When antibody prepared in rabbits against purified rat collagen is injected into the circulation of normal or adjuvant-prepared rats, it becomes fixed in the basement membranes of the renal glomeruli and, to a lesser extent, of the tubules. This antibody was identified in frozen kidney sections by fluorescence occurring where the rabbit globulin associated with the fixed collagen antibody reacted with fluorescein-labeled duck anti-rabbit globulin (1). Preliminary studies indicated that the collagen antibody fixation was not limited to the kidney, but was also widely distributed in connective tissues of many organs of the rat. On the basis of these findings, studies were undertaken to investigate the exact sites at which the antibody was fixed, to see whether these were limited to collagen or also included reticulin, and to establish the immunologic specificity of the reaction by appropriate controls.

**Procedure**

Tissues taken from the same young adult black and white hooded rats of the Whalen strain used in the previous study of the kidney and preserved were utilized (1). Rats were of both sexes and weighed 180 to 310 gm. These normal or Freund adjuvant-prepared rats had been injected intravenously or intracardially with normal rabbit serum or rabbit anti-collagen serum or globulin. The specific treatment for each group of rats and the time of sacrifice are shown in Table I. Detailed description of the methods used for preparing the collagen and anti-collagen sera (2, 3), absorbing the antisera (3), and separating the globulin fraction (4) have been previously reported. The rabbit anti-rat collagen sera used fixed complement in titers of 1:128 to 1:256; the anti-fish collagen serum had a titer of 1:512. The nitrogen concentrations of the globulin fractions ranged from 10.9 to 20.6 mg/ml; titers could not be established because these fractions are anticomplementary.

Duplicate blocks of tissue were taken. One set was fixed in Zenker-formol for paraffin sections and stained with hematoxylin and eosin or by the periodic acid–Schiff reaction; the other set was rapidly frozen in petroleum ether chilled to −70°C, as described by Tobie (5), blotted dry, and stored in stoppered glass tubes at −70°C. Frozen sections were cut at 3 to 5 μ thickness in a cryostat at −20°C. Their subsequent treatment and the preparation of the antisera

* This investigation was supported by the National Heart Institute, Research Grant H-1138 (C9), Public Health Service.
conjugated with fluorescein isothiocyanate have been reported in detail (1). The general procedure was to apply fluorescein-conjugated anti-rabbit globulin from ducks to mounted sections from the rats which had been injected with anti-collagen sera. As controls for the specificity of the fluorescence, the blocking technic of Coons and Kaplan (6) and heterologous conjugated globulin were applied to adjacent sections. Tissues from normal uninjected rats were also treated with the conjugated anti-rabbit globulin.

For examination of the sections, the ultraviolet light source was a high pressure mercury arc lamp, Philips CS 150 W. An immersion darkfield condenser (D 1.20 A, E. Leitz, Inc.) without a funnel stop and a monocular microscope for fluorescence were used with appropriate light filters (1).

### TABLE I

*Immunofluorescence in Rat Tissues after Injection of Collagen Antibodies*

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Material injected</th>
<th>No. of injections</th>
<th>Total volume</th>
<th>Time of sacrifice, after last injection</th>
<th>Specific fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>13†</td>
<td>Anti-rat collagen serum</td>
<td>1 to 3</td>
<td>2.0 to 7.5</td>
<td>7 days</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>“ “ globulin</td>
<td>1</td>
<td>1.0 to 2.5</td>
<td>7 “</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>“ “ “</td>
<td>1</td>
<td>2.0</td>
<td>45 min.</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>“ “ “</td>
<td>1</td>
<td>2.0</td>
<td>92 days</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Normal rabbit serum</td>
<td>3</td>
<td>8.0</td>
<td>7 “</td>
<td>0</td>
</tr>
<tr>
<td>4§</td>
<td>Anti-fish collagen serum</td>
<td>3</td>
<td>8.0</td>
<td>7 “</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>“ “ globulin</td>
<td>1</td>
<td>2.0</td>
<td>7 “</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Anti-rat collagen serum absorbed with rat collagen</td>
<td>2</td>
<td>4.0</td>
<td>7 “</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Anti-rat collagen serum absorbed with fish collagen</td>
<td>2</td>
<td>4.0</td>
<td>7 “</td>
<td>+</td>
</tr>
</tbody>
</table>

*This table is condensed from Table I in reference 1.*

† 8 of these rats received preparatory treatment with adjuvant.

