IMMUNOLOGIC RELATIONS AMONG VARIOUS ANIMAL COLLAGENS*

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Plates 29 to 31

(Received for publication, April 16, 1965)

Collagens from rat tail tendon and carp swim bladder have been found to be immunologically distinct. Antibody prepared in rabbits to purified acid soluble rat collagen was not removed by absorption with native or purified carp collagen; homologous absorption removed the antibody completely (1). Antibody to carp collagen injected intravenously into rats did not cause reverse anaphylaxis (2), induce renal glomerular lesions in adjuvant-prepared animals (3), or become fixed either in the basement membranes of renal glomeruli (4) or in other sites where reticulin or collagen is present (5); however, when antibody to rat collagen was injected into rats, each of these phenomena ensued.

Although vertebrate collagens are indistinguishable histologically and by electron microscopy and have a characteristic wide-angle x-ray diffraction pattern, the immunologic differences between rat and carp collagen prompted the study of collagens from other animals. This paper presents the results of complement fixation and *in vivo* immunofluorescence tests using anticollagen sera and collagens from rat, mouse, guinea pig, man, chicken, and carp as antigens to determine whether collagens from animals other than rat and carp can also be differentiated immunologically.

Materials and Methods

Collagens from rat or mouse tail tendons, guinea pig or chicken leg tendons, and carp swim bladder were prepared by the method previously described (1). Human collagen was prepared from the skin of dead-born infants. The epidermis and subcutaneous adipose tissue were removed by scraping with a sharp blade. Subsequent procedures took place at 4°C. Thirty to 40 gm of corium were finely minced with scissors, washed 3 times with cold acetone-ether, 1:1, once with 0.9 per cent NaCl solution, 3 times with 0.3 M Na_2HPO_4 solution, and then with distilled water until all phosphate had been removed, as shown by the absence of precipitate upon the addition of 10 per cent $AgNO_3$ to the wash water. The moist tissue particles were

* This investigation was supported by The National Heart Institute, Research Grant H-01138 (C13) Public Health Service, and The Arthritis Foundation, New York Chapter.

again minced in a Waring blender for approximately 1 minute. From this step on, the procedure was essentially the same as for the other collagens except as follows: acetic acid, pH 3.2, was used for extraction instead of pH 3.8; filtration, preceded by glass wool straining out of large particles, was done through a Celite No. 545 pad on 589-1H Schleicher and Schull filter paper in a Buchner funnel; the final solution of collagen was dialyzed against several changes of acetic acid, pH 3.8, for 48 hours before the material was frozen and dried.

The hydroxyproline content of collagen was determined by the method of Neumann and Logan (6).

The preparation of antisera and the technique of complement-fixation tests have been previously described (1). Anti-chicken collagen serum was absorbed with sheep erythrocytes to remove heterophile antibodies before either complement fixation or injection into animals.

For absorption with collagen, aliquots of rabbit anticollagen serum were mixed with thoroughly washed, finely minced native collagen in the proportion of 3 parts serum to 1 part packed collagen (30 ml serum to 10 gm collagen, wet weight). The mixtures were incubated for 2 hours at 37°C with frequent mixing, stored overnight at 4°C, and centrifuged at 3500 RPM in the cold to remove large particles of collagen, and then centrifuged again for 90 minutes at 40,000 RPM at 0°C. The sera were tested before and after absorption.

For the immunofluorescence studies, rabbit anticollagen sera were injected intravenously into rats, mice, and chickens and intracardially into guinea pigs. Divided doses were given on consecutive days, and the animals were sacrificed 7 days after the last injection unless otherwise stated.

A series of 17 young black and white hooded Whalen or Long-Evans strain rats, weighing 180 to 320 gm, was given 2 injections, total volume 4.0 ml, of antisera. Four rats were given anti-rat collagen serum, complement fixation titer 1:128; 2, anti-mouse collagen serum, titer 1:128; 4, 1 of which died after 4 days, anti-guinea pig collagen serum, untitered; 3, anti-human collagen serum, titer 1:64; 2, anti-chicken collagen serum, titer 1:128; and 2, anti-carp collagen serum, titer 1:512. Of 10 additional rats, 4 were given anti-rat collagen serum absorbed with rat collagen; 2, the same serum absorbed with guinea pig collagen; 2, anti-guinea pig collagen serum absorbed with guinea pig collagen; and 2, the same serum absorbed with rat collagen. The rats given anti-guinea pig collagen serum absorbed with guinea pig collagen received 4.5 ml divided into 3 doses.

Five mice of the Swiss-Webster albino strain, weighing 15 to 20 gm, were given 2 injections, total volume 1.5 ml, of anti-mouse collagen serum, titer 1:256, and 6 were given anti-rat collagen serum, titer 1:256.

