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Characterization of Antibodies In Human Urine *

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Human urine contains proteins that have been demonstrated to be antigenically identical with serum immunoglobulins (1–8). The immunoglobulins usually present in urine have been 7 S γ-globulin and β_{2A}-globulin (3,7), whereas β_{2M}-globulin has been found in only a few cases (9). A low molecular weight protein, antigenically related to 7 S γ-globulin, has also been observed (2, 4–8). This low molecular weight γ-globulin (γ_L) [1] has been reported to be identical to the light (L) polypeptide chains that form part of the 7 S γ-globulin molecule, and it has been suggested that they were free L chains representing the normal counterparts to Bence Jones proteins (10–12).

Antibody activity in urine has been demonstrated against several microorganisms. Agglutinins reacting with *Vibrio cholera* and *Salmonella typhi* (13) and precipitins against diphtheria and tetanus toxoid (14), as well as neutralizing activity against polio virus (15), have been described. Usually, the antibodies responsible for these activities have not been further characterized immunologically or physicochemically. The reports of Remington, Merler, and their colleagues (16, 17), however, present evidence that precipitating activity against tetanus toxoid and neturalizing activity against polio virus reside in a protein fraction of a molecular weight of 12,900. These authors found no antibody activity in material that was antigenically identical with serum 7 S γ-globulin.

There has been increasing interest in recent years in relating antibody activity to different structural units of the γ-globulin molecule. This study was undertaken to determine whether antibodies are indeed present in the urine in the low molecular weight proteins. If this were so, the immunological characterization of this naturally occurring γ_L-protein with antibody activity might provide further information relating structure to function within the γ-globulin molecule. Our results showed that antibody activity in urinary proteins resides mainly in 7 S γ-globulin with some activity in fractions of lower molecular weight. These latter fractions were shown to contain L chains and material related to H chains. The L chains alone did not show antibody activity.

Methods

Source of samples

Urine was obtained from eleven patients with systemic lupus erythematosus and from eight patients with a mixed type of connective tissue disease. These individuals showed antinuclear activity in their sera as demonstrated by the immunofluorescent technique (Table I). Most of these patients were being treated with steroids during the time of the urine collections. Patients with normal and abnormal amounts of proteinuria were included in the study. The proteinuria is listed in Table I, together with the antinuclear activity found in the sera and urines.

A group of hyperimmunized individuals consisted of one of the above-mentioned patients with a mixed type of connective tissue disease (WM), four patients with multiple myeloma (RS, CA, LG, BL), four patients with rheumatoid arthritis (MG, MH, RC, TC), one with osteoarthritis (LT), and one individual lacking β_{2A}-globulin in his serum (LH). LH and one of the patients with rheumatoid arthritis were given one injection of 1 ml, subcutaneously, of blood group B substance.[2] All of the others were immunized by repeated subcutaneous injections of 10^{11} T_2 phages.[3] Before use

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[1] The designation γ_L-globulin used in earlier papers (8, 10) will be used for all the low molecular weight proteins in urine related to γ-globulin. It will be shown that this fraction of urine contains proteins identical to L chains as well as proteins related to other parts of the 7 S γ-globulin molecule.

[2] Knickerbocker Biologicals, New York, N. Y.

[3] Prepared as described by Jesaitis (18) and under his kind direction.

in humans, the purified phages were tested in bacteriological cultures for sterility. They were also first tested in rabbits and in guinea pigs and found to have negligible amounts of pyrogens.

Preparation of urine concentrates

Usually, several 24-hour urine collections were obtained from each patient. To avoid infection of the urine samples, the genitalia were cleansed with pHisohex before voiding, and the urine samples were kept in sterile bottles at + 4° C, with the addition of chloroform. This preservative does not inactivate T_2 phages. By this procedure most final urine concentrates were found to be sterile; only occasionally a scarce growth of *Escherichia coli* or *Proteus* was observed.

For the concentration of the urines, ultrafiltration through Visking tubings (23 to 32 inches) as described by Berggård (19) was used. A concentration of 500- to 1,000-fold from the original volume was usually employed for these studies.

Separation techniques

1. *Zone electrophoresis* on starch or on Pevikon was performed in the manner previously described (20, 21).

2. *Gel filtration* of the urine concentrates was done on Sephadex G-100 [4] as described by Flodin (22). The column was of the dimension 4.5 × 70 cm, and for the elution a 0.01 M Tris-HCl buffer, pH 8.2, with 0.5 or 1.0 M NaCl was used. Urine concentrates containing up to 2 g of protein were easily separated on this column. The filtrate was collected in 6-ml fractions with a drop counting fraction collector. The optical density of the fractions at 280 mμ was read in a Beckman DU spectrophotometer. Material antigenically related to γ-globulin was located in the eluates by precipitation in capillaries with an anti-γ-globulin antiserum. Fractions were then pooled, concentrated in collodion bags,[5] and finally dialyzed against physiological saline.

3. Separation of the urinary γ-globulins of different molecular weight was also obtained by *ultracentrifugation in a sucrose density gradient* (23, 24). A gradient of 5 to 25% sucrose was used in runs at 35,000 rpm for 18 hours.

4. Immunoglobulins with antinuclear activity were isolated from concentrated urine by *adsorption on calf thymus nuclei.* The method used has been described previously (25), but certain modifications were employed. Isolated preparations of calf thymus nuclei were allowed to react with urine concentrates at 37° C for 1 hour, followed by 48 hours at 4° C. The nuclei were separated by centrifugation and repeatedly washed in cold saline. To the isolated nuclei, 1 or 2 ml of DNAse [6] solution, containing 0.4 mg DNAse per ml, was added, and digestion was carried out at 37° C for 1 hour. The digests were cleared by centrifugation and dialyzed against saline. Immunoglobulins with high titer antinuclear activity were isolated in this fashion.

