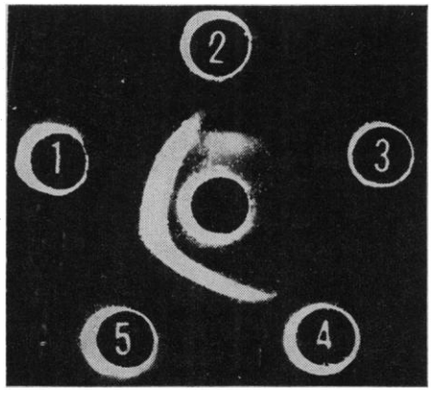

Fig. 2. Immunodiffusion. Comparison of group 2 Bence Jones proteins and autologous myeloma proteins. Well No. 1, Bence Jones protein 2–20; No. 2, Bence Jones protein 2–24; No. 3, γ_2-myeloma protein 2–24; No. 4, normal γ-globulin; No. 5, γ_2-myeloma protein 2–20. The center well contained a specific group 2 Bence Jones (2–20) antibody, absorbed with 5 mg normal serum and 2 mg of γ-globulin per milliliter. Protein concentration in the antigen wells was 0.1 percent.

ancy between BJ 2–24 and its corresponding myeloma protein was detected when a group-specific antibody directed against the Bence Jones protein of another patient was used. It is not clear whether other antibody systems would detect this apparent deficiency of the myeloma protein.

Some hereditary characteristics of γ-globulin molecules have been localized to specific polypeptide chains of the parent molecule. Certain of these characteristics, the Inv factors, have been localized to L-chains and in addition have been identified on some Bence Jones proteins (*10*). Franklin *et al.* (*11*) have previously reported that some papain-split A and C fragments may possess greater degrees of Inv activity than are present in the parent γ-globulin molecule. The fact that these subunits contain genetic information in greater quantities than the parent molecules raises the possibility whether there is partial masking of the Inv activity in the parent γ-globulin molecule which is unmasked when A and C fragments or L-chains are split out. It is possible that hidden antigenic sites made available when L-chains are cleaved from normal γ-globulin may in some cases be related to these Inv determinants. If this is true, we may expect to find Inv-positive Bence Jones proteins and Inv-negative myeloma proteins in the same patient. Indeed, Harboe *et al.* (*12*) reported a patient with an Inv-negative myeloma protein and an Inv-positive Bence Jones protein. There may also be greater degrees of Inv activity on some Bence Jones proteins when compared to the Inv-positive autologous myeloma protein.

The fact that hidden antigenic determinants on normal L-chains react with Bence Jones antibodies suggests that similar hidden sites are present on the excreted Bence Jones protein. It is of interest that one Bence Jones protein tested had antigenic determinants which were absent in the myeloma protein of the same patient. It is possible that this Bence Jones protein contains antigenic determinants which are inaccessible or hidden when it is fully incorporated into the myeloma protein as L-chains. The possibility that the Bence Jones protein in this case differs structurally from the autologous myeloma protein has not, however, been ruled out. Mannik and Kunkel have previously reported a patient with an immunologically dissimilar Bence Jones and myeloma protein (*1*). The two proteins in our case were immunologically both group 2.

Thus at least a part of the immunologic difference noted between Bence Jones proteins and normal pooled γ-globulin may be related to the unmasking of hidden antigenic determinants of L-chains when they are either not incorporated into or cleaved from the parent γ-globulin molecule. These hidden sites are present on Bence Jones proteins and on normal L-chains.

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Gamma Globulin Antigenic Types Defined by Heavy Chain Determinants

Abstract. *Two populations of immunologically distinguishable 7S gamma globulins in normal human serum and two corresponding antigenic types of myeloma 7S gamma globulins have been detected with rabbit antiserums to proteins associated with pathological conditions, the differences being related to the H-chains of 7S gamma globulin. No relationship exists with type I and type II antigenic classification, determined by L-chains. Human serums with various hereditary gamma globulin (Gm) specificities contain both types of 7S gamma globulin.*

