

# TWO TYPES OF $\gamma$ -MYELOMA PROTEINS, $\beta_{2A}$ -MYELOMA PROTEINS, $\gamma_1$ -MACROGLOBULINS, AND BENCE JONES PROTEINS IDENTIFIED BY TWO GROUPS OF COMMON ANTIGENIC DETERMINANTS

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## TWO TYPES OF $\gamma$ -MYELOMA PROTEINS, $\beta_{2A}$ -MYELOMA PROTEINS, $\gamma_1$ -MACROGLOBULINS, AND BENCE JONES PROTEINS IDENTIFIED BY TWO GROUPS OF COMMON ANTIGENIC DETERMINANTS

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The serum myeloma proteins are characteristic products of malignant plasma cells, and individual myeloma proteins provide an important source of information on the protein-producing capacities (and limitations) of plasma cell clones. The globulins produced by malignant plasma cells are closely related to the normal  $\gamma$ -globulins, but immunochemical studies of the antigenic determinants present on myeloma proteins have uniformly shown that myeloma proteins are antigenically deficient when compared to normal  $\gamma$ -globulins (1-8). Similar studies (9-13) have shown that macroglobulinemic macroglobulins are antigenically deficient when compared to the normal  $\gamma_1$ -macroglobulins. Antigen deletion has been demonstrated to occur with malignant transformation (14), and antigenic deficiency of myeloma proteins could be viewed as reflecting a plasma cell change associated with malignancy. Alternatively, myeloma protein differences may simply reflect differences between normal  $\gamma$ -globulin molecules (and between normal plasma cells). Immunochemical studies have not settled this question, although marked variability in the antigenic determinants on individual myeloma proteins has been noted. A number of studies, however, have emphasized systematic differences among the proteins formed in malignant plasma cells. Such proteins are now identified as Bence Jones proteins,  $\gamma$ -myeloma proteins,  $\beta_{2A}$ -myeloma proteins, or  $\gamma_1$ -macroglobulins on the basis of specific immunochemical and physicochemical properties (15-18). Systematic differences of another type, however, may also occur within these four major groups. Two antigenically different groups of Bence Jones proteins have been identified in several laboratories (19-23). Two types of  $\gamma$ -myeloma proteins were identified by Korngold and Lipari (7), and Franklin (24) found two types

of  $\beta_{2A}$ -myeloma proteins. The molecular differences within major  $\gamma$ -globulin groups has received less attention than the  $\gamma$ -,  $\beta_{2A}$ -,  $\gamma_1$ -, and BJ-grouping, and the presence of two general subdivisions has not been established as a consistent feature of all of the anomalous  $\gamma$ -globulin groups.

In the course of immunochemical studies of  $\gamma$ -globulins, differences were observed in the antigenic composition of S pieces obtained by papain digestion of individual myeloma proteins (25, 26). In view of previous observations (16, 27, 28) that the antigenic determinants common to all  $\gamma$ -globulin groups were present in the S pieces from  $\gamma$ -globulin molecules and that Bence Jones proteins were antigenically related to the S pieces, studies were undertaken to determine whether all four classes of proteins formed in malignant plasma cells could be divided into two groups on the basis of readily identifiable antigenic differences.

Two forms of molecules (types I and II) differing in antigenic composition were identified in each anomalous protein group (29). Further studies demonstrated that two sets of antigenic determinants (types I and II) were common to all the  $\gamma$ -globulin groups, but that the relative frequency of types I and II molecules differed in the various anomalous  $\gamma$ -globulin groups. Molecules having types I and II antigenic determinants were compared to see whether other molecular parameters varied in association with two types of antigenic determinants. These observations form the basis of the present report.

### MATERIALS AND METHODS

**Proteins.** Twenty-six  $\gamma$ -myeloma proteins and ten  $\beta_{2A}$ -myeloma proteins were isolated by DEAE-cellulose chromatography, or by a combination of zone electrophoresis and chromatography of myeloma sera (30). Macro-

globulins were isolated from the serum of ten patients with primary (Waldenström's) macroglobulinemia by preparation of macroglobulin-rich serum fractions by repeated precipitation through dialysis against distilled water, by 35% ammonium sulfate precipitations, or by zone electrophoresis and subsequent purification by DEAE-cellulose chromatography or Sephadex G-200 filtration (31). Bence Jones proteins were prepared by DEAE-cellulose chromatography from urine concentrated by ultrafiltration, or by addition of 650 g of ammonium sulfate per L of urine to precipitate the urinary proteins. Bence Jones proteins were obtained from ten patients with no other anomalous protein, and from eleven patients with  $\gamma$ -myeloma protein, three with  $\beta_{2A}$ -myeloma proteins, and five with macroglobulinemia.

Each of the proteins was characterized by analytic ultracentrifugation in a Spinco model E ultracentrifuge, by paper electrophoresis and starch gel electrophoresis, and by immunoelectrophoresis using antiserum reacting with Bence Jones proteins, 6.6 S  $\gamma$ -globulins,  $\beta_{2A}$ -globulins, and  $\gamma_1$ -macroglobulins, as well as antiserum reacting specifically with each of the last three globulin groups (30, 32). Each of the proteins was classified on the basis of its immunochemical and ultracentrifugal properties (33). The  $\gamma$ -myeloma proteins sedimented as 6.6 S components in the ultracentrifuge. The  $\beta_{2A}$ -myeloma proteins frequently contained 9, 11, and 13 S components as well as 6.6 S components. The macroglobulins were composed of 18, 24, and 32 S components. Bence Jones proteins had sedimentation coefficients between 2.8 and 4.2 S, and had typical heat precipitation properties when tested by the methods of Putnam and associates (34). Hexose analyses were performed by a modification (35) of the thymol-sulfuric acid method of Shetlar and Masters (36).

