COMPLEMENT FIXATION IN DISEASED TISSUES

I. FIXATION OF GUINEA PIG COMPLEMENT IN SECTIONS OF KIDNEY FROM HUMANS WITH MEMBRANOUS GLOMERULONEPHRITIS AND RATS INJECTED WITH ANTI-RAT KIDNEY SERUM*,†

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PLATES 57 TO 60

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The means by which interaction of antigen and antibody may lead to disease is not well understood. Arthus (1) observed that repeated subcutaneous injections of horse serum in rabbits elicited progressively severe local inflammatory reaction. From clinical observations on serum disease in man von Pirquet (2) suggested "that a disease might be due indirectly to an antibody" and he coined the term allergy to mean "a changed reactivity" that occurs as a result of the injection of foreign serum or exposure to other antigenic substances. Schick (3), impressed by the similarity of some of the manifestations of serum disease to the Nachkrankheiten of scarlet fever, suggested that the latter were allergic reactions. From experiments on local anaphylaxis, as seen in the Arthus phenomenon, Opie (4) concluded that the cutaneous inflammatory reaction is a result of antigen meeting with antibody in the tissues. For many years it has been suggested and recently emphasized that antibodies may arise to antigenic components of an individual’s own tissues and produce cytotoxic effects (5, 6).

To elucidate the role of antibodies and allergic reactions in disease it would seem important to identify antigen-antibody complexes in tissues. Globulin of anti-kidney serum has been demonstrated in glomeruli of animals injected with nephrotoxic antiserum (7, 8). Human globulin has been identified in renal glomeruli of patients with a variety of diseases affecting the kidneys (9–11). In these investigations the deposits of globulin, identified by means of the fluorescent antibody technique, have been interpreted as antibodies localized in glomerular capillary walls. However, it cannot be concluded from the immunohistologic investigations on human tissues that the deposits of globulin represent antibody in combination with antigen.

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A complement fixation test applied to sections of tissue and carried out in parallel with immunohistologic tests for deposits of globulin would provide a means by which antigen-antibody aggregates in tissues could be identified by their avidity for complement. The purpose of this report is to demonstrate the fixation of guinea pig complement to glomerular sites of localized rabbit antibody-globulin in sections of kidney from rats injected with rabbit anti-rat kidney serum and in glomerular sites of localized human globulin in sections of biopsied kidneys from humans with the nephrotic syndrome.

**Materials and Methods**

Sprague-Dawley white rats were injected intravenously with 0.2 cc. of rabbit anti-rat kidney serum. Other rats of the same stock were similarly injected with normal rabbit serum for the purpose of control study. Specimens of rat kidney were obtained from anesthetized animals during perfusion with saline 3½ hours after injection of the nephrotoxic serum. Specimens of kidney from the patients with the nephrotic syndrome were obtained by percutaneous needle biopsy. One portion of each biopsied specimen was immediately placed in 10 per cent formalin fixative for embedding in paraffin, sectioning, and staining with hematoxylin and eosin. The other portion of each specimen was frozen in a matrix of gelatin, stored in a freezer at -20°C for periods varying between 2 and 10 days, and then sectioned in the frozen state and placed directly on glass slides as described elsewhere (12). In many instances serial sections were obtained from the frozen specimens. These sections were placed in a bath of anhydrous acetone for 10 minutes, air-dried for about 15 minutes, and then stored in glass jars in a refrigerator for periods not exceeding 24 hours. It was found that longer periods of storage of sections of several of these kidneys resulted in deterioration of their structure and complement-fixing properties. Sections of the tissues to be examined were washed three times for 10 minutes each in a bath of saline buffered at pH 7.4 with 0.01 M phosphate. The excess saline was wiped from the slide around the section and then the appropriate test serum applied to the sections with a capillary pipette.

The antisera used in this investigation were obtained from several sources. For identification of rabbit globulin in the sections of rat kidney an immune serum against rabbit antibody was prepared by immunization of ducks with sheep red cell stroma sensitized with rabbit amboceptor. For identification of human globulin in case 3 an antiserum prepared in the horse against Colin globulin fraction II of human serum and the fluorescein-conjugated globulin fraction of this antiserum were used. For identification of human globulin in the three other cases an antiserum was prepared in rabbits against the globulin fraction of human serum precipitated by half-saturation with ammonium sulfate. Immunoelectrophoretic analysis of the latter immune serum showed precipitins against gamma, beta, and alpha globulins and albumin. The precipitins against human albumin and alpha 1 globulins were no longer demonstrable, however, when the immune serum was diluted 1:5 or after it was conjugated with fluorescein isothiocyanate. Use of the fluorescein-conjugated globulin fraction

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1. The rabbit anti-rat kidney serum was obtained from Dr. Beatrice Seegal, Columbia College of Physicians and Surgeons, New York.
2. The kidney biopsies were performed by Dr. E. L. Becker and Dr. E. Ginn of the Department of Medicine, The New York Hospital, New York.
3. Kindly supplied as the serum and as the globulin fraction conjugated with fluorescein isothiocyanate by Dr. Paul Klein, Dusseldorf, West Germany.
4. Purchased from Sylvana Co., Orange, New Jersey.
of the horse and of the rabbit antiserum against human globulin have given the same reaction
with human globulin in the tissues.

