It has been recently established that the diminution or extinction of the serum complement titer which sometimes occurs in the course of infectious disease is coincident with a decrease or disappearance of primarily C'4 and secondarily C'2 and C'1 (1). It was also found that prognosis was grave for those patients whose complement titers fell to zero or near-zero. The mechanism of masking, inactivation, or interference with the production of these components, whatever the case may be, has not as yet been fully investigated, nor has the manner in which the diminution of titer may contribute to greater susceptibility to infection.

In cases of kidney disease, in which sometimes a lowering of serum complement has also been observed, the excretion of protein in the urine presents a situation distinct from that generally found in cases of uncomplicated infectious disease. While not always reflecting itself in a decrease of serum complement titer, for reasons discussed below, a loss of complement by means of urinary excretion nevertheless could take place in kidney disease, and could be a contributory factor to decreased resistance to superimposed infection.

The present study is therefore concerned with the analysis, chiefly with respect to the complement components, of a series of urine specimens obtained from normal individuals, from cases of infectious disease, and from patients with kidney disease.

**METHODS**

1. **Preparation of urine specimens.** Urine samples, consisting of single voidings, were usually processed within a few hours after collection. Specimens were measured for volume, adjusted to pH of about 6.6 with either 0.1 N NaOH or 0.1 N HCl, filtered or centrifuged to remove insoluble material, and chilled to 1°C. One of 2 methods, both of which were designed to preserve complement activity and both of which gave comparable results, was then used to prepare urine or urinary protein solutions for testing. These methods are as follows.

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1 Aided by a grant from the Commonwealth Fund.
dialyzed urine or the urinary protein solution was tested by both slide and test tube methods for isohemagglutinin type against A, B, and O cell suspensions.

4. Electrophoresis. Several samples of treated urines were subjected to electrophoresis using a veronal buffer of pH 7.8 and ionic strength of 0.1.

RESULTS

1. Complementary activity

Table I summarizes the significant data obtained with respect to complement activity of the urinary proteins. In the table a given complement component activity is expressed in the number of units per gram of protein found in, or isolated from, the urine, which caused complete hemolysis of the standard dose of sensitized sheep red cells in the presence of an added excess of the other 3 components. As a basis for comparison, the table includes the number of such units of each of the components which would be found in a normal serum of average complement titer (33.3 units per ml. of serum) and protein content (7.0 per cent). Since the complement components are associated with the globulins, their units could more properly be expressed per gram of globulin, but even such designation would be arbitrary, and accordingly is made only in 2 instances.

a. Normal urines. Urine specimens from 3 normal individuals were treated according to Method (b). In each case a very minute amount of protein was obtained which showed no complement component activities.

b. Infectious diseases. The urines of 2 patients with meningococcal meningitis, one with scarlet fever, and one with both rheumatic fever and Type XII pneumococcal meningitis, were studied. Although all of the urines contained some protein, no complement component activities were detected in any of them. The data obtained with respect to the rheumatic fever-meningitis patient, because of the relatively high urinary excretion of protein, are included in Table I.

c. Nephrotic syndrome; lipoid nephrosis. Urine specimens from 3 children were treated and tested; complement components were found in the urinary proteins of each case at one time or another.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Date of urine sample</th>
<th>Total protein in urine</th>
<th>Approximate number of units of C' component isolated from the urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>grams per cent</td>
<td>C'1</td>
</tr>
<tr>
<td>D. C.</td>
<td>Rheumatic fever; Type XII pneumococcal meningitis</td>
<td>3-3-45</td>
<td>0.44</td>
<td>0</td>
</tr>
<tr>
<td>D. M.</td>
<td>Lipoid nephrosis</td>
<td>2-5-45</td>
<td>0.58</td>
<td>100</td>
</tr>
<tr>
<td>D. M.</td>
<td>Lipoid nephrosis</td>
<td>2-13-45</td>
<td>0.32</td>
<td>0</td>
</tr>
<tr>
<td>A. S.</td>
<td>Lipoid nephrosis; upper respiratory infection</td>
<td>2-5-45</td>
<td>0.33</td>
<td>0</td>
</tr>
<tr>
<td>A. S.</td>
<td>Lipoid nephrosis; upper respiratory infection</td>
<td>2-13-45</td>
<td>0.56</td>
<td>0</td>
</tr>
<tr>
<td>J. G.</td>
<td>Lipoid nephrosis</td>
<td>2-5-45</td>
<td>0.42</td>
<td>40</td>
</tr>
<tr>
<td>J. Q.</td>
<td>Chronic glomerulonephritis; nephrotic syndrome; upper resp. infect.</td>
<td>4-21-44</td>
<td>0.54</td>
<td>0</td>
</tr>
<tr>
<td>L. B.</td>
<td>Acute glomerulonephritis</td>
<td>5-2-44</td>
<td>0.61</td>
<td>0</td>
</tr>
<tr>
<td>E. M.</td>
<td>Acute glomerulonephritis; nephrotic syndrome</td>
<td>2-27-45</td>
<td>1.73</td>
<td>0</td>
</tr>
<tr>
<td>M. P.</td>
<td>Arteriosclerotic heart disease; diabetes mellitus</td>
<td>2-22-45</td>
<td>0.22</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Date of urine sample</th>
<th>Total globulin in urine</th>
<th>Units C' component isolated from urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>grams per cent</td>
<td>C'1</td>
</tr>
<tr>
<td>D. M.</td>
<td>Lipoid nephrosis</td>
<td>2-13-45</td>
<td>0.08</td>
<td>0</td>
</tr>
<tr>
<td>E. M.</td>
<td>Acute glomerulonephritis; nephrotic syndrome</td>
<td>2-27-45</td>
<td>0.79</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Units C' component per gram of protein in an average normal serum (approximate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,275</td>
</tr>
<tr>
<td>10,300</td>
</tr>
</tbody>
</table>

