ANTIGEN-ANTIBODY REACTION IN THE PATHOGENESIS OF BILATERAL RENAL CORTICAL NECROSIS*

By LEUNG LEE,‡ M.D.

(From the Department of Pathology, New York University School of Medicine, New York)

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The generalized Shwartzman reaction has become a valuable laboratory model for studying the pathogenesis of bilateral renal cortical necrosis because of the close similarity between the experimental lesions and those of human disease (1).

The phenomenon has classically been produced in the rabbit by bacterial endotoxins (2) and results from the widespread occlusion of glomerular capillaries by dense fibrin deposits (3–5). Two basic conditions (3) appear essential for the development of the lesion: (a) the slow activation of intravascular coagulation with the formation of fibrin aggregates in the circulating blood, and (b) the inhibition of the protective mechanism concerned with the intravascular removal of fibrin. In the rabbit it has recently been shown that the phagocytic clearance mechanism of the reticuloendothelial system is largely responsible for the elimination of circulating fibrin aggregates, and that unless reticuloendothelial blockade occur concurrently with intravascular coagulation, fibrin is not generally deposited in sufficient quantity to produce occlusive vascular lesions (3, 6). In view of these findings it has been suggested (3) that bacterial endotoxins produce the generalized Shwartzman reaction by virtue of their capacity not only to initiate intravascular coagulation (7) but also to cause profound depression of reticuloendothelial function (8).

There is evidence to suggest that antigen-antibody interaction may also result in the activation of blood coagulation (9, 10). However, bilateral renal cortical necrosis has not been observed as a direct consequence of immune reactions *in vivo* despite their capacity to produce other forms of tissue damage such as acute serum sickness or the Arthus reaction. It is possible that any fibrin aggregates formed in the blood stream as the result of antigen-antibody interaction are efficiently removed by an active reticuloendothelial system. From these considerations it was expected that lesions resembling those of the

generalized Schwartzman reaction would develop under these circumstances, provided the reticuloendothelial system were blockaded. This has indeed proved to be the case, and in the present report similarities in the production of bilateral renal cortical necrosis by antigen-antibody reaction and by bacterial endotoxin are described, and the implications of this interrelationship are discussed.

Materials and Methods

Animals.—Hybrid albino rabbits of either sex weighing approximately 1.4 to 1.8 kilos were used.

Antigens.—Crystallized bovine serum albumin (BSA) was obtained from Armour Pharmaceutical Co., Kankakee, Illinois, and hen ovalbumin, twice recrystallized, was obtained from Worthington Biochemical Corp., Freehold, New Jersey. The materials were dissolved in pyrogen-free physiologic saline to make a 1 per cent solution for parenteral injection.

Immunization of Animals.—In experiments employing actively immunized rabbits, immunization with BSA was accomplished in the following manner. Each rabbit received an initial intramuscular injection of 10 mg of BSA incorporated in complete Freund's adjuvant (Difco Laboratories, Inc., Detroit) followed at weekly intervals by intramuscular injections of 20 mg of BSA without adjuvant for a total of 4 injections. Four days after the last inoculation, the antibody response to BSA was determined in each animal by the qualitative precipitin technique carried out in glass capillary tubes. By comparing the amount of precipitate formed at equivalence against precipitin reactions using antiserum to BSA of known antibody content, an approximation of the amount of antibody in the circulation of the test animals was obtained. The majority of rabbits were estimated to have antibody concentrations of approximately 1 to 2 mg of antibody protein per ml of serum. These animals were used for experimentation within 1 week after the last immunizing injection.

Antiserum.—Antiserum against BSA was prepared in a group of 20 rabbits immunized over a 2 month period following the procedure described in the previous section. The animals were bled by cardiac puncture 5 days after the last injection of antigen. Using sterile precautions the sera were pooled, inactivated at 56°C for 30 minutes, and stored in the frozen state until use. The pooled antiserum was analyzed for antibody content by the quantitative precipitin technique (11), and was found to contain 4.9 mg antibody protein per ml.