Twenty-two short-haired English guinea pigs, weighing 250 to 350 gm, were injected with antisera into the left heart under light ether anesthesia on consecutive days. None showed evidence of anaphylactic shock, but 3 died from cardiac tamponade shortly after an injection. Eight guinea pigs were given anti-guinea pig collagen serum; 1 had a single injection of 2.0 ml and died 45 minutes later; 6 had a total of 2.0 to 4.5 ml in 2 injections; 2 of these were sacrificed 45 minutes after the 2nd injection; 1 was given 4 injections, total volume 11.0 ml. Anti-rat collagen serum, titer 1:64 to 1:128, was given to 4 guinea pigs; 3, 1 of which died after the 2nd injection, had 4.0 ml in 2 doses; 1 was given 3 doses, 6.0 ml, and died after the 3rd injection. Four guinea pigs were given 4.0 ml of anti-mouse collagen serum, titer 1:128, in 2 doses. Two guinea pigs were given 4.5 ml of anti-human collagen serum, titer 1:128, in 2 injections. Two guinea pigs were given 4.0 ml of anti-chicken collagen serum, titer 1:128, in 2 injections. Two guinea pigs were given 4.0 ml of anti-carp collagen serum, titer 1:64, in 2 injections. Of 11 additional guinea pigs, 2 were given 2 intracardiac injections, total volume 3.5 ml, of anti-rat collagen serum absorbed with rat collagen and 2, the same serum absorbed with guinea pig collagen. Of 3 guinea pigs given anti-guinea pig collagen serum absorbed with guinea pig collagen in 2 injections, 2 received 4.0 ml and 1, 4.5 ml. The anti-guinea pig collagen serum ab-

sorbed with rat collagen was given to 4 guinea pigs in 2 injections, total volume 4.0 ml; 1 of these died from cardiac tamponade a few minutes after the 2nd injection.

Of 10 Plymouth Rock chickens, weighing 1.4 to 1.8 kg, 2 were given 6.0 ml divided into 2 injections of each of the following antisera: anti-rat collagen serum, titer 1:256; anti-mouse collagen serum, titer 1:256; anti-human collagen serum, titer 1:128; and anti-carp collagen serum, titer 1:128. Anti-chicken collagen serum, titer 1:128, was given to 2 chickens in the same way, but the total volume was 6.5 ml. Of 8 additional chickens, 2 were given 6.0 ml in 2 injections of each of the following antisera: anti-chicken collagen serum absorbed with chicken collagen or with human collagen and anti-human collagen serum absorbed with human collagen. Anti-human collagen serum absorbed with chicken collagen was given in a single injection of 4.0 ml to 1 chicken which died 1 day later and to a 2nd chicken in 2 injections, total volume 6.5 ml.

At autopsy of the animals, duplicate blocks of tissue were removed from kidney, liver, and spleen. 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 was rapidly frozen in petroleum ether and stored at −70°C. After frozen sections were cut in a cryostat at −20°C, they were treated with fluorescein-conjugated anti-rabbit globulin from ducks or goats.[1] The details of preparing the sections and examining and photographing them under ultraviolet light have been reported previously (4). As controls for the specificity of the fluorescence, the blocking technique of Coons and Kaplan (7) and heterologous conjugated globulin were applied to adjacent sections. Tissues from normal uninjected animals were also treated with conjugated anti-rabbit globulin.

EXPERIMENTAL OBSERVATIONS

Complement-Fixation Reactions.—Rat, mouse, guinea pig, human, chicken, and carp collagens as antigens and rabbit antisera to rat, mouse, human, chicken, and carp collagens were used for the complement fixation tests shown in Table I. Rabbit antiserum to guinea pig collagen was invariably anticomplementary and could not be tested by this method. Much study failed to reveal a satisfactory explanation for this effect. Each collagen fixed complement most strongly with its homologous antiserum. In addition, several cross-reactions were observed. Mouse and rat collagen fixed complement to a similar degree with both anti-rat and anti-mouse collagen sera. Guinea pig collagen also fixed complement with both anti-rat and anti-mouse collagen sera but in a lower titer. Human and chicken collagen showed marked reciprocal cross-reactions. Slight reaction occurred between rat collagen and anti-human collagen serum. The very slight reaction (plus-minus) in two tubes with human collagen and anti-mouse collagen serum is probably not significant. Carp collagen, alone of those tested, showed no cross-reactions. The results of these experiments suggest that the collagens of the rat and mouse are closely related antigenically and that guinea pig collagen is related to both, but less closely. The cross-reaction between human and chicken collagen suggests that these collagens also are antigenically related to each other.

Immunofluorescence Reactions.—Tissues from rats, mice, guinea pigs, and

[1] Goat anti-rabbit serum was supplied by the courtesy of Dr. Leonhard Korngold.

TABLE I

Complement Fixation
Various Vertebrate Collagens with the Corresponding Antisera

Rabbit antiserum prepared against collagen from:	Antigen: 1 mg/ml diluted 1:											Serum control
	1	2	4	8	16	32	64	128	256	512	1024	
Rat collagen												
Rat	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	−	−
Mouse	++	+++	++++	+++±	++++	++++	++++	++±	+±	±	−	−
Man	±	±	+	+	+	−	−	−	−	−	−	−
Chicken	−	−	−	−	−	−	−	−	−	−	−	−
Carp	−	−	−	−	−	−	−	−	−	−	−	−
Mouse collagen												
Rat	++++	++++	++++	++++	++++	++++	++++	++++	+±	±	−	−
Mouse	++++	++++	++++	++++	++++	++++	++++	++++	+++	+	−	−
Man	−	−	−	−	−	−	−	−	−	−	−	−
Chicken	−	−	−	−	−	−	−	−	−	−	−	−
Carp	−	−	−	−	−	−	−	−	−	−	−	−
Guinea pig collagen												
Rat	−	−	+	+++	++±	+±	−	−	−	−	−	−
Mouse	−	−	±	+±	++	+	−	−	−	−	−	−
Man	−	−	−	−	−	−	−	−	−	−	−	−
Chicken	−	−	−	−	−	−	−	−	−	−	−	−
Carp	−	−	−	−	−	−	−	−	−	−	−	−
Human collagen												
Rat	−	−	−	−	−	−	−	−	−	−	−	−
Mouse	±	±	−	−	−	−	−	−	−	−	−	−
Man	++++	++++	++++	++++	++++	++++	++++	+++	+	±	±	−
Chicken	+	++++	++++	++++	++++	++++	+++	−	−	−	−	−
Carp	−	−	−	−	−	−	−	−	−	−	−	−
Chicken collagen												
Rat	−	−	−	−	−	−	−	−	−	−	−	−
Mouse	−	−	−	−	−	−	−	−	−	−	−	−
Man	+++	++	++	++	+	±	−	−	−	−	−	−
Chicken	++++	++++	++++	++++	++++	++++	++	±	−	−	−	−
Carp	−	−	−	−	−	−	−	−	−	−	−	−
Carp collagen												
Rat	−	−	−	−	−	−	−	−	−	−	−	−
Mouse	−	−	−	−	−	−	−	−	−	−	−	−
Man	−	−	−	−	−	−	−	−	−	−	−	−
Chicken	−	−	−	−	−	−	−	−	−	−	−	−
Carp	++++	++++	++++	++++	++++	++++	+++	+++	+	±	−	−

