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DEMONSTRATION OF ANTIBODY TO RAT COLLAGEN IN THE RENAL GLOMERULI OF RATS BY FLUORESCENCE MICROSCOPY

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In a previous study, diffuse lesions in renal glomeruli of rats prepared with Freund adjuvant were found after the injection of rabbit serum containing antibodies to rat collagen (1). However, neither the antibody nor adjuvant alone induced the injury. It was considered that these renal lesions might result from two antigen-antibody reactions: the fixation of the rabbit anti-rat collagen serum to an antigen in the glomeruli, presumably collagen, and the reaction of this antigen-antibody complex with anti-rabbit globulin produced in the rat by the stimulation of the adjuvant. That a reaction with the antibody to collagen did take place, however, in rats not prepared with adjuvant was demonstrated by the occurrence of reverse anaphylaxis following the intravenous injection of serum containing antibodies to rat collagen (2).

The fluorescent antibody method developed by Coons and his associates (3, 4) provides a means of locating the site of specific antibody or antigen in tissues, and it was, therefore, utilized to determine whether the antibody to rat collagen localized in the renal glomeruli.

The study here reported shows that injected collagen antibody does localize in the basement membrane of renal glomeruli, the site of injury in the adjuvant-prepared rats. Injected antibody was also found in the renal glomerular basement membrane of rats not given adjuvant, although no injury was apparent in their kidneys.

Materials and Methods

The preparation of collagen and rabbit anti-collagen sera, as well as the technic of the complement fixation tests, have been previously reported (5). All sera used for injection of rats were heated to 56°C. for 30 minutes to reduce primary toxicity. The method used for absorption of anti-collagen sera has also been described (2). The globulin fraction of the sera was precipitated out with 20 per cent sodium sulfate (6).

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Black and white hooded rats of the Whalen strain, of both sexes, weighing 180 to 310 gm. were used. A group of 11 rats were prepared with Freund adjuvant (7), a water-in-paraffin oil emulsion in which the aqueous phase was physiological saline alone and the oil phase, arlacel A® (mannide monolaurate), bayol F®, and *Mycobacterium butyricum*. One ml. of adjuvant, divided among several sites, was given subcutaneously each week for 3 weeks. One week after the last adjuvant injection subsequent treatment with serum was started. A second group of 22 rats was not prepared with adjuvant.

Both adjuvant-prepared and normal rats received 2 or 3 daily intravenous injections of normal rabbit or rabbit anti-collagen serum. Single intracardiac injections of serum or globulin fractions were made into the left heart of normal rats under light ether anesthesia. Unless otherwise stated, the rats were sacrificed with ether anesthesia 7 days after the last injection of serum or globulin. Two or more adjacent blocks, approximately 5 × 10 × 10 mm., were rapidly cut from each tissue to be examined. One block was fixed in Zenker-formol for paraffin sections which were stained with hematoxylin and eosin and by the periodic acid-Schiff reaction. The other block was quickly frozen in petroleum ether (reagent) previously chilled to −70°C in a dry ice–alcohol bath, as described by Tobie (8). The frozen tissues were removed from the petroleum ether, blotted dry, placed in tightly stoppered pyrex glass tubes, and stored at −70°C. in a dry ice freezer.

For sectioning the frozen tissue, blocks were mounted with a few drops of water on object holders precooled with dry ice and cut at 2 to 4 μm with an American Optical rotary microtome No. 815 at −20°C. in a temperature controlled cryostat. Hamilton Watch oil, No. T 3358 (Hamilton Watch Co., Lancaster) was used to lubricate the microtome at this low temperature. The frozen sections were mounted on chilled glass slides, 1 mm. thick, and thawed quickly by placing the under surface of the slide against the hand at room temperature. The slides were air dried for 10 to 15 minutes at room temperature, fixed in 95 per cent alcohol for 30 seconds, washed twice in 0.01 M phosphate buffered saline (pH 7.2) for 10 minutes each, drained, and wiped dry except over the sections. These preparations were kept in closed containers at −10°C. for 24 to 48 hours before they were stained with fluorescent antibody.

