Reports

Localization of Two Genetic Factors to Different Areas of γ-Globulin Molecules

Abstract. Gm(a) and Gm(b) factors are present in 7S γ-globulin molecules and absent in 19S γ-globulins, β_{2A}-globulins and Bence-Jones proteins, whereas the Inv(b) factor was demonstrated in all four kinds of proteins. The Inv type was identical in isolated 7S and 19S γ-globulins of six normal sera. After papain splitting of 7S γ-globulin, Gm determining sites were present only in the fast (F) split product and Inv determining sites were present only in the slow (S).

Recent observations on human γ-globulin have indicated that a variety of genetic types occur in different individuals. Of the seven factors known, only four are considered in this paper: the Gm(a) factor is present in 52.6 percent of the American white population, and the Gm(b) factor is present in 91.5 percent. The responsible genes, Gm^a and Gm^b, behave as codominant alleles. The Inv(a) factor is present in about 20 percent and the Inv(b) factor in about 99 percent of the same population; the responsible genes also behave as codominant alleles apparently being present at another independent locus (*1–4*).

All determinations were made by the hemagglutination inhibition reaction using red cells coated with incomplete anti-D and agglutinators of well-known and high degree of specificity. The Gm types were determined by a slide technique using anti-Gm(a) Smejsa with anti-D J.J., and anti-Gm(b) A.Berg with anti-D N.B. The Inv types were determined by a tube technique using anti-Inv(a) Travnikova with anti-D Roehm, and anti-Inv(b) Lucas with anti-D Ham. All readings were performed with a blind technique, and the results were easily reproducible.

Myeloma proteins were purified by starch block electrophoresis and classified immunologically as belonging to the more complete or more deficient 7S γ-globulin type or to the β_{2A} class. Macroglobulins were similarly purified from sera of patients with macroglobulinemia Waldenström. The macroglobulins and isolated Bence-Jones proteins were tested for purity by ultracentrifugation and immunological methods.

Four myeloma proteins were selected for splitting by papain because they fell into the antigenically more complete 7S γ-globulin type and were of relatively low electrophoretic mobility. Isolated γ-globulin of low electrophoretic mobility from a selected normal serum (45502) was used in one experiment. After treatment with papain (*5*), two main antigenic fragments were distinguished by immunoelectrophoresis (*6*) and subsequently separated by starch block electrophoresis. The degree of separation of the slow moving (S) and the fast (F) split products varied somewhat. A high degree of purity was, however, obtained in the segments corresponding to the slowest portion of S and the fastest portion of F. A typical example of a test for purity, using double diffusion in agar, is shown in Fig. 1. The slightly curved lines of both S and F isolated split products fused with marked spur formation with the straight lines of unsplit material. The S and F lines passed through each other with no evidence of cross-reaction. Each of the split products gave a single line indicating that there was very little or no contamination with unsplit material or the other split fraction.

None of the purified S preparations inhibited anti-Gm(a) Smejsa in the highest concentration tested (1 mg/ml). The isolated F split product of those proteins which possessed the Gm(a) character inhibited the agglutination completely in seven or eight successive, twofold serial dilutions, starting at the same protein concentration. If the starting material was of type Gm(a—), none of the split fractions showed inhibition. Tests with two additional anti-Gm(a) sera (1604 and Kouba) gave virtually identical results. Preparation 45502 was Gm(b+); again the inhibiting capacity was confined to the F fragment. Neither of the split fragments of two Gm(b—) preparations had any inhibiting capacity.

Inv(a) testing of preparation 45502 showed that the inhibiting capacity was present in the S fragment, whereas F did not inhibit. One of the myeloma proteins was Inv(a+); again the inhibiting capacity was confined to the S fragment. Three of the myeloma proteins and preparation 45502 were Inv(b+). In all cases the inhibiting capacity was present in the S fragment and absent in F. In the Inv tests, the F fragments had no inhibiting capacity at a concentration of 1 mg/ml, whereas the S fragments inhibited the agglutination completely in at least three doubling dilutions starting at the same protein concentration.