− Indicates complete hemolysis; ± to +++ indicates intermediate degrees of hemolysis; and ++++ indicates no hemolysis with complete fixation of complement.

chickens injected with antisera prepared to rat, mouse, guinea pig, human, chicken, or carp collagens were studied by the immunofluorescence technique (Table II). Although only the findings in the kidney are tabulated, the liver and spleen of each animal were also examined. Fluorescence indicating the fixation of antibody to its antigen in the liver was generally less intense than in the kidney and spleen. Paraffin sections of these organs showed no abnormalities. With the divided doses used, anaphylactic shock was not observed.

In the rat renal glomeruli, the antibodies to both rat and mouse collagens fluoresced intensely (Figs. 1, 2, and 3); that to guinea pig collagen was fixed, but less intensely (Fig. 4); antibody to human collagen was also definitely fixed (Fig. 5). Fixation of the antibody to chicken (Fig. 6) and carp collagens

TABLE II

Immunofluorescence of Injected Collagen Antibodies in Renal Glomeruli of Various Animals

Rabbit antiserum prepared against collagen from:	Animal in which antiserum was tested*			
	Rat	Mouse	Guinea pig	Chicken
Rat	++++	++++	++±	−
Mouse	++++	++++	+	−
Guinea pig	++	n.d.	++++	n.d.
Man	+++	n.d.	−	+++
Chicken	−	n.d.	−	+++
Carp	−	n.d.	−	−

* Intensity and extent of fluorescence in kidney sections is indicated on a ++++ to ± scale; − indicates no fluorescence; n.d. indicates not done.

was not detected. The mouse kidney sections showed strong fixation of antibody to either rat or mouse collagen (Figs. 7 and 8). Because of the great similarity between the rat and mouse collagens by immunofluorescence and complement fixation, no further immunofluorescence studies were performed in mice.

The rabbit anti-guinea pig collagen serum was found to contain antibody which was strongly fixed by its antigen in the guinea pig renal glomeruli, liver, and spleen (Figs. 9, 13, and 14). This finding provided evidence that guinea pig collagen was antigenic, since rabbit antisera to guinea pig collagen could not be tested by complement fixation. Antibody to rat collagen was also fixed in the guinea pig (Table II, Fig. 10), but not as strongly as antibody to homologous collagen. Antibody to mouse collagen was visible in the renal glomeruli (Fig. 11), but little or none was present in the liver or spleen. No fixation of antibody to human (Fig. 12), chicken, or carp collagen was found.

In chickens fluorescence indicating the presence of fixed antibody was found

in renal glomerular basement membranes of those animals given antibody to chicken or human collagen (Table II, Figs. 15 and 16). Strong fluorescence was also present in liver and spleen of these animals. No cross-reaction was observed with antibody to rat, mouse, or carp collagen. These findings parallel the results of the complement-fixation reactions.

Cross-Absorption Studies—The cross-reactions between rat, mouse, and guinea pig collagens, and between human and chicken collagens made further investigation of the antigenic specificity of these collagens desirable.

Anti-rat and anti-mouse collagen sera were reciprocally cross-absorbed with rat and mouse collagens and then tested by complement fixation against each collagen. Both unabsorbed antisera reacted equally well with both antigens. All detectable antibody was removed from both antisera by either rat or mouse collagen (Table III). These results indicate more conclusively that mouse and rat collagens are closely similar or identical antigens.