The 7*S* γ-globulin molecule consists of two pairs of polypeptide chains, designated in the human as L- (light) and H- (heavy) chains. Both normal and myeloma 7*S* γ-globulins of man have recently been divided into two molecular classes on the basis of antigenic and structural characteristics of the L-chains and their counterparts, the urinary Bence Jones proteins (*1*). Hitherto, the only evidence for differences in the H-chains (*2*) has been in the nature of the Gm γ-globulin genetic factors localized in these chains (*3*). In the present work two new immunological types of normal and pathological 7*S* γ-globulins have been defined through determinants localized in the H-chains. The classification is done through use of antiserums prepared against the recently discovered proteins excreted in a lymphoma-like disorder described by Franklin *et al.* (*4*). In this disease the abnormal proteins are related to the H-chain of 7*S* γ-globulin. They are of low molecular weight (53,000), are antigenically and structurally similar to the papain-produced F (fast) fragment of 7*S* γ-globulin, and are wholly dissimilar to Bence Jones proteins (*5*).

The proteins were isolated by precipitation with ammonium sulfate from the urines of the patient Cr described by Franklin *et al.* (*4*), and of the patient Zu described by Osserman and Takatsuki (*6*). Comparative mapping of the peptides of these two proteins and of the F (fast) fragment of 7*S* γ-globulin demonstrated the presence of some twenty peptides in common; there were also differences between the Cr- and Zu-protein. However, these proteins were similar both in sedimenta-

tion coefficient (3.8*S*) and amino acid content.

The Cr- and Zu-proteins were further purified by ion-exchange chromatography on diethylaminoethyl-Sephadex, and rabbit antiserums were produced against the purified Cr-protein and the purified Zu-protein. The antiserums showed a partial antigenic difference between these proteins (Fig. 1*a*) and reacted with 7*S* γ-globulin but not with β_{2A}- or β_{2M}-globulin. After absorption of the antiserum to Cr with the Zu-protein, the absorbed antiserum still reacted with the Cr-protein and also with normal 7*S* γ-globulin, but not with the Zu-protein. Likewise when antiserum to Zu was absorbed with the Cr-protein, the absorbed antiserum reacted with Zu and 7*S* γ-globulin but not with Cr-protein. These results indicate that the Cr- and Zu-proteins share a common moiety, but that in addition each has at least one specific antigenic determinant that is also present in normal 7*S* γ-globulin.

In immunodiffusion experiments the antiserum to Cr produced one line with normal 7*S* γ-globulin or with normal human serum, whereas two precipitation lines were formed by the antiserum to Zu (Fig. 1*b*). The line close to the antigen well (HS) gave a reaction of identity with the Zu-protein (Fig. 1*b*); hence, this line corresponded to γ-globulin molecules bearing determinants specific for Zu. From experiments with antiserum to Cr, absorbed with the Zu-protein, it was found that the other precipitation line was due to γ-globulin molecules with determinants specific for Cr. The antigens taking part in these reactions were both detected in the γ-globulin region by immunoelectrophoresis (Fig. 1, *c* and *f*).

The relative position of the two precipitation lines obtained in the reaction of antiserum to Zu with normal human serum indicated that, in human serum, two populations of 7*S* γ-globulin exist which share some antigenic determinants. The major population possesses determinants specific for the Cr-protein. The minor population lacks the determinants for Cr and has determinants specific for Zu.

This assumption was supported by two other findings. One of these is the formation of only one precipitation line between normal human serum and antiserum to Cr (Fig. 1*b*). This occurs because the concentration necessary for precipitation is obtained closer to the antiserum well for the Cr-type molecules (which represent the major population of 7*S* γ-globulin molecules) than for the Zu-type molecules. Hence, the Cr-type molecules bind both the Cr-specific antibodies as well as the antibodies against the common determinants. This prevents the formation of a second precipitation line with the Zu-type molecules. Since the latter type of molecules forms a minor population, it is clear that a second precipitation line will be formed between normal human serum and antiserum to Zu.

The second support was found by antigenic typing of the abnormal 7*S* γ-globulins in the serums of patients with multiple myeloma. Since myeloma proteins have their physiological counterparts in the γ-globulin system of normal human serum, forty-nine 7*S* γ-globulins from myeloma patients were tested by immunodiffusion and immunoelectrophoresis for the presence of specific Cr- or Zu-determinants. In four of these serums the abnormal protein turned out to be of the Zu-type; in the remaining forty-five, the abnormal protein was of the Cr-type (Fig. 1, *d*, *e*, *g*, and *h*). This frequency ratio is in accord with the assumed presence of a relatively small number of Zu-type γ-globulin molecules in normal human serum. Six of the Cr-type myeloma globulins in this series lacked one or more of the Cr-specific antigenic determinants as indicated by spur-formation in immunodiffusion. Whether these proteins represent a subgroup is under study. As would be predicted, the only serums to give two lines in immunodiffusion experiments with antiserum to Cr were serums containing myeloma 7*S* γ-globulin of the Zu-type.