Antisera against fixed guinea pig complement were produced in rabbits by a series of
injections with sensitized sheep red cell stromata (SA) persensitized with guinea pig comple-
ment (SAC') as described elsewhere (13). These anti-SAC' sera were absorbed with sheep red
cell stromata and with sensitized stromata. The antisera against fixed guinea pig complement,
hereafter referred to as anti-gpC', were then tested for the specificity of immunologic reaction
with components of guinea pig complement fixed to sensitized erythrocytes (13, 14). The
anti-gpC' agglutinated sensitized sheep erythrocytes that had been exposed to complement
(EAC') and the fluorescein-conjugated globulin fraction of the anti-gpC' reacted with per-
sensitized sheep erythrocytes (EAC'). Sensitized cells (EA) or sensitized cells exposed to
heat-inactivated, chelated, or decomplemented guinea pig serum and sensitized cells exposed
to complement reagents lacking in C'1 or C'4 gave negative results in agglutination and
fluorescence tests with the anti-gpC' serum. In a previous report it was shown that this fluo-
rescein-conjugated anti-gpC' could be used to detect sites of uptake of guinea pig complement
fixed in vitro in sections of kidney taken from rats injected with rabbit anti-rat kidney serum
(15).

All of the globulin fractions of the immune sera that were conjugated with fluorescein-
isothiocyanate were absorbed at least once with beef liver powder before use.

Fresh guinea pig serum was obtained periodically by cardiac puncture of guinea pigs
lightly anesthetized with ether. The blood was clotted in vasceline-coated tubes. Aliquots of
the guinea pig serum were stored in sealed ampuls at \(-20^\circ\text{C}\).

Detection of Globulin in Sections of Kidney.—To detect sites of localized rabbit globulin in
sections of rat kidneys one drop of conjugated duck anti-rabbit amboceptor was applied to
washed sections. The sections of human kidneys were treated with a drop of fluorescein-
conjugated anti-human globulin to detect sites of localized human globulin. These sections
were incubated for 30 minutes in a moist chamber at room temperature, washed for 30 minutes
in buffered saline, and then mounted while still wet in 2 drops of buffered glycerine under
coverslips.

Control Procedures for the Detection of Globulin.—Several control procedures were carried
out to determine the specificity of the reactions observed in the above tests. Sections of the
human kidneys were incubated with unconjugated anti-human globulin for 1 hour prior to
incubation for 20 minutes with the conjugated anti-human globulin. Blocking of the uptake
of conjugated antibodies by such prior treatment with non-conjugated antibodies could be
demonstrated. Other sections of human kidneys were incubated with the fluorescein-con-
jugated anti-rabbit amboceptor to show lack of uptake of antibodies against heterologous
globulin. Finally, still other sections were incubated with conjugated anti-human globulin
that had previously been absorbed with human serum. By such absorption prevention of the
uptake of conjugated antibodies against human globulin could be demonstrated.

Specific fluorescence was then defined as that yellow-green fluorescence that was greatly
diminished by the blocking procedure or that was completely abolished by either the blocking
or absorption procedure.

Detection of Guinea Pig Complement Fixed in the Tissues.—Three drops of guinea pig serum
diluted 1:30 with saline buffer, containing calcium and magnesium ions (Mayer's buffer)
(16), were placed on sections of the kidneys and the slides incubated in a moist chamber in
a 37°C oven for 1 hour. The slides were then rinsed in two changes of buffered saline for 30
minutes. The excess saline was wiped from the slides up to and around the tissue sections
and then a drop of fluorescein-conjugated anti-gpC' applied to the wet section. Incubation
was repeated for 30 minutes in a moist chamber at room temperature. Finally, the slides
were rinsed and mounted as previously described.
Control Procedures for Complement Fixation in Tissues.—Control preparations were made to determine whether the conditions for fixation of material from guinea pig serum to "sensitized" tissues paralleled the conditions for fixation of guinea pig complement to sensitized red cells. Prior to treatment with fluorescein-conjugated anti-gpC', sections of kidney were, therefore, incubated respectively with guinea pig serum diluted 1:30 with Mayer's buffer and inactivated at 56°C for 30 minutes or with guinea pig serum diluted 1:30 with citrate-saline containing 0.01 M ethylenediaminetetraacetate. Other sections were treated with conjugated anti-gpC' alone to find out if non-specific fluorescence or cross-reactions between anti-gpC' and rat or human serum components would occur. In the case of kidney sections from rats other control experiments were performed in which kidney sections were incubated first with a complement reagent lacking in C1 and then in a second step with conjugated anti-gpC'.

