# PHYSICOCHEMICAL AND IMMUNOLOGIC STUDIES OF GAMMA GLOBULINS OF NORMAL HUMAN URINE *

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Normal human urine has been shown to contain up to 150 mg. of protein in a 24 hour aliquot. Electrophoretic analyses of these proteins by a number of observers (1–3) have demonstrated the presence of components corresponding to each of the five major fractions present in serum. In general, however, the resolution of these peaks has been poor, and significant amounts of protein migrating more rapidly than albumin have been demonstrated. Immunoelectrophoretic studies of normal human urine by Grant (4) and Patte, Baldassaire and Loret (5) have revealed the presence of at least eight components which reacted with an antiserum to normal human serum and frequently gave reactions of identity with the corresponding proteins present in serum. Among the proteins identified by these observers were albumin, four α-globulins, two β-globulins and γ-globulin (4).

In spite of the qualitative similarities of the serum and urine proteins, quantitative differences in the relative amounts of the major fractions have been demonstrated. Thus, whereas albumin is the major protein component of serum, the globulin fractions predominate in urine (1, 3). The presence in urine of γ-globulin at a relative concentration equal to and frequently greater than that in serum cannot be explained by current concepts concerning the origin of urinary proteins by a process of glomerular filtration of serum proteins (6, 7) followed by a nonselective tubular reabsorption (8). Such a mechanism would lead to a preferential excretion in the urine of proteins of low molecular weight and would result in an increase in their concentration relative to that of the larger proteins. Since the molecular weight of the major fraction of γ-globulin in serum is approximately two and one-half times that of albumin, the presence in urine of relatively greater amounts of protein with the mobility of γ-globulin suggested the existence of additional protein species which have not been detected in serum. The present study presents evidence for the existence in normal urine of proteins with a mobility similar to γ-globulin and an approximate molecular weight of 35,000, and which are antigenically closely related to the γ-globulin fraction of serum. While this work was in progress, low molecular weight γ-globulin fractions have also been demonstrated in normal human urine by Webb, Rose and Sehon (9).

## MATERIAL AND METHODS

Twenty-four hour urines were collected from 12 normal males and stored in the cold at 4° C. Following filtration to remove formed elements and insoluble precipitates, the urines were dialyzed against running tap water for 24 hours in the cold and concentrated to a volume of 10 to 15 ml. by pervaporation at 0 to 4° C. In a number of instances protein was precipitated by the addition of 650 Gm. ammonium sulfate to each liter of urine. Following centrifugation at 3,000 rpm for one hour, the precipitate was dialyzed against running tap water to remove the salt, and the protein solution was concentrated as above. Both methods yielded similar results. For large scale physicochemical studies, 5 to 10 L. urine from a single subject were processed.

*Electrophoresis.* This was performed using starch or polyvinyl chloride resin 427 (Goodrich Chemical Company) as the supporting medium and barbital buffer, pH 8.6, $\mu = 0.05$ or 0.10 as described by Kunkel (10). Protein was eluted from one-half inch segments by displacement filtration and its concentration estimated by the modified Folin tyrosine method (11). Fractions corresponding in mobility to the γ-globulin peak of a control serum separated on the same block were pooled and concentrated by ultrafiltration through collodion membranes [1] with a vacuum of approximately 260 mm. mercury.

*Ultracentrifugation.* This was done in a Spinco Model E ultracentrifuge at a temperature of 20° C. us-

[1] Schleicher and Schuell Company, Keene, N. H.

ing cells with double sector centerpieces (12). Quantitative measurements were carried out with a comparator as described by Trautman (12). Molecular weights were determined by the technique of Archibald (13) at speeds of 20,410 to 24,630 rpm. The partial specific volume ($\overline{V}$) for the urinary $\gamma$-globulin was assumed to be similar to that of serum $\gamma$-globulin and equal to 0.74.

