References and Notes

1. Burroughs, Welcome and Co., Inc., Tuckahoe, N.Y.
2. F. L. Dunn and F. L. Lambrecht, *J. Parasitol.* **49**, 316 (1963); J. A. Porter, Jr., C. M. Johnson, L. De Sousa, *ibid.*, in press.
3. R. S. Bray, *Ergeb. Mikrobiol. Immunitatsf. Exp. Therap.* **36**, 168 (1963).

4. Work aided by the Medical Research and Development Command, Office of the Surgeon General, U.S. Army (grant DA-MD-49-193-65-G165). We thank George Hitchings, Burroughs, Welcome and Co., Inc., for the immunosuppressant drugs and for advice on dosage.

3 July 1966

Antigenic Heterogeneity of Human Immunoglobulin A Proteins

Abstract. *Two types of human IgA-myeloma proteins were distinguished by immunochemical tests. Seven of 51 IgA-myeloma proteins contained an antigenic determinant that was not detected in the other 44 proteins. The distinctive antigenic site was not demonstrated on either the heavy or light polypeptide chains.*

Four classes of immunoglobulin molecules, designated IgG, IgA, IgM, and IgD, are present in normal human serum. Two of these classes, IgG (*1*) and IgM (*2*), have been divided into subclasses on the basis of antigenic differences detected among molecules within each class. This report presents the results of experiments that demonstrate antigenic differences among the IgA molecules and shows that IgA, like IgG and IgM, may be divided into at least two antigenic subclasses.

Antiserums to normal serum IgA and IgA-myeloma proteins were prepared in rabbits and rhesus monkeys. One monkey antiserum (M-90) distinguished two categories of IgA molecules. This antiserum resulted from the immunization of a monkey with a crude preparation of normal human serum IgA, which had been obtained by a combination of zone electrophoresis and anion exchange chromatography (*3*). The monkey was repeatedly injected with this IgA preparation emulsified in complete Freund's adjuvant. Only bleedings obtained during the period 2 to 3 months after primary immunization contained antibodies with the specificity described below. Antiserum M-90 also contained antibodies to many serum proteins other than IgA and was rendered specific for IgA by absorption with human serum that was normal except for a marked deficiency of IgA-globulin.

A number of human serums containing IgA-myeloma proteins were then studied by double diffusion in gel with the absorbed antiserum (Fig. 1, bottom). Some of the precipitin bands spurred over others, indicating that this antiserum detected antigenic differences among the IgA-myeloma proteins.

The antiserum was further absorbed with whole serum from patient Sa until the precipitin reaction between the antiserum and IgA-myeloma protein Sa was abolished. When this absorbed antiserum was retested with the same IgA-myeloma serums, precipitin bands were obtained with some, but not all, of the serums (Fig. 1, top). This indicated that IgA-myeloma proteins could be divided into two groups: those that precipitated with this antiserum and those that did not.

Two IgA-myeloma serums, Fu and Ma, were selected as prototypes for further studies described below. IgA-myeloma proteins that precipitate with the absorbed antiserums will be referred to as Fu type, and the others will be referred to as Ma type.

A survey of 51 serums containing IgA-myeloma proteins showed that seven serums (14 percent) were of the Fu type while 44 serums (86 percent) were of the Ma type (Table 1).

An antiserum specific for Ma type IgA has not been prepared. Since Ma type myelomas are defined by failure to precipitate with the antiserum to Fu, it is not known how many other antigenic types may be included in the Ma group.

Immunoelectrophoretic studies of serum Co (containing Fu type IgA-myeloma protein) indicated that the precipitin band developed by the absorbed monkey antiserum involved only the IgA-protein (Fig. 2). Thus, the absorbed antiserum was reacting with antigenic determinants confined to IgA-myeloma molecules.

IgA molecules consist of heavy and light polypeptide chains which are held together by covalent disulfide bonds and other noncovalent forces. The heavy polypeptide chains carry class-specific antigenic determinants which permit IgA to be distinguished from other immunoglobulin molecules. The light polypeptide chains occur in two antigenic forms, designated κ- and λ-chains. Either κ- or λ-chains may be associated with the heavy chains of an IgA molecule.

Studies were performed to determine whether the Fu type antigenic determinants were on the light or heavy polypeptide chains. IgA-myeloma protein Fu (λ-type) was isolated by zone electrophoresis and anion exchange chromatography (*3*). The protein was reduced with 0.1*M* dithioerythritol and alkylated with 0.22*M* iodoacetamide at *p*H 8.2. The reduced and alkylated protein was acidified with propionic acid and sep-

Table 1. Precipitin reactions of 51 IgA-myeloma containing serums. All serums were tested with the same monkey antiserum which had been absorbed with serum Sa. (+) indicates precipitin reaction in gel; (−) indicates absence of precipitin reaction.