The cross-reaction between rat and guinea pig collagens was also investigated by absorption studies. Because of the anticomplementary effect of the anti-guinea pig collagen serum, it could be tested only by immunofluorescence. Unlike the results with rat and mouse collagens, guinea pig collagen in the presence of anti-rat collagen serum fixed complement only weakly and with a prozone effect. Furthermore, although the homologous absorption was complete, the heterologous absorption of anti-rat collagen serum by guinea pig collagen only moderately reduced the antibody titer when tested against its homologous rat collagen but was complete when tested against the heterologous guinea pig collagen (Table IV). By the immunofluorescence method complete reciprocal cross-absorption studies were possible. Rabbit anti-rat and anti-guinea pig collagen sera, unabsorbed, absorbed with rat collagen or guinea pig collagen, were tested in rats and in guinea pigs (Table V). The results with the anti-rat collagen serum confirm those found with complement fixation using the same antisera; that is, antibody from the unabsorbed serum was fixed in the renal glomeruli of rats and, somewhat less strongly, of guinea pigs; all antibody was removed by absorption with homologous collagen; but antibody was only reduced in rats and not detectable in guinea pigs when the serum had been absorbed with the heterologous guinea pig collagen. With the unabsorbed anti-guinea pig collagen serum, antibody was fixed in both rats and guinea pigs, and absorption with the homologous guinea pig collagen completely removed all antibody so that no fixation occurred in either rats or guinea pigs. However, after absorption with heterologous rat collagen, antibody was still detectable in both the rats and guinea pigs. The reason for the incomplete absorption of the anti-guinea pig collagen serum by rat collagen when tested in the rat is not clear, but most likely is quantitative since reabsorption completely removed the antibody when tested in both rats and guinea pigs.

The cross-reaction found between human and chicken collagens and their

TABLE III

Complement Fixation

Cross-Absorption between Rat and Mouse Collagens and Antisera

Rabbit antiserum		Antigen: 1 mg/ml diluted 1:											Serum control
Prepared against collagen from:	Absorbed with collagen from:	1	2	4	8	16	32	64	128	256	512	1024	
Rat collagen													
Rat	—	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	−	−
"	Rat	−	−	−	−	−	−	−	−	−	−	−	−
"	Mouse	−	−	−	−	−	−	−	−	−	−	−	−
Mouse	—	++	+++	++++	+++±	++++	++++	++++	++±	+±	±	−	−
"	Mouse	−	−	−	−	−	−	−	−	−	−	−	−
"	Rat	−	−	−	−	−	−	−	−	−	−	−	−
Mouse collagen													
Mouse	—	++++	++++	++++	++++	++++	++++	++++	++++	+++	+	−	−
"	Mouse	−	−	−	−	−	−	−	−	−	−	−	−
"	Rat	−	−	−	−	−	−	−	−	−	−	−	−
Rat	—	++++	++++	++++	++++	++++	++++	++++	++++	+±	±	−	−
"	Rat	−	−	−	−	−	−	−	−	−	−	−	−
"	Mouse	−	−	−	−	−	−	−	−	−	−	−	−

− Indicates complete hemolysis; ± to +++ indicates intermediate degrees of hemolysis; and ++++ indicates no hemolysis with complete fixation of complement.

TABLE IV

Complement Fixation

Absorption of Anti-Rat Collagen Serum with Rat and Guinea Pig Collagens

Rabbit antiserum		Antigen: 1 mg/ml diluted 1:											Serum control
Prepared against collagen from:	Absorbed with collagen from:	1	2	4	8	16	32	64	128	256	512	1024	
Rat collagen													
Rat	—	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	−	−
"	Rat	−	−	−	−	−	−	−	−	−	−	−	−
"	Guinea pig	+++	++±	++	+++	++±	++	+±	+	±	±	−	−
Guinea pig collagen													
Rat	—	−	−	+	+++	++±	+±	−	−	−	−	−	−
"	Rat	−	−	−	−	−	−	−	−	−	−	−	−
"	Guinea pig	−	−	−	−	−	−	−	−	−	−	−	−

− Indicates complete hemolysis; ± to +++ indicates intermediate degrees of hemolysis; and ++++ indicates no hemolysis with complete fixation of complement.

respective antibodies was also further investigated by complement fixation and immunofluorescence after reciprocal cross-absorption of the antisera. The results of these complement-fixation reactions are shown in Table VI. Absorption of both anti-human and anti-chicken collagen sera with the homologous collagen removed all antibody whether tested against human or chicken collagen. However, when anti-human collagen serum was absorbed with chicken collagen, all antibody directed toward chicken collagen was completely removed but the titer was not significantly reduced for its homologous antigen. Similarly, the antibody to human collagen was completely removed from the anti-chicken collagen serum by absorption with human collagen, but again the titer was not significantly reduced when tested against chicken collagen, its homologous anti-

TABLE V

Immunofluorescent Reactions in Renal Glomeruli of Rats and Guinea Pigs after Cross-Absorption of the Corresponding Anticollagen Sera

Rabbit antiserum		Animal in which antiserum was tested*	
Prepared against collagen from:	Absorbed with collagen from:	Rat	Guinea pig
Rat	—	++++	++±
"	Rat	—	—
"	Guinea pig	+++	—
Guinea pig	—	++	++++
"	Guinea pig	—	—
"	Rat	++	+++

* Intensity and extent of fluorescence in kidney sections is indicated on a ++++ to ± scale; — indicates no fluorescence.

gen. By immunofluorescence the absorption studies were necessarily limited to chicken collagen and parallel those found by complement fixation. Chickens were given rabbit anti-chicken collagen serum absorbed with chicken or human collagen, and anti-human collagen serum absorbed with human or chicken collagen. Unabsorbed anti-chicken collagen serum was strongly fixed in the renal glomeruli of chickens. When the antiserum had been absorbed with homologous chicken collagen, no specific fluorescence occurred, but after absorption with heterologous human collagen fluorescence was only slightly reduced. The antiserum to human collagen, which also fluoresced strongly in the chicken when unabsorbed, had all detectable antibody removed by absorption with either chicken or human collagen. From these studies it is apparent that human and chicken collagens are related and contain common antigens, but they can be easily differentiated immunologically in contrast to rat and mouse collagen.

