Specific localized tissue damage resulting from an interaction between a tissue antigen and injected serum containing antibodies against that antigen has been repeatedly demonstrated in animals (1). The demonstration that collagen, a fibrous connective tissue protein, is antigenic has made it possible to investigate tissue injury by antibodies against this protein. When purified soluble collagen prepared from rat tail tendons is injected into rabbits, it induces complement-fixing antibodies (2). Although rabbit serum containing antibodies to rat collagen causes reverse anaphylactic shock, when injected into rats, neither single lethal nor multiple sublethal injections results in detectable injury to tissue collagen (3). However, collagen antibody has been found to have a specific effect on collagen in vitro. The addition of rabbit anti-rat collagen serum prevents the reconstitution of rat collagen fibers from solution, whereas the addition of normal rabbit serum results in the formation of fibers with the characteristic periodicity of native collagen (2). Rabbit anti-chicken collagen serum has also been shown to inhibit fibrogenesis in tissue cultures of chick dermis (4). Therefore, further attempts were made to determine whether anti-collagen serum would induce collagen injury in vivo.

This report deals with the induction of renal glomerular lesions by the use of rabbit anti-rat collagen serum in rats prepared with a water-in-paraffin oil adjuvant emulsion.

**Materials and Methods**

Young male and female, black and white hooded rats of the highly inbred Whelan strain, weighing between 90 and 150 gm. were used. Their diet consisted of "big red" dog pellets, bread, and milk daily, greens twice weekly, and water ad libitum.

The complete adjuvant as described by Freund (5) consisted of a water-in-paraffin oil emulsion containing killed mycobacteria. The aqueous phase was 20 ml. of normal rabbit, *This work was supported by grants-in-aid from the National Heart Institute of the National Institutes of Health, United States Public Health Service; the Helen Hay Whitney Foundation; and the Arthritis and Rheumatism Foundation.*

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chicken, or horse serum or saline. The oil phase was an autoclaved mixture of 1.5 ml. of arlacel A\textsuperscript{1} (mannide monoleate), an emulsifying agent, and 8.5 ml. of bayol F\textsuperscript{2}, a paraffin oil of low viscosity. To this mixture, 8.0 mg. of killed and dried \textit{Mycobacterium butyricum} was added. As the aqueous phase was added by drops to the oil phase, the mixture was emulsified by repeatedly filling and emptying a syringe in it. The incomplete adjuvant was prepared in the same manner without the addition of mycobacteria.

The preparation of the collagen and the rabbit anti-collagen sera as well as the technique of the complement fixation tests have been described (2). Rabbit anti-\textit{Limulus polyphemus} hemocyanin serum (precipitin titer over 1:10,000) was supplied by Dr. Jules Freund of the National Institutes of Health, Bethesda, Maryland. The rabbit anti-Group B streptococcus serum against strain D156C was obtained from Dr. Rebecca C. Lancefield of The Rockefeller Institute, New York. The precipitin titer of this serum was 1:128 with a crude hydrochloric acid extract of the same strain used as antigen; this is considered a potent anti-streptococcal serum. Rabbit anti-rat kidney and rabbit anti-rat glomerular sera were supplied by Dr. Beatrice C. Seegal of the Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York. Each of these sera readily induced nephrotic nephritis in rats.

Rabbit serum containing antibody to rat serum was prepared by intravenous injection of pooled normal rat serum in doses of 2 to 4 ml. daily, 5 days each week over a 4 week period. The rabbits were bled 5 days after the last injection. Rat serum containing antibody to rabbit serum was prepared by injecting 1.0 ml. doses of normal rabbit serum intravenously or intraperitoneally daily for 5 days each week over a 6 week period. The rats were bled from the heart 5 days after the last injection. Precipitin titers of each of these two sera were determined by adding constant amounts of antisemum to 2-fold serial dilutions of antigen in capillary tubes, after the method of Swift, Wilson, and Lancefield (6).

Adjuvant was injected subcutaneously into the rats in the nuchal region and along both sides, 2 cm. from the spine. One ml., divided among several sites, was given each week for 3 weeks.

Intravenously injected serum or saline was given in 3 to 7 daily doses, beginning 1 week after the last subcutaneous injection. All sera used for intravenous injection were heated at 56\textdegree{}C. for half an hour, to diminish the primary toxicity.

The animals were sacrificed by ether anesthesia 7 days after the last injection, unless otherwise stated. Tissues were fixed in Zenker-formol or 10 per cent neutralized formalin. Paraffin sections were stained as routine with hematoxylin and eosin and, in many instances, with the Goldner-Foot modification of Masson's trichrome stain for connective tissue, Weigert's differential stain for fibrin, periodic acid-Schiff reaction, and Giemsa or methylene blue. Frozen sections were stained for fat with Scharlach R.

**EXPERIMENTAL OBSERVATIONS**

\textit{Attempts to Induce Specific Tissue Injury by Anti-Collagen Serum}.—Since serum containing antibodies to rat collagen reacts specifically with collagen \textit{in vitro}, it appeared reasonable to assume that rats injected with these antibodies would show damage to tissue containing collagen. However, varying amounts of anti-collagen serum failed to produce alteration of the collagen fibers (Table I).

Because it has been possible to produce injury to organs by injecting tissue

\textsuperscript{1} Obtained from the Atlas Powder Co., Delaware, Maryland.