Immunological techniques

Double diffusion analyses in agar gel were performed as described earlier (26) as were the immunoelectrophoretic experiments (27). Quantitative precipitation was done as described by Kabat and Mayer (28). The protein content was determined by a modified Folin-Ciocalteau method, using Cohn Fraction II γ-globulin as a standard.

Antisera and special antigens

Antisera against whole γ-globulin was obtained from rabbits immunized with Cohn Fraction II. Antisera against slow-moving (S) fragments of enzymically split 7 S γ-globulin (29) were produced by immunization of rabbits with S fragments, prepared by degradation of Fraction II with pepsin (30). Antisera against the fast-moving (F) fragment of 7 S γ-globulin, split by papain (29), were made by absorption of anti-γ-globulin sera with S fragments. An antiserum reacting with the H (heavy) chains of 7 S γ-globulin (11) was prepared by absorption of an anti-γ-globulin serum with Bence Jones proteins of the antigenic groups I and II (31). Antiserum specific for β_{2A}-globulin was prepared by adsorption of an anti-immunoglobulin serum with whole serum from an individual lacking in β_{2A}-globulin. L chains were prepared as described by Fleischman, Pain, and Porter (32).

Techniques for antibody detection

1. *Antinuclear activity.* The immunofluorescent technique of Coons and Kaplan (33) was employed to determine the antinuclear activity of urinary proteins. Sections of frozen mouse kidney, cut in a cryostat at 4 to 6 μ, were used as the source of nuclei. After being washed in two changes of buffered saline (0.02 M phosphate, pH 7.0) for 10 minutes, the section was reacted with the urine sample for 30 minutes at room temperature. The sections were then washed for 10 minutes and stained with fluorescein-conjugated rabbit antihuman Fraction II globulin for 30 minutes. Antinuclear activity was detected by specific fluorescence of tubular and glomerular cell nuclei in the mouse kidney.

2. *Antibodies against the T_2* phages were determined by the agar layer method of Adams (34). Of a suspension of 2×10^5 T_2 phages in a nutrient broth, 0.2 ml was incubated with 0.2 ml of the test sample for 1 hour at 37° C. The reaction was interrupted by dilution to 1/100 with ice-cold broth. One-tenth ml of this dilution was plated by addition to melted 0.5% agar infected with *E. coli* B.[7] The number of plaques was recorded after incubation overnight. Duplicates of plates were always made. As a unit of antibody activity the amount of material needed to neutralize 50% of the introduced phage (PhN_{50}) was used. The calculations of PhN_{50} U per

[4] Pharmacia, Uppsala, Sweden.

[5] Membrangesellschaft AG, Göttingen, Germany.

[6] Worthington Biochemicals, Freehold, N. J.

[7] Kindly supplied by Dr. M. Jesaitis.

TABLE I

Subjects studied for urinary antibodies

Diagnosis	Patient	Antinuclear factor*		Proteinuria
		Serum	Urine†	
				g/24 hours
	A. Subjects with autoimmune disease			
Systemic lupus erythematosus	MB	3+	1+	0.2
	SS	4+	3+	0.2
	WJ	4+	2+	<0.1–4.0
	PA	3+	2+	0.1
	CT	4+	1+	0.2
	BR	4+	1+	0.1
	NN	1+	Negative	0.1
	HM	3+	2+	0.1
	BSl	3+	1+	<0.1–.5
	BSa	1+	Negative	1.0–1.5
	ES	1+	Trace	<0.1
Mixed type of connective tissue disease	HS	1+	Negative	0.1
	WM	3+	2+	0.2
	GR	2+	1+	0.2
	ML	1+	Negative	0.2
	DD	2+	Negative	0.5
	MA	3+	1+	<0.1
	EO	Trace	Negative	<0.1
	LW	2+	Negative	0.2
	B. Subjects immunized with T_2 phage anti-T_2 titers			
Miscellaneous conditions	WM	340	66	0.2
	LT	170	2	0.1
	RS	110	4	<0.1–0.5
	CA	120	6	0.1
	LG	105	15	0.5–2.4
	BL	50	36	0.2
	MG	100	40	0.1
	MH	20	6	0.2
	RC	90	15	0.1
	TC	50	10	<0.1
	C. Subjects immunized with blood group B substance anti-B titers			
Miscellaneous conditions	JC	1/8,000	1/2,500	0.4–0.8
	LH	1/1,000	1/3	<0.1

* Given in arbitrary units from trace to 4+.
† Urines concentrated 1,000-fold.

ml were made according to Barlow, Van Vunakis, and Levine (35).

Neutralization of the T_2 phages was also obtained after degradation of antibody-containing γ-globulin from hyperimmunized individuals by papain or pepsin in the presence of cysteine.

3. *Antibodies against blood group B substance* were detected by agglutination on glass slides. When fractions were examined that might contain antibodies of low molecular weight, the test was performed as an indirect Coombs test with addition of Coombs serum to the washed B cells that had been incubated with the test sample.

Results

Antibody activity in whole urine concentrates. Antinuclear activity was observed by the immunofluorescent technique in concentrated urines from the majority of the patients in the connective tissue disease group (Table I). The presence of antinuclear activity in the urine was seen mainly in those patients with high titer activity in serum. Control samples from individuals lacking the activity in their serum showed no activity in their urine concentrates. Urine concentrates from individuals immunized with T_2 phages all showed neutralization of the phage to varying extent (Table I). Such inactivation was not obtained with urine concentrates from nonimmunized individuals or from urine samples collected before immunization. Agglutination of B cells, but not of A cells, was obtained with the urine concentrates from the individuals immunized with B substance (Table I).