§ 3 of these rats received preparatory treatment with adjuvant.

In photographing sections in which fluorescence was present, exposure times of 35 to 120 seconds were satisfactory; but when fluorescence was absent, as in the control sections, greatly prolonged exposure times of 24 to 30 minutes were necessary to obtain any image. A 35 mm Leica camera with a Mikas micro attachment and Ansco super hypan speed film were used.

**EXPERIMENTAL OBSERVATIONS**

*Sites of Fixation of Injected Antibody to Rat Collagen in Tissues of the Rat.—*

The collagen antibody was demonstrated by fluorescence microscopy in the renal glomerular basement membranes of rats injected with rabbit anti-rat collagen antibody (1). This same method was applied to the study of collagen antibody in other tissues from the rats used for the study of the antibody localization in renal glomeruli. For the purposes of the former study, some of the rats had been prepared with Freund adjuvant before the injection of the antibody. Although renal lesions occurred only in the adjuvant-prepared rats, no
difference between normal and prepared rats could be detected in antibody fixation; for that reason, the two groups are not separated in this report.

A group of 24 rats was injected intravenously or intracardially, 13 with rabbit anti-rat collagen sera and 11 with the globulin fraction of these sera. They were sacrificed 7 days later, with the exception of one sacrificed after only 45 minutes and another after 92 days. The serum was given in 1 to 3 doses with a total volume of 2.0 to 7.5 ml; the globulin was given in a single injection of 1.0 to 2.5 ml (Table I). Blocks for both paraffin and frozen sections were taken from heart, lung, liver, spleen, adrenal, kidney, jejunum, axillary lymph nodes, thymus, peripheral nerve, aorta, skeletal muscle, and brain. Blocks of only synovia and eye were taken from 2 additional normal rats, not included in the table, which were given 6.5 ml of rabbit anti-rat collagen serum intravenously in 3 doses and sacrificed 7 days later.

Paraffin sections revealed no pathologic changes except the glomerular lesions in the kidneys of the adjuvant-prepared rats and occasional granulomata in their lungs and rarely in the lymph nodes; these lesions have been described in detail (7).

All rats of this group had fixed collagen antibody identifiable under ultraviolet light by the brilliant yellow-green fluorescence characteristic of fluorescein isothiocyanate.

**Heart:** Most sections from the heart were taken through the ventricular myocardium. In the right ventricle the muscle fibers were of loose structure, in the left they were more compact. As illustrated (Fig. 1), the connective tissue sheath of the myocardial fibers showed the fluorescence indicating the fixation of the injected antibody. The muscle fibers themselves showed no fluorescence whatever.

**Lung:** Compression of the tissue in many lung sections, with obliteration of the alveolar sacs, made structural details often difficult to identify in the fluorescent sections. However, the specific yellow-green fluorescence appeared as a close-meshed fibrillar network in the alveolar walls (Fig. 3). In places, the fluorescent fibers could clearly be seen to surround the capillaries of the alveolar septa. The visceral pleura appeared as a dense layer of parallel fibers with intense fluorescence, similar to that in the capsule of other organs. No specific yellow-green fluorescence was present in control sections of the lung, but fine fibers in the bronchial and alveolar walls showed the typical blue autofluorescence of elastic fibers (Fig. 4). In the walls of the arterioles adjacent to the bronchioles, the yellow-green fluorescence could be distinguished in both the medial and adventitial layers. The intersegmental veins also were outlined by specific fluorescence.

**Liver:** In sections of the liver, intense fluorescence in the capsule and interlobular dense connective tissue was continuous with the intralobular network of reticular fibers surrounding and running parallel to the long axis of the sinusoids. The walls of the central veins of the hepatic lobules also showed fluorescence (Fig. 5). No fluorescence was visible within the hepatic or Kupffer
cells. Blue autofluorescence was visible in the internal elastic membrane of the arterioles, but beneath the intima the yellow-green fluorescence of the fixed collagen antibody could sometimes be distinguished.