\textsuperscript{2} Obtained from Esso Standard Oil Co., New York City.
# Table I

Results of Attempts to Produce Tissue Injury in Rats by Rabbit Anti-Rat Collagen Serum

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Preparatory treatment</th>
<th>Inciting agent*</th>
<th>Serum titer†</th>
<th>No. of injections</th>
<th>Time of sacrifice, days after last injection</th>
<th>Total volume</th>
<th>Renal lesions</th>
<th>Granulomata</th>
<th>Lungs</th>
<th>Lymph nodes</th>
</tr>
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<tr>
<td>12</td>
<td>None</td>
<td>Rabbit anti-rat collagen serum</td>
<td>1:128-1:256</td>
<td>3-7</td>
<td>8.0-24.5</td>
<td>1-20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>3</td>
<td>Purified rat tail collagen in adjuvant§</td>
<td>None</td>
<td>1:256</td>
<td>-</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Normal rabbit serum</td>
<td>Rabbit anti-rat collagen serum</td>
<td>1:256</td>
<td>4</td>
<td>10.0</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Rabbit anti-rat collagen serum in adjuvant¶</td>
<td>None</td>
<td>1:256</td>
<td>-</td>
<td>-</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Rabbit anti-rat collagen serum**</td>
<td>Rat anti-rabbit serum</td>
<td>1:128</td>
<td>4</td>
<td>15.0-40.0</td>
<td>7-12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td>22</td>
<td>Normal rabbit serum in adjuvant§</td>
<td>Rabbit anti-rat collagen serum</td>
<td>1:64-1:256</td>
<td>4-5</td>
<td>3.4, 8.0-16.0</td>
<td>1, 7-8</td>
<td>2211</td>
<td>13</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>“ “</td>
<td>Rabbit anti-fish collagen serum</td>
<td>1:128</td>
<td>5-6</td>
<td>9.6-10.3</td>
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<td>0</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>“ “</td>
<td>Rabbit anti-group B streptococcus serum</td>
<td>1:128</td>
<td>6</td>
<td>11.4-12.4</td>
<td>7</td>
<td>0</td>
<td>2</td>
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</tr>
<tr>
<td>4</td>
<td>“ “</td>
<td>Rabbit anti-hemocyanin serum</td>
<td>&gt;1:10000</td>
<td>4-6</td>
<td>3.5-9.4</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>“ “</td>
<td>Rabbit anti-rat serum</td>
<td>1:40728</td>
<td>7</td>
<td>8.5</td>
<td>7</td>
<td>0</td>
<td>39</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>“ “</td>
<td>Normal rabbit serum</td>
<td>1:128</td>
<td>4-5</td>
<td>11.8-16.0</td>
<td>7-9</td>
<td>0</td>
<td>10</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>“ “</td>
<td>Saline</td>
<td>-</td>
<td>4</td>
<td>16.0</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>“ “</td>
<td>None</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>14</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>“ “</td>
<td>Rabbit anti-rat collagen serum absorbed with rat collagen</td>
<td>1:128</td>
<td>4-5</td>
<td>8.4-9.0</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

* Daily intravenous injections beginning 1 week after last preparatory injection.
† Titers of anti-collagen sera were determined by complement fixation. Titers of other antisera were determined by the precipitin method.
§ Three weekly subcutaneous injections of 1.0 mL each.
¶ Four 1.0 mL intravenous injections each week for 3 weeks.
‖ Four weekly subcutaneous injections of 1.0 mL each; serum titer 1:256.
** Three daily intravenous injections of 3.0 mL each; serum titer 1:64.
†† One rat did not develop nephritis; it had been sacrificed only 1 day after receiving 3.4 mL of antiserum.
|| Lungs of 2 animals not examined.
antigens mixed with the Freund adjuvant (5), mixtures of purified solutions of collagen from rat tail tendons and adjuvant were injected subcutaneously into rats in an attempt to induce collagen damage. Microscopic study of tissues from these animals showed no injury except granulomatous lesions with giant cells of the Langhans’ type in the lungs and in regional and distant lymph nodes (Table I). Rist (7) and Casals and Freund (8) have described similar lesions in these organs caused by subcutaneous injection of killed mycobacteria in oil.

Further attempts to induce injury to collagen were based upon Kay’s observation that nephritis did not appear in rabbits as a direct effect of duck antirabbit kidney serum, but occurred only after the rabbits had developed antibodies to the duck serum (9). Since the capacity of rats to form precipitins is relatively poor, attempts were made by various means to increase antibody formation to normal rabbit serum. For this purpose, rats were immunized with normal rabbit serum before they were injected with the anti-collagen serum. In another series of rats, the rabbit anti-rat collagen serum was incorporated in adjuvant and given subcutaneously. In a third series, passive transfer of antibody to rabbit serum produced in rats was employed following the injection of the anti-collagen serum. Histologic examination showed no evidence of collagen injury in any of these experiments (Table I). But the animals given antibody by passive transfer showed some proliferation of cells of the renal glomeruli. While this was not considered significant because it was slight, not consistently seen, and only transient, it did suggest that, with higher titered antibody to normal rabbit serum, definite injury might be found in the renal glomeruli.

Injection of rats with normal rabbit serum in adjuvant consistently developed anti-rabbit serum antibodies in higher precipitin titer than any other method employed. Furthermore, the average titer of 1:512 was sustained for several weeks. When rats so prepared were injected intravenously with rabbit anti-rat collagen serum daily for 4 to 6 days and sacrificed 7 days after the last injection, a diffuse injury to the glomeruli of the kidneys was observed.