### TABLE I

**Clinical and Pathologic Data at Time of Biopsy**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Duration of Illness</th>
<th>Edema</th>
<th>Blood pressure</th>
<th>Albuminuria</th>
<th>Hematuria</th>
<th>RBC/400x</th>
<th>BUN</th>
<th>Urea Clearance, per cent of normal</th>
<th>A/G</th>
<th>Excretion of protein/24 hrs</th>
<th>Serum cholesterol</th>
<th>L.E. Preparation</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>M</td>
<td>5</td>
<td>+130/80</td>
<td>4++</td>
<td>1-2</td>
<td>13-9</td>
<td>77</td>
<td>1.8/2.0</td>
<td>18.8</td>
<td>380</td>
<td>Neg.</td>
<td>2X</td>
<td>Membranous glomerulonephritis</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>M</td>
<td>5</td>
<td>+120/80</td>
<td>4++</td>
<td>5-20</td>
<td>15</td>
<td>79</td>
<td>2.1/2.3</td>
<td>8.5-13</td>
<td>500</td>
<td>Neg.</td>
<td>3X</td>
<td>Membranous glomerulonephritis</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>F</td>
<td>16</td>
<td>+120/70</td>
<td>4++</td>
<td>8-10</td>
<td>76-94</td>
<td>7.1</td>
<td>1.7/1.6</td>
<td>9.6-11</td>
<td>490</td>
<td>Neg.</td>
<td>5X</td>
<td>Chronic membranous glomerulonephritis</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>F</td>
<td>5</td>
<td>+15/60</td>
<td>3-</td>
<td>8-10</td>
<td>57</td>
<td>3/19</td>
<td>419</td>
<td></td>
<td>Neg.</td>
<td>2X</td>
<td>Probable membranous glomerulonephritis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In cutting frozen sections of the human tissues each sixth or tenth section was immediately fixed on a glass slide in Zenker-5 per cent acetic acid solution for staining with hematoxylin and eosin. Examination of these stained sections permitted selection of sections most suitable for the immunohistologic tests. The sections treated with conjugated sera and mounted in buffered glycérin were examined and photographed with Zeiss equipment for fluorescence microscopy. The source of illumination was an HBO 200W mercury vapor lamp. Barrier filters BG 23 and GG 4 or GG 4 alone were used in combination with exciter filter UG 2 for color photomicrography. Super Anscochrome tungsten-type film was used and exposures varied from 30 to 60 seconds. After the sections had been systematically examined with the fluorescence microscope they were then prepared for staining with hematoxylin and eosin or the periodic acid-Schiff reaction. Coverslips were floated off the slides and the glycérin mounting medium dissolved by placing the slides on edge in a jar of buffered saline. The slides were then rinsed for about 10 minutes in a solution of one-half anhydrous diethyl ether and one-half 70 per cent ethanol to dissolve the immersion oil. The slides were taken from this bath, drained of excess fluid, and then placed in Zenker-5 per cent acetic acid fixative for 15 to 20 minutes. After fixation the slides were washed in tap water and cleared of Zenker crystals in iodine solution and sodium thiosulfate solution for staining with hematoxylin and eosin or by the periodic acid-Schiff reaction. By this means the same structures examined for fluo-
rescence could very often be reexamined and photographed after staining with hematoxylin and eosin.

Brief summaries of the clinical and pathologic data pertaining to the patients from whom specimens of kidney were obtained by biopsy are given below and are listed in Table I.

Case 1.—(History No. 865633, The New York Hospital) A 33 year old man with history of hay fever and many episodes of pharyngitis gradually developed periorbital and ankle edema that began 5 months prior to renal biopsy and was associated with blood pressure of 160/90 mm of mercury and albuminuria. He was treated with diuretics, including mercuhydrin, and 1 month prior to biopsy with antibiotics because of pleuritis. Glomeruli in the biopsied specimen showed normal or occasionally slightly increased cellularity with widely patent capillary loops, diffuse slight thickening of the capillary basement membranes and focal intercapillary deposits of moderate size that showed a positive periodic acid–Schiff (PAS) reaction. There were occasional small foci of adherence of glomerular tufts to Bowman’s capsule. A diagnosis of membranous glomerulonephritis was made.

Case 2.—(History No. 845713, The New York Hospital) A 33 year old woman with history of “St. Vitus’ dance” at age 11 and uncomplicated pregnancy at age 23, developed periorbital and pedal edema 5 months prior to renal biopsy. At that time her blood pressure and BUN were normal but albuminuria was present (1 plus) and the serum cholesterol was elevated (469 mg per cent). The serum albumin–globulin ratio was 2.3/2.6 and the urea clearance was 79.2 per cent of normal. Glomeruli in the biopsied specimen showed slight increase in cellularity of somewhat lobulated tufts that filled Bowman’s space and were focally adherent to Bowman’s capsule. The glomerular capillary basement membranes were diffusely and moderately thickened with considerable narrowing of capillary loops by focal intercapillary deposits that showed a positive PAS reaction. Some tubular atrophy was noted. A diagnosis of membranous glomerulonephritis was made. After biopsy the patient was treated with metacorten and 6 months later 4 plus albuminuria and edema were still present. In this period her blood pressure varied from 120/90 to 150/80.