**Immunochemical procedures.** Antisera prepared by immunization of rabbits, goats, horses, or sheep with whole normal  $\gamma$ -globulin, normal 6.6 S  $\gamma$ -globulin, normal 18 S  $\gamma_{1M}$ -globulins,  $\gamma$ -myeloma proteins,  $\beta_{2A}$ -myeloma proteins,  $\gamma_{1M}$ -macroglobulins, and Bence Jones proteins were tested for their capacity to detect type I and type II antigenic determinants. Most of the studies to be described utilized antisera prepared in rabbits by immunization with  $\gamma$ -globulins prepared by zone (block) electrophoresis of normal serum, with normal 6.6 S  $\gamma$ -globulins prepared by DEAE-cellulose chromatography, or with purified urinary Bence Jones proteins. Rabbit antiserum against type II Bence Jones proteins appeared to be specific for this type of protein, but the available rabbit anti-type I Bence Jones protein antisera were absorbed with type II Bence Jones protein or type II myeloma proteins to insure specificity. Specific anti-I and anti-II antisera also were prepared by absorption of rabbit anti-normal 6.6 S  $\gamma$ -globulin antiserum with type I or type II  $\gamma$ -myeloma proteins.

Purified proteins were compared by Ouchterlony tests at protein concentrations of 0.1 to 0.2 mg per ml (Bence Jones protein), 0.2 to 0.3 mg per ml (myeloma proteins), and 0.4 to 0.5 mg per ml ( $\gamma_1$ -macroglobulins) in  $3\frac{1}{2} \times 4$ -

inch plates of agar in barbital buffer of 0.045 ionic strength and pH 8.6 containing 0.01 M sodium azide. Immuno-electrophoresis was carried out in similar plates. Dark-field photographs were made at 24, or 48 hours, or both.

## RESULTS

*Two types of Bence Jones proteins, myeloma proteins, and macroglobulins.* Two types of Bence Jones proteins, differing in antigenic determinants, were readily seen on Ouchterlony agar double-diffusion tests (Figure 1A). The precipitin lines of Bence Jones proteins *a* and *d* intersected, indicating that the two proteins differ in antigenic determinants. A similar intersection is seen with the precipitin lines formed by Bence Jones proteins *c* and *f* (Figure 1A). Bence Jones proteins *a*, *b*, *c*, and others with similar antigenic determinants are termed type I. Bence Jones proteins *d*, *e*, *f*, and proteins with similar antigenic determinants are termed type II. The basis for this nomenclature is discussed below, but it may be noted here that type I corresponds to type B of Korngold and Lipari (22) and type II corresponds to their type A. The antigenic differences between types I and II Bence Jones proteins can also be demonstrated with specific antisera, as shown in Figure 1B, where precipitin lines are formed only between a Bence Jones protein and its corresponding specific antiserum, i.e., between type I Bence Jones protein and anti-I antiserum.

The antigenic differences shown in Figure 1 are type-specific and are not protein-specific. Individual proteins in each Bence Jones protein group are closely related antigenically, as shown by the precipitin lines formed by individual Bence Jones proteins that fuse (evidence for antigenic similarity) when tested against rabbit antinormal  $\gamma$ -globulin antiserum. This is seen in Figure 1 for three Bence Jones proteins of each type.

$\gamma$ -Myeloma proteins were divided into two groups on the basis of antigenic differences. As shown in Figure 2A, precipitin lines of two  $\gamma$ -myeloma proteins intersected, indicating that each had antigenic determinants not present on the other protein. These differences were revealed by a mixture of two antisera prepared against types I and II Bence Jones proteins, respectively. The distinctive antigenic determinants of each type of  $\gamma$ -myeloma protein were also identified by means of antisera specific for the type I or II anti-

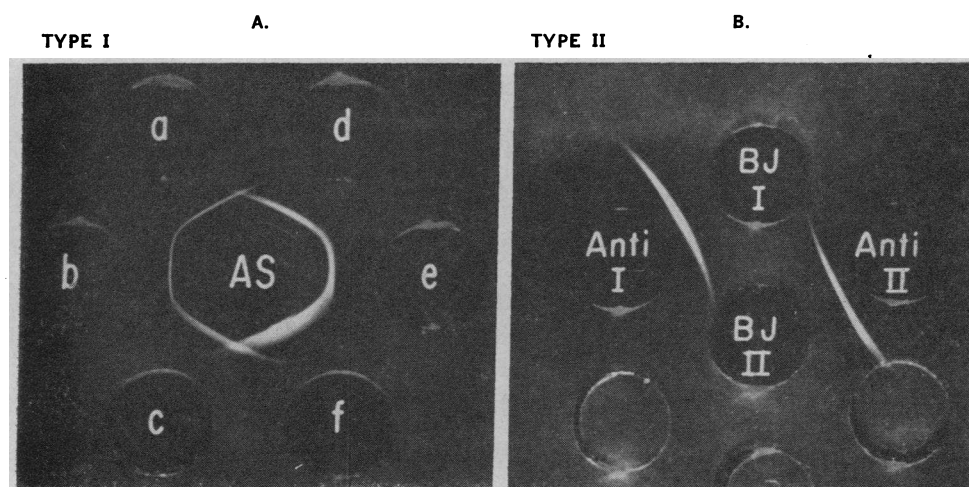


FIG. 1. A. TWO TYPES OF BENCE JONES PROTEINS. Purified Bence Jones proteins *a*, *b*, and *c* from patients (E.B., M.T., and G.Y.) have similar antigenic properties, as revealed by fusion of the precipitin lines formed in an Ouchterlony plate using rabbit antinormal human 6.6 S  $\gamma$ -globulin antiserum (AS). These were termed type I Bence Jones proteins. Another group of Bence Jones proteins *d*, *e*, and *f* from patients (Z.O., S.E., and R.F.) shared another set of antigenic determinants, and were designated type II Bence Jones proteins. The intersection of the precipitin lines formed by adjacent types I and II Bence Jones proteins (as between *a* and *d*) served to distinguish these two types of proteins. B. TWO TYPES OF BENCE JONES PROTEINS REVEALED BY TESTS WITH SPECIFIC ANTISERA. Type I Bence Jones protein J.H. (BJ I) and type II protein D.R. (BJ II) were tested against specific antisera prepared by absorption of rabbit antinormal  $\gamma$ -globulin antiserum with appropriate type I or II myeloma proteins. Similar results were obtained with antiserum prepared by immunization of rabbits with type I or II Bence Jones proteins and absorption of the rabbit serum, where necessary, with appropriate Bence Jones protein.

genic determinants. Such antisera were prepared by immunization of rabbits with type I or II Bence Jones proteins, or by absorption of rabbit antinormal 6.6 S  $\gamma$ -globulin antiserum with type I or II  $\gamma$ -myeloma proteins. As shown in Figure 2B, each  $\gamma$ -myeloma protein reacts only with the corresponding specific antiserum.