To avoid fatal anaphylactic shock, it was found essential to give the rabbit serum in small intravenous doses at first, 0.1 to 0.3 ml., to rats prepared with normal rabbit serum-adjuvant emulsions. Although slight shock did occur even with these small amounts, the rats became refractory after the first injection and subsequent doses up to 3.0 and 3.5 ml. could be given safely.

Pathologic Findings.—The most conspicuous changes occurred in the kidneys, but only in rats which had received injections of water-in-paraffin oil emulsion, followed later by rabbit serum containing antibodies to rat collagen. However, all rats which had received adjuvant showed lesions similar to those described by Freund (5) at the sites of injection of adjuvant, in lymph nodes draining these sites, and in distant nodes, spleen, and lungs.

The kidneys from rats which had received adjuvant and the anti-rat collagen serum were enlarged and pale. The capsules were tense but stripped easily. On hemisection, cortical stria-
tions were usually indistinct. Histologic section showed almost all of the glomeruli to be altered (Figs. 2 to 8); they were enlarged and relatively avascular, with intense swelling, shredding, and fusion of the basement membranes (Fig. 2). Within the tuft, these membranes were fused with loss of the normal glomerular lobulation. Frequently Bowman’s space was obliterated by fusion of the visceral and capsular membranes. Proliferation and swelling of the constituent cells of the glomerular tuft were marked, and numerous mitotic figures were seen. These cells protruded into the capillaries, blocking the lumina (Fig. 2). The cellular proliferation was sometimes focal, but usually it was found throughout the glomerular tuft. Hyaline or fibrinoid material occasionally was found in the glomerular capillaries (Figs. 3 and 4). Eosinophilic deposits within a necrotic focus of the tuft were also noted (Fig. 7). But most striking were the multinuclear giant cells within the glomeruli (Figs. 3, 4, 5, 7, and 8). These giant cells were numerous, and on rare occasions were found within the lumina of the proximal convoluted tubules (Fig. 5). Proliferation of the capsular epithelium fused with the visceral portion and formed typical crescents. Occasionally, Bowman’s space was dilated and filled with pink-staining material (Fig. 6), and the convoluted and collecting tubules were dilated and filled with this eosinophilic substance (Figs. 6 and 8). Strikingly absent were polymorphonuclear leukocytes and fibrin thrombi in the glomerular capillaries; no fat droplets were seen in sections stained with Scharlach R. Furthermore, the interstitial tissue was normal, and the interlobular blood vessels and tubular epithelium showed very little change.

At the sites of adjuvant injection, the skin was intact; on section, oily material drained from these areas. Histologic section showed granulomatous lesions in the subcutaneous tissue. These lesions consisted of many mononuclear cells of the epithelioid type, lymphocytes, plasmacytes, and giant cells with multiple, peripheral nuclei. In many instances, oil droplets were seen within the giant cells, as were free vacuoles of material which also stained with Scharlach R. The tissue about the granuloma was edematous and contained foci of polymorphonuclear leukocytes.

The lymph nodes draining the sites of adjuvant injection, and also tracheo-bronchial and abdominal para-aortic nodes, were enlarged, and on section the surfaces were firm with no evidence of necrosis or abscess formation. Microscopic examination revealed the normal cellular structure to be infiltrated by foci of granulomatous lesions like those described at the sites of injection (Fig. 10). Many of the large multinuclear giant cells had phagocytized oil droplets. No caseation, necrosis, or bacteria were found. Culture of these nodes on appropriate media revealed no growth of mycobacteria or other bacteria.

No gross changes were found in the lungs, but microscopic study revealed the same non-caseating granuloma in the subpleural spaces and, frequently, deep within the lung parenchyma about bronchioles (Fig. 9).

The spleens of all animals receiving adjuvant were markedly enlarged and firm, and, when they were cut, Malpighian corpuscles were prominent. No granuloma were seen on histologic study, but there was a marked increase of large mononuclear cells and foci of plasmacytes. The sinuses were engorged with blood. The liver also contained foci of plasmacytes and lymphocytes in the perportal areas. Ascites, pleural or pericardial effusion, or edema was not observed. No abnormalities of the heart, pancreas, salivary glands, intestine, adrenals, gonads, bone marrow, or knee joints were seen in the gross or on microscopic examination.

A small group of rats was studied at longer intervals after the injections. One or two animals were sacrificed at 1, 3, 6, 9, and 12 months respectively after being prepared with adjuvant and subsequently injected with rabbit anti-rat collagen serum. At no time did these animals develop any apparent edema or ascites, but those sacrificed at 9 and 12 months had lost weight and were less alert than control uninjected litter mates. Granulomata like those found in the lungs of rats killed earlier were not present in animals sacrificed after 3 months, but those in lymph nodes were present without significant change at 1 year.
Kidneys from rats sacrificed at 1 and 3 months showed gross changes similar to those seen in rats killed 7 to 9 days after injection; after 6, 9, and 12 months, the kidneys showed finely pitted surfaces, but were still enlarged. The capsules of these were removed with some difficulty and, on section, both the cortical striations and the cortico-medullary junction were indistinct. Microscopically, the glomerular lesions in rats sacrificed at 3 months showed no giant cells, but early hyalinization was apparent in practically all of the glomeruli (Fig. 11). After longer intervals, the hyalinization became progressively more marked until at 12 months the glomeruli appeared as fibrous masses (Figs. 12 and 13). Fibro-epithelial crescents were prominent between the glomerular tuft and the capsular epithelium (Fig. 14). Masson's trichrome stain and the periodic acid-Schiff preparations showed that the fibrous tissue consisted of new collagen fibers. Changes in the interstitial tissue and tubules were apparent in animals killed as early as 3 months after injection, but became progressively more severe in the older rats. The interstitial tissue showed areas of fibrosis with lymphocytic cellular infiltrates (Fig. 15), but no focal deposition of polymorphonuclear leukocytes. The tubular epithelium in both cortex and medulla was atrophic, and the tubules were filled with hyaline homogeneous casts.