Hydroxyproline Determinations.—Since hydroxyproline is an amino acid pres-

TABLE VI

Complement Fixation

Cross-Absorption between Human and Chicken Collagen and Antisera

Rabbit antiserum		Antigen: 1 mg/ml diluted 1:											Serum control
Prepared against collagen from:	Absorbed with collagen from:	1	2	4	8	16	32	64	128	256	512	1024	
						Human collagen							
Man	—	++++	++++	++++	++++	++++	++++	++++	+++	+	±	±	−
"	Man	−	−	−	−	−	±	±	±	−	−	−	−
"	Chicken	++++	++++	++++	++++	++++	++++	++++	++	+	±	−	−
Chicken	—	+	++++	++++	++++	++++	++++	+++	−	−	−	−	−
"	Chicken	−	−	−	−	−	−	−	−	−	−	−	−
"	Man	−	−	−	−	−	−	−	−	−	−	−	−
						Chicken collagen							
Chicken	—	++++	++++	++++	++++	++++	++++	++	±	−	−	−	−
"	Chicken	−	−	−	−	−	−	−	−	−	−	−	−
"	Man	++++	++++	++++	++++	++++	++++	±	−	−	−	−	−
Man	—	+++	++	++	++	+	±	−	−	−	−	−	−
"	Man	−	−	−	−	−	−	−	−	−	−	−	−
"	Chicken	−	−	−	−	−	−	−	−	−	−	−	−

− Indicates complete hemolysis; ± to ++ indicates intermediate degrees of hemolysis; and ++++ indicates no hemolysis with complete fixation of complement.

ent in relatively large amounts only in collagen, it appeared important to see whether the hydroxyproline content of the various collagens showed significant species differences which might correlate with the immunologic findings. The average per cent of hydroxyproline in the purified acid soluble collagen preparations were: rat 12.8, guinea pig 13.2, human 11.7, chicken 12.1, and carp 9.4. These findings show that the hydroxyproline content of carp collagen is lower than that of the chicken and mammalian collagens which vary by only small amounts. It is of interest that carp collagen was also the only one tested that showed no immunologic cross-reactions.

DISCUSSION

The findings reported in this paper show that collagens from various animals exhibit a pattern of antigenicity characteristic of species specificity (8). Tissue specificity, or an antigen common to all of the collagens, was not demonstrated. By complement fixation, *in vivo* immunofluorescence, and cross-absorption methods, rat and mouse collagens were found to be so closely related antigenically as to be indistinguishable; guinea pig collagen was related to both rat and mouse collagens, but less closely. Rat collagen also reacted with antiserum to human collagen, but the reverse cross-reaction did not occur. Human and chicken collagens were antigenically related to each other. Carp collagen was distinct from the others. The complete absorption of antibody from antisera to rat or guinea pig collagen and from antisera to human or chicken collagen by the homologous antigen and the incomplete absorption by the respective heterologous antigen was consistent with cross-reactions found in species specificity (9).

The general agreement in the results with the two different immunologic tests, complement fixation and *in vivo* immunofluorescence, adds significance to the findings of species specificity. The two methods also complemented each other in this study since rabbit anti-guinea pig collagen serum was invariably anticomplementary and could not be tested by complement fixation but could be by immunofluorescence, and *in vivo* tests were not practical with man or fish, but their collagens could be tested by complement fixation.

The cross-reactions may represent either quantitative or qualitative differences in the chemical groupings responsible for the antigenicity of collagen or may be the result of multiple antigens, some of which may be common to more than one collagen. The viscosity and spontaneous precipitation of the acid soluble collagens at pH's approaching neutrality or in the presence of various salts and polysaccharides prevented the use of agar diffusion and immunoelectrophoretic techniques to determine whether multiple antibodies to collagen or whether antibodies to other antigens are present in the antisera. It may be possible to alter the physical state of collagen without affecting its antigenicity so that these methods can be applied (10). Earlier studies (1) have shown the

purified collagen to be free of serum proteins and, although a minimal amount of polysaccharide was present, antibody to it could not be demonstrated in the anti-collagen serum by complement fixation.

Other reports on species specificity of collagen have recently appeared. By complement fixation and inhibition of fiber reconstitution from collagen solutions, Hisa and Suzuki (11) found species specificity of collagens from rat tail tendon and bovine Achilles's tendon. Steffen, Timpl, and Wolff (12) have also found that acid soluble collagens from the skin or periarticular tissue of calf and rabbit showed species specificity. They also found that acid soluble collagen, hydroxylamine-treated collagen, and gelatin all prepared from the same source, differed in antigenicity.

Species specificity among collagens presupposes chemical differences among them. Indeed, numerous studies (13–15) have established that collagens from a wide variety of animals show quantitative differences in a number of amino acids. An application of immunologic methods to the problems of the molecular structure of collagen is shown by Schmitt and his associates (16) who have reported that the antigenicity of tropocollagen from calf skin depends on "telopeptides" external to the helical molecular structure, and that removal of these appendages by proteases abolishes the antigenicity of the tropocollagen. Whether these telopeptides are the only source of collagen antigenicity and whether they vary from one species to another is not yet known.

Whenever injected antibody to collagen, homologous or heterologous, was fixed in the rat, mouse, guinea pig, or chicken, the antibody showed specific localization in the basement membranes of the renal glomeruli and also at sites where collagen or reticulin fibers are normally present in the liver and spleen, as previously reported with homologous antibody in the rat (4, 5). This finding supports the assumption that collagen in some form is present in the renal glomerular basement membranes.