The location of the determinants in this classification was studied by immunodiffusion experiments of papain-digested 7*S* γ-globulins. This enzyme splits the γ-globulin molecule into two fragments. According to the electrophoretic mobilities, these pieces are named F (fast) fragment and S (slow) fragment, respectively. The F fragment consists of a large part of the H-chain,

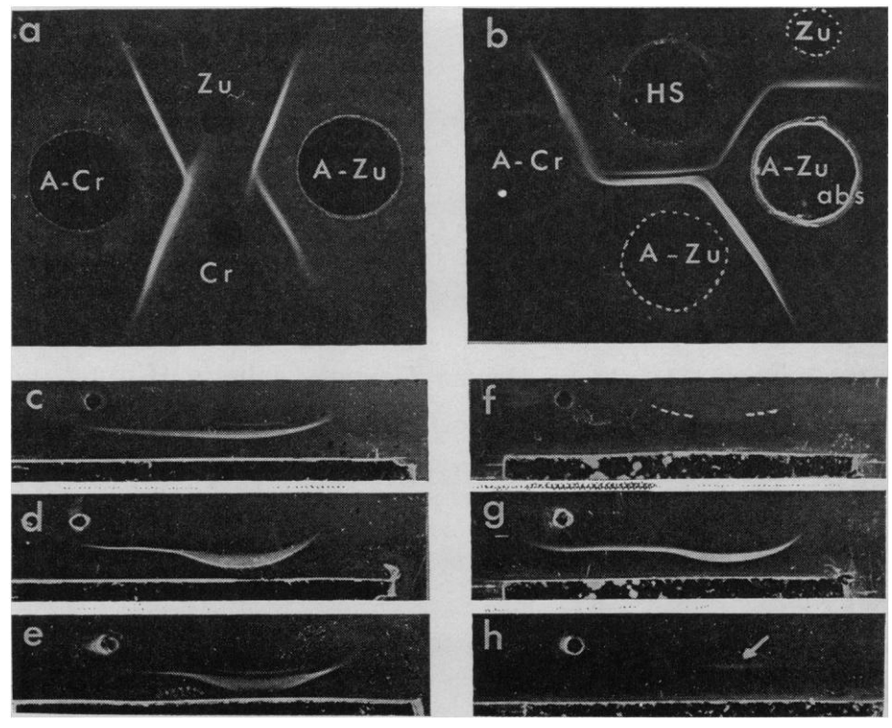

Fig. 1. All antiserums used were rabbit antiserums. *a*, Spur formation in immunodiffusion between the Cr-protein (Cr, lower well) and the Zu-protein (Zu, upper well). Antiserums: antiserum to Cr (A-Cr, left well) and antiserum to Zu (A-Zu, right well). *b*, Immunodiffusion with normal human serum (HS) and Zu-protein (Zu) as antigens. Antiserums: anti-Cr (A-Cr), anti-Zu (A-Zu), and anti-Zu absorbed with Cr-protein ($A\text{-}Zu_{abs}$). *c-h*, Immunoelectrophoresis; anode at the left. *c* and *f*, Antigen is normal human serum. *d* and *g*, Antigen is a myeloma serum containing an abnormal 7*S* γ-globulin of the Zu-type. *e* and *h*, Antigen is myeloma serum containing an abnormal 7*S* γ-globulin of the Cr-type. Antiserum in *c*, *d*, and *e* is anti-Zu. Antiserum in *f*, *g*, and *h* is anti-Zu absorbed with Cr-protein.

whereas the S fragment contains the L-chain and part of the H-chain (*7*). The specific Cr-determinants and Zu-determinants were found on the F (fast) fragment. Since the Cr- and Zu-proteins differ markedly in carbohydrate content, further study may implicate this moiety in the antigenic differences.

Serums of 150 normal individuals were examined, and all contained both populations of γ-globulin. Several of these serums were known to be of different Gm specificities. The results indicated that both antigenic types of 7*S* γ-globulins (Cr and Zu) are present, regardless of the Gm type of the serum.