*Spleen:* The capsule, trabeculae, and the fine reticular framework outlining the sinuses and forming a pericellular network all showed the specific bright yellow-green fluorescence (Fig. 7). Detailed study revealed no fluorescence in the cytoplasm or nuclei of any splenic cells. Fluorescence was not limited to the areas where collagen is known to be present, but was equally clear around and within the Malpighian bodies, where only reticular fibers occur (Fig. 9).

*Adrenal:* The fibrous capsule, trabeculae, and the reticulum between the cortical cell cords showed the specific fluorescence, but the cells of the cortex contained none (Figs. 10, 11).

*Kidney:* As reported in the previous study (1), characteristic fluorescence was present in the basement membranes of the glomeruli and faintly in those of the tubules, but not in the glomerular or tubular cells (Fig. 13).

*Jejunum:* Sections through the intestine in the region of the jejunum consistently showed fluorescence in the submucosa extending up into the villi and particularly marked at the lamina propria (Fig. 15). It was also present between the muscular coats and in the serosa. Neither epithelial cells nor smooth muscle fibers were stained (Fig. 17).

*Lymph node:* The nodes sectioned were from the axillae and showed essentially the same pattern of fluorescence as the spleen, that is, in the capsules, trabeculae, and fine reticular network, with the fluorescence limited to fibers and not present within cells (Fig. 18).

*Thymus:* The thick walled capsule of the thymus showed intense fluorescence. Fine fluorescent fibrils formed a network through the cortex, but were especially concentrated about the small blood vessels (Fig. 20). No fluorescence was seen within the cells of this organ.

*Synovia:* Blocks containing the capsule and synovial membrane were removed from knee joints of the rats. Folds of synovial membrane containing villi revealed intense specific fluorescence in irregularly placed fibers (Fig. 22). Deeper layers of the subsynovial membrane and capsule showed a more regularly arranged pattern of fiber fluorescence (Fig. 24). Walls of blood vessels in the capsule and of the capillaries in the villi also showed fluorescence.

*Nerve:* Sections of peripheral nerves showed well defined fluorescence in the thick epineurium; less intense fluorescence was visible within the nerve, between and around individual myelinated nerve fibers (Fig. 25).

*Aorta:* Blocks for study were taken from the thoracic aorta. The clearest yellow-green fluorescence was present in the adventitia, but between the elastic membranes with their blue autofluorescence there were fine fibers of the specific fluorescence (Fig. 26). In the control sections (Fig. 27), only the autofluorescence was visible.
Blood vessels: The medium-sized and small arteries in all tissues showed blue autofluorescence in the elastic membranes, but outside of this could be distinguished the yellow-green fluorescence; the veins and capillaries were outlined by fluorescent fibers in their walls without any autofluorescence.

Skeletal muscle: Sections were taken from the muscles and the attached tendon of the ventral portion of the neck. The closely packed fibers of the tendon appeared intensely fluorescent and were continuous with the interlacing fluorescent fibers surrounding the individual muscle fibers (Fig. 28). No fluorescence was evident within the muscle fibers proper.

Eye: Sections of the posterior segment of the eye revealed heavy fluorescent staining of the fibers of the sclera, but not of the layers of the retina (Fig. 30). Small blood vessels in the choroid showed slight yellow-green fluorescence in their walls.

Brain: Sections from the brain had no fluorescence in the cerebral tissue itself, but it was present in the blood vessel walls and the choroid plexus. The meninges showed intense fluorescence.

The close proximity of the fluorescent connective tissue separating the individual fibers in muscle or nerve (8) made it impossible to determine whether the sarcolemma or neurolemma, respectively, was also fluorescent. For this reason the terms sarcolemma and neurolemma have not been used in the present study.

The observations on these various organs and tissues have shown that specific fluorescence, indicating the presence of the injected antibody to collagen, was consistently present at sites where collagen or reticulin are normally found.