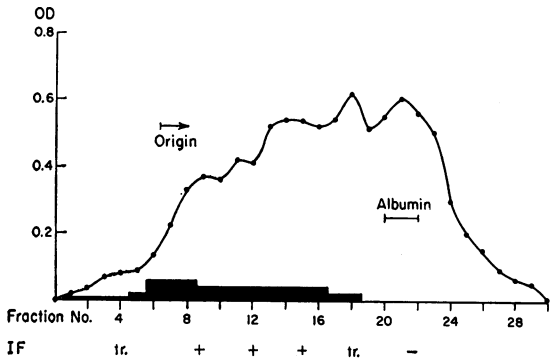

FIG. 1. ZONE ELECTROPHORESIS ON STARCH OF URINE CONCENTRATE FROM PATIENT WITH SYSTEMIC LUPUS ERYTHEMATOSUS. Material reacting with anti-γ indicated in the shaded area; antinuclear factor demonstrated by immunofluorescence (IF). Anode to the right (tr. = trace).

Separation of the urine proteins by zone electrophoresis in starch or Pevikon showed that the activities were localized mainly in the γ-globulin region (Figure 1). In five cases of strong activity positive results were also obtained with fractions in the β and α_2 areas. In all of these regions material antigenically related to γ-globulin could be demonstrated by immunological techniques (Figure 1).

Separation and characterization of the 7 S and γ_L-globulins. With gel filtration through Sephadex G-100, a group separation of the urine proteins was obtained. The chromatograms obtained varied somewhat for the 12 different individuals studied in this way, but not in a consistent manner. After the void volume a first peak was obtained, and thereafter one large or sometimes two smaller peaks were noted (Figure 2). Repeated experiments in the same individual gave very similar patterns.

In nine instances zone electrophoresis in starch or Pevikon was employed for further purification of the immunoglobulins. As all γ-globulin-related material was included in this study and such material was found also in the β- and α-globulin areas of the electrophoretogram (Figure 1), only a partial purification was obtained. This was also the case with purification by precipitation with ammonium sulfate, as a saturation of up to 80% had to be used to precipitate all the γ_L-globulins.

A localization in the Sephadex chromatogram of the immunoglobulin-related material was made first by the screening procedure of precipitation in capillary tubes with anti-γ-globulin serum and then by quantitative precipitation with anti-S and anti-F sera (Figure 2). Two fractions of immunoglobulin-related material were found. The first fraction was located in the descending limb of the first peak. Anti-S and anti-F sera tested with this first fraction showed corresponding curves suggesting that the S and F antigenic sites were on the same molecule. The fraction consisted mostly of material identical with 7 S γ-globulin in Ouchterlony plates and some material identical with β_{2A}-globulin. Two lines were seen in immunoelectrophoresis, one corresponding to γ-globulin and one to β_{2A}-globulin (Figure 3c).

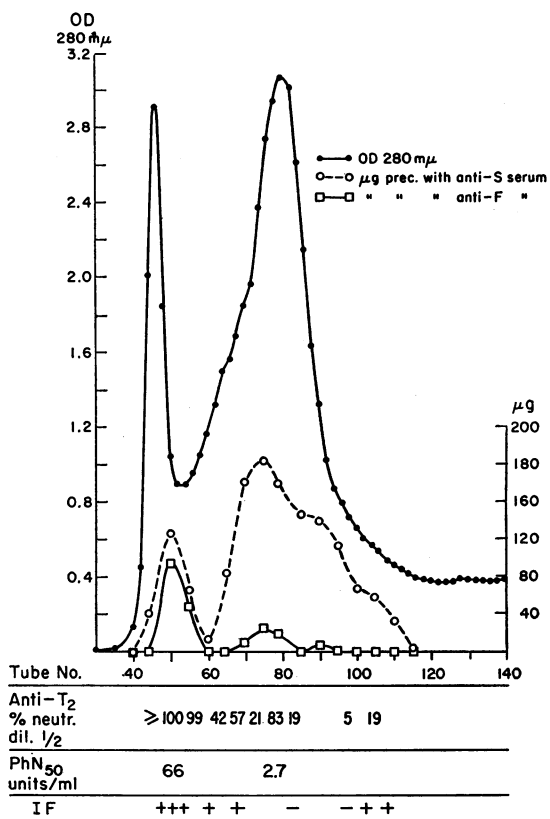

FIG. 2. CHROMATOGRAMS OBTAINED BY GEL FILTRATION ON SEPHADEX G-100 OF A WHOLE URINE CONCENTRATE (PATIENT WM). Material precipitable (prec.) by anti-S and anti-F sera indicated. Antibody activity against T_2 phages given in per cent of inactivation by different fractions, and when possible, in PhN_{50} U per ml (PhN_{50} = amount of material needed to neutralize 50% of the introduced phage.) Antinuclear factor demonstrated by immunofluorescent technique is also indicated.