The two types of  $\gamma$ -myeloma proteins were compared with the two types of Bence Jones proteins (Figure 2C). Type I  $\gamma$ -myeloma proteins and type I Bence Jones proteins shared antigenic determinants (see fusion of precipitin lines in Figure 2C). Similar observations indicated that type II Bence Jones proteins and type II  $\gamma$ -myeloma proteins shared another set of antigenic determinants. These findings demonstrated that the two types of  $\gamma$ -myeloma proteins were antigenically related to the two types of Bence Jones proteins. Therefore, additional studies were undertaken to determine whether similar molecular types occurred in other  $\gamma$ -globulin classes.

Purified  $\beta_{2A}$ -myeloma proteins were found to

differ in antigenic determinants, since intersecting precipitin lines developed when appropriate proteins were compared (Figure 3A). The two types of  $\beta_{2A}$ -myeloma proteins were identified as type I or II molecules by means of Ouchterlony tests using specific anti-I or anti-II antiserum (Figure 3B).

Two types of macroglobulins were identified with similar reagents. Precipitin lines formed by the two types of  $\gamma_1$ -macroglobulins intersected (Figure 4A), indicating that each possessed antigenic determinants not present on the other protein. The  $\gamma_1$ -macroglobulins were identified as type I or II molecules, i.e., as proteins having either type I or II antigenic determinants, by Ouchterlony tests employing specific antisera (Figure 4B).

*Antigenic determinants of individual molecules.* The tests with specific antisera (Figures 1 to 4) revealed that individual anomalous proteins reacted with either specific anti-I or anti-II antiserum, but not with both. This was confirmed in studies of

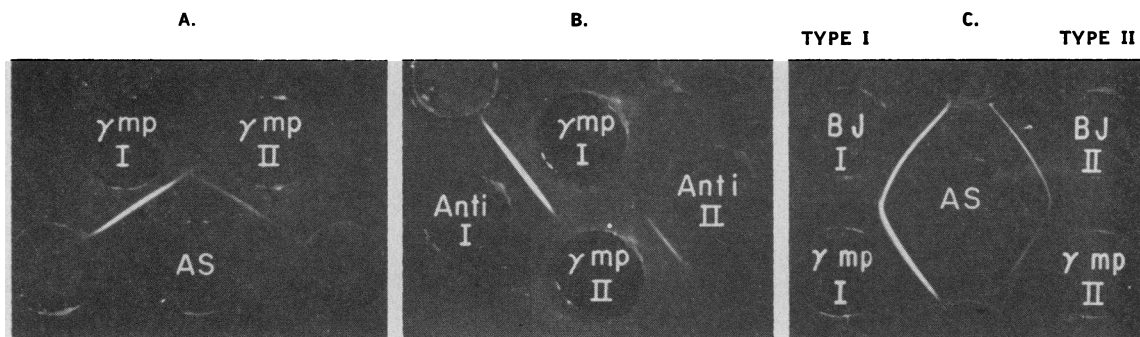


FIG. 2. A. TWO TYPES OF  $\gamma$ -MYELOMA PROTEINS. Intersection of the precipitin arcs occurred when  $\gamma$ -myeloma proteins from patients N.S. and T.N. were tested against pooled rabbit antisera (AS) prepared by immunization with types I and II Bence Jones proteins. B. TWO TYPES OF  $\gamma$ -MYELOMA PROTEINS REVEALED BY SPECIFIC ANTISERA. N.S. and T.N.  $\gamma$ -myeloma proteins tested against specific anti-I and anti-II antisera prepared as for Figure 1B. C. RELATION OF TWO TYPES OF  $\gamma$ -MYELOMA PROTEINS TO TWO TYPES OF BENCE JONES PROTEINS. A type I Bence Jones protein J.H. (BJ I) was seen to share common antigenic determinants with  $\gamma$ -myeloma protein N.S. ( $\gamma$ mp I), as revealed by fusion of the BJ I precipitin line with the precipitin line formed by the  $\gamma$ -myeloma protein when tested against rabbit antinormal human  $\gamma$ -globulin S fragment antisera (AS). Similarly, the precipitin line formed by type II Bence Jones protein L.E. fused with that formed by  $\gamma$ -myeloma protein S.J. ( $\gamma$ mp II), indicating a close antigenic relationship between these types of Bence Jones proteins and  $\gamma$ -myeloma proteins.

74 purified proteins, including 28 Bence Jones proteins, 26  $\gamma$ -myeloma proteins, 10  $\beta_{2A}$ -myeloma proteins, and 10  $\gamma_1$ -macroglobulins. Evidently, each anomalous protein has only type I or II antigenic determinants, but not both types.

*Antigenic determinants common to all  $\gamma$ -globulin classes.* The observations made with specific antiserum indicated that types I and II antigenic determinants were represented in each of the anomalous  $\gamma$ -globulin classes, i.e., among the Bence Jones proteins,  $\gamma$ -myeloma proteins,  $\beta_{2A}$ -

myeloma proteins, and  $\gamma_1$ -macroglobulins. In a more direct comparison, representative proteins from each class were tested by the Ouchterlony double-diffusion techniques (Figure 5). When tested with specific antisera (Figure 5), the precipitin line formed by each type I protein fused with that of the adjacent protein. This evidence indicates that type I proteins from all  $\gamma$ -globulin classes share common antigenic determinants. Similarly, type II proteins of all four  $\gamma$ -globulin classes share another set of antigenic determinants