The immunologic specificity of the fluorescence was controlled by treating duplicate sections of each preparation in two ways. To one slide, unlabeled duck anti-rabbit globulin was applied first, and then, after washing, the labeled duck anti-rabbit globulin; this is the blocking or inhibition test. To another slide, heterologous conjugated rabbit anti-duck globulin was applied instead of the homologous globulin. In the blocking controls, extinction of the fluorescence was complete in most instances, but there was occasionally slight residual fluorescence. The sections treated with the heterologous conjugated globulin had no specific fluorescence (Figs. 2, 4, 6, 8, 12, 14, 16, 19, 21, 23, 27, 29, and 31). These controls indicate the specificity of the in vitro portion of this method, the fluorescence occurring where the conjugated anti-rabbit globulin becomes attached to the rabbit globulin associated with the collagen antibody.

The Specificity of the Fixation of the Collagen Antibody to Its Antigen.—

A control group of 7 rats was injected with normal rabbit serum or rabbit anti-fish collagen serum or globulin, and sacrificed after 7 days. 2 were given a total of 8.0 ml of normal rabbit serum in 3 injections; 4 were given a total of 8.0 ml of rabbit anti-fish collagen serum in 3 injections; and 1 was given 2.0 ml of rabbit anti-fish collagen globulin in a single injection.
Tissues from these animals were sectioned and prepared like those from the rats given antibody to rat collagen. In paraffin sections no lesions were apparent, except the occasional granulomata seen in lymph nodes and lungs of adjuvant-treated rats (7). In the frozen sections treated with the conjugated anti-rabbit globulin, no specific fluorescence was seen (Table I).

This experiment reveals that normal rabbit serum or antibody to fish collagen does not become fixed in vivo as the homologous antibody to rat collagen does.

*Fluorescence after Injection of Absorbed Anti-Collagen Serum.*—When anti-rat collagen serum from which the antibody had been removed by absorption with homologous native rat collagen was injected into rats, the specific fluorescence was not found in the kidney; however, in rats injected with serum absorbed with heterologous fish collagen, the fluorescence in the kidney was similar to that found when unabsorbed serum was used (1).

2 rats were given 2 intravenous injections of a total of 4.0 ml of rabbit anti-rat collagen serum absorbed with native rat collagen, and 2 were given 2 intravenous injections of a total of 4.0 ml of the same serum but absorbed with native fish collagen. All were sacrificed 7 days later.

In tissues from the rats given the serum absorbed with fish collagen, that is, serum in which the anti-rat collagen antibodies were still present, the fixed antibody showed the characteristic distribution of fluorescence. No specific fluorescence was seen in any tissues from the rats given the serum from which the antibodies to rat collagen had been removed by absorption with the homologous collagen.

*In Vitro Fixation of Collagen Antibody to Purified Reconstituted Collagen Fibers.*—As a further investigation of the specificity of the reaction of the collagen antibody with its antigen, experiments were undertaken with purified collagen fibers reconstituted from dilute acetic acid solutions (2).

To precipitate the fibers from solution, several drops of buffered saline (pH 7.2) were added to an equal volume of acetic acid solution of collagen (1 mg/ml) on a slide. The mixture was spread evenly over the slide, air-dried, fixed in 95 per cent alcohol for 30 seconds, washed in buffered saline, and air-dried again. Slides so prepared were treated with unlabeled rabbit anti-collagen serum, 1:128, for 45 minutes, washed for 10 minutes in buffered saline, and then covered with fluorescein-conjugated duck anti-rabbit globulin for 30 minutes, washed twice in buffered saline, mounted in buffered glycerine, covered with a coverslip, and studied under ultraviolet light. The usual blocking and heterologous globulin controls showed no fluorescence.