Absence of Renal Lesions in Control Rats.—To determine the specificity of rabbit serum containing antibody to rat collagen in inducing renal lesions, rabbit sera containing antibodies to a number of different antigens were employed (Table I). Anti-fish collagen serum was chosen because fish collagen is a tissue antigen morphologically similar to rat collagen, but immunologically distinct from it (2, 3). Two sera to antigens unrelated to collagen, Group B streptococcus and Limulus polyphemus hemocyanin, were tested. The rabbit serum containing antibodies to rat serum was also employed to establish whether the renal lesion might result from a reaction between any rat tissue antigen and its corresponding antibody. Since most rat tissues contain collagen, serum was selected as another rat antigen; moreover, rat serum had previously been shown to be serologically unrelated to collagen (1). To complete this control series, rats were given normal rabbit serum or saline intravenously, or no intravenous injection at all. All of the rats used as controls were prepared with subcutaneous injections of normal rabbit serum incorporated in adjuvant.

As shown in Table I, no renal lesions developed in any of these control rats. Anaphylactic shock occurred in all of them after the initial intravenous injection of rabbit antiserum, thus indicating that they had developed antibodies to the normal rabbit serum incorporated in the adjuvant. This also showed that shock was not a factor in inducing renal injury. All of the rats in these experiments developed granulomata in lymph nodes and, in many instances, in the lungs. The absence of glomerular lesions suggested that, under the conditions of the experiments, both the rabbit anti-rat collagen serum and the normal rabbit serum-adjuvant mixture were requisites for the production of kidney lesions.

Effect of Rabbit Anti-Rat Collagen Serum Absorbed with Native Rat Collagen.—To find out whether the antibody to rat collagen was specifically necessary for the production of the glomerular lesions, absorption studies were undertaken.
Pooled rabbit anti-rat collagen serum, inactivated at 56°C. for 30 minutes, was mixed with thoroughly washed, finely minced native rat collagen in the proportion of 3 parts serum to 1 part packed collagen (30 ml. of serum to 10 gm. collagen, wet weight). The mixture was incubated for 2 hours at 37°C., stored overnight at 4°C., and centrifuged at 3,500 R.P.M. in the cold to remove large particles of collagen and then centrifuged again for 90 minutes at 40,000 R.P.M. at 0°C. Samples of the pooled serum were tested in the absorbed and unab sorbed states, both by complement fixation and by intravenous injection into rats previously prepared by subcutaneous administration of normal rabbit serum incorporated in adjuvant.

From the results shown in Table II, the native rat collagen completely absorbed the complement-fixing antibodies. With the removal of the anti-collagen antibodies, the capacity to induce the renal lesions was also removed, as shown in Table I. This observation provides further evidence that the antibody which fixes complement in the presence of homologous collagen antigen is an essential factor for the production of these glomerular lesions in rats.

**TABLE II**

<table>
<thead>
<tr>
<th>Antiserum prepared against purified rat collagen</th>
<th>Antigen: purified rat collagen 1 mg./cc. diluted 1:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unabsorbed: control</td>
<td>1  2  4  8  16  32  64  128  256  512</td>
</tr>
<tr>
<td>Absorbed with rat collagen</td>
<td>+++  ++++  ++++  ++++  ++++  ++++  ++++  ++++  +--</td>
</tr>
</tbody>
</table>

--- indicates complete hemolysis; + to +++ indicates intermediate degree of hemolysis; and ++++ indicates no hemolysis with complete fixation of complement.

Normal Chicken or Horse Serum Incorporated in Adjuvant, as the Preparatory Agent.—The question arose whether normal serum from species other than rabbit could be substituted for the normal rabbit serum in the preparatory injections. For this purpose, chicken and horse sera were selected. Rats were prepared with subcutaneous injections of normal chicken serum in adjuvant, and then given intravenous injections of rabbit anti-rat collagen serum, normal rabbit serum, or normal chicken serum. Other rats were prepared with normal horse serum in adjuvant and then intravenously given rabbit anti-rat collagen serum, normal rabbit serum, or normal horse serum. Mild shock was noted at the initial injection of all of the sera except the normal rabbit serum. From the findings shown in Table III, nephritis occurred in the rats prepared with either horse or chicken serum and then challenged with rabbit anti-rat collagen serum, whereas renal lesions failed to develop in those challenged with the normal rabbit, chicken, or horse serum. The granulomata due to adjuvant were found in all the animals. These results indicate that normal rabbit serum is not a
requisite for the production of the renal lesion and raised the question, whether any antigen is required in the adjuvant.