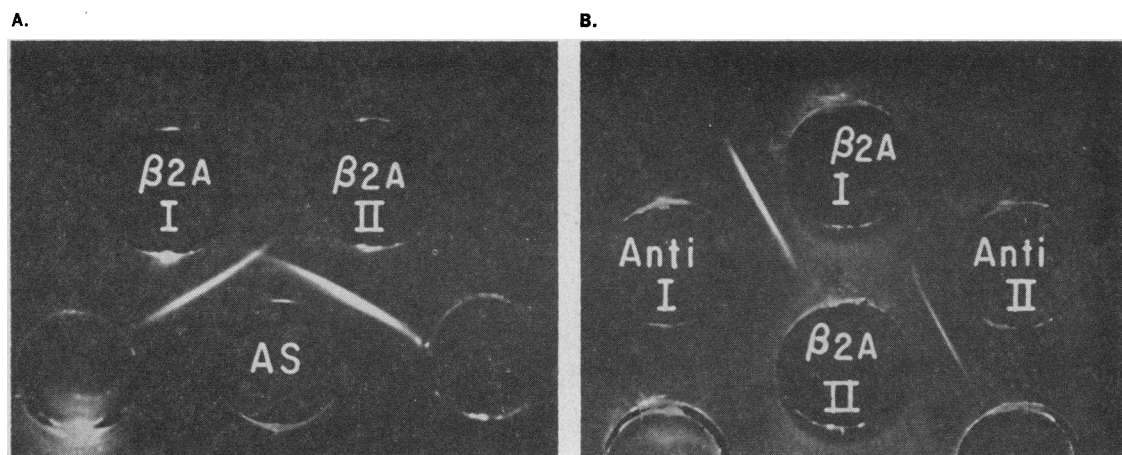


FIG. 3. A. TWO TYPES OF  $\beta_{2A}$ -MYELOMA PROTEINS. Purified  $\beta_{2A}$ -myeloma proteins from patients J.B. ( $\beta_{2A}$  I) and W.H. ( $\beta_{2A}$  II) were compared using rabbit antinormal  $\gamma$ -globulin antiserum (AS). B. TWO TYPES OF  $\beta_{2A}$ -MYELOMA PROTEINS REVEALED BY TESTS WITH SPECIFIC ANTISERA. Purified proteins from W.J. and G.F. were tested against specific anti-I and specific anti-II antisera as in Figure 1B.

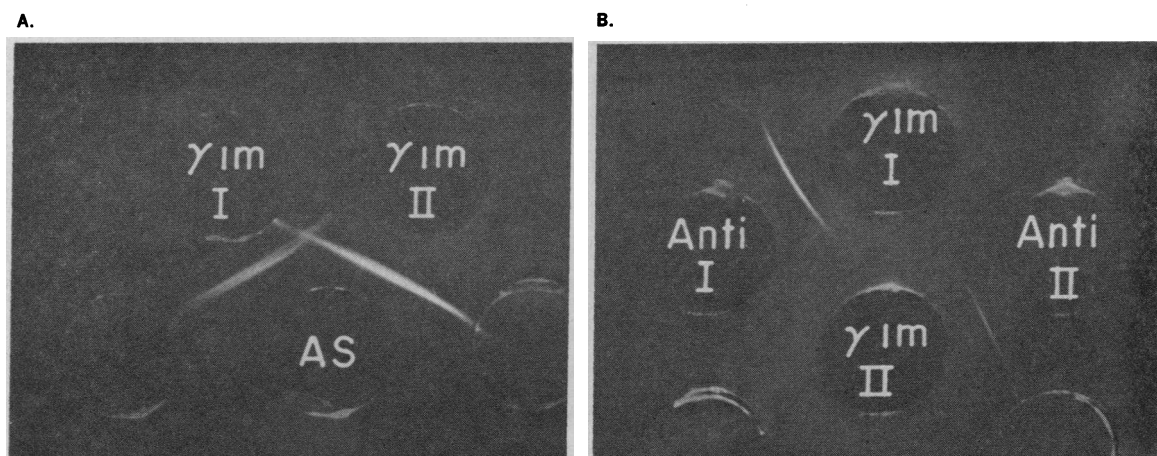


FIG. 4. A. TWO TYPES OF  $\gamma_1$ -MACROGLOBULINS. Purified  $\gamma_1$ -macroglobulins from patients with Waldenström's macroglobulinemia, J.B. ( $\gamma_{1m}$  I) and F.R. ( $\gamma_{1m}$  II), were compared using rabbit antinormal  $\gamma$ -globulin antiserum (AS). B. ANTIGENIC DIFFERENCES IN TYPE I AND TYPE II MACROGLOBULINS REVEALED BY SPECIFIC ANTISERA. Purified proteins from J.W. (I) and F.R. (II) were used in the test.

(see fusion of adjacent precipitin lines when tested with specific anti-II antiserum, Figure 5).

**Properties of type I and II molecules.** The electrophoretic mobility of the 122 types I and II proteins is graphically illustrated in Figure 6. Types I and II molecules are found to extend throughout the entire electrophoretic range from the slow  $\gamma$ -globulin region to the  $\alpha$ -globulin region. Although the anomalous  $\gamma$ -globulin groups differed in range of mobility—i.e.,  $\beta_{2A}$ -globulins generally migrated faster than  $\gamma$ -myeloma proteins—the types I and II molecules of each group were found to have approximately the same electrophoretic distribution.

Ultracentrifugal studies revealed that the types I and II Bence Jones proteins had approximately the same sedimentation coefficients (Table I). Types I and II  $\gamma$ -myeloma proteins sedimented at about 7 S. Both types I and II  $\beta_{2A}$ -myeloma proteins usually were composed of 6.6, 9, 11, and 13 S components.  $\beta_{2A}$ -Myeloma proteins having primarily 9 S components were represented among the proteins of each type.

Starch gel electrophoresis showed that the Bence Jones proteins were heterogeneous, but the relative number of components did not correlate with the Bence Jones protein type.  $\gamma$ -Myeloma proteins may appear homogeneous, or show electro-