Antisera for fluorescent conjugation were obtained by immunization of ducks or rabbits with globulin fractions of normal sera. Three weekly intramuscular injections of 4 ml. of normal rabbit globulin incorporated in Freund adjuvant were given to ducks at multiple sites. One week later the antibody titer was tested. If this was not yet sufficiently high, subsequent courses of globulin were given. Rabbits were immunized in the same way with duck globulin. Antibody titers were determined by the capillary precipitin method (9) by adding constant amounts of antibody to twofold serial dilutions of antigen. Titers of 1:1024 to 1:4096 were found to be satisfactory for the preparation of conjugated globulin.

Globulin fractions were prepared from these immune sera and adjusted to a concentration of 20 mg. of protein per ml. for the conjugation with fluorescein isothiocyanate by the methods of Riggs et al. (10) and Marshall et al. (11). To remove unconjugated fluorescein from the conjugated globulins, they were dialyzed at 4°C. against 0.01 M phosphate buffered saline (pH 7.2). Two to 3 ml. portions of the conjugate were frozen without preservative and stored at −72°C. Before use, non-specific fluorescence was removed by absorption twice with rat liver powder, as described by Coons, Leduc, and Connoley (12). Particulate matter was removed by centrifugation at 16,000 R.F.M. at 4°C. and filtration through a Millipore filter, pore size 0.45 μ. These fluors were employed for staining of the rat tissue sections described above. Samples of unconjugated globulin antibody were kept at −72°C. for control blocking tests, described by Coons and Kaplan (4).

1 Obtained from the Atlas Powder Co., Delaware, Maryland.
2 Obtained from Esso Standard Oil Co., New York.
Mounted tissue sections were covered with a drop of the appropriate fluorescent-labeled globulin, kept for 30 minutes in a moist chamber at room temperature, then rinsed with buffered saline (pH 7.2) for 10 minutes, wiped around the section, air-dried, mounted in buffered glycerine (pH 7.2), and covered with No. 1 glass coverslips for fluorescent microscopic study.

A Leitz ortholux universal research monocular microscope for fluorescence was equipped with apochromatic 12X, 24X, and 40X objectives and a 10X eyepiece. The ultraviolet light source was a high pressure mercury arc lamp, Philips CS 150 W. An immersion dark field condenser (D 1.20 A, E. Leitz, Inc.) without a funnel stop was used.

An euphos protective filter (Leitz), thickness 1.5 mm., was fitted in the ocular. A heat-absorbing filter, a fluid filter of 3 per cent copper sulfate to absorb any red light, and an ultraviolet transmitting filter UG1, 2 mm. thick, were used for the visual and photographic studies.

For photography of fluorescent sections, a 35 mm. Leica camera with a Mikas micro attachment and Ansco super hypan speed film was employed with exposure times of 35 to 50 seconds, depending on image intensity.

EXPERIMENTAL OBSERVATIONS

Detection in Renal Glomeruli of Collagen Antibody Injected into Adjuvant-Prepared Rats.—To determine whether collagen antibody was present at the site of the injury in renal glomeruli of adjuvant-prepared rats injected intravenously with rabbit serum containing antibodies to rat collagen, it was necessary to apply the two-step, or indirect, procedure of immunofluorescence. Preliminary attempts to identify antibody by injecting conjugated anti-collagen serum had been unsuccessful. High-titered antisera are necessary for visualization of the conjugated proteins; the titer of collagen antibody, not high initially, was lowered even further by conjugation. However, since the two-step procedure permitted the use of very high-titered serum for conjugation, this method was adopted.

The first step of the indirect procedure was to inject into the rats unlabeled rabbit serum containing antibody to the rat collagen; the second step was to stain tissue sections from these rats by conjugated duck globulin containing antibodies to normal rabbit globulin. If the antibody to rat collagen is fixed by its homologous antigen, this antigen-antibody complex can then be identified by its reaction with the fluorescent-labeled antibody to rabbit globulin.

Five rats, prepared with Freund adjuvant, were given 3 daily intravenous injections of rabbit anti-rat collagen serum, titer 1:128 or 1:256, in a total volume of 7.0 to 7.5 ml. (Table I). Seven days later they were sacrificed and tissues obtained for examination.