The double diffusion tests in agar indicated that the purified preparations did not contain unsplit material. Ultra-

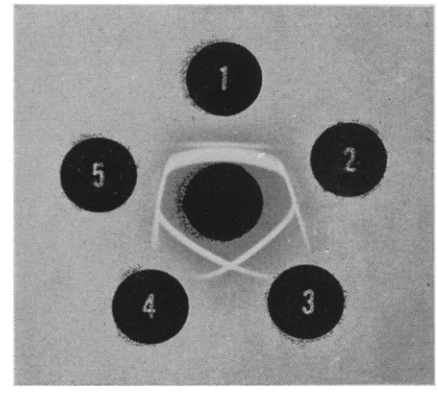

Fig. 1. Control of purity of isolated S and F fragments after papain splitting of 7S γ-globulin. Center well: Rabbit anti-human 7S γ-globulin No. 509; well 1, whole split; wells 2 and 5, unsplit; well 3, isolated slow (S) and well 4, isolated fast (F).

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

centrifugation, using a 5 to 10 percent sucrose density gradient, gave more direct evidence that the inhibition was caused by components with a sedimentation coefficient smaller than 7S. Ten fractions were obtained from each tube, and inhibiting activity was recovered from top fractions of the split material, while corresponding fractions of control tubes with unsplit 7S γ-globulin contained no protein or inhibiting activity.

The β_{2A} myeloma protein was isolated from ten sera. Tests for purity were made by double diffusion tests in agar and showed that the preparations' contamination with 7S γ-globulin were less than 2 percent of the total protein content. All ten β_{2A}-globulins were clearly Gm(a—b—), whereas seven possessed the Inv(b) character. Five isolated 19S γ-globulins from macroglobulinemia sera were all Gm(a—b—), whereas two of them were Inv(b+). Finally, six Bence-Jones proteins were Gm(a—b—), whereas two of them possessed the Inv(b) character.

Since the 19S γ-globulins possessed the Inv(b) character, it seemed of interest to isolate these from individual normal sera to determine whether they were of the same type as the 7S γ-globulins. Euglobulin preparations were dissolved in glycine HCl buffer at *p*H 3.0 and subjected to density gradient ultracentrifugation. Selected fractions were carefully dialyzed against saline and tested immunologically for purity before Gm and Inv typing. Bottom fractions were obtained from six individuals with 19S γ-globulins free of 7S γ-globulin; the corresponding top fractions contained 7S γ-globulin and no 19S material. The Gm characters were present in the 7S fraction, whereas the fractions containing 19S γ-globulin all were Gm(a—b—). Two of the 7S γ-globulin fractions were Inv(a+b+) and four Inv(a—b+). Identical results were obtained in Inv typing of the fractions containing 19S γ-globulins.

It has previously been known that Gm and Inv determining sites are present on 7S γ-globulin molecules (*2*, *3*). Evidence has also been presented which indicates that 19S γ-globulins and β_{2A}-globulins lack the Gm characters (*7*). No evidence has been available concerning the presence of Inv determining sites on these proteins.

The present experiments confirmed that Gm determining sites are present only in 7S γ-globulins, whereas Inv determining sites are present also in 19S γ-globulins, β_{2A}-globulins, and Bence-Jones proteins. These findings and the results of the splitting experiments provide evidence for a common genetic makeup of a part of all four types of proteins. This is particularly interesting as this part of the 7S γ-globulin molecule is known to contain the antibody combining sites (*8*). The findings are in agreement with previous evidence from this laboratory (*9*) and elsewhere (*10*) which indicates that the immunological cross-reaction between 7S γ-globulin and the three other proteins is due to common antigenic determinants present on the S fragment after papain splitting of 7S γ-globulin, whereas antigenic determinants present on the F fragment appear not to be shared by the other proteins.