Preparation of Soluble Immune Complexes.—Soluble complexes were prepared by the addition to antiserum of 5 times the amount of antigen used to precipitate at equivalence. To 500 ml of the pooled antiserum containing 4.9 mg antibody protein per ml was added 1750 mg of BSA dissolved in 100 ml of sterile physiologic saline solution. Slight precipitation occurred in the reaction mixture after incubating overnight at 4°C. The suspension was centrifuged in the cold, and the clear supernatant containing the soluble complexes was collected for intravenous administration.

Reticuloendothelial Blockade.—Blockade of the reticuloendothelial system was produced by injecting intravenously 3 ml of thorotrast per kilo of body weight (12). This preparation was obtained from Testagar & Co., Detroit, and consisted of a sterile colloidal suspension containing 24 to 26 per cent thorium dioxide.

Anticoagulant.—Liquaemin sodium (Organon, Inc., West Orange, New Jersey) was an aqueous solution of heparin solution containing 1000 units per ml.

Demonstration of "Heparin-Precipitable Fibrinogen".—Four ml blood samples were withdrawn from each animal by cardiac puncture into syringes containing heparin in a concentration of 100 units per ml of blood. The plasma was collected in small tubes and placed in an ice bath. At the end of 2 hours, the amount of precipitate formed was estimated visually.

Immunohistochemical Studies.—The details in the preparation and absorption of sheep

antiserum against rabbit fibrin, the conjugation with fluorescein isothiocyanate, the controls employed for determining the specificity of staining, and the preparation and staining of tissue sections were described in an earlier communication (6). A sheep antiserum directed specifically against rabbit gamma globulin and conjugated with flourescein isothiocyanate according to the procedure described by Mellors (13) was kindly furnished by Dr. Robert T. McCluskey.

Routine Histological Studies.—Autopsies were performed immediately following sacrifice of the animal or as early as possible when death had occurred during the night. Blocks of tissue from the kidney, lung, spleen, and liver were fixed in 10 per cent neutral formalin, and sections were stained with hematoxylin-eosin, periodic acid–Schiff, and phosphotungstic acid–hematoxylin.

EXPERIMENTAL

Effect of Reticuloendothelial Blockade on Immunized Rabbits Challenged with Antigen.—Thirty-two rabbits immunized against BSA were estimated to have

TABLE I

The Occurrence of Bilateral Renal Cortical Necrosis in Blockaded Immunized Rabbits Challenged with Antigen

Antigen injected	Status of rabbit	Reticulo-endothelial system	No. of rabbits	No. dead	No. with bilateral renal cortical necrosis
BSA	Immunized against BSA	Normal	10	2	0
"	" " "	Blockaded	14	6	8
Ovalbumin	" " "	"	8	0	0
BSA	Normal	"	8	0	0

in their circulation a specific antibody concentration of approximately 1 to 2 mg of antibody protein per ml of serum. In 14 of these animals reticuloendothelial blockade was produced by the intravenous injection of 3 ml of thorotrast per kilo of body weight. Four hours later each rabbit was challenged intravenously with 50 mg of BSA given slowly over a period of 15 minutes. As controls, 10 immunized rabbits with normal reticuloendothelial function were challenged with the same dose of BSA; an additional 8 immunized animals were also pretreated with thorotrast but challenged with 50 mg of a heterologous antigen, ovalbumin; and lastly, 8 rabbits without prior immunization were subjected to reticuloendothelial blockade and subsequent challenge with BSA. The survivors were sacrificed 24 hours later. The results are presented in Table I.