**Adjuvant, without Serum or Mycobacteria, as the Preparatory Agent.**—In further investigation of the serum-adjuvant used in the preparation of the

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Preparatory treatment*</th>
<th>Inciting agent‡</th>
<th>Serum titer§</th>
<th>No. of injections</th>
<th>Total volume</th>
<th>Time of sacrifice, days after last injection</th>
<th>No. of rats with pathologic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Normal chicken serum in adjuvant</td>
<td>Rabbit anti-rat collagen serum</td>
<td>1:64-1:128</td>
<td>4-5</td>
<td>10.0</td>
<td>7</td>
<td>11 8 11</td>
</tr>
<tr>
<td>8</td>
<td>“ “</td>
<td>Normal rabbit serum</td>
<td>—</td>
<td>5</td>
<td>10.0-12.1</td>
<td>7-9</td>
<td>0 2 8</td>
</tr>
<tr>
<td>6</td>
<td>Normal horse serum in adjuvant</td>
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<td>—</td>
<td>5</td>
<td>9.7</td>
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<td>0 6</td>
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<td>—</td>
<td>—</td>
<td>5</td>
<td>8.7</td>
<td>7</td>
<td>0 1 6</td>
</tr>
<tr>
<td>12</td>
<td>Adjuvant without serum</td>
<td>—</td>
<td>—</td>
<td>5</td>
<td>8.7</td>
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<td>Incomplete adjuvant without serum</td>
<td>—</td>
<td>—</td>
<td>5</td>
<td>8.7</td>
<td>7</td>
<td>0 1 6</td>
</tr>
</tbody>
</table>

* Three weekly subcutaneous injections of 1.0 ml. each.
‡ Daily intravenous injections beginning 1 week after last preparatory injection.
§ Titer of anti-collagen sera were determined by complement fixation.
|| Incomplete adjuvant indicates adjuvant without mycobacteria.

TABLE III

**Effect of Varying the Content of the Adjuvant on the Production of Renal Lesions**

Rats, serum, or mycobacteria, or both were omitted. Rats were injected subcutaneously with (a) adjuvant without serum, or (b) normal rabbit serum in incomplete adjuvant, i.e., without mycobacteria, or (c) incomplete adjuvant without serum, and were subsequently given intravenous rabbit anti-rat collagen serum. The results of this experiment are shown in Table III, and provide evidence that neither serum nor mycobacteria are essential in the preparatory subcutaneous injection for the production of the glomerular lesions. Giant cells
of the foreign body type were less numerous, but were present in the renal lesions as well as in granulomata of the lungs and lymph nodes of the rats not given mycobacteria.

Although the renal lesions were present, they were less severe when the serum or mycobacteria or both had been omitted from the adjuvant. A comparison of the degree of renal involvement in rats prepared in different ways is shown in Table IV. There is little apparent difference between normal rabbit and chicken serum used in the adjuvant to prepare rats for the injection of the anti-collagen serum; lesions were severe with either. The two rats tested with horse serum showed less severe renal lesions, but more experiments would be needed for a valid comparison. When serum is omitted altogether from the adjuvant, the severity of lesions is only slightly less than with chicken or rabbit serum. However, the use of incomplete adjuvant, whether combined with rabbit serum or not, leads to lesions of distinctly less severity.

**Anti-Rabbit Serum Precipitins in Rats Given Rabbit Anti-Rat Collagen Serum.**—Antibodies to normal rabbit serum had been induced in the rats in which the renal lesions were first observed by the incorporation of rabbit serum in the adjuvant before the rabbit anti-rat collagen serum was given. Wood (10) reported that when adjuvant and antigen were given to rabbits by separate routes, antibody formation was potentiated. Similar observations were made in 1942 by Freund (11), who injected saline incorporated into aquaphor and bayol F adjuvant intracutaneously or subcutaneously, and soluble protein antigens or killed typhoid bacilli intravenously. He found antibody formation higher in

**TABLE IV**

*Comparison of the Severity of Renal Lesions Induced by Rabbit Anti-Rat Collagen Serum in Rats Prepared by Various Combinations of Adjuvant and Normal Serum*

<table>
<thead>
<tr>
<th>Preparatory materials</th>
<th>Severity of renal lesions in individual rats*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal serum</td>
</tr>
<tr>
<td>Rabbit... Complete‡</td>
<td>++</td>
</tr>
<tr>
<td>Chicken... &quot;</td>
<td>++</td>
</tr>
<tr>
<td>Horse... &quot;</td>
<td>++</td>
</tr>
<tr>
<td>None§... &quot;</td>
<td>++</td>
</tr>
<tr>
<td>Rabbit... Incomplete</td>
<td>++</td>
</tr>
<tr>
<td>None§... &quot;</td>
<td>++</td>
</tr>
</tbody>
</table>

* Each reading is an estimate in a single rat of the severity of the renal lesions on a ++++++ to + scale; 75 to 100 per cent involvement of glomeruli was recorded as ++++++; 50 to 75 per cent as +++; 25 to 50 per cent as ++; less than 25 per cent as +-; no lesions as 0.

‡ Complete adjuvant consists of arlacel A, paraffin oil, and killed mycobacteria; mycobacteria are omitted from the incomplete adjuvant.