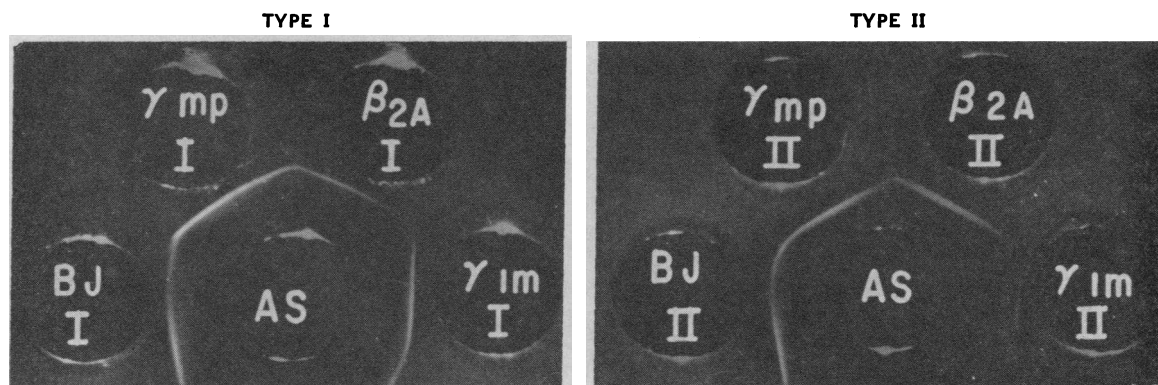


FIG. 5. COMPARISON OF TYPE I AND TYPE II PROTEINS FROM SEVERAL  $\gamma$ -GLOBULIN CLASSES. Bence Jones proteins,  $\gamma$ -myeloma proteins,  $\beta_{2A}$ -myeloma proteins, and  $\gamma_1$ -macroglobulins from J.H., R.P., W.J., and J.B., respectively, were used as type I proteins; those from E.L., T.N., G.F., and F.R. were used as type II proteins. Specific anti-I and anti-II antisera prepared from rabbit antinormal  $\gamma$ -globulin antiserum were used (AS).

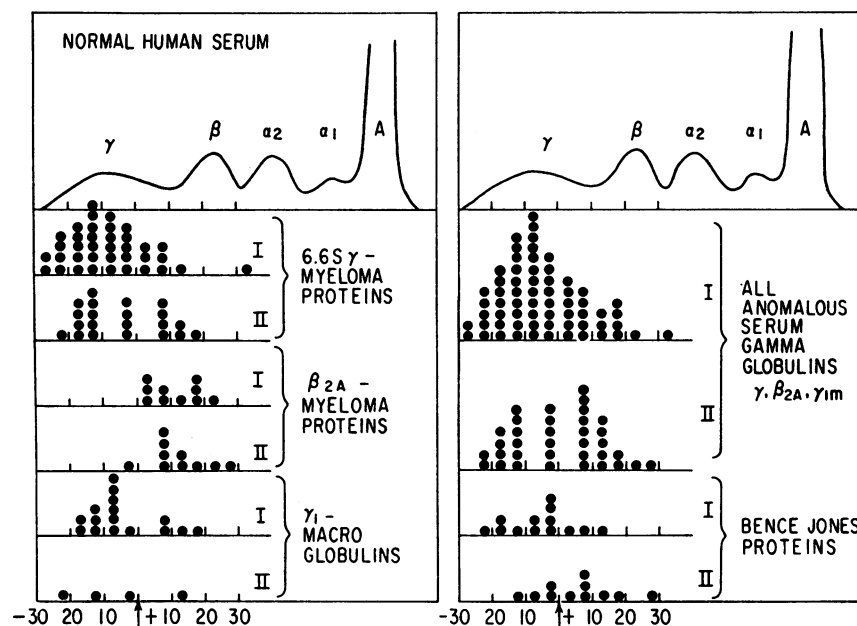


FIG. 6. ELECTROPHORETIC DISTRIBUTION OF TYPE I AND TYPE II MYELOMA PROTEINS, MACROGLOBULINS, AND BENCE JONES PROTEINS. Electrophoretic mobility was determined by zone electrophoresis of serum, urine, or purified proteins on filter paper strips with the Durrum-Spinco apparatus and barbital buffer at pH 8.6, ionic strength 0.09. Mobility values are plotted in relation to the point of sample application, and a normal serum electrophoretic pattern is included for orientation.

TABLE I  
Some physicochemical properties of type I and type II molecules\*

Type of protein†	Ultracentrifugation† <i>S</i> (20, <i>w</i> )	Electrophoretic mobility	Starch gel electrophoretic pattern	Hexose
Bence Jones proteins	<i>S</i>	<i>mm from origin on paper</i>		<i>% of protein</i>
I	3.71 [11]	− 3 [13]	100% } Heterogeneity	
II	3.71 [10]	+ 7 [10]	100% }	
γ-Myeloma proteins				
I	6.96 [8]	−10 [36]	63% [43] } Electrophoretic	1.5 [8]
II	7.04 [2]	− 5 [21]	68% [19] } heterogeneity‡	1.6 [7]
β <sub>2A</sub> -Myeloma proteins				
I	6.6, 9, 11, 13 [10]	+12 [10]	70% [10] } Polymeric	3.7 [4]
II	6.6, 9, 11, 13 [11]	+11 [10]	82% [11] } heterogeneity‡	3.0 [2]
γ <sub>1</sub> -Macroglobulins				
I	18, 24, 32 [17]	−10 [16]	25% [ 4] } Anomalous protein	6.1 [4]
II	18, 24, 32 [ 4]	−10 [ 4]	12% [17] } band in gel	6.0 [1]

\* On naming of types, see Discussion. Numbers in brackets indicate number of samples tested.

† Analysis of isolated Bence Jones proteins and  $\gamma$ -myeloma proteins and of whole serum for  $\beta_{2A}$ -myeloma proteins and  $\gamma_1$ -macroglobulins. Mean values are recorded.

‡ Starch gel electrophoretic patterns indicating heterogeneity on the basis of multiple components differing in net electrical charge (electrophoretic heterogeneity) or in size (polymer-type heterogeneity) have been described in detail (33).