*Immunochemical studies.* Antisera to Fraction II $\gamma$-globulin (Lederle C-380) and to the urinary $\gamma$-globulin fraction from two normal subjects were prepared in rabbits by the subcutaneous route with Freund adjuvant. The antiserum against Fraction II had previously been shown to be specific for the fraction of $\gamma$-globulin with a sedimentation coefficient of 7S (7S $\gamma$-globulin) (14). Antigens were examined by double diffusion in 0.5 to 1.0 per cent agar according to the method of Ouchterlony (15), by immunoelectrophoresis by the method of Williams and Grabar (16) and by quantitative precipitin analysis as previously described (14). The amount of protein precipitated was determined by the modified Folin technique (11) and converted to milligrams of nitrogen according to a standard curve prepared with human $\gamma$-globulin. Supernate analyses with 7S $\gamma$-globulin were carried out in capillary tubes and by double diffusion in agar.

*Radioactive studies.* In order to determine the source of the $\gamma$-globulin present in urine, studies were performed with $I^{131}$-labeled human $\gamma$-globulin. Fraction II $\gamma$-globulin (Lederle C-543) was iodinated by standard methods (17).[2] The iodinated material was passed through a

[2] We are indebted to Drs. M. Rothchild and M. Oratz of the Veterans Administration Hospital, New York, N. Y., for their assistance in carrying out this phase of the study.

Dowex-1 ion-exchange column to remove free iodide and through a Seitz filter and cultured for sterility before use. The resultant material contained less than 2 per cent nontrichloracetic acid (TCA)-precipitable radioactivity; 50 to 100 $\mu$c. of this $I^{131}$-labeled $\gamma$-globulin with a specific activity (SA) of 10 $\mu$c. per mg. of protein was given by intravenous injection to four normal male subjects. Urines were collected for the first eight and following 16 hours, and serum was drawn eight and 24 hours following injection. The urines were dialyzed for 48 hours against running tap water to remove free iodide, concentrated by pervaporation and separated with the corresponding sera by starch zone electrophoresis. Radioactivity on the recovered fractions was assayed in a well-type scintillation counter with a sensitivity of 0.9 $\times$ $10^6$ counts per minute (cpm) per microcurie of $I^{131}$ above a background of 150 cpm.

## RESULTS

Representative electrophoretic patterns obtained from one normal urine and the corresponding serum are shown in Figure 1. Compared with the distinct resolution of the major protein components obtained in the serum pattern, the peaks in the urine appeared to be broader and more nonhomogeneous, thus making it difficult to define individual components. In the majority of urines examined, the concentration, relative to the other components, of the protein fraction with the mobility of $\gamma$-globulin was greater than in the corresponding serum. In general the urines contained significant amounts of protein with a
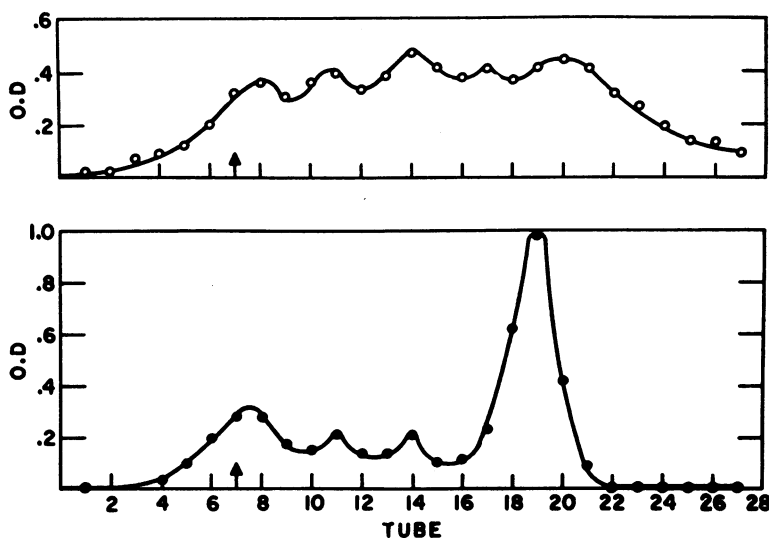


FIG. 1. ELECTROPHORETIC PATTERNS OF ONE NORMAL 24 HOUR URINE SPECIMEN (ABOVE) AND SIMULTANEOUSLY RUN SERUM (BELOW)

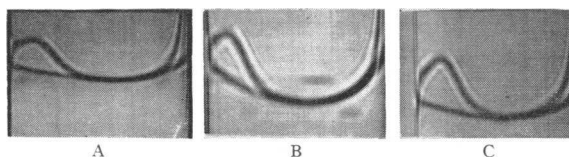Separation carried out in starch, barbital buffer, pH 8.6, $T/2 = 0.05$.

