

The instrument was able to detect a 2 percent deviation from the absorption and emission characteristics that would be displayed by a black body at the same temperature and emissivity. A culture of the same bacteria on agar was examined after vacuum drying. The dried film was approximately 250 μ thick and was completely absorbing beyond 5.8 μ , but there were no recognizable emission features. A film of dried *Penicillium notatum* of approximately 12 μ had no pronounced absorption features and no emission features.

The epidermis of plants of the cactus family has been of interest because of an outer layer that inhibits loss of water; such a layer would be useful in a Martian organism. The recent work of Rea (3) has shown that such epidermis is very similar to paraffin in absorption and reflection. The epidermis from each of two local cacti were dried in a vacuum and examined. The dried epidermis was approximately 60 μ thick and translucent. Both showed better than 60 percent absorption at 5 to 6.6 μ with some characteristic features, though none were as pronounced as those found in the plastic films. The absorption spectrum of one of the species is shown in Fig. 4. No characteristic features were detectable in emission.

It appears, from the results described, that emission spectra of organic solids will have recognizable features for only a very limited set of conditions. For a significant emission characteristic to appear, the specimen must be thin and it must have specific absorption. The background must not radiate strongly in the 5- to 6.6- μ region; therefore, it must be a substance of lower emissivity, lower temperature, or a combination of these characteristics. The dilution of emission characteristics in the 50- μ sample of film H is striking when compared with the strong specific characteristics of the 6 μ Mylar.

The effect is readily understandable in the light of Kirchhoff's law. The thin specimens emit, as a function of wavelength, in proportion to their absorptivity in the linear manner of a substance whose optical depth is less than one. As the thickness is increased, the optical depth of the specimen becomes high at all wavelengths and the radiant flux loses characteristic spectra.

These results would seem to make the prospect of detection of life on the surface of Mars by emission spectra in the 5- to 6.6- μ region remote. It is

difficult to imagine organisms on a planetary surface meeting the extreme conditions necessary to produce recognizable emission features.

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References

1. W. M. Sinton, *Astrophys. J.* **126**, 231 (1957); *Science* **130**, 1234 (1959).
2. W. Fastie, *J. Opt. Soc. Am.* **42**, 641 (1952).
3. D. G. Rea, T. Belsky, M. Calvin, *Science* **141**, 923 (1963).

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Antibody Activity in Six Classes of Human Immunoglobulins

Abstract. *Antibody activity to thyroglobulin was identified in six classes of immunoglobulins in man, that is, in type I and type II, 6.6S γ -globulins; in type I and type II, β_{2A} -globulins; and in type I and type II, γ_{1M} -macroglobulins. These observations indicate that antibody-activity sites are separate from the part of the H chains of immunoglobulin molecules responsible for specific properties of 6.6S γ -globulin, β_{2A} -globulin, or γ_{1M} -globulin, and, also, are separate from the part of L chains responsible for type I or type II characteristics of immunoglobulin molecules.*

Normal human serum contains six immunoglobulin groups (type I, γ -; type II, γ -; type I, β_{2A} -; Type II, β_{2A} -; type I, γ_{1M} -; and type II, γ_{1M} -globulins) (1). There are about 8 mg of type I γ -globulins and 4 mg of type II γ -globulins per milliliter in normal serum, and lesser amounts of the types I and II β_{2A} -globulins and γ_{1M} -globulins (2). Antibody activity has been identified in the γ , β_{2A} , and γ_{1M} subdivisions of the immunoglobulins. Our studies were undertaken to determine if antibody activity was present in the type I and type II molecules of each group, that is, in all six classes of immunoglobulins.

Serums with antibody activity against thyroglobulin were obtained from seven patients with chronic thyroiditis (3) and serums with antibodies against insulin were obtained from two patients with diabetes mellitus (4). Each serum was tested for 6.6S γ -, β_{2A} -, and γ_{1M} -globulin antibodies to thyroglobulins (3) or insulin (4) by radioimmuno-electrophoresis (5). After electrophoresis of these serums in agar, specific antisera (6) were added to antiserum

troughs to obtain immune precipitates of individual immunoglobulins. Labeled antigen (I^{131} -thyroglobulin or I^{131} -insulin) was then added to the troughs and diffused through the agar to combine with antibody molecules contained in the immunoglobulin precipitates. The labeled antigen and specific antibody were detected by autoradiography (Fig. 1). Thyroglobulin was taken from the α -globulin fraction obtained by zone (block) electrophoresis fractionation of thyroglobulin preparations (3). Labeling with I^{131} was done by the technique of McFarlane (7).