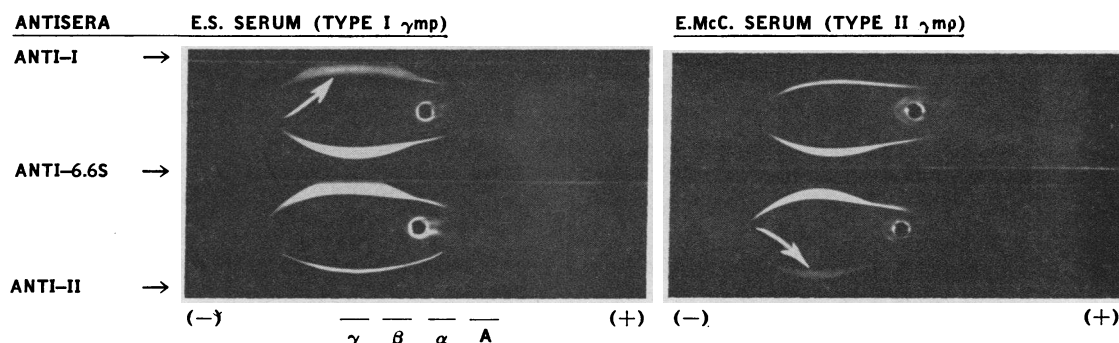


FIG. 7. IDENTIFICATION OF TYPES I AND II MYELOMA PROTEINS BY IMMUNOELECTROPHORESIS OF WHOLE SERUM. Serum from patient E.S. was used in the first set of immunoelectrophoretic analyses, and serum from E.McC. in the second. After electrophoresis in agar, specific anti-I antiserum was added to the upper trough, specific anti-6.6 S  $\gamma$ -globulin antiserum to the middle trough, and specific anti-II antiserum to the lower trough. The arrows indicate the anomalous protein revealed by specific anti-I and anti-II antisera. As may be seen in the myeloma sera, the normal  $\gamma$ -globulins react with both antisera, but the characteristic bowing of the myeloma globulin is found only with homologous antiserum.

phoretic heterogeneity on starch gel electrophoresis (33). Both homogeneous and heterogeneous  $\gamma$ -myeloma proteins were represented within each type. Sixty-three per cent of type I  $\gamma$ -myeloma proteins showed electrophoretic heterogeneity, as did 68% of the type II  $\gamma$ -myeloma proteins.

The hexose content of purified protein was measured (Table I). Types I and II  $\gamma$ -myeloma proteins had similar hexose contents, i.e., about 1.5%. A wider range of hexose values (2 to 4.5%) was found with  $\beta_{2A}$ -myeloma proteins, but no significant difference was noted between the two types of  $\beta_{2A}$ -globulins. Both types I and II  $\gamma_1$ -macroglobulin contained approximately 6% hexose.

*Relative frequency of types I and II molecules.* Identification of anomalous types I and II proteins in serum and urine was facilitated by immunoelectrophoretic procedures using specific anti-I or anti-II antiserum in the antibody troughs. Typical results for two myeloma sera are shown in Figure 7. The E.S. serum contains a 6.6 S  $\gamma$ -myeloma protein (intense precipitin arc with specific anti- $\gamma$ -globulin antiserum in center trough) that reacts with specific anti-I antiserum (upper trough), but not with anti-II antiserum (lower trough), identifying the protein as a type I  $\gamma$ -myeloma protein. The E.McC. serum also contains a  $\gamma$ -myeloma protein which, in contrast, fails to react with anti-I antiserum, but does react with spe-

cific anti-II antiserum, i.e., a type II  $\gamma$ -myeloma protein.

Serum and urine proteins from 120 patients with multiple myeloma or macroglobulinemia were examined to determine the relative frequency of molecules having the types I and II antigenic determinants (Table II). Seventy-five purified proteins, representing all four classes of  $\gamma$ -globulins, were tested by Ouchterlony analysis (Figures 1 to 4), and additional sera or urine were tested by immunoelectrophoresis. The frequency of type I molecules was 82% among the  $\gamma_1$ -macroglobulins, 67% among 6.6 S  $\gamma$ -myeloma proteins, 53% for Bence Jones proteins, and 50% for the  $\beta_{2A}$ -myeloma proteins. The difference between the frequency of type I protein occurrence in the 6.6 S  $\gamma$ -myeloma protein group and in other anomalous protein groups was not statistically significant ( $p > 0.05$  in each case).

TABLE II  
Frequency of type I and type II molecules in multiple myeloma and macroglobulinemia\*

Proteins	Purified proteins		Whole serum		Total percentage	
	I	II	I	II	I	II
6.6 S $\gamma$ -Myeloma proteins [66]	19	7	25	15	67	33
$\beta_{2A}$ -Myeloma proteins [22]	4	6	7	5	50	50
$\gamma_1$ -Macroglobulins [22]	9	1	9	3	82	18
Bence Jones proteins [30]	16	12		2	53	47

\* Numbers in brackets indicate the number of samples tested. On naming of types, see Discussion.



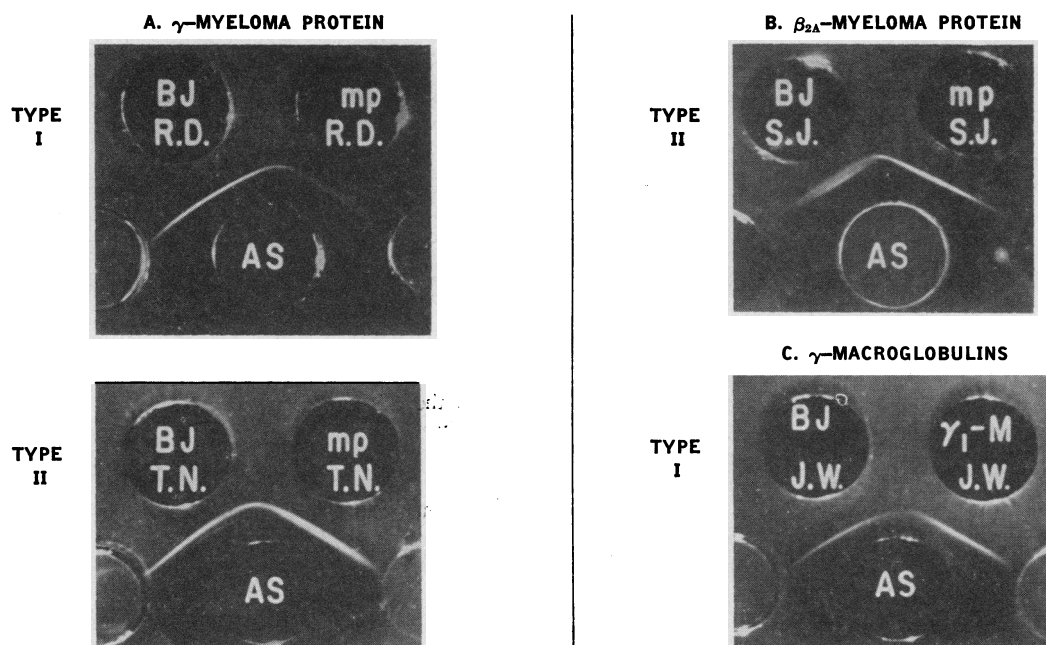


FIG. 8. COMPARISON OF BENCE JONES PROTEIN AND ANOMALOUS SERUM GLOBULIN OF THE SAME PATIENT. Ouchterlony tests were conducted with specific antisera, and only positive results are shown, as with R.D.'s Bence Jones protein and  $\gamma$ -myeloma protein, which reacted only with specific anti-I antiserum (AS) and not with anti-II antiserum.