In previous studies it was found that a single injection of endotoxin or a slow infusion of thrombin ordinarily produced few pathologic alterations in the normal animal, while the administration of these same substances in combination with reticuloendothelial blockade regularly led to massive fibrin deposition

and bilateral renal cortical necrosis (3). It is evident from Table I that the injection of specific antigen into actively immunized rabbits achieved results which closely paralleled those described following endotoxin or thrombin administration, and that in the presence of reticuloendothelial blockade the renal lesion produced (Fig. 1) appeared identical with that of the generalized Shwartzman reaction. The necrosis of convoluted tubules and the hemorrhage into the interstitial tissue were apparently the result of widespread deposition of dense, eosinophilic fibrinoid material occluding the glomerular capillaries (Fig. 2). The material appeared either homogeneous or somewhat fibrillar depending on the compactness of the deposits, and like fibrin, reacted characteristically with periodic acid–Schiff and phosphotungstic acid–hematoxylin stains. Further resemblance to the fibrin deposits of the generalized Shwartzman reaction (14) was shown by the birefringence of this material when glomeruli teased from fresh specimens were examined with polarized light.

From other experiments it was found that the fibrinoid material was first deposited as a dense layer along the inner wall of glomerular capillaries and could be detected as early as 2 hours after the challenge with specific antigen. Over the next several hours there was progressive accumulation of this material which eventually filled or markedly narrowed the capillary lumina, and by the 8th hour early necrotic changes in the convoluted tubules became evident. Histopathologic changes similar to those in the kidneys were occasionally observed in the liver, spleen, and lungs, but in these organs the lesions were less conspicuous and sparsely distributed.

In all of the immunized rabbits which failed to exhibit renal cortical necrosis following the challenge with specific antigen, small clumps of amorphous eosinophilic material were occasionally seen in the lumen of glomerular capillaries. Unlike the fibrin deposits of the generalized Schwartzman reaction, the material did not line endothelial surfaces in the characteristic "wire loop" pattern.

In the present experiment the majority of deaths occurred 4 or more hours after the injection of antigen. In most instances, the cause of death could not be satisfactorily explained on the basis of the autopsy findings.

Effect of Soluble Complexes in Rabbits with Reticuloendothelial Blockade.— Preformed soluble antigen-antibody complexes have been shown to exert a variety of biological effects including the acceleration of the clotting process *in vitro* (10). It was therefore of interest to determine whether normal animals infused with soluble complexes would also develop lesions similar to those of the generalized Schwartzman reaction.

Each of 20 normal rabbits received intravenously 30 ml of a solution containing BSA rabbit anti-BSA soluble complexes prepared as described. The solution was slowly infused into the marginal ear vein over a period of 1 hour. In 12 of these rabbits thorotrast had been given 3 hours previously to produce reticuloendothelial blockade. As controls, a group of 8 blockaded

rabbits received intravenous infusions of an equal volume of antiserum alone, while 8 others were infused with a preparation containing BSA mixed with normal serum. Those animals surviving to the 24th hour were sacrificed.

The results are shown in Table II.

The hemorrhagic necrosis in the kidneys occurring after the intravenous infusion of soluble complexes was entirely comparable both in the gross (Fig. 3) and histologically (Fig. 4) to the lesions elicited by the administration of endotoxin or thrombin in normal rabbits or by the injection of antigen into immunized animals. As expected, blockade of the reticuloendothelial system was required for the development of the renal lesion. In this and the preceding experiment, acute glomerulonephritis and arteritis were not observed in any of the tissues examined.

TABLE II

The Occurrence of Bilateral Renal Cortical Necrosis in Blockaded Rabbits Infused with Soluble Antigen-Antibody Complexes

Material infused	Reticuloendothelial system	No. of rabbits	No. dead	No. with bilateral renal cortical necrosis
BSA anti-BSA serum	Normal	8	1	0
" " "	Blockaded	12	3	6
BSA normal serum	"	8	0	0
Anti-BSA serum	"	8	0	0