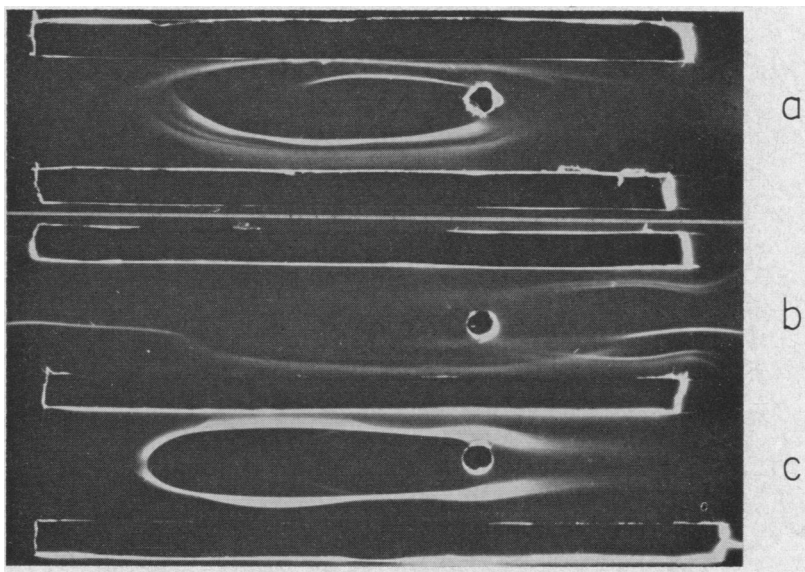

FIG. 3. IMMUNOELECTROPHORESIS OF A WHOLE URINE CONCENTRATE (A), γ_L-FRACTION (B), AND 7 S FRACTION (C), OBTAINED BY GEL FILTRATION. The whole urine concentrate (a) was developed with an antiserum known to react strongly with β_{2A}-globulin (upper trough) and an antiserum to human Fraction II (lower trough). The γ_L-fraction (b) was developed with the same antihuman Fraction II (lower trough) and with this antiserum absorbed with S fragment of γ-globulin (upper trough). The excess of S fragment used for absorption formed a precipitate between the two antiserum troughs. The urine 7 S fraction (c) was developed against anti-Fraction II in both troughs.

The β_{2A}-line was not clearly seen in this photographic reproduction. In comparison, whole urine concentrates (Figure 3a) showed four lines where the two closest to the antiserum basin corresponded to γ_L-globulins and the others to 7 S γ- and β_{2A}-globulins (7, 8). It appeared that the γ_L-globulins were absent from this first fraction obtained from Sephadex chromatography.

The second fraction reacting with anti-γ-globulin sera was broader and consisted mainly of material reacting with anti-S (Figure 2). There was usually a small amount of material reacting with anti-F. In some experiments an indication of two peaks was seen with the anti-F reacting material, whereas the anti-S reacting material usually formed one broad inhomogeneous peak. Immunodiffusion studies showed that in all areas within the second peak, material was present that gave reaction of partial identity with 7 S γ-globulin or with S fragments, but complete identity with L chains (Figure 4a). In addition, many of these fractions gave one and in some cases two pre-

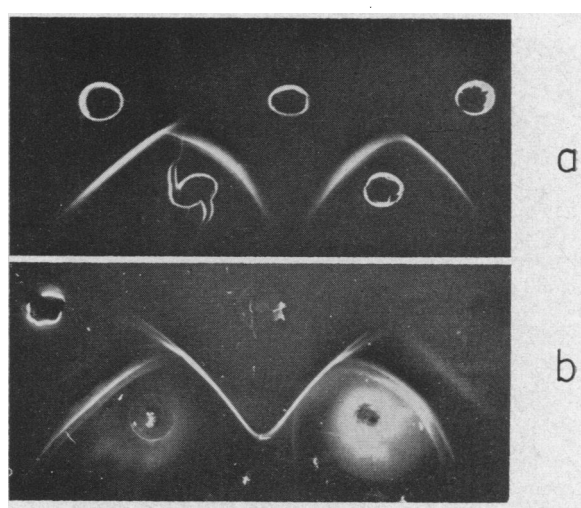

FIG. 4. (A) COMPARISON OF A γ_L-GLOBULIN FRACTION (UPPER CENTRAL WELL) WITH AN S FRAGMENT (LEFT UPPER WELL) AND L CHAINS OF 7 S γ-GLOBULIN (RIGHT UPPER WELL) USING AN ANTI-γ-GLOBULIN SERUM (LOWER WELLS). (B) COMPARISON OF A γ_L-GLOBULIN FRACTION (UPPER LATERAL WELLS) WITH AN F FRAGMENT (UPPER CENTRAL WELL), USING ANTI-F SERUM (LOWER LEFT WELL) AND ANTI-H SERUM (LOWER RIGHT WELL).