Case 3.—(History No. 834539, The New York Hospital) A 28 year old woman developed generalized edema 16 months prior to renal biopsy and was treated with steroids and diuretics. She developed grand mal seizures 10 months prior to biopsy and marked fatigue, myalgia, nausea, and an acute depressive reaction 3 months prior to biopsy. The biopsied specimen of kidney showed marked replacement of glomerular cells and narrowing or collapse of glomerular capillaries by intercapillary fibrillar and hyaline material that showed a positive PAS reaction. The resulting lobulated, sometimes shrunken glomeruli showed numerous adhesions to slightly or moderately thickened fibrous and occasionally fibrocellular glomerular capsules. A diagnosis of advanced or chronic membranous glomerulonephritis was made. Two months after the biopsy albumin (3 plus), 5 to 10 red cells and 15 to 20 white cells per high power field, and mixed casts were present in the urine. During the subsequent 6 months she developed anemia, the BUN rose to 116 mg. per cent and the blood pressure rose to 215/115. The patient was reported to have been admitted to another hospital where she died.

Case 4.—(History No. 863276, The New York Hospital) A 22 year old nurse with history of recurrent salpingitis for 3 years had sudden onset of ankle edema 5 months prior to renal biopsy. Albuminuria and urinary casts were noted. Her blood pressure and BUN were normal. She was treated with prednisone for 1 month. The biopsied specimen showed slight increase in cellularity of glomerular tufts due chiefly to proliferation of endothelial cells. There were occasional foci in which glomerular capillary loops were adherent to Bowman’s capsule. There was rather marked focal thickening of glomerular capillary walls that in some areas was associated with partial obliteration of the capillary lumina. A few contracted glomeruli consisted partially or wholly of hypocellular and fibrillar material that showed a positive PAS reaction. A diagnosis of probable membranous glomerulonephritis was made. During the 2
months following renal biopsy this patient's clinical status remained unchanged and she continued to have 4 plus albuminuria.

RESULTS

Results of this investigation are illustrated in Figs. 1 to 28. 3/4 hours after intravenous injection of rats with 0.2 cc of rabbit anti-rat kidney serum it was found that sections of the rat's kidney, exposed to fluorescein-conjugated anti-rabbit amboceptor, showed yellow-green fluorescence confined in a delicate pattern to the glomerular capillary walls (Fig. 1). No specific fluorescence was emitted from tubules, interstitial tissue, or arteries. The sections of rat kidney treated first with guinea pig serum and then with fluorescein-conjugated anti-gpC' showed yellow-green fluorescence in glomerular capillary walls in a delicate pattern very similar to that described above (Fig. 2). No specific fluorescence was found elsewhere in these sections. Sections of rat kidney exposed to conjugated anti-gpC' alone or to either heat-inactivated guinea pig serum, chelated guinea pig serum, R1, or decomplemented guinea pig serum and then in a second step to conjugated anti-gpC' showed no specific yellow-green fluorescence. Sections of kidney from normal rats or rats injected with normal rabbit serum showed no uptake of conjugated anti-rabbit globulin and no fixation of guinea pig complement. It was evident that the sites of localized rabbit globulin in the glomeruli of rats injected with nephrotoxic serum were the same sites in which guinea pig complement was fixed in vitro.

The diagnosis of nephrotic syndrome due to membranous glomerulonephritis was made in the first two human cases studied. After treatment with conjugated antihuman globulin, the sections of biopsied kidneys in these two cases showed bright specific fluorescence in an often sharply defined, occasionally interrupted or coarsely beaded pattern that followed the contour of the capillary walls throughout each glomerulus (Figs. 5 and 7). In addition a smudged or sometimes granular pattern of specific fluorescence was observed in swollen endothelial cells that nearly or completely plugged the capillary lumina (Figs. 3, 5, 7, and 9). No specific fluorescence was observed in the Bowman’s capsules, tubules, tubular casts, arteries, or the interstitium in these sections. Uptake of conjugated antihuman globulin was greatly reduced in sections of case 2 and completely blocked in sections of case 1 by previous exposure of the sections to non-conjugated antihuman globulin; and it was completely prevented in both cases by previous absorption of the conjugated anti-human globulin with human serum. Sections treated with fluorescein-conjugated hyperimmune globulin against rabbit antibody showed no specific fluorescence. Use of the immunohistologic complement fixation test resulted in both cases in bright yellow-green fluorescence in a sharply defined, frequently interrupted or beaded pattern that followed the contour of glomerular capillary walls (Figs. 6, 8, 11, and 13). Smudged and granular fluorescence observed in swollen endothelial cells was of somewhat less intensity and not as uniform in distribution as that observed in sections treated with con-
jugated anti-human globulin. Sections treated first with heat-inactivated (Fig. 16) or chelated guinea pig serum and then with conjugated anti-gpC' showed no fluorescence or only occasional mere traces of fluorescence in glomerular capillary walls. Sections treated with conjugated anti-gpC' alone showed no yellow-green fluorescence (Fig. 15). The latter control indicated absence of any cross-reaction of the anti-gpC' with constituents of human serum.