The differences in antigenicity found among these animal collagens show that immunologic methods may provide a useful method for further studying fibrogenesis, developmental changes, and pathologic alterations of collagen.

SUMMARY AND CONCLUSIONS

By the use of complement fixation and *in vivo* immunofluorescence with cross-absorption studies it was shown that acid soluble collagens prepared from rat, mouse, guinea pig, chicken, carp, and man exhibit species specificity. Rat and mouse collagens were found to be indistinguishable and to cross-react with guinea pig collagen. Cross-reactions also occurred between the collagens of rat and man and chicken and man. Tissue specificity, or an antigen common to all of the collagens, was not demonstrated. There was complete agreement in the results of the two immunologic methods. The findings in this study support the conclusion that collagen in some form is present in the renal glomerular basement membranes.

It is a pleasure to acknowledge the able technical assistance of Miss Mildred Schleimer, Mrs. Evelyn Merson, Miss Blanche A. Gelman, and Mr. George Frederick Walker.

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EXPLANATION OF PLATES

Sections were taken from rats, mice, guinea pigs, or chickens injected intravenously or intracardially with rabbit anticollagen serum and sacrificed 7 days later. Frozen sections of kidney, liver, and spleen were treated with fluorescein-conjugated duck or goat anti-rabbit globulin. By ultraviolet light, specific fluorescence appears yellow-green to the eye, but white in the photographs. Exposure times were 2 to 10 minutes except as noted for Figs. 6 and 12.

PLATE 29

FIG. 1. Glomerulus from the kidney of a rat injected with anti-rat collagen serum. Fluorescence at the site of antigen-antibody fixation outlines the basement membrane. The adjacent tubules can be seen but do not show specific fluorescence. × 333.

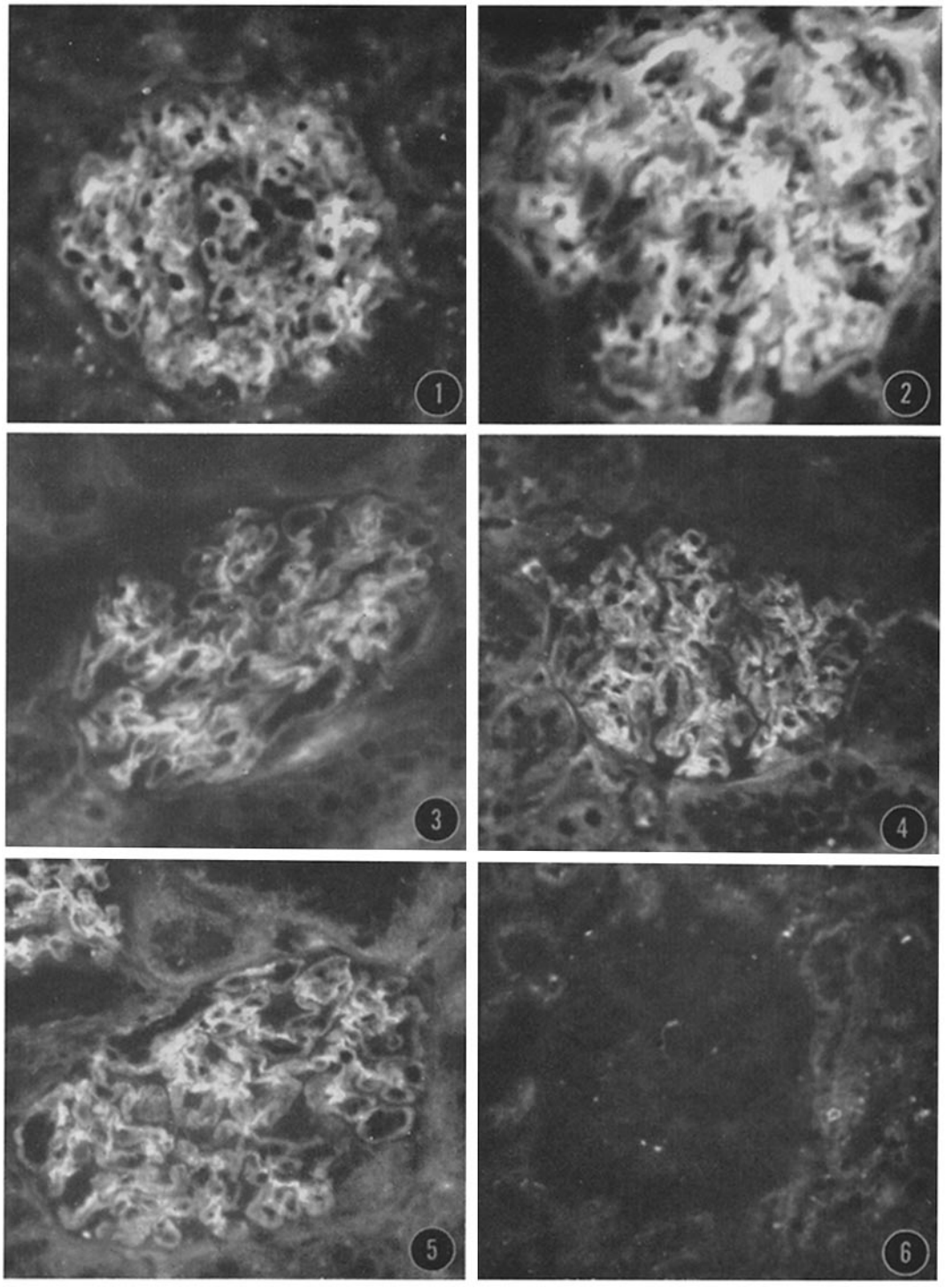
FIG. 2. Glomerulus from the kidney of another rat treated like the rat in Fig. 1 shows intense fluorescence of the basement membranes. × 416.