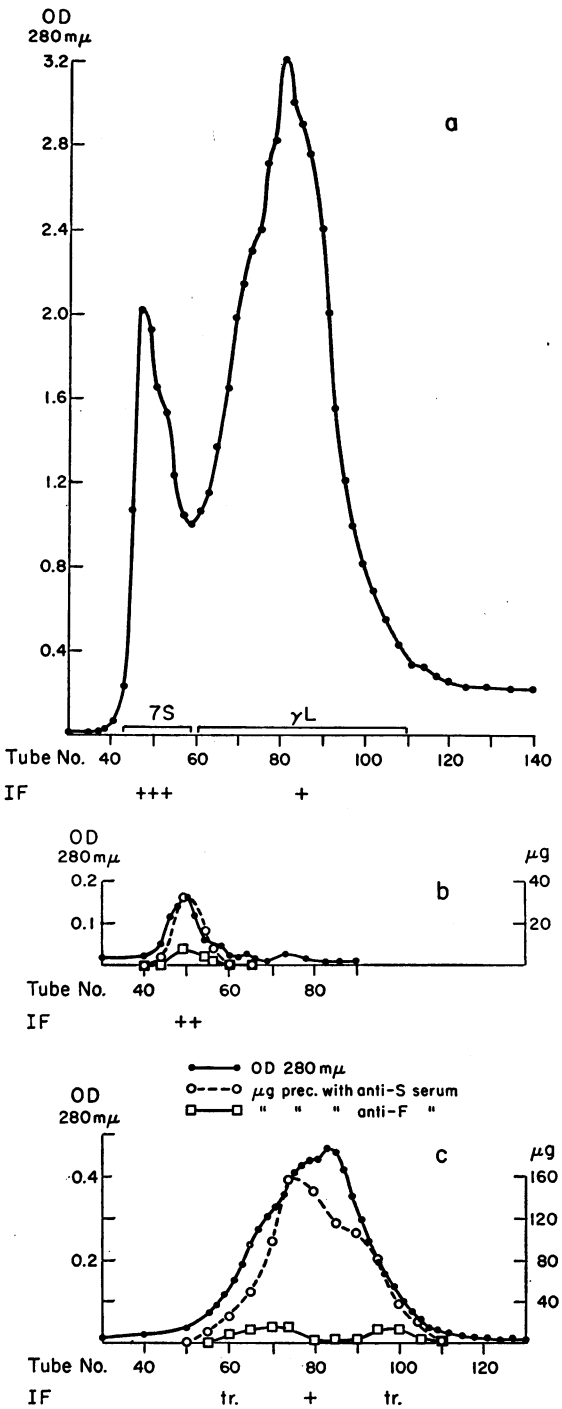

FIG. 5. (A) CHROMATOGRAM OF GEL FILTRATION ON SEPHADEX G-100 OF A WHOLE URINE CONCENTRATE. Antinuclear activity indicated in the 7 S and γ_L-fractions. (B) RECHROMATOGRAPHY OF THE 7 S AND (C) γ_L-FRACTIONS AFTER PURIFICATION BY ZONE ELECTROPHORESIS ON PEVIKON. Material precipitable with anti-S and anti-F sera indicated as well as antinuclear activity.

cipitates with anti-F sera (Figures 3b and 4b). These F-related precipitates were not due to the presence of complete H chains, as they gave reactions of partial identity with 7 S γ-globulin using anti-H serum. This material was also usually only partially related to F fragments (Figure 4b).

Fractions of urine separated by gel filtration were rechromatographed separately and recovered in the same regions, thus showing little contamination with each other (Figure 5a-c). Ultracentrifugal density gradient studies showed that the first fraction corresponded to 7 S γ-globulin in sedimentation, and the middle part of the lighter fraction approached the sedimentation of a Bence Jones protein used as a reference (Figure 6). These findings were consistent in 14 experiments using material from eight individuals.

Antibody activity in the two immunoglobulin fractions. Antinuclear activity was studied in the two chromatographic fractions, and in eight patients the first peak, containing 7 S γ-globulin and some β_{2A}-globulin, consistently gave a strong staining of nuclei with the immunofluorescent technique (Figure 7a). Material from the broad second peak of γ_L-globulin also showed nuclear staining, but definitely weaker staining reaction than seen with material from the first peak (Figure 7b). Often the staining was noted also very far out in the γ_L-globulin fraction (Figure 2).

The presence of 7 S γ-globulin as a contaminant in the γ_L-fractions that showed antibody activity was excluded by rechromatography and density gradient studies as shown in Figures 5 and 6. Immunodiffusion analysis also showed that the material in the γ_L fraction was antigenically deficient to whole 7 S γ-globulin (Figure 4).

Studies in four patients of the phage neutralizing activity in the immunoglobulin-containing peaks showed that the 7 S fraction contained antibodies in titers from 2 to 66 PhN_{50} U. This was observed in patients both with and without evidence of renal disease. In one case no phage neutralizing activity could be shown in the γ_L-globulin fraction. In two other cases, some of the undiluted γ_L-fractions contained less than 1 PhN_{50} U, figures of questionable significance since they fall within the error of the technique. Only with the γ_L-globulin fractions of one patient (WM) was significant inactivation of the phages obtained (Fig-

ure 2). The amount of inactivation was low, but consistently reproducible. One portion of the γ_L-fraction had 2.7 PhN_{50} U per ml, whereas the 7 S fraction had 66.

Density gradient ultracentrifugation studies were performed on the urine from the patient with the high titer of anti-B antibodies (JC, Table I). It was found that antibody activity resided in material sedimenting similar to or somewhat faster than serum 7 S γ-globulin. The γ_L-globulin prepared from the urine by gel filtration showed titers of 1/20 with a Coombs technique. The significance of these low titers may be questionable. In contrast to this the first gel filtration fraction of this urine gave an anti-B titer of 1/5,000.

Studies of the urinary antibodies eluted from nuclei. Immunodiffusion studies of the urinary antibodies eluted from urines reacted with calf thymus nuclei showed in twelve experiments the presence of significant amounts of L chains as well as of 7 S γ-globulin, giving a pattern similar to that of the whole urine concentrate with an anti-γ-globulin serum.

Antinuclear antibodies were demonstrated in these eluates by immunofluorescence. The fractions of a density gradient separation of the eluates usually showed strong activity in the 7 S region and weak activity in a low molecular weight protein fraction. Figure 8 shows antinuclear activity present in one such eluate (III), which on density gradient ultracentrifugation showed only low molecular weight proteins sedimenting slightly faster than ribonuclease.

Absorption experiments. In the low molecular weight fractions where antibody activity was demonstrable, L chains were present as well as material related to H chains. To try to relate one or both of these two types of polypeptide chains to the antibody activity, absorption experiments were carried out. Antisera against H chains and F fragments were employed. Attempts to use antisera against L chains were unsuccessful because in the absorption experiments so much antiserum had to be added for a complete absorption that the relatively weak antibody activities in the γ_L-fractions were diluted out.