The patient in case 3 was considered to have had the nephrotic syndrome for about 16 months and a diagnosis of chronic membranous glomerulonephritis was made on microscopic examination of the renal biopsy. Sections of kidney treated with fluorescein-conjugated anti-human globulin showed bright specific fluorescence in a few sharply defined thin segments of glomerular capillary walls. The greatest amount of fluorescence, however, was noted in smudged, thickened segments of the glomerular capillary walls that partially or completely obliterated the lumina. The portions of glomeruli that appeared partially fibrosed emitted either no or only slight specific fluorescence. Solid and coarsely granular tubular casts showed very intense yellow-green fluorescence. Sections treated first with guinea pig serum and thereafter with conjugated anti-gpC' showed specific fluorescence in delicate and sometimes thickened or slightly smudged walls of peripheral glomerular capillary loops (Fig. 17). The more delicate of these areas showed fluorescence in a beaded pattern in capillary walls. The cytoplasm in a few large endothelial or epithelial cells showed a coarse granular pattern of yellow-green fluorescence. Some of the tubular casts showed yellow-green fluorescence of only slight intensity. As in the two previous cases sections treated by the control procedures were negative.

In the fourth case, diagnosed as probable membranous glomerulonephritis, the pattern of fluorescence observed in the treated kidney sections was quite different from the patterns described in the previous three cases. Sections exposed to conjugated anti-human globulin showed specific fluorescence in short thin segments of glomerular capillary walls (Fig. 19). This was noted to be confined almost entirely to peripheral portions of the glomeruli. Rarely a slightly swollen endothelial cell and associated segment of capillary wall showed some specific fluorescence. In general most of each glomerulus was free of the great amount of yellow-green fluorescence observed in the previous three cases. No specific fluorescence was observed in tubules, arteries, or the interstitium in these sections or in sections treated with the appropriate control tests described above. In this case the immunohistologic complement fixation test showed fewer areas of fluorescence than were observed in sections treated with conjugated anti-human globulin. In sections treated first with guinea pig serum and in a second step with fluorescein-conjugated anti-gpC' yellow-green fluorescence, sometimes in a finely beaded pattern, was emitted from both thin and thickened short segments of glomerular capillary walls. In addition in one glomerulus yellow-green fluorescence was emitted from a segment of a large thickened peripheral capillary loop (Fig. 21) resembling the glomerular lesion (Fig. 22) of
lupus erythematosus. The remaining portions of these glomeruli were dark and showed no uptake of the conjugated anti-gpC after treatment of the sections with guinea pig serum. The control tests as performed in the other cases were negative (Figs. 23 and 24).

For the purpose of additional control studies tests for localized globulin and for the fixation of guinea pig complement were carried out on sections of kidney from a number of other patients with a variety of diseases. (a) Sections, taken from the normal portion of a kidney surgically removed from a 10 month old boy with nephroblastoma (Wilms' tumor), were treated with fluorescein-conjugated anti-human globulin and showed yellow-green fluorescence limited to occasional tiny specks in glomeruli and occasional segments of peripheral glomerular capillaries. Barely discernible traces of fluorescence, interpreted as negative, were observed in tiny specks scattered in the periphery of some glomeruli of sections treated with guinea pig complement and thereafter with conjugated anti-gpC. (b) Sections of kidney, obtained at autopsy from a 5 month old boy with arrested hydrocephalus and history of recent septicemia, were treated with conjugated anti-human globulin and showed bright yellow-green fluorescence in occasional short thin segments of glomerular capillary loops and in occasional small foci in walls of arterioles. The immunohistologic complement fixation test was completely negative. (c) Sections of kidney were obtained at autopsy from a 79 year old woman with history of resected carcinoma of the breast and in whom carcinoma of the pancreas with metastases, rheumatic mitral stenosis, arteriosclerotic cardiovascular disease, and arterionephrosclerosis were found. The sections of kidney treated with conjugated anti-human globulin showed bright yellow-green fluorescence in a coarse granular pattern in the cytoplasm of occasional groups of proximal tubular epithelial cells, in globular tubular casts, and in amorphous deposits along the surface of arterial endothelium. Glomeruli were negative in this test. The complement fixation test was completely negative. (d) Sections of kidney were obtained at autopsy from a 53 year old man with rheumatic aortic stenosis, and (e) from a 73 year old woman with arteriosclerotic and hypertensive cardiovascular disease, healed myocardial infarct, and arterionephrosclerosis, and (f) from a normal portion of a kidney with renal carcinoma removed surgically from a 73 year old woman. In these last three cases sections of the kidneys treated with conjugated anti-human globulin showed traces to moderate amounts of yellow-green fluorescence in sites corresponding to eosinophilic hyaline areas in walls of small arterioles. In addition a slight degree of yellow-green fluorescence was observed in a diffuse and delicate pattern in glomerular capillary walls. In these three cases the complement fixation test was negative with respect to all structures in the sections of kidneys.