Rat collagen fibers treated with the homologous rabbit anti-rat collagen serum, or fish collagen fibers treated with the anti-fish collagen serum showed specific yellow-green fluorescence like that seen in tissue sections; whereas each collagen preparation treated with the heterologous antibody had no fluorescence. These observations indicate that the material to which the antibody becomes fixed in the tissue sections is antigenically similar to the purified reconstituted collagen.
DISCUSSION

The results of this study have revealed that the antibody to rat collagen, prepared by rabbits and injected into rats, can be identified in collagen and reticulin fibers in all organs and tissues so far examined; i.e., heart, lung, liver, spleen, adrenal, kidney, intestine, lymph node, thymus, joint synovia, peripheral nerve, aorta, striated muscle, scleral coat of eye, meninges, choroid plexus, and walls of blood vessels in the brain. No collagen antibody has been found within cells. It was seen in tissues of an animal sacrificed as early as 45 minutes after injection, and it was still present but somewhat less intense in another examined after 92 days. The synovia and eye, however, were studied only in rats sacrificed after 7 days.

The specificity of the fixation of injected antibody to rat collagen was controlled by the substitution of normal rabbit serum or heterologous antibody and by the removal of homologous antibody by absorption with homologous, but not heterologous, collagen. The specificity of the fluorescent staining procedure was controlled by pretreatment with unlabeled antibody to rabbit globulin to block the reaction and by the use of heterologous labeled rabbit antiduck globulin.

The antibody to collagen was found where collagen and reticulin are normally present: for example, in the spleen the specific yellow-green fluorescence occurred in the capsule and trabeculae, which contain collagen fibers, and also in the fine fibers within the pulp and around the Malpighian corpuscles, which are considered to be reticulin (Figs. 7 and 9). These findings confirm our previous observations in the kidney, from which it was concluded that collagen and reticulin may have a common antigen. In the normal mammalian renal glomerular capillary basement membrane, a number of workers using electron microscopy have been unable to find fibrils with the periodicity characteristic of collagen or reticulin (9-13). However, in a recent study using a new embedding technic for preparation of sections for electron microscopy, such fibers have been illustrated in the glomeruli of normal rats (14). Also, the hydroxyproline content of normal dog (15) and human (16) glomeruli has been found to be compatible with the presence of collagen. In the present study, the clear fluorescent staining of undoubted collagen and reticulin fibers in other organs, strongly suggests that the similarly stained material in the renal glomerular basement membranes is some form of collagen or reticulin. Furthermore, the in vitro fixation of the homologous collagen antibody to the purified reconstituted collagen fibers, as demonstrated by their fluorescence, supports the observation that the antigen with which the collagen antibody reacts in the tissues is also collagen.

The general pattern of fluorescent staining of the injected antibody to collagen has both remarkable points of similarity to and differences from that found with the nephrotoxic antibodies. Injected nephrotoxic antibody has been
ANTIGENICITY OF RAT COLLAGEN

identified by fluorescence in glomerular and tubular basement membranes in the kidney and in adrenal structures (17, 18). In a more extensive study, antibody to rat kidney was found in high concentration in kidney, adrenal, ovary, and spleen, in lower concentration in thyroid, lymph node, and liver, and not at all in testis, lung, skin, brain, and heart (19). By an in vitro method, applying conjugated antibody against rat glomeruli or lung directly to tissue sections, fluorescence was seen in basement membranes and reticulum of a variety of organs, in sarcolemma of skeletal muscle, and neurolemma of peripheral nerves; but it was not present in collagen of the various organs or of tendon, in elastic fibers, or in cartilage (20, 21). These workers were unable to define the nature of the antigenic substance with which their antibodies reacted, but considered that it might be a mucopolysaccharide.

The antigens used for preparing the nephrotoxic sera, whether obtained from whole kidney, glomeruli, placenta, or lung (22), are undoubtedly a mixture of antigenic substances, none of which has been specifically defined. However, the collagen used for preparation of our antibody has been purified from the native state, and by immunological tests, enzymatic analyses, and electron microscopy the antibody to it has been shown to be directed to the collagen fiber itself and not to other tissue proteins or polysaccharides (2). Furthermore, cross-complement fixation tests have shown that antiserum to rat kidney or glomeruli differs from that to rat collagen (7). It is not surprising, then, that the pattern of fluorescent staining with the two types of antisera should differ. Although differences in methods prevent valid detailed comparisons, in general it seems that nephrotoxic sera react with reticular fibers, and anti-collagen sera with both reticular and collagen fibers in rat tissues. It is noteworthy that the widespread fixation of either antibody may occur without pathologic changes in extrarenal sites detectable by light microscopy.