Effect of Heparin on the Production of Bilateral Renal Cortical Necrosis by Antigen-Antibody Interaction.—One consequence of the injection of antigen into immunized rabbits or the infusion of preformed soluble complexes into normal animals might be the intravascular deposition of antigen-antibody aggregates (15, 16). This deposition might be further enhanced by blockade of the reticuloendothelial system (17) which was shown to function efficiently in the clearance of antigen-antibody complexes from the circulation (18). These considerations raised the question whether the renal cortical necrosis produced under the present circumstances was indeed due to intravascular coagulation or was simply the result of massive deposition of antigen-antibody aggregates causing obstruction of the glomerular circulation. In the next experiment an attempt was made to discriminate between these alternative mechanisms by studying the effect of heparin on the occurrence of the renal lesion. It was reasoned that if renal cortical necrosis were due to the deposition of antigen-antibody precipitates, rendering the blood incoagulable by the administration of heparin would not interfere with the development of the lesion. If, on the other hand, intravascular coagulation were the central mechanism in the

pathogenesis of this phenomenon, then inhibiting the conversion of fibrinogen to fibrin with heparin would effectively prevent the production of occlusive vascular lesions.

Accordingly, three groups of rabbits, immunized against BSA and subjected to reticuloendothelial blockade, were challenged intravenously with 50 mg of BSA. Just prior to the injection of antigen, one group of animals was given heparin intravenously in a dose of 3000 units per kilo of body weight. In the second group heparin was not given to the animals until 4 hours after the challenge with antigen. The third group of immunized rabbits served as controls and did not receive heparin. The experiment was terminated at the 24th hour, and the survivors were sacrificed.

The results are presented in Table III.

It was found that none of the immunized rabbits pretreated with heparin had developed renal cortical necrosis following the injection of antigen. Fur-

TABLE III

Effect of Heparin on the Occurrence of Bilateral Renal Cortical Necrosis in Blockaded Immunized Rabbits Challenged with Antigen

BSA injected at zero time	No. of blockaded immunized rabbits*	No. with bilateral renal cortical necrosis
Heparin given immediately before BSA	10	0
" " 4 hrs. after BSA	7	3
" omitted	6	3

* Each rabbit was previously immunized against BSA and subjected to reticuloendothelial blockade prior to challenge with the specific antigen.

thermore, histologic examination of the kidneys of these animals did not reveal the deposition of fibrinoid material within the glomerular capillaries. By contrast, when the administration of heparin was omitted or was delayed until 4 hours after the injection of antigen, the occurrence of renal cortical necrosis was not prevented. The results of this experiment were therefore in accord with the hypothesis that intravascular coagulation was directly responsible for the production of the lesions observed in the present investigation.

Immunohistochemical Studies on the Nature of the Fibrinoid Deposits Produced by Antigen-Antibody Interaction.—It still remained to be shown that the material occluding the glomerular capillaries was in fact fibrin. To this end, the kidneys from several immunized rabbits which had developed cortical necrosis after the challenge with antigen were studied with the fluorescent antibody technique.

When the tissue sections were treated with fluorescein-labeled sheep anti-rabbit fibrin (or fibrinogen) antiserum, it was found that in almost every glomerulus (Fig. 5) the capillary loops were filled and distended by large

deposits of apple-green fluorescent material which corresponded in quantity and distribution to the fibrinoid deposits observed in routine histological preparations. The specificity of the immuuological staining was shown by the virtually complete absence of specific fluorescence produced by the prior application to the tissue section of unconjugated anti-rabbit fibrin antiserum. The staining was not prevented, however, by the prior application of unconjugated sheep antiserum containing antibodies against rabbit gamma globulin and several other serum proteins.