DISCUSSION

The uptake of fluorescein-conjugated antibodies against rabbit amboceptor in glomerular capillary walls in sections of kidney from rats injected with rabbit anti-rat kidney serum indicates that rabbit antibody had localized in vivo, presumably by aggregating with glomerular antigens. In sections of kidney from four patients with membranous glomerulonephritis fluorescein-conjugated anti-human globulin was taken up in glomerular capillary walls in a pattern
similar to that described by other investigators (9–11). This immunohistologic demonstration of human globulin in the diseased kidneys cannot in itself, however, be considered identification of localized human antibodies in the way that immunohistologic demonstration of rabbit globulin in sections of kidney from rats injected with rabbit anti-rat kidney serum can be considered identification of rabbit antibody localized at sites of rat kidney antigen.

The uptake of fluorescein-conjugated antibodies against guinea pig complement (anti-gpC') in glomerular capillary walls in sections of altered rat and human kidneys that were previously incubated with fresh guinea pig serum indicated that material in the guinea pig serum had fixed in the tissues during the incubation. Failure of uptake of conjugated anti-gpC' in sections of human or rat kidney that were previously incubated with heat-inactivated or chelated guinea pig serum indicated that the material fixable from fresh guinea pig serum failed to be fixed in the sections of kidney under conditions whereby guinea pig complement also fails to be fixed to sensitized red blood cells. This fixable material from guinea pig serum was, therefore, considered indistinguishable from complement. The pattern and distribution of the fixed guinea pig complement were essentially the same as the respective pattern and distribution of rabbit globulin in the kidneys of rats injected with rabbit anti-rat kidney serum and of human globulin in the diseased kidneys of the four patients with the nephrotic syndrome. This immunohistologic demonstration of fixation of guinea pig complement to sites of localized globulin can be considered identification of complexes that react in the tissues like antibodies in complement-fixing antigen-antibody complexes. The beaded pattern seen along the glomerular capillary walls in sections of kidney from the first three human cases may be significantly related to the nodular, dense thickenings and "moth-eaten" appearance of the glomerular capillary basement membrane observed in electron micrographs of sections of kidney from patients with various forms of "nephrosis" (17–19).

The failure of uptake of fluorescein-conjugated anti-gpC' in sections of kidney not previously incubated with guinea pig serum indicated that antibodies against guinea pig complement showed no cross-reactions with components of rat or human serum. Absence of complement fixation in areas of the sections that did not contain demonstrable deposits of globulin indicated that complement fixed only to sites of localized globulin. On the other hand, all deposits containing globulin in human kidneys do not behave like complement-fixing complexes as evidenced by failure of fixation of complement in tubular casts and in sections of kidney from five patients with a variety of other diseases. It must be considered that antibody globulin may occur in tissues in antigen-antibody complexes that are not avid for guinea pig complement. It must also be considered that deposits of globulin may form in tissues by metabolic or physiologic means other than antibody-antigen reaction and be able to fix guinea pig complement in the absence of demonstrable antigen.
Whether the complement-fixing complexes in the glomeruli of the patients studied in this investigation comprise aggregated globulin or autoantibodies combined with glomerular antigen or foreign antigen and specific antibody that combine either in the glomeruli or in the blood and then are deposited in the glomeruli has not been determined.

The fixation in vitro of guinea pig complement in glomeruli of the rat and human kidneys in the experiments reported in this investigation suggests that homologous complement might fix in vivo in the same sites. The aggregation in tissues of antibodies with antigens and an enzymatic or lytic action of complement on the “sensitized” tissue might lead to histologic and physiologic alterations observed in experimental animals and in man with diseases in which it is presumed that allergic reactions play an important role. At the present time it has not been possible by immunohistologic means to satisfactorily identify homologous complement fixed to antigen-antibody complexes. Determination of the avidity of localized globulin for homologous and heterologous complement is the object of further efforts to explain the pathogenesis of nephritis and other disease in which antigen-antibody reactions are likely to be of pathogenetic significance.