Paraffin sections of kidneys stained with hematoxylin and eosin or periodic acid–Schiff reaction showed the renal glomerular lesions which have been previously reported (1). Basement membranes of glomeruli were swollen, shredded, and fused; there were crescent formation, cellular proliferation, numerous multinuclear giant cells, and capillary hyaline thrombi (Figs. 3, 4, 6 to 8).
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Frozen sections prepared from adjacent blocks were stained with the fluorescein-conjugated duck globulin containing antibody to normal rabbit globulin. By ultraviolet illuminated microscopy, brilliant yellow-green fluorescence, characteristic of fluorescein isothiocyanate, was seen in the renal glomerular basement membranes and faintly in the basement membranes of the tubules (Figs. 1, 2, 5, 9, and 10). The fluorescence looked like sharply defined lines, which appeared to be extracellular. No fluorescence was present in the cells of the glomeruli or tubules. The elastic tissue of the interlobular arteries could be recognized by its bright blue autofluorescence which obscured any specific fluorescence that may have been present in the adventitia.

Duplicates of each section were prepared for control studies on the immunological specificity of the fluorescence. The blocking test, as described by Coons and Kaplan (4), was performed by pretreatment of the tissue with unlabeled duck anti-rabbit globulin before the labeled globulin. The second control consisted of staining with a heterologous fluor; i.e., conjugated rabbit globulin

TABLE I
Summary of Experimental Data on the Immunofluorescent Identification in the Kidney of Collagen Antibody Injected into Rats

| No. of rats | Preparatory treatment | Rabbits serum or globulin injected into rats | Serum titer* | Globulin nitrogen | Injections | Time of sacrifice, after last injection | No. of rats with:
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Adjuvant§</td>
<td>Anti-rat collagen serum 1:128-1:256</td>
<td>i.v. 3</td>
<td>7.0-7.3</td>
<td>7 days</td>
<td>5</td>
<td>§</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>Anti-fish collagen serum 1:128</td>
<td>i.e. 1</td>
<td>2.0</td>
<td>7 &quot;</td>
<td>3</td>
<td>§</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>Anti-rat collagen serum 1:128-1:256</td>
<td>i.v. 3-3</td>
<td>4.7-7.0</td>
<td>7 &quot;</td>
<td>0</td>
<td>§</td>
</tr>
<tr>
<td>9</td>
<td>&quot;</td>
<td>Anti-rat collagen globulin 1:10.9-20.1</td>
<td>i.e. 1</td>
<td>1.0-2.5</td>
<td>7 &quot;</td>
<td>9</td>
<td>§</td>
</tr>
<tr>
<td>1</td>
<td>&quot;</td>
<td>Normal rabbit serum 1:512</td>
<td>i.v. 3</td>
<td>8.0</td>
<td>7 &quot;</td>
<td>0</td>
<td>§</td>
</tr>
<tr>
<td>1</td>
<td>&quot;</td>
<td>Anti-fish collagen serum 1:10.9-20.1</td>
<td>i.e. 1</td>
<td>2.0</td>
<td>7 &quot;</td>
<td>0</td>
<td>§</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>Anti-rat collagen serum absorbed with rat collagen</td>
<td>0</td>
<td>4.0</td>
<td>7 &quot;</td>
<td>0</td>
<td>§</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>Anti-rat collagen serum absorbed with fish collagen</td>
<td>1:128</td>
<td>4.0</td>
<td>7 &quot;</td>
<td>0</td>
<td>§</td>
</tr>
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* Titers of anti-collagen sera were determined by complement fixation.
† Route of injection was intravenous (i.v.) or into the left heart (i.e.).
§ Three weekly subcutaneous injections of 1.0 ml. each.
¶ After absorption.
containing antibodies to duck globulin. The absence of fluorescence in either of these control sections demonstrated the immunologic specificity of the identification of the collagen antibody.