Seven serums containing antibodies to thyroglobulin fixed sufficient amounts of the labeled thyroglobulin so that an autoradiograph could be obtained. In five cases, both type I and type II γ -globulins had antibody activity (Fig. 1). The 6.6S γ -globulins were isolated from two serums by DEAE (dimethylaminoethyl) cellulose chromatography (8). On radioimmuno-electrophoresis antibody activity was found in the type I and type II globulins. In two additional cases, type I γ -globulins fixed a small amount of labeled thyroglobulin but none was detected in the type II γ -globulins. This difference may have been due to a relative deficiency of type II antibodies since type II γ -globulins were present in lower concentrations than those of type I and the type II may not have been detected under the conditions of the experiment. In two serums containing insulin antibodies, both type I and type II γ -globulins bound radioiodinated insulin (Fig. 1). Normal serum, however, did not bind I^{131} -insulin or I^{131} -thyroglobulin.

Our observations indicate that γ -globulins of both types I and II may have antibody activity. The relative amounts of type I and type II antibodies (as judged by the intensity of the autoradiograph lines) varied from a preponderance of type I to about equal amounts of type I and type II antibodies. Further studies were undertaken to determine whether type I and type II γ_{1M} -macroglobulins and β_{2A} -globulins contain antibody activity.

Two serums (HM and LH) known to have antithyroglobulin activity in γ_{1M} -macroglobulin molecules, were fractionated by a combination of block electrophoresis and filtration through Sephadex G-200 to obtain the 18S γ_{1M} -macroglobulin fraction (6). By radioimmuno-electrophoresis and Ouchterlony diffusion with labeled antigen, the type I and type II γ_{1M} -globulins from

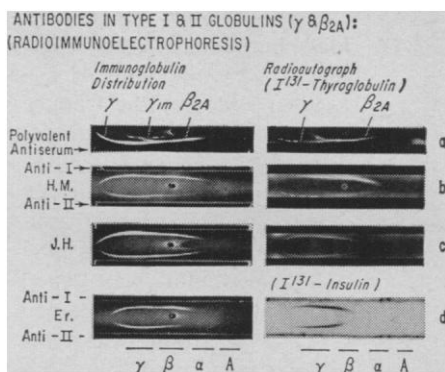


Fig. 1. Radioimmuno-electrophoresis of type I and type II γ -globulin antibodies. Protein distribution is seen on the left and autoradiographic demonstration of antibody activity is on the right. In Fig. 1a, 6.6S γ , γ_{1M} , and β_{2A} -globulins are identified in HM serum on the left, and γ - and β_{2A} -antibodies are evident on the right. Figure 1b shows types I and II immunoglobulins and antibodies (γ and β_{2A}) in HM serum. Figure 1c shows types I and II γ -globulins and antibodies to thyroglobulin in JH serum. Figure 1d shows types I and II γ -globulins and antibodies to insulin in ER serum.