SUMMARY AND CONCLUSIONS

Antibody to rat collagen, prepared in rabbits and injected into the circulation of normal or adjuvant-prepared rats, becomes fixed to its antigen and can then be identified in tissue sections under ultraviolet light by its fluorescence after application of fluorescein-conjugated anti-rabbit globulin. In heart, lung, liver, spleen, adrenal, kidney, jejunum, lymph node, thymus, joint synovia, peripheral nerve, aorta, skeletal muscle, eye, and brain, the antibody was found at all sites where collagen and reticulin are normally present, but except for the kidneys of the adjuvant-prepared rats, no pathological abnormalities were demonstrated. It was not found within cells.

Specific fluorescence was absent from tissues of rats injected with normal rabbit serum or rabbit anti-fish collagen serum or rabbit anti-rat collagen serum after absorption with rat collagen, but was present when the anti-rat collagen serum had been absorbed with fish collagen. The reaction could be
blocked by pretreatment of sections with unlabeled anti-rabbit globulin and did not occur with heterologous labeled anti-duck globulin.

After serial treatment in vitro with homologous antibody to collagen and the conjugated anti-rabbit globulin, purified reconstituted collagen fibers showed the same fluorescence as fibers in the tissues; no fluorescence of the fibers occurred when heterologous antibody to collagen was applied.

These findings indicate that the antibody to rat collagen is directed toward an antigen present in both collagen and reticulin.

It is a pleasure to acknowledge the able technical assistance of Mrs. Bonita Grossman.

BIBLIOGRAPHY


EXPLANATION OF PLATES

All sections showing specific fluorescence were treated with fluorescein-conjugated duck anti-rabbit globulin. All control sections were treated with heterologous rabbit anti-duck globulin. Photographs were taken by ultraviolet light with exposure times of 35 to 120 seconds for fluorescent sections and 24 to 30 minutes for the controls.

PLATE 38

Fig. 1. Section of the columnae carneae of the right ventricle from a rat given a single injection of rabbit anti-rat collagen globulin into the left heart and sacrificed 7 days later. Viewed by ultraviolet light, the fluorescence at the site of antigen-antibody reaction, appearing white in the photograph, outlines the connective tissue about the myocardial fibers. × 325.

Fig. 2. Control section of heart adjacent to that in Fig. 1. The muscle fibers can be seen, but show no fluorescence. × 333.

Fig. 3. Lung from a rat given 3 intravenous injections with a total volume of 7 ml of rabbit anti-rat collagen serum and sacrificed 7 days later. In the area shown are compressed alveolar walls with fluorescence of the closely meshed fibers. In the upper right corner is a bronchus with intense fluorescence in its wall. × 333.

Fig. 4. A control section of lung adjacent to that in Fig. 3 shows no specific fluorescence, but the autofluorescence of elastic fibers in the alveolar walls can be seen. × 333.

Fig. 5. Liver from a rat given a single injection of rabbit anti-rat collagen globulin into the left heart and sacrificed 45 minutes later. The central vein is outlined by fluorescence in its walls. Radiating from it are fibers running along the sinusoids. × 188.

Fig. 6. A control section adjacent to that in Fig. 5. The hepatic cell structure is visible but without any fluorescence. × 275.
PLATE 39

Fig. 7. Spleen section from the same rat as in Fig. 1. The capsule, trabeculae, and fine pericellular fibers all show intense fluorescence. × 333.

Fig. 8. A control spleen section adjacent to that in Fig. 7 illustrates the absence of fluorescence in any structures. × 325.

Fig. 9. Malpighian body from the same spleen section as in Fig. 7 is surrounded by a fine network of fluorescent fibers. × 172.

Fig. 10. Adrenal from the same rat as in Fig. 1 shows fine fluorescent fibers outlining the cortical cell cords. × 315.