Absorption of the whole urine concentrates with anti-F showed in Ouchterlony plates that only the L chains and β_{2A}-globulin were left. Anti-T_2

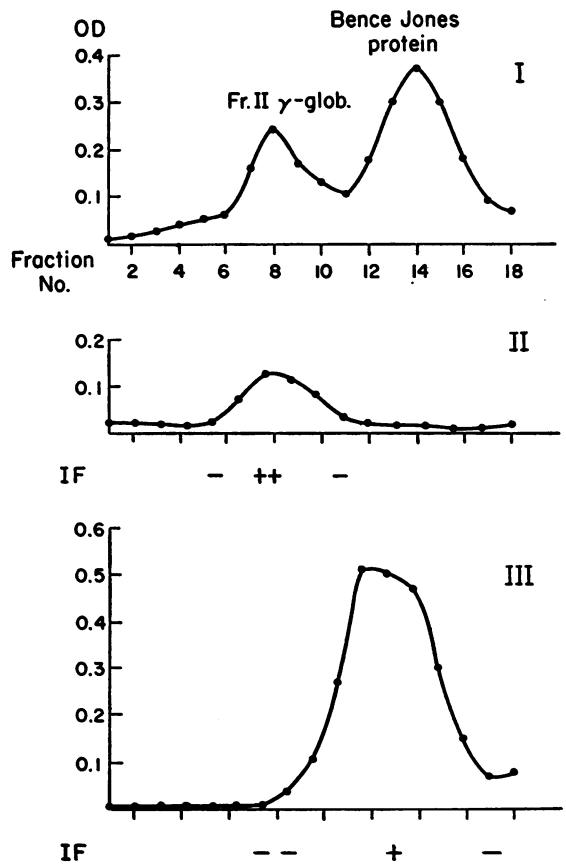

FIG. 6. DENSITY GRADIENT ULTRACENTRIFUGATION OF THE RECHROMATOGRAPHED 7 S (II) AND γ_L-FRACTIONS (III) IN FIGURE 5. Antinuclear activity indicated. Uppermost curve (I) shows Fraction II γ-globulin and a Bence Jones protein run simultaneously as a reference. Top fractions to the right.

activity in such absorbed samples was usually completely abolished (Table II). In one case (WM), however, some activity was still present. This could be due to β_{2A}-globulin. WM was the same patient that showed activity in the gel filtration γ_L-fraction, but who also had β_{2A}-globulin that showed some neutralizing activity. Absorption of the whole urine concentrate of WM with an anti-B_{2A}-globulin serum diminished the activity by a small but probably significant amount.

Anti-H serum was added to the only two low molecular gel filtration fractions giving a significant neutralization of the T_2 phage. In both cases a complete abolishment of the activity was obtained (Table II). The absorptions were complete;