FIG. 2. ULTRACENTRIFUGAL PATTERNS OF γ-GLOBU-LINS OBTAINED FROM URINES OF THREE DIFFERENT SUBJECTS

Pictures were taken after 80 minutes at a speed of 52,640 rpm. Sedimentation proceeds from left to right.

mobility greater than albumin. This fraction could not be detected in the sera under the conditions of this study.

Figure 2 shows the ultracentrifugal patterns of the proteins migrating with the mobility of γ-globulin obtained from the urines of three different subjects. Two of these (A and C) were prepared by pervaporation, while the middle one (B) was obtained by ammonium sulfate precipitation. In each instance there was a major broad nonhomogeneous peak with an approximate $s_{20,w}$ of 1.5–1.6S.[3] In a number of instances there were small amounts of a second more rapidly sedimenting peak with an approximate $s_{20,w}$ of 7S. In the γ-globulin fractions prepared from normal urines this additional component, which appears to correspond to the 7S γ-globulin of serum, never exceeded 10 per cent of the total protein present. In the urines of five patients with glomerulonephritis, pyelonephritis and nephrosclerosis whose urinary protein excretion amounted to 5 to 10 Gm. per day, this more rapidly sedimenting component increased in absolute and relative concentration and constituted more than 75 per cent of the total γ-globulin fraction.

Table I lists the $s_{20,w}$ at a protein concentration of 0.1 to 0.3 per cent of the urine γ-globulins of eight subjects. Observed sedimentation coefficients were corrected to water as a solvent and expressed in Svedberg units. Because no appreciable effect of concentration on the $s_{20,w}$ was found, the values given are uncorrected for protein concentration. The observed $s_{20,w}$ in seven of the subjects ranged from 1.1 to 1.9S, with a mean of 1.6S. One subject (A.L.) had an observed $s_{20,w}$ of 2.5 and 2.6 on two occasions. Nine different samples were examined from one subject (E.F.) and the

[3] Sedimentation coefficient in water at 20° C., expressed in Svedberg units (S).

sedimentation coefficient varied from 1.2 to 1.9S, with a mean of 1.6S.

In order to rule out the possibility that this slowly sedimenting peak was a result of protein breakdown during the prolonged preparative procedures, control experiments were performed: Cohn Fraction II γ-globulin which did not contain detectable amounts of protein sedimenting more slowly than the 7S peak, or 1 to 2 ml. normal serum, was diluted in 2,000 ml. normal saline or normal urine and handled in a manner identical to a simultaneously prepared urine. There was no evidence of selective loss of the 7S peak or breakdown of this peak to the low molecular weight component during handling. Similarly, addition of pepsin, plasmin and streptokinase to sera or γ-globulins under these conditions did not result in breakdown of 7S γ-globulin to smaller fractions.

Molecular weights were calculated during the "approach to sedimentation equilibrium" by the method of Archibald (13). The values of four different samples from one subject ranged from 23,800 to 38,000 and are listed in Table I. Because most of the preparations examined were not completely homogeneous, the significance of the one low value cannot be fully evaluated. Since this technique initially emphasizes the heavy components of a polydisperse solution, it appears possible that the lower value was due to the greater purity of this particular preparation. However, the possibility also exists that this may represent an even smaller fraction of γ-globulin than the

TABLE I

Sedimentation coefficients and molecular weight determinations of urine γ-globulins

| Subject | No. of examinations | $s_{20,w}$* | Molecular weight |
|---|---|---|---|
| E. F. | 9 | 1.6 (1.2–1.9)† | |
| O. S. | 1 | 1.1 | |
| M. F. | 1 | 1.1 | |
| S. I. | 1 | 1.7 | |
| B. A. | 1 | 1.7 | |
| A. L. | 2 | 2.6 (2.5–2.7)† | |
| S. N. | 1 | 1.8 | |
| A. N. | 1 | 1.8 | |
| E. F. | 1 | 1.6 | 30,000 |
| E. F. | 1 | 1.7 | 38,000 |
| E. F. | 1 | | 35,600 |
| E. F. | 1 | 1.6 | 23,800 |