§ When no serum was used, saline was substituted.
these rabbits than in others receiving no adjuvants, but not as high as in rabbits injected with antigens incorporated in the adjuvant. Rats given intravenous normal rabbit or rabbit anti-rat collagen serum in 3 to 7 daily doses without adjuvant failed to develop precipitins to rabbit globulin 7 days after the last injection. It therefore seemed important to determine whether antibodies to normal rabbit serum were induced by intravenous injection of the rabbit anti-

TABLE V

<table>
<thead>
<tr>
<th>Rat no.</th>
<th>Rat antiserum</th>
<th>Antigen: normal rabbit serum diluted 1:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control: intravenous rabbit anti-rat collagen serum when rabbit serum was omitted from the preparatory adjuvant.</td>
</tr>
</tbody>
</table>

Blood from 6 normal rats was obtained by cardiac puncture 1 week before they were given 3 weekly subcutaneous injections of 1.0 ml. each of complete adjuvant without serum; 1 week after the third injection, each rat was injected intravenously with rabbit anti-rat collagen serum in daily doses of 1.0, 3.0, 3.0, and 3.0 ml., respectively. Serum was obtained when the rats were sacrificed 7 days after the last injection. All these rats showed renal lesions. The precipitin titers against normal rabbit serum were determined and are shown in Table V.

Anti-rabbit serum precipitins were found in all of the rats following injection with the anti-rat collagen serum.

These findings strongly suggest that all rats which had developed nephritis
had also developed antibodies to the normal rabbit globulin contained in the rabbit anti-rat collagen serum and that both antigen-antibody systems, i.e., collagen–anti-collagen and rabbit globulin–anti-rabbit globulin, probably play a part in the pathogenesis of these renal lesions.

**Relationship between Nephrototoxic Nephritis in Rats and the Renal Lesions Described in the Present Study.**—Since the nephrototoxic nephritis described by Masugi (12) is induced in rats by rabbit antibody to tissue antigen or antigens present in whole rat kidney emulsion, it was important to establish its relationship to the renal lesions found in this investigation. Dr. Beatrice Seegal allowed us to study microscopic sections of rats with nephrototoxic nephritis sacrificed at about the same interval after the intravenous injection of the antibody as those injected with the anti-rat collagen serum.

The nephrototoxic lesion at this stage is characterized by the exudative component of inflammation with fibrin thrombi in the glomerular capillaries, infiltration by polymorphonuclear leukocytes, and necrosis in the glomerular tufts; the interstitial tissue is edematous and infiltrated with lymphocytes, plasmacytes, and polymorphonuclear leukocytes. On the other hand, the renal glomerular lesions in rats injected with the anti-rat collagen antibody are more extensive and show little exudative, but marked proliferative, cellular changes in the glomeruli with little interstitial involvement. Although bizarre giant cells were reported in some of the glomeruli of rats with nephrototoxic nephritis (13), those found in the present study were more numerous, conspicuous, and consistently observed.

Histologically, therefore, lesions of these two experimental kidney diseases appear to be different.

### TABLE VI

**Complement Fixation Reactions of Anti-Rat Collagen, Anti-Rat Glomeruli, and Anti-Rat Kidney Sera with Rat Collagen and Rat Kidney**

<table>
<thead>
<tr>
<th>Antisera prepared against</th>
<th>Antigen: purified rat collagen 1 mg./ml. diluted 1:</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>128</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat kidney ................</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>&quot; glomeruli ..............</td>
<td>+++++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>± +</td>
</tr>
<tr>
<td>&quot; collagen ..............</td>
<td>+++++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>± +</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antigen: rat kidney 10 mg./ml. diluted 1:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat kidney ................</td>
</tr>
<tr>
<td>&quot; glomeruli ..............</td>
</tr>
<tr>
<td>&quot; collagen ..............</td>
</tr>
</tbody>
</table>

− indicates complete hemolysis; ± to + + + indicates intermediate degrees of hemolysis; and + + + + indicates no hemolysis with complete fixation of complement.
In vitro immunologic studies were also used to determine whether the rabbit anti-rat collagen serum was in any way related to the rabbit anti-rat kidney serum. Complement fixation reactions against rat collagen and rat kidney were carried out with rabbit anti-rat collagen, anti-rat glomeruli, and anti-rat kidney sera. The results of these tests are shown in Table VI. Antibody to rat kidney or glomeruli failed to fix complement in the presence of rat collagen; antibody to rat collagen fixed complement with the rat collagen, its homologous antigen. Antibody to rat kidney or glomeruli fixed complement in the presence of its homologous antigen, an emulsion of whole rat kidney. However, antibody to rat collagen also fixed complement in a much lower titer in the presence of rat kidney. Since antibody to rat collagen reacts with native collagen (1), it is believed that this slight cross-reaction with rat kidney is due to the native collagen in the whole kidney emulsion. However, collagen in the insoluble state, such as that contained in the kidney emulsion, does not stimulate the formation of antibody to collagen, and the lack of this antibody explains the failure of anti-rat kidney or glomerular serum to fix complement in the presence of rat collagen. The results of these in vitro experiments are in accord with the concept that the anti-collagen serum reacts, in vivo, specifically with its homologous antigen present in the kidney glomeruli and that the anti-collagen antibody is unrelated to the antibody involved in the production of nephrotoxic nephritis. From these studies, it appears that nephrotoxic nephritis in rats differs from the renal lesions observed in the present study.