*Bence Jones protein and anomalous globulin in the same patient.* Both urinary Bence Jones protein and an anomalous serum protein were available from 20 patients. Ouchterlony tests, as shown in Figure 8, showed fusion of the precipitin lines formed by the two anomalous proteins in each case, indicating that the two anomalous proteins from each patient were of the same type (I

or II). The anomalous serum proteins, tested against Bence Jones proteins, include 6.6 S  $\gamma$ -myeloma proteins,  $\beta_{2A}$ -myeloma proteins, and  $\gamma_1$ -macroglobulins of types I and II, as shown in Table III.

#### DISCUSSION

The demonstration of two separate groups of antigenic determinants, common to all  $\alpha$ -globulin groups—i.e., in the  $\gamma$ -myeloma proteins,  $\beta_{2A}$ -myeloma proteins,  $\gamma$ -macroglobulins, and Bence Jones proteins—, which permits a division of each group into two types, confirms and extends the observations of Korngold and Lipari (7), who identified three types of myeloma proteins on the basis of differences in antigenic determinants and designated them as types I, II, and III. Subsequently, myeloma proteins of type III were identified as  $\beta_{2A}$ -myeloma proteins (18). Both types I and II myeloma proteins of Korngold and Lipari (7), however, were closely related antigenically to normal  $\gamma_2$ -globulins. Both types shared some common antigenic determinants, but each type had antigenic determinants not present in the other group. Our own observations on the two groups of

TABLE III  
*Relationship of Bence Jones protein to anomalous globulin in the same patient*

Anomalous serum protein type*	Urinary Bence Jones protein type	
	I	II
$\gamma$ -Myeloma proteins		
I	6	0
II	0	5
$\beta_{2A}$ -Myeloma proteins		
I	2	0
II	0	1
$\gamma_1$ -Macroglobulins		
I	5	0
II	0	1

\* On naming of types, see Discussion.

$\gamma$ -myeloma proteins agree with those of Korngold and Lipari (7), and we have used the same terminology, i.e., types I and II, for the specific antigenic determinants distinguishing the two types of  $\gamma$ -myeloma proteins.

Identifications of two antigenically distinct types of Bence Jones protein also agree with those of Korngold and Lipari (22), whose type B is equivalent to our type I and whose type A is the same as our type II. The present studies and the recent studies of Männik and Kunkel (37), however, extend the earlier work of Korngold and Lipari (22) and Franklin (24) to show that both groups of distinctive antigenic determinants are represented in all classes of anomalous proteins. Migita and Putnam (38) also identified types I and II  $\gamma$ -myeloma proteins on the basis of antigenic determinants common to one of the two types of Bence Jones proteins. These authors (38) recognized that  $\beta_{2A}$ -myeloma proteins and  $\gamma$ -macroglobulins also had limited antigenic composition, but the proteins in these latter two classes available to them were all type I globulins.

We have referred to the two groups of antigenic determinants common to all  $\gamma$ -globulin groups as types I and II antigenic determinants, in accord with Korngold and Lipari's terminology for  $\gamma$ -myeloma proteins (7), and (because molecules with type I antigenic determinants occur more frequently) have used the same designation for all  $\gamma$ -globulin groups, i.e., in the Bence Jones proteins,  $\gamma$ -myeloma proteins,  $\beta_{2A}$ -myeloma proteins, and  $\gamma_1$ -macroglobulins.

Many of the observations reported here emphasize the antigenic differences between types I and II  $\gamma$ -myeloma proteins. These two types of  $\gamma$ -myeloma proteins also share common antigenic determinants, i.e., those characteristic of 6.6 S  $\gamma$ -globulins. Similarly, types I and II  $\beta_{2A}$ -myeloma proteins share antigenic determinants characteristic of  $\beta_{2A}$ -globulins, and types I and II  $\gamma_1$ -macroglobulins share the specific determinants of  $\gamma_{1M}$ -globulins.

The presence of 6.6 S  $\gamma$  antigenic determinants on  $\gamma$ -globulins complicated the demonstration of types I and II molecule differences. Antisera reacting with 6.6 S  $\gamma$  antigenic determinants and with types I and II determinants often showed spur formation of the precipitin lines formed by types I and II myeloma proteins

in adjacent wells on Ouchterlony plates, but did not readily distinguish the complete separateness of the types I and II antigenic determinants. Antisera reacting specifically with type I or II antigenic determinants were obtained by immunization of rabbits with purified type I or II Bence Jones proteins. For example, rabbit antisera prepared against type II Bence Jones proteins reacted with the type II antigenic determinants in all  $\gamma$ -globulin groups, but did not react (to form precipitin lines) with type I proteins. Specific antisera could also be prepared by using type I or II  $\gamma$ -myeloma proteins to absorb a potent rabbit antinormal  $\gamma$ -globulin antiserum. These absorbed antisera reacted with type I or II antigenic determinants in all  $\gamma$  groups (6.6 S  $\gamma$ -,  $\beta_{2A}$ -, and  $\gamma_{1M}$ -globulins, and Bence Jones proteins). The number of antigenic sites detected by specific anti-I or anti-II antisera has not been determined. Precipitin reactions formed readily, however, as is consistent with the presence of two or more determinants on the antigenic molecule, and the types I and II antigenic determinants may represent two groups of individual antigenic determinants.