These findings are consistent with the assumption that in normal human serums at least two populations of 7*S* γ-globulins are present which differ in part of the antigenic structure of their H-chains. To date it is impossible to state whether any more different types of H-chains of 7*S* γ-globulins exist, as appropriate antiserums to study this problem have not been available. However, an antiserum prepared against the reduced and alkylated 7*S* γ-globulin of one individual gave two lines in immunodiffusion experiments with 7*S* γ-globulin, which fused with the two lines of the reaction of antiserum to Zu with normal human serum.

The two antigenically distinguishable types of H-chains in normal and myeloma 7*S* γ-globulins may be compared to the two types of L-chains in these molecules. However, neither Cr-determinants nor Zu-determinants are confined to one or the other of the antigenic type I or type II classes.

It is proposed that these two populations of normal and myeloma 7*S* γ-globulins be designated 7*S* γ_{Cr} and 7*S* γ_{Zu}, at least until a functional name is applicable.

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Antibody to Hereditary Human Gamma-Globulin (Gm) Factor Resulting from Maternal-Fetal Incompatibility

Abstract. *Multiple samples of serum from a Gm(a−) female mated to a Gm(a+) male were obtained before, during, and after each of four normal uncomplicated pregnancies and tested for antibody to human gamma globulins of differing genetic types. An agglutinator for the Gm(a) factor first appeared in the mother's serum during the third trimester of the fourth pregnancy. The newborn (male) was genotypically Gm(a+), since his serum contained, in addition to maternal Gm(a−) gamma globulin, small amounts of Gm(a+) gamma globulin.*

Antigenic differences in human 7*S* γ-globulin, detectable by inhibition of agglutination methods, are determined by codominant alleles at two independent loci (Gm and Inv) (*1*). Antibodies to γ-globulin, specific for one or another of these hereditary antigens, have been found in the serums of multiply transfused individuals (*2*), in some Gm(−) offspring of Gm(+) mothers (*3*), in children given multiple injections of pooled Cohn fraction II for "allergy" (*4*), and in children studied 1 to 2 years after exchange transfusion for hemolytic disease of the newborn (*4*). Most reports state that the 7*S* γ-globulin in the human fetus is acquired from the mother by transplacental passage, so that the γ-globulin phenotype (Gm type) of mother and newborn child are identical (*1, 5*); however, endogenous production of small amounts of γ-globulin (0.1 to 1.0 percent of the total present in cord blood) by the fetus has recently been demonstrated in this laboratory (*6*) with the use of selected SNagg (agglutinating serums from nonarthritic patients) reagents (*7*). In such systems Gm(+) normal serums still show inhibitory activity when diluted up to 500- to 2000-fold with saline or Gm (−) serum, and Gm(+) cord serums inhibit at 1:2 to 1:20 dilutions.

These findings suggested that "anti–γ-globulins" might result from immunization of the mother by fetal γ-globulin bearing hereditary Gm determinants elaborated by genes inherited from the father but not present in the mother. In our study, the Gm types of the parents (the authors) (Table 1) made it likely that some of their offspring would be positive for the Gm(a) factor present in the father (HHF) but absent in the mother (BRF). For this reason, multiple samples of serum were obtained from the mother when she was nulliparous and also during and after each of her four pregnancies and stored in portions in the frozen state. After each pregnancy a portion of each of the available samples was thawed and tested simultaneously for anti-Gm activity. Such activity did not appear in the mother's serum during or after the first three pregnancies, but these offspring are Gm(a−)—confirmed by Gm typing at 3 years of age—(Table 1). The fourth pregnancy was uncomplicated and the mother received no transfusions or injections during its course. Her serum did not contain rheumatoid factor (negative results in sensitized sheep-cell and latex-fixation tests). During the fourth pregnancy, however, an antibody to γ-globulin demonstrable by agglutinating systems was detected in the maternal serum at the beginning of the third trimester (Table 2) and persisted until the 35th week of gestation. This agglutinator was specific for the Gm(a) factor; it agglutinated Rh-positive red cells sensitized by Gm(a+) "incomplete" anti-Rh reagents but did not agglutinate cells coated with Gm(a−) anti-Rh. That the agglutinator was specific for Gm(a) was documented by the inhibition obtained with a panel of 25 standard serums of known Gm and Inv types; these serums were used to inhibit the agglutination system established with the mother's serum and Rh-positive cells sensitized by a Gm(a+)