Fig. 11. The same adrenal section as Fig. 10 shows also the intense fluorescence of the heavier fibers in the capsule and trabeculae. × 325.

Fig. 12. Control section of adrenal adjacent to that in Figs. 10 and 11 shows no fluorescence in the capsule or cortex. × 325.
PLATE 40

Fig. 13. Kidney section from the same rat as in Fig. 1 shows white lines of fluorescence outlining the basement membranes of the glomerulus. The faint fluorescence around the tubules is not shown in this photograph. × 340.

Fig. 14. Control kidney section adjacent to that in Fig. 13 shows no specific fluorescence. The white spot is an artifact. × 333.

Fig. 15. Jejunum section from the same rat as in Fig. 1 shows fluorescence in the lamina propria within the core of two villi cut longitudinally. No fluorescence is visible within the epithelial cells. × 350.

Fig. 16. Control jejunum section adjacent to that in Fig. 15 shows portions of two villi with no specific fluorescence. × 350.

Fig. 17. The same jejunum section as in Fig. 15 shows intense fluorescence in the connective tissue of the submucosa continuous with the connective tissue in the lamina propria surrounding the villi, which here are cut in cross-section. The muscular layer at the bottom of the photograph shows no fluorescence. × 168.

Fig. 18. Peripheral lymph node from a rat given 2 intravenous injections with a total volume of 4.7 ml of rabbit anti-rat collagen serum and sacrificed 7 days later. The fluorescent fine pericellular network of fibers is well shown. At the left is a trabecula with coarser fibers and at the right, a vein with fluorescent fibers in its wall. × 333.

Fig. 19. Control lymph node section adjacent to that in Fig. 18 shows no specific fluorescence. × 333.
(Rothbard and Watson: Antigenicity of rat collagen)
PLATE 41

Fig. 20. Thymus section from the same rat as in Fig. 18 shows in the capsule a thick band of fluorescent fibers and in the cortex a fine network of fluorescent fibers outlining the small blood vessels and surrounding the cells. X 333.

Fig. 21. Control thymus section adjacent to that in Fig. 20 shows no fluorescence. X 333.

Fig. 22. Section of synovial villus from the knee joint of a rat given 3 intravenous injections with a total volume of 6.5 ml of rabbit anti-rat collagen serum and sacrificed 7 days later. Fluorescence outlines the margins of the villus and the irregularly arranged fibers within it. X 500.

Fig. 23. Control section of synovia adjacent to that in Fig. 22 shows no specific fluorescence. X 520.

Fig. 24. The same synovial section as in Fig. 22 shows the more regularly arranged fluorescent fibers in the subsynovial membrane and joint capsule. X 580.

Fig. 25. Cross-section of a peripheral nerve from the same rat as in Fig. 18 shows intensely fluorescent fibers in the epineurial tissue and fainter fluorescence within the nerve around the individual nerve fibers. X 333.
(Rothbard and Watson: Antigenicity of rat collagen)
PLATE 42

Fig. 26. Aorta from the same rat as in Fig. 1 has fibers showing specific fluorescence in the adventitia and a few between the autofluorescent wavy elastic lamellae of the media, shown in the lower left corner. X 350.

Fig. 27. Control aorta section adjacent to that in Fig. 26 shows only the autofluorescent elastic lamellae. X 340.

Fig. 28. Section of muscle with attached tendon from the same rat as in Fig. 18 shows intense fluorescence in the closely packed tendon fibers in the lower portion of the figure. These are continuous with the fibers surrounding the individual muscle fibers within which no specific fluorescence is evident. X 333.

Fig. 29. In the control muscle section adjacent to that in Fig. 28, no tendon is present. Fluorescence is absent about the muscle fibers. X 333.

Fig. 30. Section of the eye from the same rat as in Fig. 22 is taken through the sclera, choroid, and retina. Only the connective tissue fibers of the scleral coat show fluorescence in this photograph. X 358.

Fig. 31. Control eye section adjacent to that in Fig. 30 shows no fluorescence. X 358.
(Rothbard and Watson: Antigenicity of rat collagen)