* Sedimentation coefficient in water at 20°C. expressed in Svedberg units (S).
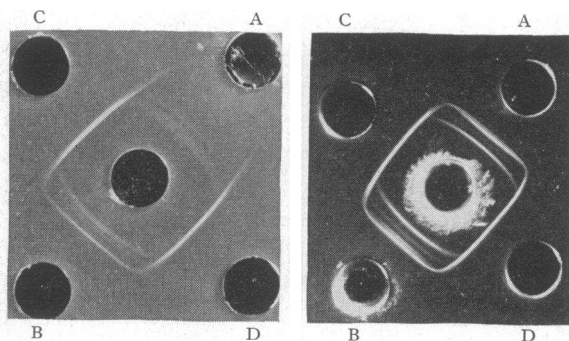† Mean and (range).

FIG. 3. DOUBLE DIFFUSION IN AGAR OF URINE γ-GLOB-
ULINS (A AND B) AND SERUM 7S γ-GLOBULIN (C AND
D) AGAINST AN ANTISERUM TO SERUM 7S γ-GLOBULINS

three samples whose molecular weights are dis-
tributed around a mean of approximately 35,000.

*Immunological studies*

When tested with an antiserum to normal hu-
man γ-globulin by the capillary precipitin tech-
nique, the urine component with the mobility of
γ-globulin invariably gave a precipitate at the in-
terphase. However, in general, the precipitate was
slower in appearance and finer in consistency than
that obtained with the homologous γ-globulin
from serum.

Figure 3 illustrates the reactions obtained when
urine γ-globulins and serum 7S γ-globulins react

in agar with an antiserum prepared against Cohn
Fraction II γ-globulin. In all instances only a
single sharp line appeared between the antiserum
and the serum γ-globulin component. In the case
of the γ-globulins prepared from urine, two or
three lines were usually present. The line closest
to the antigen well showed a reaction of complete
identity with a line formed by the 7S γ-globulin
and probably represented small amounts of this
protein frequently detected also by ultracentrifu-
gation. In only one specimen (Figure 3A), which
was ultracentrifugally free of 7S material, was
this line absent. All of the urine specimens
showed the presence of one or two additional lines
which had diffused further from the source of anti-
gen. These lines never crossed the 7S line and
appeared to show a reaction of partial identity with
it. In several preparations of urine γ-globulin
(wells A and B) there was no change in the curva-
ture or position of these lines following a 10-fold
increase in the 7S γ-globulin fraction, thus sug-
gesting that they were indeed due to smaller, more
rapidly diffusing substances which cross-reacted
with the 7S component.

In order to exclude the possible presence in
Fraction II of small amounts of low molecular
weight components similar to those isolated from
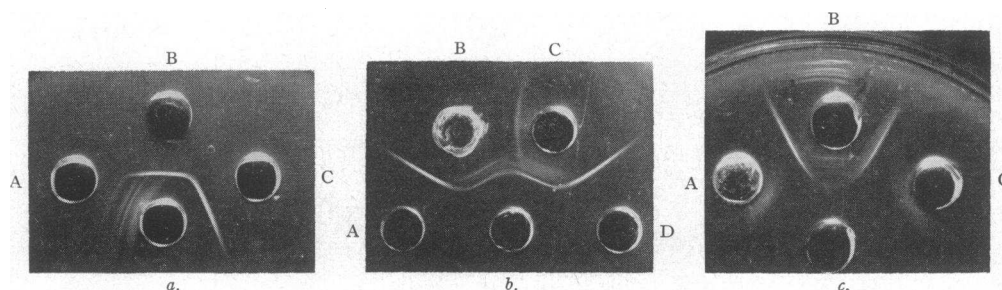urine, and to obtain preparations of "pure" 7S