SUMMARY

An immunohistologic complement fixation test has been used in an effort to detect immune complexes in sections of kidney from rats injected with rabbit anti-rat kidney serum and in sections of biopsied kidneys from four humans with membranous glomerulonephritis. Sections of the rat and human kidneys were treated with fluorescein-conjugated anti-rabbit globulin or anti-human globulin respectively. Adjacent sections in each case were incubated first with fresh guinea pig serum and then in a second step were treated with fluorescein-conjugated antibodies against fixed guinea pig complement to detect sites of fixation of the complement. It was demonstrated that the sites of rabbit globulin in glomerular capillary walls of the rat kidneys and the sites of localized human globulin in thickened glomerular capillary walls and swollen glomerular endothelial cells of the human kidneys were the same sites in which guinea pig complement was fixed in vitro. It was concluded from these studies that rabbit nephrotoxic antibodies localize in rat glomeruli in complement-fixing antigen-antibody complexes. Furthermore, it was concluded that the deposits of human globulin in the glomeruli of the human kidneys behaved like antibody globulin in complement-fixing antigen-antibody complexes. The significance of demonstrating complement-fixing immune complexes in certain diseased tissues is discussed in regard to determination of the causative role of allergic reactions in disease.

BIBLIOGRAPHY

EXPLANATION OF PLATES

PLATE 57

FIG. 1. Frozen section of kidney from a rat sacrificed 3½ hours after intravenous injection with 0.2 cc of rabbit anti-rat kidney serum. Section exposed to fluorescein-conjugated antibodies against rabbit amboceptor. Yellow-green fluorescence, emitted by glomerular capillary walls, indicates sites of localized rabbit globulin. × 200.

FIG. 2. Frozen section from the same specimen of rat kidney referred to in Fig. 1, incubated first with guinea pig serum and then with fluorescein-conjugated antibodies against guinea pig complement. Yellow-green fluorescence indicates sites of fixation of guinea pig complement and subsequent uptake of conjugated antibodies against the complement. × 200.

FIG. 3. Frozen section of the kidney from the patient in case 1, referred to in Fig. 25, exposed to fluorescein-conjugated antibodies against human globulin. Yellow-green fluorescence emitted by glomerular capillary walls indicates sites of localized human globulin. × 32.

FIG. 4. Frozen section from the same specimen referred to in Fig. 3 (case 1), incubated with guinea pig serum and then with fluorescein-conjugated antibodies against guinea pig complement. Yellow-green fluorescence emitted by glomerular capillary walls indicates sites of fixation of guinea pig complement and subsequent uptake of conjugated antibodies against the complement. × 32.

FIG. 5. Frozen section from the same specimen referred to in Figs. 3 and 4 (case 1), exposed to fluorescein-conjugated antibodies against human globulin. Yellow-green fluorescence in glomerular capillary walls and swollen endothelial cells indicates sites of localized globulin. × 125.

FIG. 6. Frozen section taken adjacent to that referred to in Fig. 5 (case 1), showing the same glomerulus, incubated with guinea pig serum and then with fluorescein-conjugated antibodies against guinea pig complement. Yellow-green fluorescence indicates sites of fixation of guinea pig complement and subsequent uptake of conjugated antibodies against the complement sometimes in beaded pattern in capillary walls and in swollen endothelial cells. × 125.

FIG. 7. Frozen section of kidney from the patient in case 2, referred to in Fig. 26, exposed to fluorescein-conjugated antibodies against human globulin. Yellow-green fluorescence indicates sites of localized human globulin, sometimes in beaded pattern in glomerular capillary walls. × 200.

FIG. 8. Frozen section from the same specimen referred to in Fig. 7 (case 2), incubated with guinea pig serum and then with fluorescein-conjugated antibodies against guinea pig complement. Yellow-green fluorescence indicates sites of fixation of guinea pig complement and subsequent uptake of conjugated antibodies against the complement, sometimes in beaded pattern in glomerular capillary walls and in swollen endothelial cells. × 200.
(Burkholder: Complement fixation in diseased tissues. 1)
Fig. 9. Frozen section from the same specimen referred to in Figs. 3 to 6 (case 1), exposed to fluorescein-conjugated antibodies against human globulin. The white in the glomerulus indicates sites of human globulin localized in glomerular capillary walls and swollen endothelial cells. X 125.

Fig. 10. The identical section illustrated in Fig. 9 (case 1), restained with hematoxylin and eosin. Gray areas in thickened portions of glomerular capillary walls were eosinophilic and correspond to sites of globulin that are white in Fig. 9. X 125.

Fig. 11. Frozen section from the same specimen referred to in Figs. 3 to 6, 9, and 10 (case 1), incubated with guinea pig serum and then with fluorescein-conjugated antibodies against guinea pig complement. The white in the glomerulus indicates sites of fixation of guinea pig complement and subsequent uptake of conjugated antibodies against the complement in glomerular capillary walls and swollen endothelial cells. X 200.

Fig. 12. The identical section illustrated in Fig. 11 (case 1), restained with hematoxylin and eosin. Gray areas in thickened glomerular capillary walls and swollen endothelial cells were eosinophilic and correspond to sites that were avid for complement and are white in Fig. 11. X 200.