To increase the concentration of antibody reaching the kidney and to avoid multiple intravenous injections, a single injection of 2 ml. of rabbit anti-rat collagen serum, titer 1:128, was made into the left heart of each of 3 adjuvant-prepared rats under light ether anesthesia. Tissues obtained after 7 days showed renal glomerular lesions and fluorescence of renal basement membranes indistinguishable from those seen after the intravenous injections. The blocking test and heterologous fluor controls were negative. This experiment indicated that, after a single intracardiac injection, sufficient collagen antibody became fixed in the kidney to be detected by immunofluorescence.

To control the specificity of the interaction of the anti-rat collagen serum with its homologous antigen in the rat kidney, antibody to fish collagen was selected. Fish collagen is morphologically indistinguishable, but immunologically distinct, from rat collagen (2, 5). Rabbit anti-fish collagen serum was given intravenously to 3 adjuvant-prepared rats. Three injections of serum, titer 1:512, in a total volume of 8 ml. were used. Seven days after the last injection, tissues were prepared in the same way as in the previous experiment. No renal injury or specific fluorescence was evident (Fig. 11).

This series of experiments demonstrated the in vivo specific localization in adjuvant-prepared rats of rabbit anti-rat collagen serum in the basement membranes of the injured renal glomeruli.

Detection in Renal Glomeruli of Collagen Antibody Injected into Rats Unprepared by Adjuvant.—Experiments previously reported showed that in rats unprepared by adjuvant, anti-collagen serum would not induce renal injury (1). But whether adjuvant was necessary for fixation of the antibody to collagen in the renal glomeruli had not been determined.

Three rats, given no adjuvant, were injected intravenously daily for 2 or 3 days with rabbit anti-rat collagen serum, titer 1:128 or 1:256, in a total volume of 4.7 to 7.0 ml. (Table I).

When they were sacrificed 7 days later, tissue sections revealed no evidence of nephritis, but on fluorescent microscopy the antibody to rat collagen was clearly identified in the basement membranes of the renal glomeruli (Fig. 12). The blocking and heterologous globulin controls showed no fluorescence.

Another series of 11 normal rats was given globulin prepared from rabbit anti-rat collagen serum.

A single injection of 1.0 to 2.5 ml. of this globulin was made into the left heart (Table I). Nine of the rats were sacrificed after a 7 day interval, but one was killed 45 minutes after injection, and another not until 92 days had elapsed.
Renal injury was not apparent in the kidney sections of any of the 11 rats, but all showed bright yellow-green fluorescence in the basement membranes of the renal glomeruli (Figs. 13 to 16). Again, there was no fluorescence in the blocking and heterologous globulin controls.

As additional controls, 2 normal rats were injected intravenously with 8.0 ml. of normal rabbit serum, divided in 3 daily injections; a third rat was given 3 daily intravenous injections of rabbit anti-fish collagen serum, titer 1:512, in a total volume of 8 ml.; and a fourth, was given a single intracardiac injection of 2 ml. of rabbit anti-fish collagen globulin.

The results in these, as in the adjuvant-prepared rats given anti-fish collagen serum, showed no renal lesions and no fluorescence (Table I).

**Effect on Fluorescence of Absorbed Anti-Collagen Serum.**—Absorption of rabbit anti-rat collagen serum with native rat collagen has been found to remove the antibody so that the serum no longer produces reverse anaphylaxis in the rat, fixes complement in the presence of homologous collagen (2), or induces renal glomerular lesions in adjuvant-prepared rats (1). Absorption with heterologous fish collagen, on the other hand, has no effect.

To determine the effect on fluorescence of absorption of the serum prior to its injection into the rats, 2 unprepared rats were given 2 daily intravenous injections of a total volume of 4.0 ml. of rabbit anti-rat collagen serum absorbed with native rat collagen, and 2 other unprepared rats were given the serum absorbed with fish collagen (Table I).

The antibody titer by complement fixation of the anti-collagen serum absorbed with rat collagen was reduced to zero, but that of the serum absorbed with fish collagen remained unchanged at 1:128. The rats were sacrificed after 7 days. The results of this experiment show that no renal lesions developed in either pair of rats. Renal sections from those given the serum absorbed with fish collagen showed the characteristic fluorescence in the glomerular basement membranes, but sections from the other rats given the serum absorbed with rat collagen showed no fluorescence. These findings confirm the effect of absorption on the antibody titer by the methods previously reported and provide further evidence for the specificity of the immunofluorescent identification of the collagen antibody combined with its homologous antigen in the kidney.