It was further of interest to determine whether the deposition of antigen-antibody precipitates had contributed significantly to the obstruction of the glomerular circulation. Accordingly, additional tissue sections were treated with a conjugated sheep anti-rabbit gamma globulin antiserum. In these preparations, any reactive material demonstrated was presumed to represent antigen-antibody aggregates. It was found that only occasional glomerular capillary loops contained large clumps of specific fluorescent material. For the most part, the stained material was scanty in amount and usually appeared as a thin layer along the inner surface of glomerular capillaries or as fine granules lying free within the lumina (Fig. 6). As controls, tissue sections from normal kidneys were also stained with conjugated antiserum directed against either rabbit fibrin or rabbit gamma globulin. In these preparations, no fluorescent deposits were observed within the glomerular capillaries.

The evidence provided by immunofluorescence showed beyond doubt that it was chiefly material derived from fibrinogen rather than deposits of antigen-antibody aggregates which occluded the glomerular circulation and led to bilateral cortical necrosis. With this technique it was not possible to determine whether the material was actually fibrin or fibrinogen, since these are antigenically similar. However, in view of the fibrillar and birefringent characteristics of the glomerular deposits and the prevention of the lesions by pretreatment with heparin, it would seem far more reasonable to consider the material deposited as being fibrin rather than precipitated fibrinogen.

The Occurrence of "Heparin-Precipitable Fibrinogen" (HPF) Following Immune Reactions in Vivo.—Thomas, Smith, and von Korff (19) had previously shown in the plasma of endotoxin-treated rabbits a derivative of fibrinogen which formed a flocculent or stringy precipitate in the cold following the addition of heparin. These investigators had suggested that HPF was an intermediate stage in the conversion of fibrinogen to fibrin capable of combining with heparin to form a precipitable complex *in vitro*. More recently, it was shown that in rabbits infused with dilute thrombin solutions HPF was also present in large amounts in the circulating blood (3). While it is not clear whether HPF is the precursor of the fibrin deposited in the generalized Schwartzman reaction, the evidence does suggest that its presence in the circulation is an indication of intravascular coagulation (3, 20).

It was therefore significant that HPF was regularly demonstrated in plasma samples obtained from immunized rabbits challenged with the specific antigen or from normal rabbits infused with soluble complexes. In many of these rabbits, the response was quantitatively comparable to that observed after an injection of endotoxin. As was the case for endotoxin, HPF appeared in the circulation usually not earlier than 1 hour after the injection of antigen, reached maximal quantity 2 to 3 hours later, and was much diminished after 6 hours. The immunologic specificity of this reaction was shown by the absence of HPF both in normal animals injected with antigen and in immunized rabbits challenged with a heterologous antigen.

The possibility that the precipitates observed in these chilled heparinized specimens represented antigen-antibody aggregates was ruled out by the following considerations: (a) the failure to demonstrate HPF in serum or citrated plasma, (b) the stringy nature of the precipitate, (c) the time of appearance of HPF and the duration of its persistence in the circulation, and (d) the absence of HPF in the blood of animals pretreated with large doses of heparin.

DISCUSSION

In the present study, the intravenous injection of antigen into specifically immunized rabbits or the infusion of soluble antigen-antibody complexes into normal animals has been shown to result under appropriate conditions in the production of bilateral renal cortical necrosis. The implications of this finding may be of some importance for several reasons. In the first place, the demonstration of massive fibrin deposits in the glomerular capillaries and of "heparin-precipitable fibrinogen" in the circulation of these animals clearly indicates that *in vivo* reactions between antigen and antibody may lead to activation of the blood coagulation system. Since it has been shown that similar reactions *in vitro* accelerate the clotting process (9, 10) and result in activation of platelet factor 3 (21), it seems likely that the initiation of intravascular coagulation is indeed a primary effect of antigen-antibody complexes and as such may have considerable significance in the pathogenesis of hypersensitivity states.

Furthermore, the results of the present study have a direct bearing on the pathogenesis of the generalized Schwartzman reaction. The evidence indicates that this phenomenon is a general one, as it can be produced by bacterial endotoxins, dilute thrombin solutions, or antigen-antibody complexes. It appears that the continuous formation of circulating fibrin aggregates together with inhibition of the phagocytic fibrin-clearing mechanism is the common denominators in the production of bilateral renal cortical necrosis in the rabbit by these different agents. It is possible that the occurrence of lesions resembling the generalized Schwartzman reaction in various human disorders unrelated in etiology is explained on this basis.