FIG. 4. *a.* DOUBLE DIFFUSION IN AGAR COMPARING REACTION OF AN ANTISERUM TO URINE
γ-GLOBULIN (CENTER WELL) WITH URINE γ-GLOBULIN (A), 7S SERUM γ-GLOBULIN (B), AND
WHOLE SERUM (C)

*b.* DOUBLE DIFFUSION IN AGAR COMPARING REACTION OF URINE γ-GLOBULIN (CENTER WELL)
AND 7S SERUM γ-GLOBULIN (A AND D) WITH ANTISERA TO SERUM 7S γ-GLOBULIN (B) AND
TO URINE γ-GLOBULIN (C)

There is fusion of all of the 7S γ-globulin lines and one of the more rapidly diffusing lines
present in the urine.

*c.* DOUBLE DIFFUSION IN AGAR COMPARING REACTION OF URINE γ-GLOBULIN (CENTER WELL)
WITH AN ANTISERUM TO URINE γ-GLOBULIN ABSORBED WITH 7S γ-GLOBULIN (A); SAME ANTI-
SERUM UNABSORBED (B); SAME ANTISERUM ABSORBED WITH WHOLE SERUM (C)

The lines between the antisera represent a reaction of the antisera with the excess γ-globulin
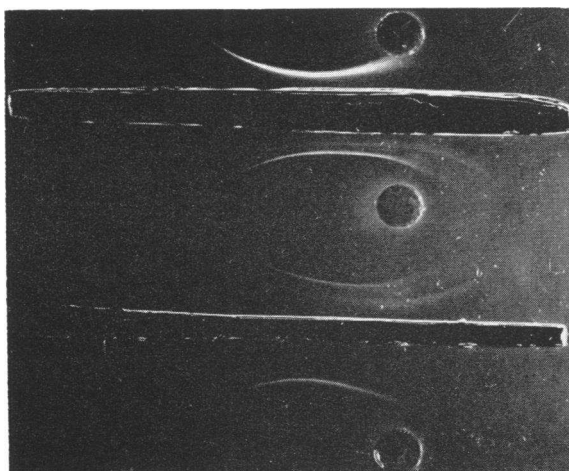used in absorption.

FIG. 5. IMMUNOELECTROPHORETIC ANALYSIS OF γ-GLOB-
ULIN FROM URINE (CENTER) AND COHN FRACTION II
(TOP AND BOTTOM)

Two additional lines with a γ mobility are present in the
urine. The anode is to the right.

γ-globulins free of any possible lighter contami-
nants, several preparations of Fraction II were
separated into eight to 10 fractions by centrifuga-
tion in a saline density gradient. The details of
this procedure have been published previously
(18). The time of centrifugation was such that
the bottom 0.5 ml. was free of albumin ($s_{20,w}$ =
4S) which had been added to a simultaneously
separated control preparation, and therefore pre-
sumably also free of lighter molecules. The top
two fractions were rich in albumin and should
also have contained any possible lighter contami-
nants. When tested with an antiserum to 7S
γ-globulin, both the top and bottom fractions gave
only a single line which fused to give a reaction
of identity. Absorption of this antiserum with
equivalence amounts of the more rapidly sedi-
menting fraction ("pure" 7S γ-globulin), removed
all antibody activity to the urinary proteins.

Confirmatory evidence was obtained with
antisera to urinary γ-globulin fractions. In
double diffusion studies in agar only a single
line appeared with whole serum or 7S γ-globu-
lin while two or three additional lines were
present when urinary γ-globulins were tested
(Figure 4a). The position and curvature of these
additional lines suggested that they were due to
low molecular weight substances. When the re-
action of the urine γ-globulins was compared with
antisera to urine and serum γ-globulins, a reaction

of identity was observed between the outer 7S lines
and one of the more rapidly diffusing lines (Fig-
ure 4b). The outer line also fused with a line
formed by 7S serum γ-globulin and each of the
antisera. This would suggest that some of the
antibodies in both antisera reacted with at least
two common antigens in the urine. Only one
line, the most rapidly migrating one in Figures 4a
and 4c, appeared to be unique to the urine since
it could not be removed by repeated absorptions
with 7S γ-globulins or whole serum (Figure 4c).
This may represent a constituent specific to urine,
possibly a mucoprotein. One line did not resolve
sufficiently to permit an adequate comparison.