FIG. 3. Glomerulus from the kidney of a rat given anti-mouse collagen serum shows the same pattern of fluorescence in the basement membrane as in Figs. 1 and 2. × 395.

FIG. 4. Glomerulus from the kidney of a rat injected with anti-guinea pig collagen serum also shows basement membrane fluorescence. × 375.

FIG. 5. A glomerulus and part of another from the kidney of a rat given anti-human collagen serum show basement membrane fluorescence. × 408.

FIG. 6. Glomerulus from the kidney of a rat given anti-chicken collagen serum shows no specific fluorescence because antibody was not fixed. The glomerular structure can be faintly seen. Exposure time was 28 minutes. × 410.



(Rothbard and Watson: Immunologic relations among collagens)

PLATE 30

FIG. 7. Two glomeruli from the kidney of a mouse given anti-mouse collagen serum show unusually clear outlines of the basement membranes. × 408.

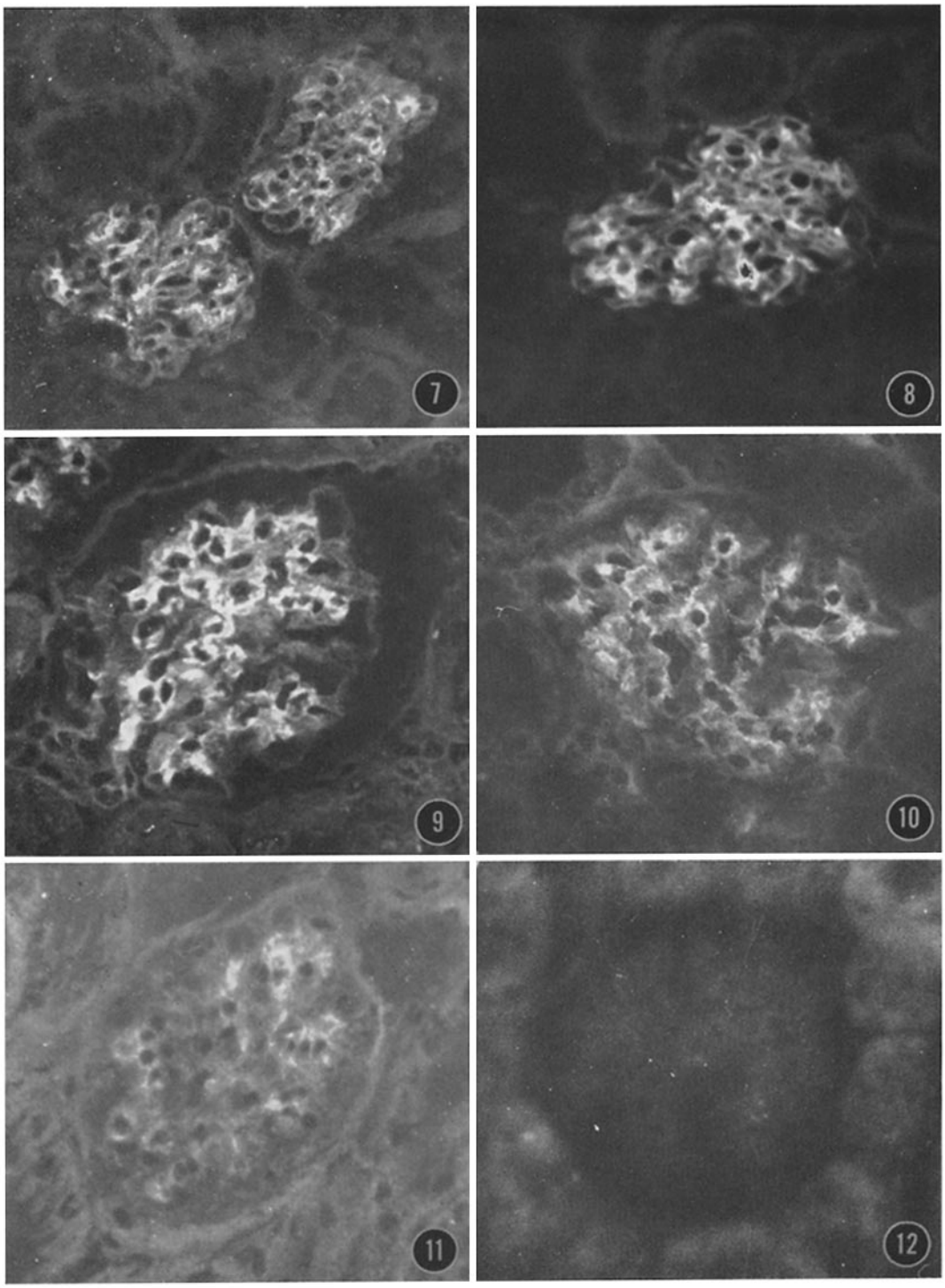
FIG. 8. Glomerulus from the kidney of a mouse given anti-rat collagen serum also shows the basement membrane outlined. × 408.