**In Vitro Localization of Anti-Collagen Serum in the Rat Kidney.**—

Frozen sections from kidneys of normal rats were treated with unlabeled rabbit anti-rat collagen serum, titer 1:128, for 45 minutes. The slides were washed for 10 minutes in buffered saline (pH 7.2), air-dried, and then layered for 30 minutes with the conjugated duck globulin containing antibody to normal rabbit globulin. After the usual washing and mounting, the slides were examined under ultraviolet illumination.

Fluorescence occurred with equal brightness in the basement membranes of the glomeruli and of the tubules (Fig. 17). The intensity and definition of the
fluorescence were less in these glomeruli than in those from rats given the collagen antibody \textit{in vivo}, but much brighter in the tubules. These differences are probably due to the difference in concentration of antibody reaching the various portions of the kidney by the two procedures. As controls for this observation, duplicate slides were prepared using normal rabbit serum instead of the anti-collagen serum; other sections treated with the anti-collagen serum were tested by the blocking and heterologous globulin tests used previously. None of these showed fluorescence. This \textit{in vitro} experiment, with its controls, revealed that the anti-collagen serum reacts at sites where its homologous antigen is present.

\textit{Distribution of Injected Collagen Antibody in Other Rat Tissues}.—Sections from lung, liver, spleen, adrenal, and lymph node of normal rats injected with rabbit anti-rat collagen serum or globulin were prepared and stained with fluorescein-conjugated duck globulin containing antibody to rabbit globulin. By this indirect staining procedure, the fluorescence indicating the presence of collagen antibody appeared in the capsules, trabeculae, and the network of fine fibers of these organs. The cytoplasm and nuclei of the cells were free of any fluorescence. These experiments have not yet been completed and will be reported later. Nevertheless, it appears from these preliminary studies that by the indirect method of fluorescent staining of collagen antibody, collagen can be demonstrated in various animal tissues.

\textbf{DISCUSSION}

The results here reported indicate that, by fluorescence microscopy, antibody to rat collagen injected into the circulation of either normal or adjuvant-prepared rats can be identified in the basement membranes of renal glomeruli, but renal injury occurs only in those rats prepared with adjuvant. The demonstration of antibody at this site confirms the supposition that the interaction of the rabbit anti-rat collagen serum with its antigen in the kidney is an essential factor in the production of the renal glomerular lesions.

The method used combines administering this antibody \textit{in vivo} with staining in the tissue \textit{in vitro} by fluorescein-conjugated anti-rabbit globulin from ducks. This procedure is essentially the same as that employed in studies in various animals on the distribution of the antibody to kidney in nephrotoxic nephritis (13–15); however, the antibody to collagen and that to rat kidney have been shown to be immunologically different (1). The immunologic specificity of the interaction between the antibody to collagen and its homologous antigen in the glomeruli has been controlled by a variety of tests. The most significant of these tests was the injection of rats with rabbit antibody to fish collagen, which demonstrated the lack of any collagen antibody fixation and the absence of consequent fluorescence. Moreover, the removal of antibody before injection, by absorption of the anti-rat collagen serum with homologous
collagen, prevented the fixation and fluorescence of antibody which did occur after the injection of untreated serum or serum absorbed with the heterologous fish collagen. Additional controls also showed the specificity of the staining by blocking the fluorescence in duplicate sections with unlabelled duck anti-rabbit globulin, and by failing to demonstrate fluorescence with conjugated rabbit anti-duck globulin. The successful fluorescent staining of collagen in normal kidney \textit{in vitro} by the serial application of specific anti-collagen serum and subsequent staining with the fluorescein-conjugated duck globulin provides an alternate method of value because only a small volume of the anti-collagen serum is required and that need not be of high titer.

Although the antibody can be demonstrated in the glomeruli for as long as 92 days after injection of a rat not prepared with adjuvant, no evident renal injury occurred. This fixation of antibody in the absence of renal injury is analogous to the observations of Seegal (15), that injection of rat or duck anti-rat aorta serum did not induce nephritis but that the antibody became localized in the glomeruli.