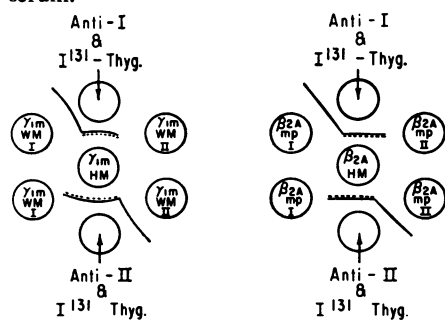


Fig. 2. Protein and autoradiographic observations on Ouchterlony analysis (with labeled antigen) of purified γ_{1M} -globulins and β_{2A} -globulins from the serum of patient HM with chronic thyroiditis. In testing the γ_{1M} -globulins, the purified serum fraction was placed in the center well of an Ouchterlony plate. Types I or II γ_{1M} -macroglobulin purified from the serum of patients with Waldenström's macroglobulinemia (γ_{1M} -WM-I or II), were placed in four adjacent wells. In the two remaining wells were placed specific antisera, reacting either with types I or II γ -globulins. After formation of the immune-precipitin arcs, the plates were washed and the precipitin line was photographed. Thyroglobulin- I^{131} was added to each of the antiserum wells. One day later saline washing of the plates was begun and, subsequently, autoradiographs were obtained. The presence of an immune precipitate band is indicated by the solid line and an autoradiograph band, which was actually superimposed on the precipitin band, is indicated by the dotted line in the drawing. The results of γ_{1M} -globulin tests are shown on the left half of Fig. 2. The results of similar tests with HM β_{2A} -globulin fractions are illustrated on the right. In this experiment types I or II β_{2A} -myeloma proteins (β_{2A} -MP-I or II) were used in the four side wells to identify the types I and II β_{2A} -globulin components in the HM serum fractions.

both serums were found to have antibody activity. The results of this Ouchterlony diffusion of HM γ_{1M} -macroglobulin are reproduced in Fig. 2.

Serum HM with β_{2A} -globulin antibodies against thyroglobulin was tested for antibody activity in the types I and II β_{2A} -globulins. As seen in the autoradiograph in Fig. 1, antibody activity was found throughout the immunoelectrophoretic β_{2A} -globulin region (as well as 6.6S γ -globulin region). The anodal extension of the β_{2A} -globulin arc of the HM serum autoradiograph contrasts with the cathodal γ -globulin arc seen in JH and ER serum (Fig. 1). When tested with antiserum specific for type I and type II immunoglobulins, HM serum revealed gull-wing shaped autoradiograph arcs for both type I and type II antibodies (Fig. 1). The cathodal arc of the gull-wing corresponds to the distribution of 6.6S γ -globulins. The anodal arc corresponds to the location of β_{2A} -globulins. The observations with radioimmuno-electrophoresis indicate that both type I and type II β_{2A} -globulins have antithyroglobulin antibody activity. Fractions rich in β_{2A} -globulin were obtained by Sephadex G-200 filtration of HM serum and refiltration of the fraction intermediate between the 18S and 6.6S peaks. β_{2A} -Globulin fractions were also prepared by DEAE-cellulose chromatography of 6.6S serum globulin fractions obtained by Sephadex G-200 filtration (6). As shown in Fig. 2, both type I and type II β_{2A} -globulins were present on Ouchterlony-diffusion test, and subsequent addition of I^{131} -labeled thyroglobulin revealed antibody activity in both types I and type II β_{2A} -globulins.

The study with HM serum demonstrates that antibody activity may be present in all six immunoglobulin groups, that is, type I and type II β_{2A} -globulins, type I and type II γ_{1M} -globulins, and type I and type II γ -globulins in a single patient. Mannik and Kunkel (9) also have identified antibodies to thyroglobulin on type I and type II γ -globulin molecules. In addition, they found antibodies to dextran and teichoic acid, Rh antibodies, and isoagglutinins in both types of γ -globulins. In several isohemagglutinin preparations evidence was obtained for antibody activity in type I and type II β_{2A} -globulins or γ_{1M} -globulins.

The identification of antibody activity in all six classes of immunoglobulins poses a problem in relating the antibody site to other specific properties of the

immunoglobulin. Two classes of polypeptide chains, L or B (molecular weight, 20,000) and H or A (molecular weight, 50,000), have been identified in the γ -globulins (10, 11). Immunological studies have shown that L polypeptide chains exist in two forms (types I or II) (12) and H chains in three forms (6.6S γ , β_{2A} , or γ_{1M}) (11, 13). Thus the L and H chains, which together constitute the immunoglobulin molecule, determine whether it is type I γ , or type II γ , or type II β_{2A} , and so forth.

It is not certain whether antibody activity is a property of only one of the two classes of polypeptide chains (L or H), or whether both chains together contribute to antibody specificity. Combination of rabbit antibody with antigenic determinants of the H chains responsible for immunochemical differentiation of the γ -, β_{2A} -, and γ_{1M} -human immunoglobulin molecules, or combination with antigenic determinants of the L chains responsible for type I and type II properties of the immunoglobulin molecules, leaves the antibody-combining site of the immunoglobulin free to react with the thyroglobulin or insulin antigen. Thus, the antibody combining site is separate from the structural configurations (antigenic determinants) responsible for highly specific antigenic differences which permit distinction between L polypeptide chains of type I and type II and between H polypeptide chains of γ -, β_{2A} -, and γ_{1M} -immunoglobulins.