DISCUSSION

The experimental data recorded in the present paper demonstrate that a progressive renal glomerular injury can be induced in rats prepared with subcutaneous adjuvant and then injected intravenously with rabbit anti-rat collagen serum. That neither the water-in-paraffin oil adjuvant emulsion nor the anti-collagen serum alone induces the lesion has been shown. Moreover, anti-rat collagen serum absorbed with native rat collagen and rabbit antisera prepared against various other antigens, including fish collagen or rat serum, failed to provoke the renal abnormality in rats previously prepared with adjuvant.

The extensive alteration of the visceral and capsular basement membranes of the glomeruli suggests that this experimental renal disease may be the result of an interaction between the collagen antibody and an antigen in the basement membranes. Farquhar, Vernier, and Good (14), using electron microscopy, were unable to find evidence of collagen in the normal glomerulus. However, Goodman, Greenspon, and Krakower (15) have reported that antibody to the parietal capsular membranes of canine glomeruli also reacts with collagen fibrils from cornea and tendon. Furthermore, by immuno-chemical reactions, Cruickshank and Hill (16), in rats, and Scott (17), in human beings, have found reticulin in renal glomeruli; and by electron microscopy, Porter (18) has shown...
that collagen and reticulin are identical. Indeed, Irving and Tomlin (19) have
observed that the characteristic silver staining of reticulin is due to a complex of
ground substance, and that collagen fibers can be made argyrophilic by adding
hyaluronic acid, blood plasma, or blood proteins. It thus seems probable that
antibody to collagen does react with a substance, presumably collagen or re-
ticulin, in the glomerular basement membrane. The limitation of the effect of
the anti-collagen serum to the kidney may be due to the renal anatomical struc-
ture and function, which expose antigen directly to a large proportion of the
injected circulating anti-rat collagen serum.

A prominent feature of the renal lesions was the frequent occurrence of multi-
nuclear giant cells in the glomeruli, strikingly similar in morphology to those
in the granulomata elsewhere in the tissues of all rats which had received ad-
juvant. This raised the question, whether the renal lesions are provoked by
insoluble material disseminated from the adjuvant at the site of injection or in
the lymph nodes or lungs. Since giant cells were observed in the glomeruli of
rats prepared without mycobacteria in the adjuvant, these bacilli can be ex-
cluded as a causative factor. However, the hydrocarbons in the oil phase of the
adjuvant are capable of promoting the formation of giant cells. The failure of
appropriate staining reactions to reveal lipoid material in the kidney lesions
does not necessarily rule out the possibility that an insoluble foreign body may
be present, although not demonstrated by the methods employed. The fact
that the giant cells were not found in the glomeruli of control rats prepared with
adjuvant, but appeared only when anti-rat collagen serum had also been in-
jected intravenously, suggests another explanation, namely, that they are
formed as part of the response to the injury of the glomeruli by an antigen-
antibody reaction. Giant cells of this type have been reported in the seminifer-
ous tubules of rats with allergic aspermatogenesis induced by injection
of testicular suspension in adjuvant (20), and have also been observed in im-
munologic reactions without adjuvant, such as in the Arthus phenomenon in
the skin of rabbits (21), in kidneys of rats, rabbits, and dogs with nephrotoxic
nephritis (13), and in spleens and lymph nodes of rabbits given horse serum (22)
or crystalline bovine serum albumin (23) as antigens.

Although anaphylactic shock developed in rats prepared with normal rabbit,
chicken, or horse serum in adjuvant and given intravenous injections of the
homologous serum, no renal lesions occurred. It thus appears unlikely that
either anaphylactic shock or serum nephritis can be an explanation of the
renal damage.

Normal rabbit serum as an antigen was incorporated in the adjuvant used to
prepare the rats in which the renal lesion was first observed. Later it was found
that normal chicken or horse serum could be substituted for this rabbit serum.
Although rabbit serum incorporated in the complete adjuvant was followed by
most severe lesions, they still appeared when no serum was added to the ad-
juvant, and also when incomplete adjuvant was used.
RENAL GLOMERULAR LESIONS

The need for the adjuvant as a preparatory agent for the development of this renal disease has been demonstrated. Current knowledge of the function of adjuvant combined with antigen in intensifying and maintaining antibody formation, altering sensitization, and provoking tissue injury has been extensively reviewed by Freund (5). A conspicuous effect of adjuvant is a proliferative inflammation in the tissues, characterized by mononuclear epithelioid cells, lymphocytes, plasmacytes, and multinuclear giant cells. This cellular reaction appears, though not to the same degree, whether or not mycobacteria or antigen is included in the adjuvant mixture. It is probable that this widespread cellular response, also known to occur to some extent following usual immunization, leads to the increased antibody production provoked by subsequent injections of an antigen. This would explain why the rats formed precipitins to the normal rabbit serum following injection of rabbit anti-rat collagen serum after preparation with adjuvant, but not when given the antiserum alone. This may also explain why adjuvant is needed to produce the renal lesion. Kay (9) has shown that rabbits given duck anti-rabbit kidney serum do not develop nephrotoxic nephritis until after a latent period during which they have produced antibody to the duck globulin. Nephritis results when this antibody to duck globulin reacts in the kidney with its homologous antigen, duck globulin, in the complex of the duck anti-rabbit kidney antibody and renal antigen. Seegal and Bevans (13) have found that with duck anti-rabbit kidney serum of greater potency there is no latent phase and the second antigen-antibody reaction is not necessary. Since the antisera to rat collagen are not of high titer, it seems likely that, as in Kay's experiments, a second antibody is required for the manifestation of the renal lesions. This second antibody, the one to rabbit globulin produced in the rats prepared with adjuvant, would then react with the rabbit globulin of the rabbit anti-rat collagen serum bound to its antigen in the kidney. Whether a more potent anti-rat collagen serum would produce the renal lesion without the need for adjuvant is not known.