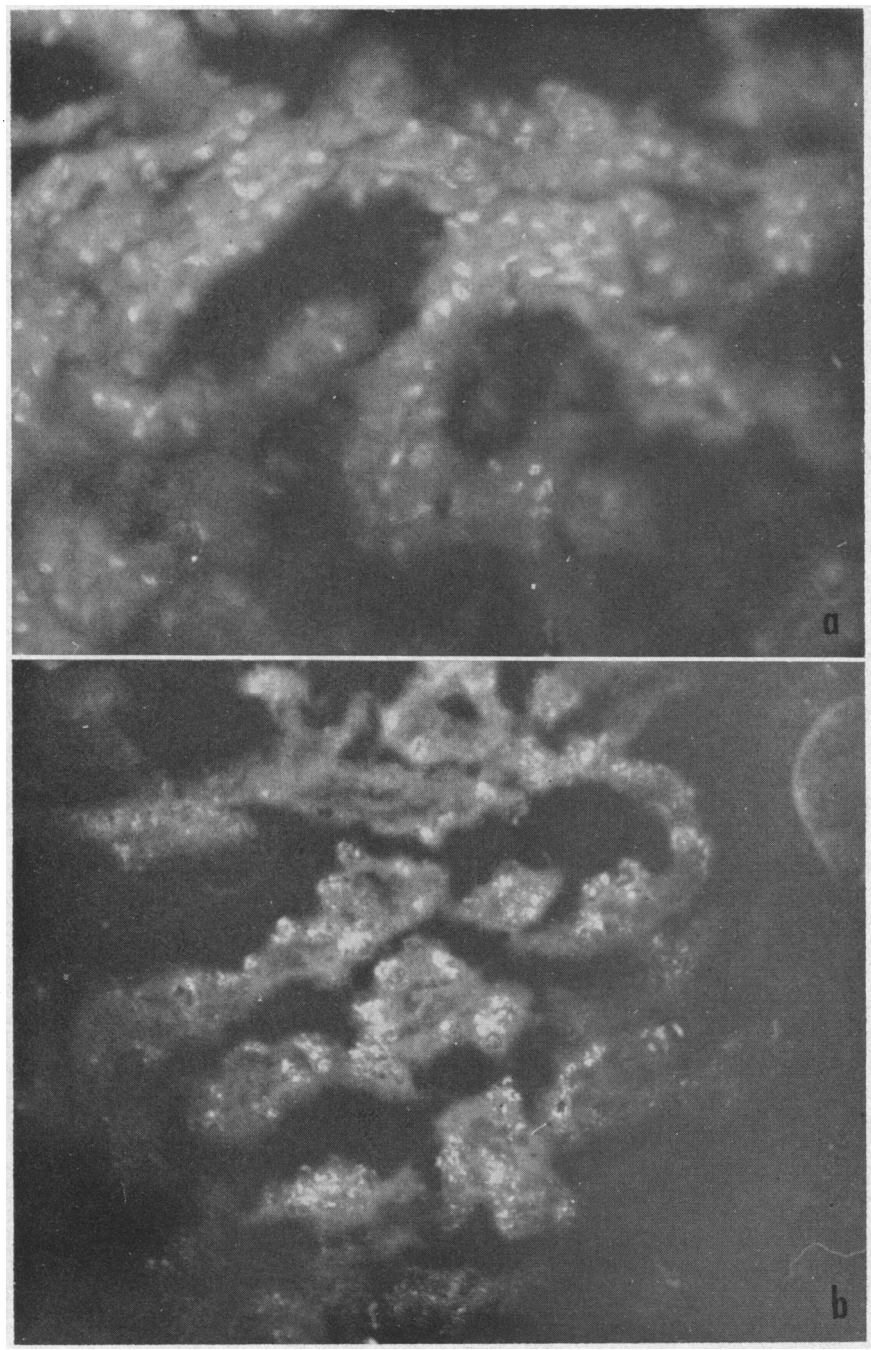

FIG. 7. (A) ANTINUCLEAR ACTIVITY IN A 7 S FRACTION AS SEEN BY FLUORESCENT STAINING OF NUCLEI IN A MOUSE KIDNEY SECTION. (B) ACTIVITY SEEN IN A γ_L-GLOBULIN FRACTION.

only L chains were left as shown by immunodiffusion analysis.

Similar absorption experiments with low molecular weight gel filtration fractions, having antinuclear activity, showed in all instances that the anti-H serum eliminated the activity (Table II). The same absorption, repeated with antibody eluted from nuclei, showed abolishment of all the

antinuclear activity with anti-H sera (Table II). In all instances immunodiffusion controls showed the residual presence of L chains after the absorptions.

Control experiments. It was thought important to find out whether or not any enzyme degradation occurred in the urine concentrates during processing, possibly giving rise to fragments of γ-globulin that could show antibody activity.

An isolated myeloma protein was added (15 to 30 mg) to the sterile bottles used for the urine collections in three different experiments and was processed together with the urine in the usual manner. After the completion of the concentration the sample was studied by immunodiffusion, using antiserum against F fragments and antiserum against the myeloma protein. No fragments reacting with anti-F serum could be demonstrated. Separation of the urine concentrate by ultracentrifugation in a sucrose gradient showed no material in the top fractions reacting with homologous antimyeloma serum made specific for the myeloma protein by absorption with Fraction II γ-globulin. Thus, no evidence for proteolytic activity against the added γ-globulin in the urine preparations could be found during the processing.

TABLE II

Loss of antibody activity in urine after absorption with anti-F and anti-H antiserum

Material	% of phage inactivation	
Whole urines	Dilution 1/20 with saline	Dilution 1/20 with anti-F
LT	54	0
TC	18	0
RC	50	3
MG	51	0
BL	31	1
WM	81	17
γ_L-globulins	Dilution 1/2 with saline	Dilution 1/2 with anti-H
WM γ_L (Tubes 66–70, Figure 2)	26	0
WM γ_L (Tubes 76–80, Figure 2)	46	0
	Antinuclear factor	
	Dilution 1/2 with saline	Dilution 1/2 with anti-H
WM γ_L (Tubes 66–70, Figure 2)	1+	Negative
WM γ_L (Tubes 101–105, Figure 2)	1+	Negative
Antibody eluate I	2+	Negative
Antibody eluate II	3+	Negative

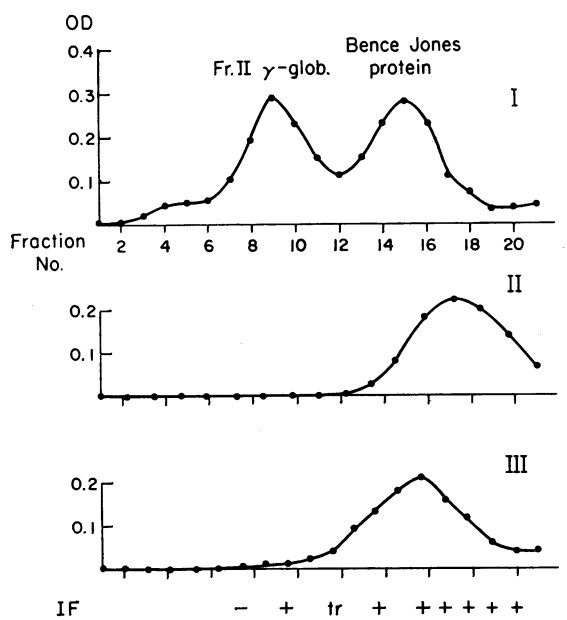

FIG. 8. DENSITY GRADIENT ULTRACENTRIFUGATION OF AN ELUATE OF URINARY ANTINUCLEAR ANTIBODIES ABSORBED TO CALF THYMUS NUCLEI (III). Antinuclear activity indicated. Fraction II γ-globulin and a Bence Jones protein (I) and ribonuclease (II) run simultaneously as references.

Discussion

Antibody activity in urine against various bacteria and viruses has been reported by several authors (13–15), and the present studies indicate that antibodies against T_2 phages could be readily demonstrated in the urine of immunized individuals. Antinuclear activity in the urine of patients with connective tissue disease has been briefly described by Hauser (36) and by Stevens and Knowles (37). It was found that antinuclear activity could be demonstrated in the urine of all patients who had a relatively strong activity in their serum (*cf.* Table I).