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References and Notes

1. M. Mannik and H. G. Kunkel, *J. Exptl. Med.* 117, 213 (1963); L. Korngold, *Intern. Arch. Allergy Appl. Immunol.* 23, 9 (1963); J. L. Fahey, *J. Immunol.* 91, 438 (1963).
2. J. L. Fahey, in *Protides of the Biological Fluids*, Proc. 11th Colloq., H. Peeters, Ed. (Elsevier, New York, in press).
3. H. Goodman, J. Robbins, E. D. Exum, in preparation. We thank J. Robbins of the National Institute Arthritis and Metabolic Diseases for a human thyroglobulin fraction prepared by ammonium sulfate precipitation.
4. Y. Yagi, P. Maier, D. Pressman, C. B. Arbesman, R. E. Reisman, A. R. Lenzner, *J. Immunol.* 90, 760 (1963). We are indebted to Y. Yagi, Department of Biochemistry, Roswell Park Memorial Institute, Buffalo, N.Y., for making available serums from FE and ER.
5. Y. Yagi, P. Maier, D. Pressman, *J. Immunol.* 89, 763 (1962); J. Morse and J. F. Heremans, *J. Lab. Clin. Med.* 59, 891 (1962).
6. J. L. Fahey and C. L. McLaughlin, *J. Immunol.* 91, 597 (1963).
7. A. S. McFarlane, *Nature* 182, 53 (1958).

8. J. L. Fahey and H. Goodman, *J. Clin. Invest.* **39**, 1259 (1960).
 9. M. Mannik and H. G. Kunkel, *J. Exptl. Med.* **118**, 817 (1963).
 10. R. R. Porter, *Basic Problems in Neoplastic Disease*, A. Gellhorn and E. Hirschberg, Eds. (Columbia Univ. Press, New York, 1962).
 11. G. M. Edelman and B. Benacerraf, *Proc. Natl. Acad. Sci. U.S.A.* **48**, 1035 (1962).
 12. J. L. Fahey, *J. Immunol.* **91**, 448 (1963).
 13. R. R. Porter, in *Protides of the Biological Fluids*, Proc. 11th Colloq., H. Peeters, Ed. (Elsevier, New York, in press); J. L. Fahey, *J. Clin. Invest.* **42**, 930 (1963), abstract.
- 20 December 1963.

Alpha-Chain of Human Hemoglobin: Occurrence in vivo

Abstract. *A minor hemoglobin component, apparently representing uncombined α -chains has been detected in the hemolysates of persons with inherited β -chain deficiency of moderate or severe degree. This finding supports the hypothesis that there is independent control of the synthesis of α -chains.*

Each type of peptide chain (α , β , γ , δ) of the normal human hemoglobin is under the control of a separate structural gene (1-3). Inherited deficiencies in the production of α -chains, known as α -thalassemias (4), result in an excess of the complementary chains β , γ or δ ; such excess can be recognized by the presence of the tetramer molecules β_2 or γ_2 , or both, and possibly also δ_2 (5). In the inherited deficiencies of β -chain production, the β -thalassemias, no surplus of α -chains has been recognized, even in homozygous individuals. This failure to observe a fraction corresponding to α -chain alone or α -chain polymers—even though α -chains are capable of separate existence in vitro (6)—has been variously interpreted. It has been suggested (i) that the presence of β - or of its equivalent γ - or δ -chains is necessary for the release of α -chains in order to permit formation

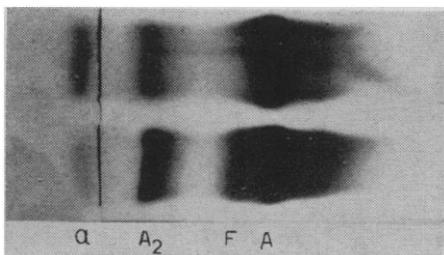


Fig. 1. Comparison of hemoglobin solution containing artificially separated α -chains (upper sample), with hemolysate from homozygous β -thalassemia. Starch-gel electrophoresis, benzidine stain; anode to the right.