FIG. 9. One glomerulus and the edge of another from the kidney of a guinea pig given anti-guinea pig collagen serum show fluorescence in the basement membranes. × 432.

FIG. 10. Glomerulus from the kidney of a guinea pig given anti-rat collagen serum shows fluorescence in the basement membranes less bright than that in Fig. 9. × 429.

FIG. 11. Glomerulus from the kidney of a guinea pig given anti-mouse collagen serum shows relatively faint, but distinct, fluorescence in the basement membrane. × 408.

FIG. 12. Glomerulus from the kidney of a guinea pig given anti-human collagen serum does not show the fluorescence seen only when antibody has been fixed. Exposure time was 40 minutes. × 391.



(Rothbard and Watson: Immunologic relations among collagens)

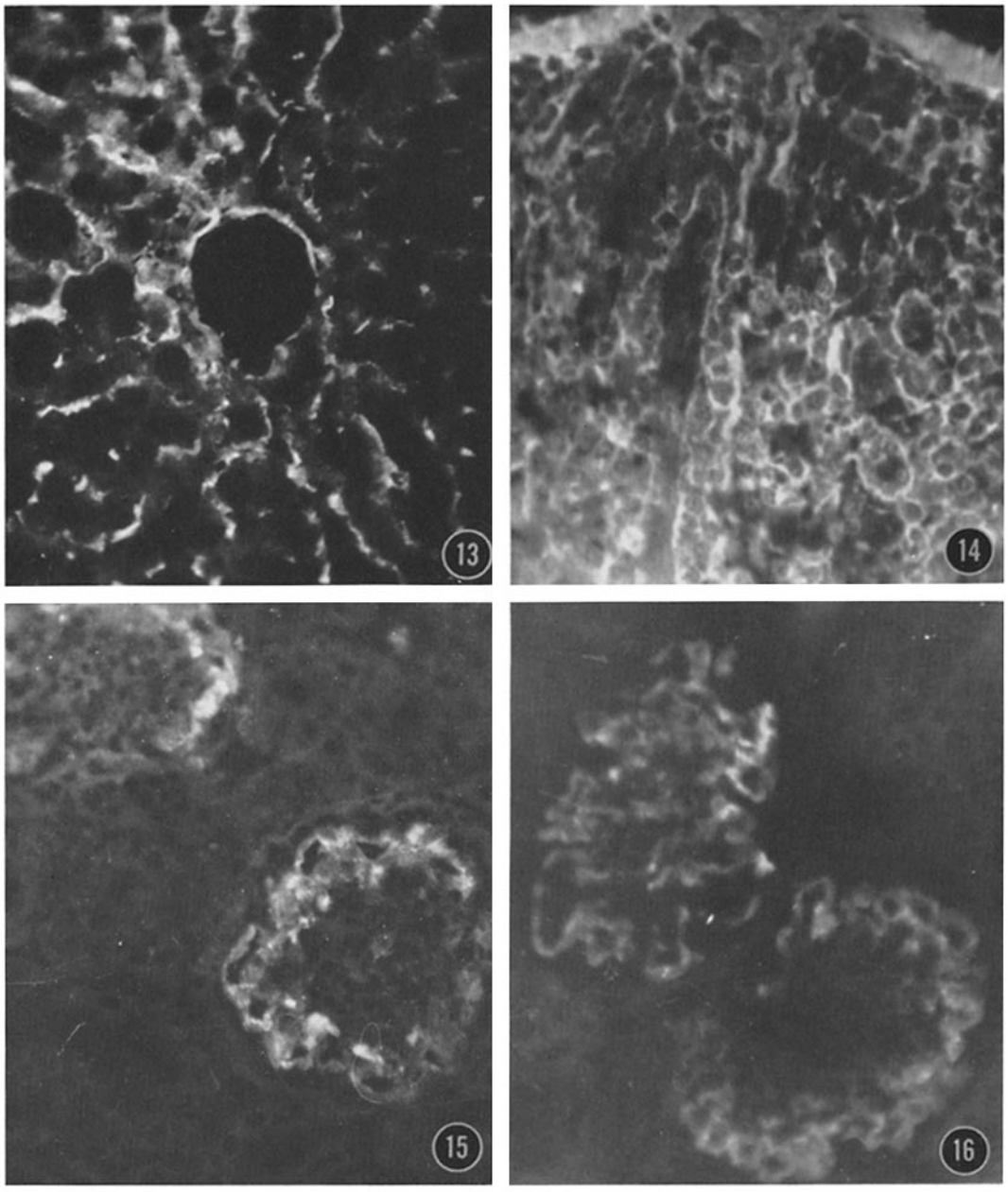
PLATE 31

FIG. 13. Liver from the same guinea pig as in Fig. 9 shows specific fluorescence outlining the walls of the central vein and in fibers along the sinusoids radiating from it. × 307.

FIG. 14. Spleen from the same guinea pig as in Fig. 9 shows specific fluorescence in fibers in the capsule at the top of the photograph, outlining sinusoids, and around the cells. × 408.

FIG. 15. Two glomeruli from the kidney of a chicken given anti-chicken collagen serum show fluorescence in the basement membranes, which in the chicken are at the periphery of the glomerulus. × 425.

FIG. 16. Two glomeruli from the kidney of a chicken given anti-human collagen serum show fluorescence in the basement membranes. The apparent location of the basement membranes in the center of the upper glomerulus is probably the result of tangential sectioning. × 408.



(Rothbard and Watson: Immunologic relations among collagens)