The location of fluorescent antibody in the present study is similar to that reported by Heller and Yakulis (16), who applied fluorescein-conjugated rabbit anti-guinea pig globulin to tissue sections from guinea pigs immunized with saline extracts of rabbit tendon incorporated in Freund adjuvant. The composition of their antigen has not yet been defined but the presence of hydroxyproline suggests that soluble collagen may be among the substances in the saline extracts of rabbit tendon.

Many antigens probably exist in the basement membranes of the renal glomeruli. The question whether collagen is one of them has not yet been established. Immunologic methods, enzymatic analysis, and electron microscopy have shown that the antibody used in this study is probably specifically directed toward collagen rather than to other possible tissue contaminants (5). It seems likely, therefore, that the anti-collagen serum used in this study does react in the renal glomerular basement membranes with an antigen which is some form of collagen. Although collagen, with its distinct periodicity, has not been demonstrated in the basement membrane of mammalian renal glomeruli (17), recent work shows that it does exist there in the frog kidney (18). Several workers (19-22) employing various methods have, however, found reticulin fibers in renal glomerular basement membranes, and it has been reported that by electron microscopy reticulin and collagen are probably identical (23). It is also possible that collagen exists in the tissues in a non-fibrous soluble state which retains the same antigenicity as collagen fibers. The identification of the antibody to collagen where collagen fibers are known to be present—in the capsules and trabeculae of the lung, liver, spleen, adrenal, and lymph node—adds to the probability that the antigen in the basement membranes of the renal glomeruli and tubules is also collagen. That the
collagen antibody was fixed and stained \textit{in vitro} in the kidney tissue from normal young rats, and that it was found as early as 45 minutes after a single injection of antibody into a normal rat, indicate that the presence of the antigen is not the result of previous tissue injury. These findings suggest that the anti-collagen serum may be used to detect homologous collagen in any tissue by fluorescence microscopy.

\textbf{SUMMARY AND CONCLUSIONS}

Rabbit serum or globulin, containing antibody to rat collagen, injected intravenously or intracardially into normal or adjuvant-prepared rats becomes fixed in the basement membranes of renal glomeruli and, to a slight extent, of the tubules. When examined by ultraviolet light, this antibody can be identified in tissue sections by the yellow-green fluorescence occurring where the rabbit globulin, associated with the fixed collagen antibody, has reacted with fluorescein-conjugated anti-rabbit globulin from ducks. The reaction of the antibody to rat collagen with its antigen in the kidney is a primary factor in the production of the renal glomerular injury which occurs in rats prepared with adjuvant. Adjacent control sections failed to fluoresce when pretreated with unlabeled anti-rabbit globulin or when treated with heterologous conjugated anti-duck globulin from rabbits. The antibody to rat collagen remains in the kidney as long as 92 days and has been detected as early as 45 minutes after injection. When normal rabbit serum, rabbit anti-fish collagen serum, or rabbit anti-rat collagen serum absorbed with rat collagen was substituted for the rabbit anti-rat collagen serum, fixed antibody could not be demonstrated by fluorescence; but absorption of the anti-rat collagen serum with fish collagen did not affect the antibody fixation.

This series of immunologic tests indicates that the anti-collagen serum reacts with its homologous antigen, presumably collagen, in the basement membranes of renal glomeruli and tubules, and that specific antibody can be used to identify collagen in other tissues of the animal body.

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EXPLANATION OF PLATES

PLATE 108

FIG. 1. Glomerulus from an adjuvant-prepared rat given rabbit anti-rat collagen serum intravenously and sacrificed after 7 days. Frozen kidney section was treated with fluorescein-conjugated antibody to rabbit globulin. Viewed by ultraviolet light, the intense fluorescence at the site of antigen-antibody union appears white in the photograph and outlines the basement membranes of the greatly distorted glomerulus. The large dark spaces probably represent giant cells or masses of hyaline material and the small ones, capillaries. × 305.