A group of representative patterns obtained by
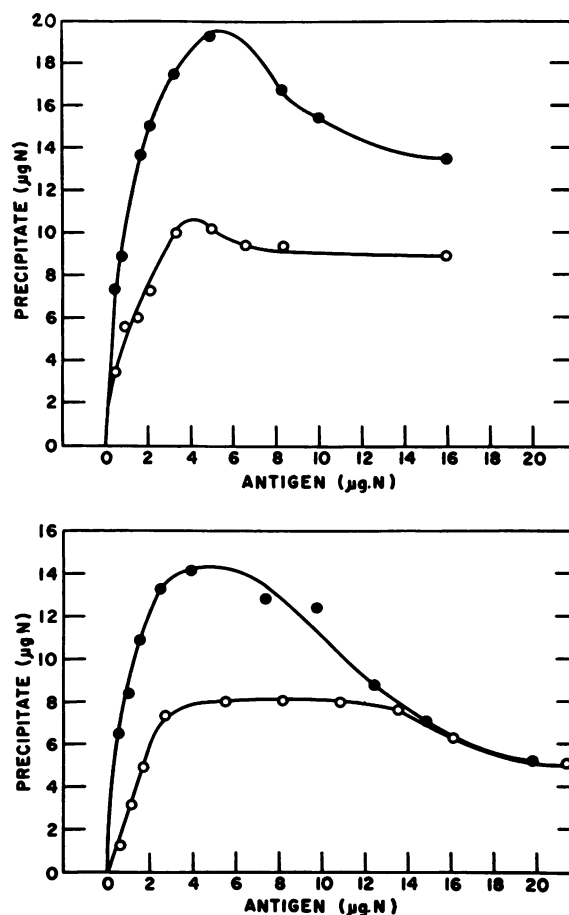immunoelectrophoresis using the same antiserum



FIG. 6. QUANTITATIVE PRECIPITIN CURVES OBTAINED
WITH AN ANTISERUM TO HUMAN γ-GLOBULIN COMPAR-
ING REACTION OF TWO URINE γ-GLOBULIN FRACTIONS
WITH 7S γ-GLOBULIN FROM SERUM

●——● serum γ-globulin. ○——○ urine γ-globulin.

Total radioactivity and specific activities of protein fractions of urine and serum from one subject after the administration of $I^{131}$-labeled gamma globulin

| Material | Time | γ-Globulin | | | β-Globulin | | | α₂-Globulin | | | Albumin | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Protein | Total activity | Specific activity | Protein | Total activity | Specific activity | Protein | Total activity | Specific activity | Protein | Total activity | Specific activity |
| | hrs. | mg. | cpm | cpm/mg. protein | mg. | cpm | cpm/mg. protein | mg. | cpm | cpm/mg. protein | mg. | cpm | cpm/mg. protein |
| Serum | 8 | 26.2 | 13,620 | 520 | 8.3 | 266 | 32 | 8.6 | 56 | 6.5 | 34.9 | 89 | 2.5 |
| Serum | 24 | 27.0 | 8,675 | 320 | 5.5 | 159 | 29 | 7.8 | 89 | 11.5 | 22.8 | 89 | 3.9 |
| Urine | 0–8 | 16.2 | 2,980 | 184 | 19.9 | 745 | 23.5 | 28.0 | 425 | 15.2 | 34.5 | 1,475 | 42.5 |
| Urine | 8–24 | 21.5 | 6,450 | 299 | 27.0 | 1,420 | 52.5 | 20.0 | 466 | 23.3 | 34.0 | 1,480 | 43.5 |

to human 7S γ-globulin is shown in Figure 5. Cohn Fraction II γ-globulin gave only a single line of precipitation with the broad distribution characteristic of γ-globulin. The urinary fraction again demonstrated two additional lines in the γ-globulin region with a mobility slightly greater than that of the simultaneously separated 7S γ-globulin.