The type I antigenic determinants reflect one structural configuration, and the type II determinants, a different configuration of these  $\gamma$ -globulins. Further studies (37-39) have shown that the types I and II antigenic determinants are present on S pieces (obtained by papain digestion) and "L" polypeptide chains (produced by reduction and alkylation) of the  $\gamma$ -globulin molecules. The F pieces and "H" chains do not have the types I and II antigenic determinants, but appear to account for the  $\gamma$ -,  $\beta_{2A}$ -, or  $\gamma_{1M}$ -specific properties of the anomalous proteins. Therefore, the myeloma protein (or other anomalous protein) may be classified on the basis of immunochemical tests that detect specific properties in two parts of the molecule. For example, a type I  $\gamma$ -myeloma protein contains the structural configurations (and polypeptide chain) characteristic of 6.6 S  $\gamma$ -globulins in one part of the molecule, while another molecular part contains a type I polypeptide chain, i.e., a polypeptide with type I antigenic determinants. The type II  $\gamma$ -myeloma protein differs in having a type II (instead of a type I) polypeptide chain, but is similar in the  $\gamma$ -globulin-specific part of the molecule. A type II  $\beta_{2A}$ -myeloma protein

is similar to the type II  $\gamma$ -myeloma protein in the common type of polypeptide chain, but differs in having a  $\beta_{2A}$ -globulin-specific polypeptide in another part of the molecule (39).

The wide range of electrophoretic mobilities encountered for both types I and II molecules indicates that the molecular configurations responsible for the two sets of antigenic determinants probably have no relation to the molecular differences responsible for electrophoretic mobility. Similarly, the hexose content, ultracentrifugal behavior, and presence or absence of electrophoretic heterogeneity (as revealed by starch gel electrophoresis) or polymer-type heterogeneity (as seen on ultracentrifugation or starch gel electrophoresis) (33) were not related to the type I or II antigenic determinants, nor, presumably, to molecular pieces represented by these two sets of antigenic determinants. These observations on the physicochemical properties of types I and II proteins support the view that the types I and II antigenic determinants are segregated in a similar part of the molecule.

Because all the anomalous proteins formed in malignant plasma cells had type I or II antigenic determinants, we may wonder whether these determinants are present as well in the normal  $\gamma$ -globulins, or whether they reflect abnormalities of malignant plasma cell metabolism. Two observations indicate that types I and II antigenic determinants are present among normal  $\gamma$ -globulins: antiserum prepared against normal 6.6 S  $\gamma$ -globulins contains antibodies against types I and II antigenic determinants (see above); and two types of molecules have been identified among normal 6.6 S  $\gamma$ -globulins, 18 S  $\gamma_1$ -macroglobulins (40) and  $\beta_{2A}$ -globulins (24). Thus, neither type I nor type II antigenic determinants represent abnormalities peculiar to products of malignant plasma cell metabolism, but rather, reflect a selection from among the antigenic determinants present in the heterogeneous normal  $\gamma$ -globulin population.

That all of 140 anomalous proteins contained either type I or type II antigenic determinants indicates that individual clones of plasma cells synthesize either type I or II antigenic determinants. Observations in 20 patients with both Bence Jones proteins and an anomalous serum globulin, showing that both proteins were of the

same antigenic type (I or II) in each case, support the view that malignant plasma cell tumors (clones) are limited to only one antigenic form of  $\gamma$ -globulin.

The possibility that the Bence Jones protein represents a  $\gamma$ -globulin subunit, identical with one of the subunits composing the larger myeloma protein molecule, is emphasized by the demonstration that the type I or II antigenic determinants on Bence Jones proteins are identical with the determinants present in the corresponding myeloma protein. This possibility, indicated by immunochemical studies (8, 22, 23, 27, 28, 37, 38, 41-44) and strengthened by the present observations, also has been supported by the physicochemical studies of Poulik and Edelman (45) and Edelman and Gally (46) demonstrating electrophoretic and other similarities between the Bence Jones protein and the "L" chain of myeloma protein from the same patients.

Tumors forming  $\gamma$ -myeloma proteins produce type I molecules more frequently than type II proteins. A possible explanation for the 2:1 ratio of type I to type II  $\gamma$ -myeloma tumors would be random malignant transformation in a heterogeneous normal plasma cell population in which type I  $\gamma$ -globulin-producing plasma cells outnumbered type II cells by a ratio of 2:1. Alternatively, type I protein-producing cells might be more susceptible to malignant transformation, or individual normal plasma cells might have the dual potentiality of forming types I and II molecules, and might lose their type II synthetic capacity more frequently on malignant transformation. It is noteworthy, moreover, that the anomalous protein in other  $\gamma$ -globulin systems (e.g.,  $\gamma_1$ -macroglobulins,  $\beta_{2A}$ -myeloma proteins, and Bence Jones proteins) also occur in type I:II ratios not significantly different from the 2:1 ratio among  $\gamma$ -myeloma proteins. Type I molecules also predominate among the normal serum  $\gamma$ -globulins (40), indicating that the predominance of type I  $\gamma$ -myeloma proteins is not a peculiarity of malignant plasma cells. This finding is also compatible with the view that malignant transformation occurs in a heterogeneous normal plasma cell population.

The selective increase of type I or II  $\gamma$ -globulin molecules is characteristic of multiple myeloma. Studies are under way to determine the diagnostic

value of immunochemical tests for discrete type I or II globulin increases in the recognition of multiple myeloma and macroglobulinemia.

#### SUMMARY

Two groups of 6.6 S  $\gamma$ -myeloma proteins, differing in antigenic composition, are described. About 67% of the  $\gamma$ -myeloma proteins have type I antigenic determinants; the remaining 33% have type II. The electrophoretic distribution, ultracentrifugal properties, hexose content, and proportion of homogeneous and heterogeneous myeloma proteins are similar for types I and II  $\gamma$ -myeloma proteins.

Two antigenically different types of  $\beta_{2A}$ -myeloma proteins,  $\gamma_1$ -macroglobulins, and Bence Jones proteins are also described.

The antigenic determinants forming the basis for identification of type I molecules appear to be the same in all  $\gamma$ -globulin classes, i.e., among the Bence Jones proteins,  $\gamma$ -myeloma proteins,  $\beta_{2A}$ -myeloma proteins, and  $\gamma_1$ -macroglobulins. Similarly, the type II proteins from each  $\gamma$ -globulin class share another set of common antigenic determinants.

One hundred forty myeloma proteins, macroglobulins, and Bence Jones proteins were examined and found to have either type I or type II antigenic determinants; no molecules had both types. When the urinary Bence Jones protein and the anomalous serum protein were compared in 19 cases, the two proteins were found to be of the same antigenic type. This evidence indicates that clones of malignant plasma cells produce either type I or type II molecules, but are unable to form both.

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