FIG. 2. Glomerulus, from the same kidney section as Fig. 1, shows fluorescent thickened basement membranes which appear to have a linear structure in some areas. × 560.

FIG. 3. Kidney section from same rat as in Fig. 1 was fixed in Zenker-formol and stained with hematoxylin and eosin for comparison with the fluorescein treated sections. Marked distortion of the glomerulus and masses of hyaline material are illustrated. × 445.

FIG. 4. Kidney section like that in Fig. 3 shows a giant cell with an adjacent mass of hyaline. × 375.
(Rothbard and Watson: Antigenicity of rat collagen)
PLATE 109

Fig. 5. Kidney section from another rat, prepared as described in legend of Fig. 1. Fluorescence appears in the greatly distorted and shredded glomerular basement membranes and faintly in the basement membranes of the tubules. × 320.

Fig. 6. Kidney section from same rat as in Fig. 5, stained with hematoxylin and eosin, shows tremendously thickened and distorted basement membranes of the glomerulus, obliteration of Bowman’s space, avascularity, and proliferation of the cellular elements. × 375.

Fig. 7. Kidney section like that in Fig. 6 shows intense cellular proliferation and avascularity. × 445.

Fig. 8. Kidney section from same rat as in Fig. 5, stained with periodic acid–Schiff reaction, illustrates the outlines of the distorted glomerular basement membranes and intense cellular reaction. Bowman’s space is completely obliterated. × 375.
(Rothbard and Watson: Antigenicity of rat collagen)
Figs. 9 and 10. Kidney sections like that in Fig. 5 show fluorescence in basement membranes of markedly distorted glomeruli. Note the fluorescence in the basement membranes of the adjacent tubules. × 345.

Fig. 11. Kidney section from an adjuvant-prepared rat given rabbit anti-fish collagen serum intravenously and sacrificed after 7 days. Frozen section was treated with fluorescein-conjugated anti-rabbit globulin. Viewed by ultraviolet light, no fluorescence was seen. The glomerular structure appears normal. To obtain this photograph, exposure time was 6 minutes as compared with the 35 to 50 seconds used when fluorescence was present. The light appearance of the tubules was due to this long exposure. The small white spots are artifacts. × 325.

Fig. 12. Kidney section from a rat not prepared with adjuvant, given rabbit anti-rat collagen serum intravenously and sacrificed 7 days later. Frozen section was treated with fluorescein-conjugated anti-rabbit globulin. The white lines of fluorescence at the site of antigen-antibody reaction show the continuous outline of the basement membranes in this normal appearing glomerulus. × 370.
PLATE 111

Fig. 13. Kidney section from a rat not prepared with adjuvant, given a single injection into the left heart of rabbit anti-rat collagen globulin and sacrificed 7 days later. Frozen section was treated with fluorescein-conjugated anti-rabbit globulin. The normal pattern of the glomerular basement membrane is shown by the fluorescence. × 400.

Fig. 14. Kidney section from same rat as in Fig. 13 was stained with hematoxylin and eosin. Note the normal architecture of the glomerulus with slight increase in cellularity. × 400.

Fig. 15. Kidney section from a rat, not prepared with adjuvant, sacrificed 45 minutes after a single injection into the left heart of rabbit anti-rat collagen globulin. Frozen section was treated with the conjugated anti-rabbit globulin. The distribution and intensity of the fluorescence is essentially the same as in Fig. 13. × 320.

Fig. 16. Kidney section from a rat, not prepared with adjuvant, sacrificed 92 days after the same treatment as the rat in Fig. 15. The pattern of fluorescence is the same as in Figs. 13 and 15, but the intensity is somewhat less. × 560.

Fig. 17. Frozen kidney section from a normal, uninjected rat was covered with unconjugated rabbit anti-rat collagen serum, washed, and then stained with fluorescein-conjugated anti-rabbit globulin. The fluorescence is distinct about the small dark capillaries within the glomerulus, but is more diffuse than when the collagen antibody was injected into the circulation. The tubular and glomerular basement membranes are stained with equal intensity; the cells of the tubules show no fluorescence. × 405.
(Rothbard and Watson: Antigenicity of rat collagen)