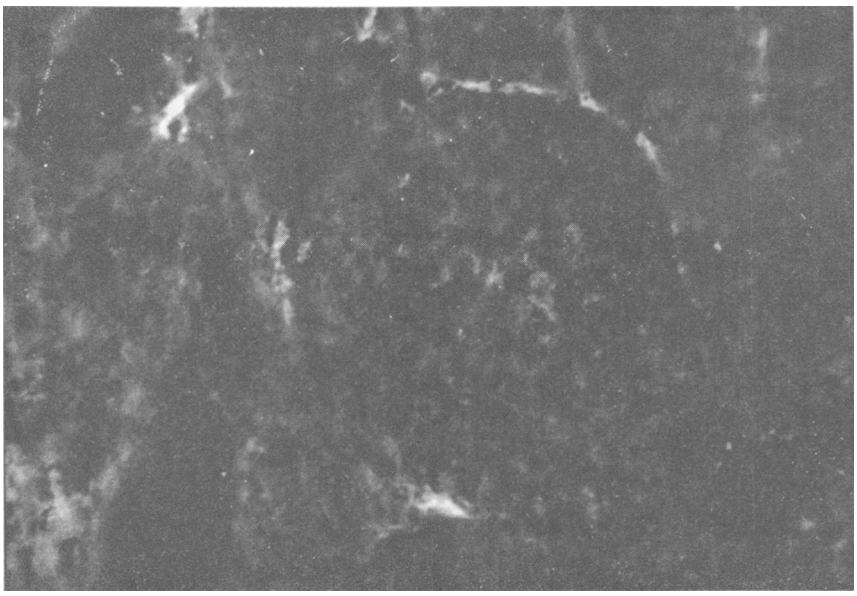

FIG. 5. This glomerulus from a nonanticoagulated animal at time of antigen elimination is stained with fluorescein isothiocyanate labeled antiserum to rabbit fibrinogen. Only minimal fluorescence confined to Bowman's space is seen. Light microscopic study showed extensive involvement of most of the glomeruli (original magnification ×500).

bly of primary importance. In others immunologic mechanisms which involve either antigen–antibody complex deposition or antiglomerular basement membrane antibodies are operative. It is known that soluble antigen–antibody complexes accelerate coagulation both *in vivo* (25) and *in vitro* (26). It might therefore be anticipated that anticoagulation would modify any possible role of glomerular fibrinogen deposition in the pathogenesis of antigen–antibody complex nephritis.

Under the conditions of our study there was no significant glomerular fibrinogen deposition in either control or anticoagulated animals at the time of maximum glomerular damage nor was it possible to modify the pathologic lesion by means of anticoagulation. The incidence of glomerular damage seen by light microscopy was slightly higher in our study than has been reported by others (10, 27) however the incidence of positive fluorescence for IgG and BSA was similar in our nonanticoagulated animals as in the study by Fish *et al.* (10). Also the lack of correlation between the findings of immune deposits and histologic changes was noteworthy in our study and is similar to that reported by these authors. Of particular interest was the finding that the anticoagulated group had a lower incidence of glomerular fluorescence for IgG and B_{1C} than was seen in the nonanticoagulated group. Whether this difference is significant is not known and a larger study group would be required in order to establish this point.

We interpret these results as indicating that although the coagulation process may be of importance in the pathogenesis of nephrotoxic serum nephritis—a condition due to antibasement membrane antibodies—it does not play a significant role in the pathogenesis of antigen–antibody complex nephritis of the acute serum sickness type.

Summary. Coagulation processes are considered important in the pathogenesis of several forms of human and experimental renal disease. The possibility was examined that anticoagulation could modify the course of acute serum sickness nephritis in rabbits.

Bovine serum albumin (BSA) was given iv to 28 rabbits. Beginning on the sixth to eighth day 15 rabbits were anticoagulated with iv warfarin sodium. Renal biopsies done

TABLE II. Fluorescence Microscopy.[a]

	B_{1C}					Gamma globulin					Fibrinogen					BSA				
	0	1	2	3	4+	0	1	2	3	4+	0	1	2	3	4+	0	1	2	3	4+
Group 1, nonanticoagulated	7		1	4	1	9		3	1		12		1			13				
Group 2, anticoagulated	13		1	1		12	2	1			12	1	2			14		1		

[a] Fluorescence was assessed on the basis of its extent and intensity and graded on a scale from 0 to 4+. The values in each column refer to the number of animals.

prior to BSA injection and at time of BSA elimination from the serum were examined by light and immunofluorescent microscopy. Most animals in both the nonanticoagulated and anticoagulated groups had significant glomerulonephritis and no differences between the groups were detected.

Although the evidence is convincing that the coagulation process is operative in the pathogenesis of nephrotoxic serum nephritis—a condition due to antiglomerular basement membrane antibodies—our data suggest that this mechanism does not play a significant role in the pathogenesis of antigen–antibody complex nephritis of the acute serum sickness type.

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Received Jan. 31, 1972. P.S.E.B.M., 1972, Vol. 140.