Finally, the capacity of antigen-antibody reactions to reproduce the general-

ized Shwartzman reaction further supports the view that certain biological effects elicited by bacterial endotoxins may be mediated by circulating antibody. Numerous reports have described similarities between the effects produced by endotoxins and by antigen-antibody complexes (9, 22–24). Moreover, the demonstration of cross-reactive antibodies to endotoxins in the serum of normal rabbits (25) may account for the lack of immunological specificity of endotoxin-induced phenomena. While it is attractive to consider the biological activity of endotoxins on the basis of their antigenicity, yet it may well be, as Stetson (26) has recently suggested, that similarities between reactions to endotoxins and to hypersensitivity merely represent final common pathways of tissue reaction triggered in different ways but expressed in a common phenomenology.

SUMMARY

In the presence of reticuloendothelial blockade, the intravenous injection of a protein antigen into specifically immunized rabbits or the infusion of soluble immune complexes into normal animals has been shown to result in the production of bilateral renal cortical necrosis. The similarity in the pathogenesis of this lesion and that seen in the classical generalized Shwartzman reaction produced by bacterial endotoxins is indicated by (a) the failure of both lesions to develop in animals pretreated with large doses of heparin, (b) by the finding of "heparin-precipitable fibrinogen" in the circulation, and (c) by the presence of massive fibrin deposits within the glomerular capillaries. These findings indicate that antigen-antibody reactions in vivo are capable of activating the blood coagulation system and that the mode of action of bacterial endotoxins may have an immunological basis.

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EXPLANATION OF PLATE 17

FIG. 1. Gross appearance of kidney exhibiting hemorrhagic necrosis from an immunized rabbit subjected to reticuloendothelial blockade and challenged intravenously with 50 mg of the specific antigen, BSA. The lesion appears identical with that produced by bacterial endotoxins. × 1.

FIG. 2. Histologic section of kidney shown in Fig. 1 stained with phosphotungstic acid–hematoxylin. The capillaries of a glomerulus are shown to be occluded by massive deposits of "fibrinoid" material. The adjacent convoluted tubules exhibit necrosis in various stages of development. × 400.

FIG. 3. Gross appearance of kidney showing cortical necrosis from a rabbit pretreated with thorotrast and given an intravenous infusion of soluble antigen-antibody complexes. The features are similar to those found in the generalized Shwartzman reaction. × 1.

FIG. 4. Histologic section of kidney shown in Fig. 3 stained with phosphotungstic acid–hematoxylin. Widespread occlusion of the glomerular capillaries by "fibrinoid" material is seen. × 400.

FIG. 5. Histologic section of kidney shown in Fig. 1 stained with fluorescein-labeled sheep anti-rabbit fibrin antiserum. Large deposits of specific fluorescent material are seen occluding the capillaries of a glomerulus. × 800.