SUMMARY AND CONCLUSIONS

Renal glomerular lesions were induced by rabbit serum containing antibody to rat collagen injected intravenously into rats prepared with subcutaneously administered Freund adjuvant. Neither the anti-collagen serum nor the adjuvant alone induced the lesion. The lesions were characterized by diffuse glomerular injury with swelling, shredding, and fusion of the basement membranes, crescent formation, cellular proliferation, numerous multinuclear giant cells, and capillary hyaline thrombi.

Various rabbit antisera, including those against fish collagen or rat serum failed to induce the renal lesion when substituted for anti-rat collagen serum. Also, anti-rat collagen serum absorbed with its homologous antigen, native rat collagen, failed to induce the lesion. Although complete adjuvant, i.e. with
mycobacteria, in which normal serum was incorporated enhanced the glomerular lesion which resulted from intravenous injection of anti-collagen serum, the incomplete adjuvant without serum was sufficient.

Comparison of the renal lesions induced by anti-collagen serum with nephrotoxic nephritis induced in rats by rabbit anti-kidney serum showed that they differ histologically. Also the antisera used to produce these two renal lesions differ immunologically.

Antibodies to normal rabbit serum developed in rats injected intravenously with rabbit anti-rat collagen serum after preparation with adjuvant, but not when adjuvant was omitted. The pathogenesis of the renal injury is discussed as a manifestation of an antigen-antibody reaction, with nephritis occurring only after the adjuvant-stimulated antibody to the rabbit globulin has been formed in the rat and has reacted with the rabbit anti-rat collagen already fixed by its homologous antigen in the kidney.

It is a pleasure to acknowledge the technical assistance of Miss Mildred Schleimer and Mr. Seymour Kaplan.

BIBLIOGRAPHY


EXPLANATION OF PLATES

PLATE 70

All sections were stained with hematoxylin and eosin.

**Fig. 1.** Kidney section from a rat given subcutaneous injections of adjuvant with normal rabbit serum followed by intravenous normal rabbit serum, and sacrificed 7 days after the last injection. Shows a relatively normal glomerulus with patent capillaries, but with slightly increased cellularity. × 436.

**Fig. 2.** Section is from the kidney of a rat given subcutaneous adjuvant with normal rabbit serum followed by intravenous rabbit anti-rat collagen serum, and sacrificed 7 days after the last injection. The glomerulus shows intense proliferation of the cellular elements with obliteration of Bowman's space, shredding and fusion of basement membranes, and avascularity. Note absence of polymorphonuclear leukocytes. × 436.

**Figs. 3 and 4.** Kidney sections from rats, treated as described in legend of Fig. 2, illustrate distortion of glomerular structure, large giant cells with multiple peripheral nuclei, and masses of hyalin material. × 323.

**Fig. 5.** Kidney section from a rat, treated as described in legend of Fig. 2, shows in the larger glomerulus a focal eosinophilic deposit in the glomerular tuft. Note the giant cell in the lumen of a proximal convoluted tubule. × 288.

**Fig. 6.** Kidney section from a rat, treated as described in legend of Fig. 2, illustrates loss of glomerular lobulation and dilated Bowman's space filled with pink-staining material, which also fills adjacent tubules. × 281.
Rothbard and Watson: Renal glomerular lesions
PLATE 71

FIG. 7. Kidney section from a rat, treated as described in legend of Fig. 2, shows a hyaline thrombus in an afferent arteriole and a multinuclear giant cell in the glomerulus. × 439.

FIG. 8. Kidney section from a rat, treated as described in legend of Fig. 2, illustrates obliteration of Bowman’s space, fusion and swelling of basement membranes, multinuclear giant cells in the glomerulus, and dilatation of tubules with atrophy of tubular epithelium and pink-staining casts in their lumina. × 281.

FIG. 9. Lung section from a rat, treated as described in legend of Fig. 2, shows a granulomatous lesion with giant cells within the parenchyma, adjacent to a bronchiole. × 88.

FIG. 10. Section of a tracheo-bronchial lymph node from a rat, treated as described in legend of Fig. 2, shows several giant cells phagocytizing oil droplets, masses of epithelioid cells, and nests of plasmacytes. × 105.
(Rothbard and Watson: Renal glomerular lesions)
PLATE 72

Fig. 11. Kidney section from a rat injected as described in legend of Fig. 2, but sacrificed 3 months later, shows early hyalinization of the upper glomerulus, and fibrotic changes in the interstitial tissue. × 355.

Fig. 12. Glomerulus from the kidney of a rat injected as described in legend of Fig. 2, but sacrificed 12 months later, shows fibrous masses and a fibrous band obliterating Bowman's space, with fibrinoid material in the lower portion of the band. × 500.

Fig. 13. Kidney section from same rat as in Fig. 12 illustrates marked interstitial fibrosis with infiltration by mononuclear cells and replacement of glomerulus by fibrous tissue. × 359.

Fig. 14. Glomerulus from the kidney of the same rat as in Fig. 12 shows a massive fibro-epithelial crescent. × 464.