in the course of the disease, but 2 of the total of 7 urine samples failed to show any component activity whatsoever. As shown in Table I, C2 and C3 chiefly were excreted, followed by C4, and in 2 of the 5 urine samples, by C1.

All 3 of these patients were edematous.

d. Glomerulonephritis. One urine sample from each of 2 patients, and 2 samples from a third patient, were studied. C2 and C4 were present in all of the urines, C3 was present in 3 of the samples, and C1 was present in none.

These patients were also edematous.

e. Arteriosclerotic heart disease and diabetes mellitus. Two urine samples obtained from one patient were studied. One of these contained about 1 per cent protein, and showed considerable C2 and C3 activities and some C4. The second sample contained relatively slight traces of protein, and was found to have no complement component.

This patient also was edematous.

f. Arteriolar nephrosclerosis. One urine obtained from a patient was examined and found to contain a very minute amount of protein with questionable traces of C1, C3, and C4 activities.

This patient did not have edema.

2. Isohemagglutinin activity

As a beginning in the study of the excretion of antibodies, the isohemagglutinin activities of a number of dialyzed urines and the urinary protein solutions were determined qualitatively. After these tests were completed, the clinical records of the patients were checked for blood types, and in every case in which such a record was available it served to confirm the urinary tests if these were at all positive. In several cases, particularly in which the content of urinary protein was low, no tests were obtained at all. Table II gives a summary of the positive results obtained by test tube titration.

3. Electrophoresis

The urinary proteins obtained in one case with nephrotic syndrome and those from one case of acute glomerulonephritis were examined in the Tiselius apparatus. In confirmation of the observations of Luetscher (3) and Blackman and Davis (4), Table III shows that the urine of this patient contained considerably more albumin than did that of the nephritic patient, and that the latter urine contained a large amount of gamma globulin. What is of particular interest, in relation to the present study, is that both of these urinary protein solutions contained complement activity and isohemagglutinins, and that the globulin fractions generally associated with these activities were shown to be present in the electrophoretic diagrams.

### Table II

<table>
<thead>
<tr>
<th>Patient</th>
<th>Date</th>
<th>Clumping by urinary proteins of cells of type</th>
<th>Blood type from urine</th>
<th>Blood type from hospital records</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. M.</td>
<td>2-13-45</td>
<td>-+++++</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>D. M.</td>
<td>2-19-45</td>
<td>-+++</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>A. S.</td>
<td>2-13-45</td>
<td>±</td>
<td>A?</td>
<td>A</td>
</tr>
<tr>
<td>A. S.</td>
<td>2-22-45</td>
<td>-</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>M. P.</td>
<td>2-20-45</td>
<td>+++++</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>E. M.</td>
<td>2-27-45</td>
<td>-++++</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>

### Table III

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Date</th>
<th>Concentrations (per cent of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Albumin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alpha</td>
</tr>
<tr>
<td>D. M.</td>
<td>Nephrotic syndrome</td>
<td>2-13-45</td>
<td>74.5</td>
</tr>
<tr>
<td>E. M.</td>
<td>Acute glomerulonephritis; nephrotic syndrome</td>
<td>2-27-45</td>
<td>53.1</td>
</tr>
<tr>
<td></td>
<td>Acute glomerulonephritis</td>
<td>2-27-45</td>
<td>74.5</td>
</tr>
</tbody>
</table>

### Discussion

In the several samples of urine obtained from normal individuals and from people suffering with uncomplicated infectious disease, no complement component activities were detected. On the other hand, the data in Table I show that in one case or another each of the 4 complement components was identified in the urinary proteins excreted in kidney disease, particularly in those cases in which there was an associated edema. In this respect, the edema probably has no significance other than as an indication of the profound reduction of the serum proteins as evidenced by the following: DM had a serum protein content of 3.3; AS, 4.3; EM, 3.62; JG, 5.6; and JQ, 4.81 per cent.