of complete hemoglobin molecules (2, 7), and (ii) that the α -chain gene may have a nonautonomous regulation, the amount of α -chains produced being dependent on the amount of β - or γ -chains available (3). According to a different interpretation, uncombined α -chains may be precipitated and form visible water-insoluble inclusion bodies within red cell precursors and young red cells, such cells dying prematurely (8); thus, no α -chains are found in the hemolysate. The latter view is strengthened by the observation that artificially separated α -chains precipitate within 24 hours when stored in dilute solution at 37°C (7). The possibility that a small amount of the unprecipitated α -chains was still present in suitable cells had yet to be tested.

Blood specimens were processed immediately after withdrawal and handled thereafter at 4° to 6°C. The cells were washed repeatedly with cold saline solution (0.9 NaCl) buffered to pH 7.2 containing 0.005M KCN; they were lysed with a very dilute buffer. Stromal material was removed by centrifugation at 15,000g for 15 minutes at 4°C. Horizontal starch-gel electrophoresis of approximately 40 to 60 μ l of the hemolysate was performed in a discontinuous barbital-TEB buffer system at pH 8.2 for 6 to 7 hours in a water-cooled apparatus. The gels were then stained with a very sensitive benzidine stain (9). The whole procedure was completed within 12 hours.

In blood samples from patients with homozygous β -thalassemia—that is, with severe β -chain deficiency—a weak, benzidine-positive band, moving more slowly than hemoglobin A₂, was detected upon electrophoresis; its position was 2 to 3 mm cathodic to the slit of insertion of the sample. No such band was seen in hematologically normal subjects or in patients with anemias, or those with pronounced reticulocytosis due to acquired conditions, or those with α -thalassemias; this band was also absent in cord blood. The band was more prominent in splenectomized patients including six β -thalassemia homozygotes, one β -thalassemia-hemoglobin E and one β -thalassemia-hemoglobin Pylos combination. In exceptional instances the zone was visible, prior to staining, as a faint pinkish area and was estimated to represent 0.5 percent of the hemoglobin content of the hemolysate. In samples from non-splenectomized cases of homozygous β -thalassemia the band was weaker;

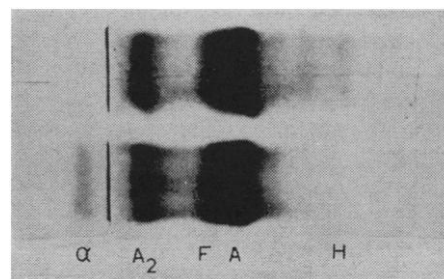


Fig. 2. The addition of pure hemoglobin H (upper sample) has caused disappearance of the cathodic minor component present in the lower sample. Hemolysate from a splenectomized case of homozygous β -thalassemia.

from the intensity of staining it was estimated to be approximately 0.1 to 0.3 percent. Even smaller amounts were found in all six instances of hemoglobin S- β -thalassemia disease. No such band has been detected so far in a few cases of simple β -thalassemia trait; we also failed to observe it in samples from one splenectomized and two out of seven nonsplenectomized cases of homozygous β -thalassemia.

The electrophoretic position of this minor component corresponds exactly to that of α -chains separated in vitro (6, 7) as demonstrated by direct comparison (Fig. 1). No catalase activity is present in this area of the electrophoretic strip. Adding, just prior to electrophoresis, either pure β_1 -component (hemoglobin H) or hemolysates containing β_1 -component to samples exhibiting this cathodic fraction always resulted in its complete disappearance (Fig. 2). Conventional treatment of the hemolysate with toluol caused complete disappearance of the band (10). Storage of the sample at 4° to 6°C led to progressive weakening of the zone, but in the samples where it had been prominent it could still be detected after 3 to 4 days of storage. When the red cells were fractionated by differential centrifugation the bottom layer contained either a smaller amount of the cathodic band than the top layer or none at all. Chemical identification of the new component has been delayed because of its extremely low concentration.

The electrophoretic position of this minor component and its ready combination with β -chains of hemoglobin as well as its occurrence in β -chain deficiencies suggest that it represents uncombined α -chains. The failure to observe this band, contrary to expectation, in a few cases of severe β -chain