Quantitative precipitin studies were carried out with an antiserum to normal human γ-globulin. Figure 6 shows two representative sets of precipitin curves, each of which compares the reaction of the γ-globulin prepared from urine with 7S γ-globulin from serum. The urine γ-globulin used in these studies contained less than 5 per cent of 7S material by ultracentrifugal analysis. In each instance the homologous antigen precipitated a larger amount of protein than the urine γ-globulin. In general the curve obtained with the urine fraction had a broader equivalence zone than was obtained with the serum γ-globulin. The decrease in the amount of precipitate, observed with similar amounts of either protein in the region of antigen excess in each set of curves, appears to preclude the possibility that the precipitate obtained with urine γ-globulin was due to the small amount of contaminating 7S γ-globulin present. When the supernates of these curves were tested with 7S γ-globulin in a capillary precipitin tube and by agar double diffusion studies, no antibody could be detected beyond the equivalence point in any of the curves. These observations would indicate a very great degree of cross-reaction between the γ-globulins of serum and urine.

### $I^{131}$-labeled γ-globulin

Fifty to 100 μc. of $I^{131}$-labeled γ-globulin was given intravenously to four normal male subjects.

Prior to and following iodination this material did not contain detectable amounts of material sedimenting more slowly than 7S γ-globulin on ultracentrifugal examination, and gave only a single line with an antiserum to 7S γ-globulin. Urines were collected for the first eight hours and from the eighth to the twenty-fourth hour, dialyzed against running tap water for 48 hours to remove all traces of free iodide, concentrated by pervaporation and fractionated by starch zone electrophoresis. The results in each of the four subjects were similar in that approximately two-thirds of the radioactivity were associated with the urinary γ-globulin fraction, while only approximately 10 per cent migrated with the albumin fraction.

The data from one representative subject are listed in Table II. Eight and 24 hours following the intravenous administration of 75 μc. of $I^{131}$-labeled γ-globulin, the bulk of the radioactivity in serum was associated with the γ-globulin fractions with a mean specific activity of 420 cpm per milligram protein. The γ-globulins prepared from the urine collected during the same period did not demonstrate a 7S peak on ultracentrifugal examination. Sixty-five per cent of the total nondialyzable protein-bound radioactivity during the second collection period was associated with the fraction of γ mobility, while only 15 per cent migrated with the albumin. The specific activity of the γ fraction was approximately 299 cpm per milligram protein, which is somewhat lower than that of the serum γ-globulin at 24 hours.

In contrast to these observations are the results of control studies with nonprotein-bound labeled iodide. $I^{131}$ was added in vitro to normal serum. The mixture was then dialyzed for 48 hours and subjected to starch zone electrophoresis

in a manner similar to the urines. In three experiments the major fraction of the radioactivity was dialyzable, and only 0.4 to 2.5 per cent of the radioactivity was recovered with the starch block eluates. Of this fraction only 0.02 to 0.9 per cent was associated with proteins having a mobility of γ-globulin, while 66 to 75 per cent migrated with the albumin fraction and 18.5 to 22.0 per cent was located ahead of the albumin peak. More than two-thirds of the radioactivity associated with the albumin in these experiments appeared to be firmly bound, since it was precipitated by trichloroacetic acid and could not be removed by further dialysis. The specific activity of the albumin in these experiments was significantly greater than that in the albumin recovered from the urine. Because of these differences in the distribution of the radioactivity noted after the *in vivo* administration of I¹³¹-labeled γ-globulin and after the *in vitro* addition of iodide to serum, it seems likely that in the *in vivo* studies virtually all of the radioactivity with the mobility of γ-globulin was protein-bound. On the other hand, some of the radioactivity migrating with the albumin may have represented free iodide. Since the latter cannot be accurately quantified by the techniques employed, the fraction of the total protein-bound radioactivity associated with the γ-globulin fraction was probably significantly greater than the figure of 65 per cent calculated above.