As seen in Table I, the consistent and high excretion of C2 is outstanding, the activity figures
often approaching that for the normal serum. In
sharp contrast are the infrequency of $C^1$ excretion,
and the relatively low value obtained for this com-
ponent even when it is present. Since the excre-
tion of a protein through the damaged kidney
may be related to its solubility, it must be pointed
out that $C^2$ is extremely soluble even in distilled
water, whereas $C^1$ behaves as a euglobulin, and
is easily precipitated. Furthermore, $C^1$ has an ap-
parent isoelectric point at about pH 6 (5), and
may be one of those more insoluble globulins, dis-
cussed by Luetscher (3), which are precipitated
from dilute solutions of pH 5.5 to 6.5, and aid in
the formation of the urinary "casts." Blackman
and Davis (4) believe that the hyaline materials
which collect in the glomeruli and tubules in pa-
tients with "progressing nephrotic nephritis," are
probably derived from globulins other than fibrin-
ogen. It may be, then, that $C^1$ is one of these
globulins, and is retained in the process of forma-
tion of the hyaline materials.

The excretion of $C^3$ and $C^4$, both of which are
in themselves relatively soluble, or at least may be
attached to soluble proteins, is fairly consistent,
though not as striking in amount as is the excre-
tion of $C^2$.

Though red blood cells were found in some of
the urine specimens tested, particularly in 2 of the
cases of glomerulonephritis, the occurrence of comple-
ment components cannot be attributed in any
great measure to the presence of whole blood,
for the following reasons: (a) In a number of
urine specimens in which complement compo-
nents were present, no red blood cells were seen
nor were positive benzidine tests obtained. (b)
Contrary to what would be expected if whole blood
were present, the entire complement complex was
not found, but only certain components. (c) The
amounts of complement components, particularly
of $C^2$ and $C^3$, found in those urines showing some
red cells, were relatively so great, that the urines
would have had to consist in large measure of
whole blood, a circumstance ruled out by the
relatively low red cell and protein contents of the
urines. From a physiologic standpoint, the dis-
covery of complement components in the urine is
extremely interesting, inasmuch as it demonstrates
that normal plasma proteins, and particularly
those with specific functions, are excreted in kidney
disease. In addition to the complement com-
ponents, the isoheamtoglobinins have been demon-
strated in the urinary protein excreted in kidney
disease. While the authors have not as yet had
the opportunity to study the excretion of anti-
bodies to infectious agents, there is no reason to
believe that these are not excreted.

The possible immunological significance of this
work now becomes apparent. It is well known
that a patient with kidney disease is particularly
subject to infection, frequently pneumococcal in
origin, and expressing itself as a septicemia, peri-
tonitis, or an upper respiratory disease. This
general lack of resistance to infection exhibited by in-
dividuals with kidney disease may in part be re-
lated to excretion of protein, as follows: (a) in
the sense of Cannon (6), by the depletion of pro-
tein reserves, with consequent reduction of ma-
trix necessary for the production of antibodies and
complement; (b) by the loss to the urine of pre-
formed antibodies, as suggested by Bell (7); (c)
by the loss over a period of time of complement
components, particularly $C^2$ and $C^4$, which to-
gether with $C^1$ are necessary for the acceleration
of opsonification of invading organisms (8);
and (d) by the diminution in the serum of any one
or more of the 4 components of complement, all
of which are necessary for bactericidal action (9).

If sufficient complement is excreted in the urine
to predispose the patient, in some degree, to in-
fecion, a concomitant reduction of the serum com-
plement should be noted. In some cases of kidney
disease a simultaneous lowering of the serum
complement titer and serum protein content has
been observed; however, this has not been the
general rule. The usual test for serum comple-
ment titer, being a test for a dissolved protein com-
plex and therefore dependent upon plasma volume,
necessarily fails to reveal the full character of
changes occurring in the plasma. Plasma volume
variations taking place with edema might tend to
offset and mask the decrease of serum protein.
Furthermore, the patients studied here have been
undergoing treatment, inclusive of transfusions of
whole blood and plasma, thus complicating even
more the expected relationship between loss of
serum protein to the urine and decrease of serum
complement titer. These considerations are the
basis for the statement made at the beginning of
this paper that loss of serum protein may not al-
ways be reflected in diminished complement titer.
SUMMARY

1. Complement component activities have not been detected in, or isolated from, the urines of 3 normal individuals, nor in those of 4 people suffering with infectious disease.

2. Complement component activities have been identified in the precipitated urinary proteins of patients showing nephrotic syndrome, and in cases of acute and chronic glomerulonephritis, as well as arteriosclerotic heart disease with a nephrotic component.

3. The excretion of C'2 was most consistent and in largest relative quantity. C'3 and C'4 were excreted in the urines of these cases with good consistency, but C'1 was seldom excreted, and then only in relatively small amount.

4. It is pointed out that C'2 is a very soluble protein, whereas C'1 is a euglobulin; and that the high excretion of the one and the retention of the other is probably related to their solubilities.

5. Isohemagglutinins have been isolated with the urinary proteins in 5 instances. Identification of these was in agreement with the blood types of the patients.

6. The physiological significance of these findings lies in the demonstration that normal plasma proteins, and particularly those with specific functions, may be excreted in the urine by patients with kidney disease.

7. The immunological significance of this study is that it may explain in part the predisposition of patients with kidney disease to infection. The loss of complement substances and antibodies, both necessary for the opsonification and killing of invading organisms, may be contributing factors to the diminished resistance of these cases.

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BIBLIOGRAPHY