After papain splitting of 7S γ-globulin, the Gm determining site was found in one of the fragments only and the Inv site in the other. Irrespective of the precise action of papain on the 7S γ-globulin molecule, these findings indicate that the sites are present on different parts of the native molecule. The results with the highly homogeneous myeloma proteins are particularly relevant in this connection where the same myeloma protein contained the two sites in the different papain fragments. The molecules of 7S γ-globulin appears to consist of several polypeptide chains linked by disulfide bonds (*11*), and it is possible that the present findings correspond to the current concept of "one gene–one polypeptide chain" as exemplified by hemoglobin. However, this remains to be proved. At any rate, one gene, which also has the code of the Gm-determining site, might control the structure of one portion of the molecule. An independent gene may control the structure of another part of the molecule which contains the antibody combining sites (*8*) and also the Inv-determining site(s) (*12*).

M. HARBOE
C. K. OSTERLAND*
H. G. KUNKEL
Rockefeller Institute, New York 21

References and Notes

1. R. Grubb and A.-B. Laurell, *Acta Pathol. Microbiol. Scand.* **39**, 390 (1956).
2. ———, *Arthritis and Rheumatism* **4**, 195 (1961).
3. C. Ropartz, J. Lenoir, L. Rivat, *Nature* **189**, 586 (1961).
4. A. G. Steinberg, J. Wilson, S. Lanset, *Nature*, in press.
5. R. R. Porter, *Biochem. J.* **73**, 119 (1959).
6. G. M. Edelman, J. F. Heremans, M.-Th. Heremans, H. G. Kunkel, *J. Exptl. Med.* **112**, 203 (1960).
7. L. Martensson, *Acta Med. Scand.* **170**, suppl. 367, 87 (1961); J. L. Fahey and S. D. Lawler, *J. Natl. Cancer Inst.* **27**, 973 (1961).
8. E. C. Franklin, *J. Clin. Invest.* **39**, 1933 (1960); J. H. Morse and J. Heremans, *J. Lab. Clin. Med.*, in press.
9. J. F. Heremans and M.-Th. Heremans, *Acta Med. Scand.* **170**, suppl. 367, 27 (1961).
10. E. C. Franklin and D. R. Stanworth, *J. Exptl. Med.* **114**, 521 (1961).
11. G. M. Edelman and M. D. Poulik, *ibid.* **113**, 861 (1961).
12. We are grateful to O. Hartmann, P. Herzog, J. C. Nielsen, C. Ropartz, and A. G. Steinberg for generous provision of reagents which made this work possible, and to A. G. Steinberg who kindly confirmed the Inv (b) character for two of the isolated proteins. This work was aided by a grant from the National Foundation.
* Fellow of the Canadian Arthritis Society.

12 January 1962

Relationship Between Temperature and the Metabolism of Experimental Ecosystems

Abstract. Short-term temperature variations of approximately 7°C above and below the normal maintenance temperature of balanced laboratory aquaria demonstrated that the metabolism of the ecosystem is practically independent of temperature. It is postulated that the closer a living system approaches the integration of a balanced ecosystem, the less it is affected by temperature.

The usual response to temperature of the metabolism of various single organisms is well known. However, the metabolic response of the balanced ecosystem, in which the food for the heterotrophs is made *in situ* by the community's autotrophs has not been studied frequently. Natural, balanced ecosystems are often large, not replicable, and temperature variation cannot be controlled. Such disadvantages may be overcome by using the microcosm method in which small replicate microecosystems are set up in the laboratory where environmental conditions can be more easily controlled than in the field. The microcosm method was used in this work.

The biota, water, and sediments for three benthic, fresh-water microecosystems studied were taken from the San Marcos River near the town of San Marcos, Texas, over a year previous to these experiments. By the time the measurements were made the communities had stabilized. The principal primary producers were *Vallisneria* and *Oedogonium*. Some of the other plants present were *Anabaena, Gleocystis, Merismopedia, Spirogyra, Cladophora, Chlamydomonas, Ankistrodesmus, Coelosphaerium, Chara, Achnanthes, Pinnularia,* and various very small green and blue-green unicellular forms. The animal contributing the greatest biomass to the microcosms was a lumbriculid oligochaete, *Sutroa* (?) whose habitat was in the sediments. The only other