and Briggs (*3*). Wightman (*4*) and Dannenburg and Liverman (*5*) have reported the conversion of tryptophan to indoleacetic acid by plant tissue. Both studies entailed the uptake of C^{14}-tryptophan and extraction of ground or homogenized tissue, and the authors assume a normal enzymic conversion by an uncharacterized system for synthesizing indoleacetic acid. However, unless *o*-dihydroxy phenols are rigorously excluded from tissues, there is the possibility that these are responsible for the conversion.

F. W. WHITMORE
R. ZAHNER
School of Natural Resources, University of Michigan, Ann Arbor

References and Notes

1. S. A. Gordon and L. G. Paleg, *Plant Physiol.* **36**, 838 (1961).
2. P. Larsen, in *Moderne Methoden der Pflanzenanalyse*, K. Paech and M. V. Tracey, Eds. (Springer, Berlin, 1955), p. 565.
3. T. A. Steeves and W. R. Briggs, *J. Exptl. Botany* **11**, 45 (1960).
4. F. Wightman, *Can. J. Botany* **40**, 689 (1962).
5. W. N. Dannenburg and J. L. Liverman, *Plant Physiol.* **32**, 263 (1957).
6. This work was supported by NSF grant G-18492 to one of us (R.Z.). Facilities at the University of Michigan Botanical Gardens were kindly provided by A. G. Norman, director.

20 April 1964

Gamma Globulin: Unmasking of Hidden Antigenic Sites on Light Chains

Abstract. *Normal light (L) chains cleaved from normal pooled human gamma globulin contain immunologic determinants not overtly present in the native unreduced molecule. These hidden determinants are shared with Bence Jones proteins. Similarly, one of two Bence Jones proteins contained immunologic determinants which were not overtly present on the autologous serum myeloma protein.*

Studies of Bence Jones (BJ) proteins have shed some light on the basic immunological, structural, and genetic nature of the human immunoglobulins. The two major antigenic groups of Bence Jones proteins have immunologic counterparts in the normal populations of γ_2-, γ_{1A}-, and γ_{1M}-globulins (*1*). In addition, Bence Jones proteins appear to be structurally identical to light (L) polypeptide chains cleaved from the autologous pathologic serum protein (*2, 3*). Using group specific antibodies to Bence Jones protein, we have previously demonstrated individual specificity of the immunizing proteins (*4*). We have also shown that Bence Jones proteins of the same group from different patients contain certain antigenic determinants not overtly present in pooled human 7*S* γ-globulin from normal individuals. The immunologic difference between Bence Jones protein and normal γ-globulin might be due not only to a quantitative change, but also to a qualitative abnormality resulting from any of the following, either individually or in combination, (i) preparative artifact; (ii) unmasking of hidden antigenic sites; or (iii) primary structural difference that might be expected of an abnormal protein. The following studies were undertaken to investigate the possibility that Bence Jones proteins contain antigenic determinants present in normal L-chains, but inaccessible when the L-chain is incorporated into the γ-globulin molecule.

Bence Jones proteins were typed (*5*) and isolated from urine by precipitation with ammonium sulfate; they were purified by dialysis, lyophilization (*6*), and finally by Pevikon block electrophoresis (*7*). Specific antibodies to group 1 and group 2 Bence Jones proteins were prepared in rabbits (*4*). Serums containing myeloma proteins were used without further separation of the protein. Immunologic analyses were performed by double diffusion in agar.

Normal L-chains (*8*), S (slow) fragments (*8*), pooled human γ-globulin (*9*), and two group 1 myeloma serum proteins were compared by means of specific antibody to group 1 Bence Jones protein (anti-BJ 1–40). The S (slow) fragments were the electrophoretically slow components of a papain digest of normal γ-globulin. One myeloma protein was a γ_2- and the other, a γ_{1A}-type. The specific antibody was absorbed with 5 mg of lyophilized normal serum to remove reactivity that arises from contamination of the immunizing antigen by slight amounts of normal serum protein. Reactivity was of significant degree with the normal L-chains and the two myeloma proteins, only slight with the normal γ-globulin, and absent with the S fragment (Fig. 1). Similar findings were noted when L-chains, γ-globulin, S fragments and group 2 myeloma proteins of γ_2-type were compared by testing with a specific antibody to group 2 Bence Jones protein. These studies indicate that immunologic information present on normal L-chains may be hidden or inaccessible in the S fragments of papain-split γ-globulin or in the undigested γ-globulin molecule.