FIG. 6. Histologic section of kidney shown in Fig. 1 stained with fluorescein-labeled sheep anti-rabbit gamma globulin antiserum. A glomerulus in shown with distended capillary loops. However, specific fluorescent material appears only as a thin layer along the inner wall of the glomerular capillaries or as granules lying within the lumina. × 800.
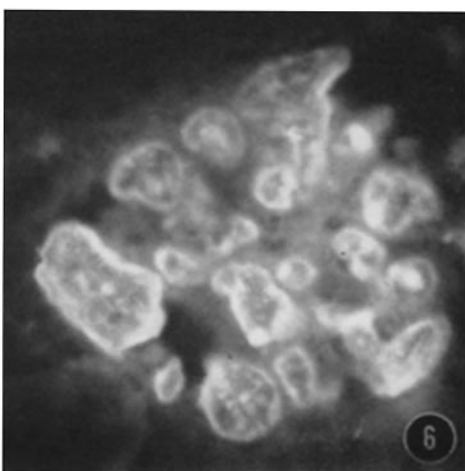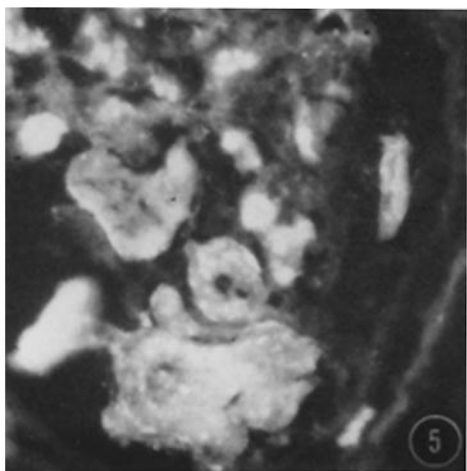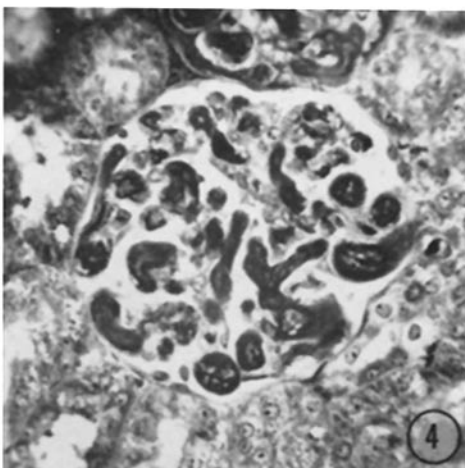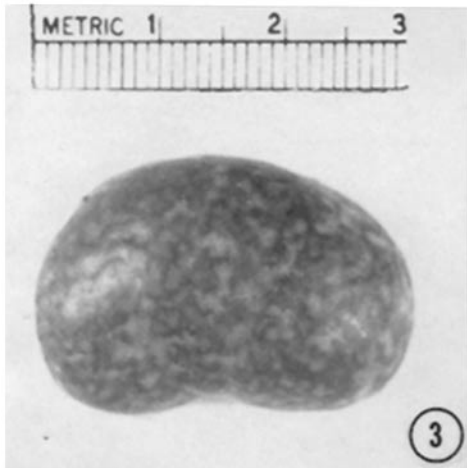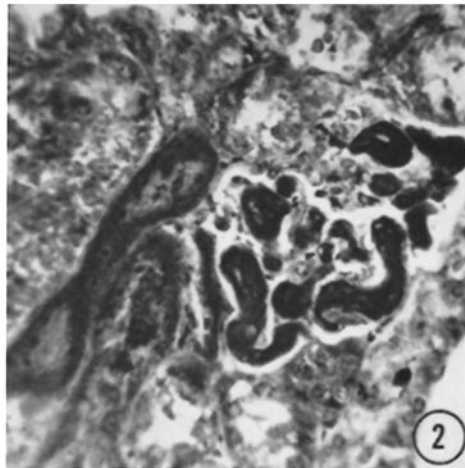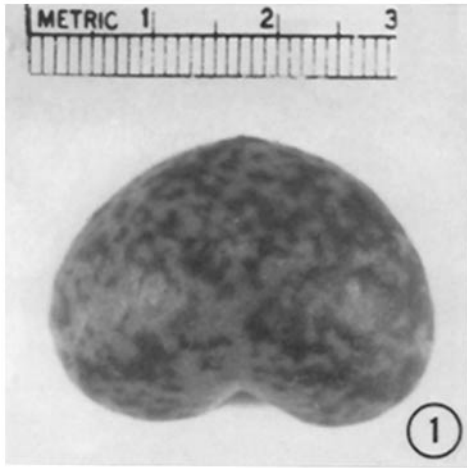
(Lee: Tissue damage by immune reactions)