In normal as well as in certain pathological proteinurias γ-globulin, β_{2A}-globulin, and the γ-related low molecular weight protein (γ_L-globulin) have been described by several authors (1–7, 38). Antibody activity demonstrable in urine could be expected to be related to any or all of these immunoglobulins. Our studies indicate that the main portion of the urinary antibodies were 7 S γ-globulin, and that some activity was also present in a low molecular weight fraction. This seemed to be the case whether the individual

studied excreted normal or abnormal amounts of protein in the urine. The phage neutralizing test indicated in the single case where significant neutralization was seen in a low molecular weight fraction that the corresponding 7 S fraction had 25 times more neutralizing activity. Of this 7 S fraction activity only a very small portion was related to β_{2A}-globulin. The immunofluorescent studies were only semiquantitative, but we consistently observed that the staining obtained with the 7 S fraction was much stronger than that obtained with the low molecular weight fractions. The results with the anti-B antibodies were similar. These findings are of interest, as earlier studies have indicated that the γ_L-globulin may be quantitatively more important than the 7 S γ-globulin in urine (2, 4, 6, 16, 17). This is also indicated by our results with quantitative precipitation of the gel filtration 7 S and γ_L-fractions with various antisera. In most cases more material was precipitated in the γ_L-fractions (*cf.* Figures 2 and 5). This relation was also indicated by preliminary studies of quantitative precipitation of I^{131}-labeled normal urinary proteins (9).

The antibody activity found in the low molecular weight fractions was usually weak but significant. It appeared from rechromatography and density gradient and immunodiffusion studies of the γ_L-fractions that this activity was not due to contamination with 7 S γ-globulin. The antibody activity could be observed in several regions of the broad gel filtration fraction of γ_L-globulin, in several cases also in material sedimenting in density gradient ultracentrifugation similar to RNAse, which has a mol wt of 13,500 (39). This finding is similar to that of Remington and associates (16, 17), who reported activity in material of a mol wt of 12,900.

In the experiments described on the characterization of the γ_L-fraction of urine, it was obvious that this material showed a pronounced heterogeneity. This has been described by other authors (6) and is apparent from the broad distribution of electrophoretic mobility and by the studies presented here in the gel filtration experiments. In quantitative precipitation analyses the γ_L-fraction obtained by gel filtration also showed a broad and inhomogeneous peak (Figures 2, 5). Cornillot, Bourrillon, Michon, and Got (40) have presented evidence that the γ_L-fraction consists of two peaks representing material of two different molecular sizes, 3.5 S and 2.2 S. Density gradient ultracentrifugation of material from different portions of our broad γ_L-fraction indicated different sizes of protein molecules. This could possibly be related to a distribution of the urinary L chains as mono- and dimers much like the report of Bernier and Putnam (41), where they demonstrated that Bence Jones proteins of group I occur as a mixture of mono- and dimers, whereas the group II Bence Jones proteins are only dimers.

Antigenic characterization of the low molecular weight fraction showed that most of the γ-related protein was similar or identical to L chains. This material contained the antigenic groupings I and II described in Bence Jones proteins and other immunoglobulins (31). This observation has also been reported by earlier investigators (6, 8). However, our studies showed in addition that material antigenically related to H chains and F fragments of 7 S γ-globulin was regularly found in small amounts in the γ_L-fraction. These components might be the normal counterpart to the H-chain–related material that recently has been reported to be excreted in the urine of a case with a lymphoproliferative disease (42).

Several investigators have recently been studying the relation of antibody activity to the H and L chains of the 7 S γ-globulin. Porter (43) did not find any activity in L chains, whereas activity was demonstrable in a fraction containing H chains. In a preliminary report Jaquet, Bloom, and Cebra (44) presented evidence for antibody activity related to the L-chain fraction. Recently, Franěk and Nezlin (45) reported findings indicating that L chains together with H chains constituted the active principle. Our γ_L-fraction contained material related to both L and H chains. It could be shown that absorption with an anti-H serum abolished the antibody activity of the γ_L-fractions. The remaining inactive fractions contained only L chains when analyzed by immunodiffusion. These results indicated that L chains alone were inactive.

The possibility also arises that the low molecular weight material could be due to enzymic degradation of the urinary immunoglobulins (2, 4), resulting in low molecular weight fragments with

retained antibody activity (30, 46–48). Recent studies, however, have given evidence that the urinary L chains, making up most of the γ_L-fraction, are not the result of a breakdown of serum 7 S γ-globulin (6, 49). They may be synthesized independently in a way similar to that shown for Bence Jones proteins (50). The material in the γ_L-fractions related to H chains might be produced by enzymic degradation but could also conceivably be synthesized like the L chains. Some evidence against enzymic breakdown could be the finding that no material completely identical with S fragments was found in the γ_L-globulin fraction, although proteolytic enzymes such as pepsin, trypsin, chymotrypsin, and a subtilisin all produce significant amounts of a fragment that is antigenically similar to the S fragments of papain-split γ-globulin (48, 51–53).

Summary

A number of antibodies in urine were studied including antibodies against T_2 phages, blood group B substance, and antinuclear antibodies in patients with connective tissue disease.

The main portion of the antibody activity resided in the 7 S γ-globulin, whereas weak activity was noted in the low molecular weight fraction related to γ-globulin (γ_L-globulin), in spite of evidence that the 7 S γ-globulin was present in smaller amounts than the γ_L-globulin.

The low molecular weight γ-globulin of urine consisted largely of material identical or similar to L chains, but some material partially identical to H chains of 7 S γ-globulin was consistently found. Evidence indicated that the latter finding was not due to contamination with whole γ-globulin. Absorption experiments with antisera showed that this portion of the H chain was essential for antibody activity of the γ_L fraction, since no activity was present when only the L chains were present.

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