Fig. 13. Frozen section of the same specimen referred to in Figs. 3 to 6 and 9 to 12 (case 1), incubated with guinea pig serum and then with fluorescein-conjugated antibodies against guinea pig complement. The white in sometimes beaded pattern in glomerular capillary walls and granular pattern in swollen endothelial cells indicates sites of fixation of guinea pig complement and subsequent uptake of conjugated antibodies against the complement. X 312.

Fig. 14. The identical section illustrated in Fig. 13 (case 1), restained with hematoxylin and eosin. The sometimes beaded, dark gray basement membranes and lighter gray, swollen endothelial cells correspond to sites that are avid for complement and are white in Fig. 13. X 312.

Fig. 15. Frozen section of the specimen of kidney from case 1 exposed only to fluorescein-conjugated antibodies against guinea pig complement. Absence of significant intensity of white in two glomeruli indicates failure of uptake of the conjugated antibodies against guinea pig complement, and therefore, absence of cross-reaction of anti—guinea pig complement with components of human serum. X 48.

Fig. 16. Frozen section of the specimen of kidney from case 1, incubated with heat-inactivated guinea pig serum and then with fluorescein-conjugated antibodies against guinea pig complement. Absence of significant intensity of white in the glomerulus indicates failure of fixation of complement from heat-inactivated guinea pig serum. X 125.
(Burkholder: Complement fixation in diseased tissues. I)
FIG. 17. Frozen section of kidney from the patient in case 3 referred to in Fig. 27, incubated with guinea pig serum and then with fluorescein-conjugated antibodies against guinea pig complement. The white in glomerular capillary loops indicates sites of fixation of guinea pig complement and subsequent uptake of conjugated antibodies against the complement. × 200.

FIG. 18. The identical section illustrated in Fig. 17 (case 3), restained with hematoxylin and eosin. Dark gray areas in obliterated peripheral glomerular capillary loops were eosinophilic and correspond to sites that were avid for complement and are white in Fig. 17. × 200.

FIG. 19. Frozen section of kidney from the patient in case 4, referred to in Fig. 28, exposed to fluorescein-conjugated antibodies against human globulin. The white in short thickened segments of glomerular capillary walls indicates sites of localized human globulin. × 125.

FIG. 20. The identical section illustrated in Fig. 19, restained with hematoxylin and eosin. × 125.

FIG. 21. Frozen section from the same specimen referred to in Figs. 19 and 20 (case 4), incubated with guinea pig serum and then with fluorescein-conjugated antibodies against guinea pig complement. The white in the glomerulus indicates sites of fixation of guinea pig complement and subsequent uptake of conjugated antibodies against complement. × 200.

FIG. 22. The identical section illustrated in Fig. 21, restained with hematoxylin and eosin. Note that only portions of the dark gray (eosinophilic), markedly thickened capillary loops here illustrated show fixation of complement in areas that are white in Fig. 21. × 200.

FIG. 23. Frozen section from the same specimen referred to in Figs. 19 to 22 (case 4), incubated with heat-inactivated guinea pig serum and then with fluorescein-conjugated antibodies against guinea pig complement. Absence of significant intensity of white in the glomerulus indicates failure of fixation of complement from heat-inactivated guinea pig serum. The white in the wall of an artery was the result of blue autofluorescence of arterial elastica. × 125.

FIG. 24. Frozen section from the same specimen referred to in Figs. 19 to 23 (case 4) incubated with guinea pig serum containing 0.01 M EDTA (chelating agent). Absence of significant intensity of white in the glomerulus indicates failure of fixation of complement from guinea pig serum deprived of divalent cations. × 125.
(Burkholder: Complement fixation in diseased tissues. I)
Fig. 25. Formalin-fixed paraffin section of biopsied kidney from a patient with the nephrotic syndrome and membranous glomerulonephritis (case 1), stained by periodic acid–Schiff reaction. Note diffuse slight thickening of capillary basement membranes and a few intercapillary foci of clustered cells associated with PA-positive material. × 258.

Fig. 26. Formalin-fixed paraffin section of biopsied kidney from a patient with the nephrotic syndrome and membranous glomerulonephritis (case 2), stained by periodic acid–Schiff reaction. There is diffuse moderate thickening of capillary basement membranes, and focally accentuated intercapillary PA-positive material that is in some areas associated with clusters of cells. × 266.

Fig. 27. Formalin-fixed paraffin section of biopsied kidney from a patient with the nephrotic syndrome and chronic membranous glomerulonephritis (case 3), stained by periodic acid–Schiff reaction. Large amounts of PA-positive material are present in intercapillary areas, replacing capillary walls. The walls of remaining capillary loops are greatly thickened by this material. There are focal adhesions between the glomerular tuft and thickened Bowman’s capsule. × 266.

Fig. 28. Formalin-fixed paraffin section of biopsied kidney from a patient with the nephrotic syndrome and subacute glomerulonephritis (case 4), stained by periodic acid–Schiff reaction. Focal thickening of glomerular capillary walls is associated in some areas with marked narrowing of the capillary lumen. × 266.
(Burkholder: Complement fixation in diseased tissues. I)