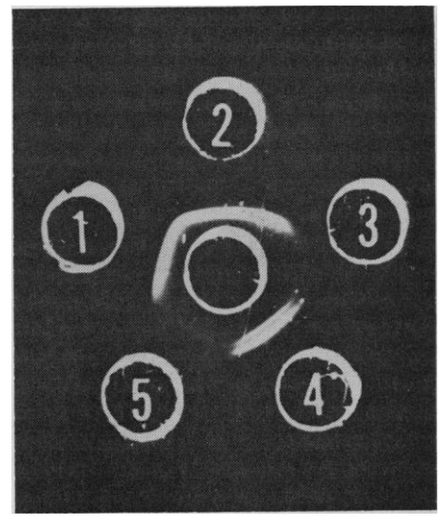

Fig. 1. Immunodiffusion: Comparison of L-chains, S fragments, γ-globulin, and myeloma proteins. Well No. 1, γ_{1A}-myeloma protein 1–40; No. 2, γ_2-myeloma protein 1–41; No. 3, normal S fragments; No. 4, normal L-chains; No. 5, normal pooled human γ-globulin. Center well contained a specific group 1 Bence Jones (1–40) antibody absorbed with 5 mg of lyophilized normal human serum per milliliter. Protein concentration in the antigen wells was 0.1 percent.

In view of these findings two Bence Jones proteins were compared in an analogous sense to the autologous γ_2 myeloma proteins by reactivity with specific antibody to group 2 (anti-BJ 2–20). In this study (Fig. 2) the antibody was absorbed with normal serum and also with 2 mg of pooled normal human γ-globulin per milliliter. The immunizing Bence Jones protein (BJ 2–20) and its autologous myeloma serum protein showed an identical degree of reactivity. Cross-reactivity of the specific antibody was noted with a Bence Jones protein (BJ 2–24) from another patient; but no reaction was apparent with the myeloma protein from this patient. These findings suggest that BJ 2–24, BJ 2–20 and myeloma protein 2–20 share antigenic determinants which are not overtly present on the myeloma protein 2–24. These determinants shared by Bence Jones proteins of the same group but from different patients represent a common core of antigenic determinants characteristic of the major immunologic group (*4*). Bence Jones proteins and myeloma proteins from the same patient should share the same antigens. The discrep-

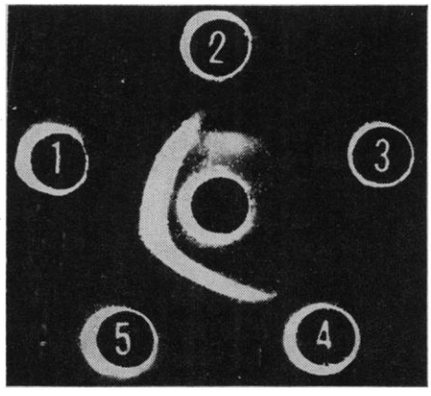

Fig. 2. Immunodiffusion. Comparison of group 2 Bence Jones proteins and autologous myeloma proteins. Well No. 1, Bence Jones protein 2–20; No. 2, Bence Jones protein 2–24; No. 3, γ_2-myeloma protein 2–24; No. 4, normal γ-globulin; No. 5, γ_2-myeloma protein 2–20. The center well contained a specific 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.

RALPH L. NACHMAN
RALPH L. ENGLE, JR.
New York Hospital–Cornell Medical Center, New York

References and Notes

1. M. Mannik and H. G. Kunkel, *J. Exptl. Med.* **116**, 859 (1962).
2. J. H. Schwartz and G. M. Edelman, *ibid.* **118**, 41 (1963).
3. D. Gross and W. V. Epstein, *J. Clin. Invest.* **43**, 83 (1964).
4. S. Stein, R. Nachman, R. Engle, Jr., *Nature* **200**, 1180 (1963).
5. L. Korngold, G. Van Leeuwen, R. L. Engle, Jr., *Ann. N.Y. Acad. Sci.* **101**, 203 (1962).
6. R. L. Engle, Jr., K. R. Woods, G. B. Castillo, J. H. Pert, *J. Lab. Clin. Med.* **58**, 1 (1961).
7. H. J. Muller Eberhard, *Scand. J. Clin. Lab. Invest.* **12**, 33 (1960).
8. Supplied by Mart Mannik and Henry Kunkel.
9. Lederle Laboratories, Pearl River, New York, lot C-763.
10. H. Fudenberg and E. Franklin, *Ann. Internal Med.* **58**, 171 (1963).
11. E. Franklin, H. Fudenberg, M. Meltzer, D. R. Stanworth, *Proc. Natl. Acad. Sci. U.S.* **48**, 914 (1962).
12. M. Harboe, C. K. Osterland, M. Mannik, H. G. Kunkel, *J. Exptl. Med.* **116**, 719 (1962).
13. Supported by grant C-1905 from the National Cancer Institute, and by USPHS training grant 2A-5337 from the National Institute of Arthritis and Metabolic Diseases.

23 April 1964

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-