These observations favor the view that under the conditions of this study, the bulk of the protein-bound radioactivity in urine and serum was associated with the fraction of γ mobility, and that the 7S γ-globulin in serum gave rise to the corresponding fractions in urine. While these experiments do not completely eliminate the presence in the iodinated globulins of low molecular weight material, in amounts too small to be detected by the techniques employed, which could then be selectively excreted by the kidney, this possibility does not appear probable. It seems likely that such proteins would be handled by the kidneys in a manner similar to the Bence-Jones proteins which are of approximately the same size. These molecules are rapidly excreted during the first 12 hours after their injection (19). Under these circumstances, the specific activity of the urine γ-globulins would be expected to be higher than

that of the corresponding serum fractions, and not lower as found in these studies.

## DISCUSSION

The present study demonstrates the presence in normal urine of proteins with the mobility of γ-globulin which are antigenically closely related to the serum γ-globulins, but differ from them in being only one-fifth to one-sixth their size. Qualitatively, similar findings have recently been reported by Webb, Rose and Sehon (9). However, the molecular weight reported by these observers is of the order of 10,600. These discrepancies and the wide range of sedimentation rates and molecular weights noted in the present study, would suggest the presence of a broad spectrum of breakdown products of different sizes derived from γ-globulin, rather than the existence of a single protein. Evidence for the presence of a number of components is further suggested by the broadness of the ultracentrifugal peak, and the presence of at least four precipitin lines in an antiserum to urine γ-globulin. Similarly, carbohydrate analyses on a number of fractions proved to be extremely high and variable, and would suggest the presence of some carbohydrate-rich contaminants, possibly similar in nature to the carbohydrate-rich material remaining at the origin reported by Rigas and Heller (1).

The results of isotopic studies reported here as well as those by Webb, Rose and Sehon (9) offer strong evidence that the urine γ-globulins are derived from the corresponding serum fractions. These studies would further suggest that in man there exists a mechanism of glomerular filtration of serum proteins similar to that demonstrated in a number of animal species by Sellers, Smith, Marmorsten and Goodman (20), Oliver, MacDowell and Lee (21) and Spector (22). Since the concentration of the γ-globulin fraction in urine relative to the other components is frequently greater than in serum, it seems probable that these fragments are formed prior to glomerular filtration and that, by virtue of their small size, they pass through the glomeruli more readily than albumin. A similar conclusion is also suggested by the appearance of larger amounts of 7S γ-globulin, without a concomitant rise in the output of the smaller fragments, in pathologic states associated with an in-

creased glomerular permeability. However, it has not been possible to date to demonstrate the presence of these components by ultracentrifugal and immunologic means in the sera of seven patients wtih acute renal shutdown of four to 10 days' duration, in whom an accumulation of these proteins would appear likely. The possibility that some of these proteins are added to the urine after completion of the process of glomerular filtration, or that they may originate in tissues of the urinary tract cannot be excluded with absolute certainty from these studies.

The exact nature and significance of these proteins is not fully understood at present. Although they resemble the pathologic Bence-Jones proteins somewhat in size, they have none of the other specific physicochemical properties characteristic of these proteins. Similarly, the isotopic studies have demonstrated that these normal urine γ-globulins appear to be derived from serum γ-globulin, whereas the Bence-Jones proteins appear to be synthesized independently (19). Recent work by Porter (23) has demonstrated that papain can break down normal rabbit γ-globulin into three types of smaller fragments. One of these retains the antigenic constituents characteristic of γ-globulin. Breakdown of human γ-globulin by similar procedures has also been observed to yield two antigenically distinct fragments (24). It seems possible that the urine γ-globulins described here represent some of the natural end products of γ-globulin metabolism *in vivo* corresponding to these fractions produced *in vitro*. The relationship of these two types of γ-globulin breakdown products is currently under study.

## SUMMARY

1. The major constituent of the γ-globulin fraction of normal human urine appears to be one-fifth to one-sixth the size of the serum γ-globulin, and has a sedimentation coefficient of approximately 1.6S.

2. This low molecular weight fragment of γ-globulin is antigenically closely related to serum 7S γ-globulin.

3. Isotopic studies have demonstrated that it is primarily derived from the serum γ-globulin fraction.

4. The possibility that the fraction represents a natural breakdown product of γ-globulin and its bearing on current theories of glomerular filtration is discussed.

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