Light chain type	Precipitin reaction	
	Fu-type	Ma-type
	(+)	(−)
κ	0	29
λ	7	15

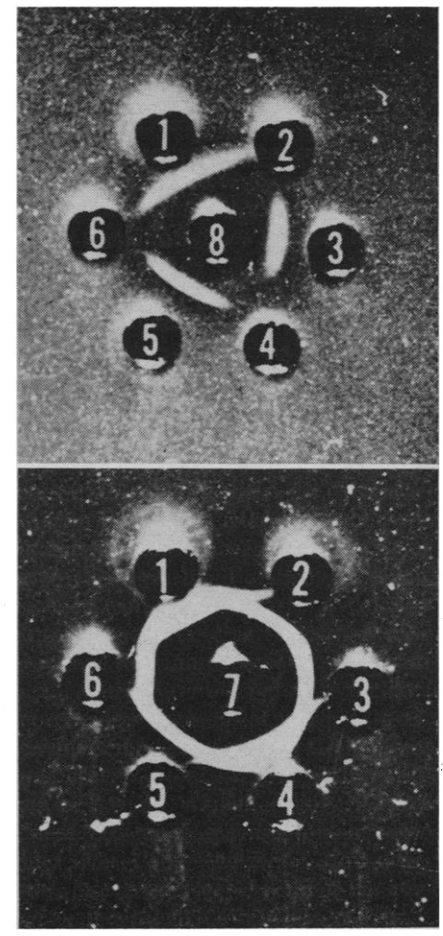

Fig. 1. Ouchterlony reactions of IgA-myeloma serums. All myeloma serums are at a 1/10 dilution. 1, Co; 2, Sa; 3, En; 4, Ra; 5, Hu; 6, Wa; 7, monkey antiserum to IgA absorbed with IgA-deficient serum; 8, the same monkey antiserum additionally absorbed with serum Sa.

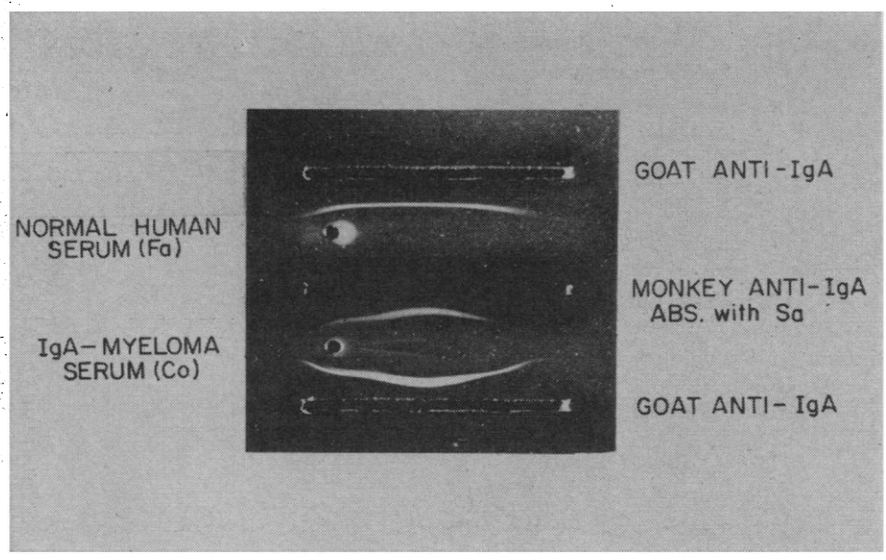

Fig. 2. Immunoelectrophoretic patterns of normal serum and IgA-myeloma serum. The IgA of the normal serum and myeloma serum are precipitated by goat antiserum to IgA. Monkey antiserum to IgA absorbed with serum Sa reacts with the Co IgA-myeloma, but not with normal serum IgA.

arated into heavy and light polypeptide chains by gel filtration through Sephadex G-200 in 1*M* propionic acid. The isolated heavy and light chains were reacted with the absorbed antiserum and neither chain gave a precipitin band in gel diffusion studies. In a separate experiment, protein Fu was reduced and alkylated as described above. The reduced, alkylated protein was not acidified. This protein preparation was tested with the absorbed antiserum and again, no precipitin band was found. Additional experiments showed that Fu-type precipitating activity could not be removed from the antiserum by absorption with Fu light chains or heavy chains. The Fu antigenic determinant, therefore, could not be localized to either the light or heavy polypeptide chain.

Analysis of the light chain types of the 51 IgA-myeloma proteins revealed that the seven Fu type proteins all had λ-type light chains, while the 44 Ma type proteins consisted of 29 with κ-chains and 15 with λ-chains (Table 1). The antiserum was not only detecting the differences between κ and λ antigens, since it reacted with some, but not all, of the λ-type IgA-proteins. The findings that all of the Fu type proteins have λ-type light chains may be due to the small number of serums studied. Additional Fu type proteins should be identified, and their light-chain type should be determined.

An attempt was made to identify Fu type IgA in normal human serum. The absorbed monkey antiserum did not give any visible precipitin bands with a human serum known to contain a normal quantity of IgA (Fig. 2). This normal serum (Fa) was the same one from which the fraction used for immunization was originally obtained. Failure of the antiserum to precipitate with normal serum IgA was confirmed by Ouchterlony studies of several other normal human serums known to contain IgA. However, molecules containing the Fu antigenic determinants must be present in normal serum since (i) the antiserum was prepared by immunization with a normal serum fraction and (ii) precipitin activity can be removed by absorption with normal serum. These facts are interpreted to mean either that only a small number of normal serum IgA molecules contain the appropriate antigenic determinant (Fu type) and that this amount of IgA is not directly demonstrable by the methods employed, or that the reaction of the antiserum with normal serum Fu type molecules leads to a soluble product.

We do not now have a specific antiserum to Ma. Therefore, neither Fu nor Ma type IgA can positively be identified in normal human serum, although both are assumed to be present.

The above findings indicate the existence of at least two antigenically distinct kinds of IgA-myeloma molecules. The chemical nature and molecular localization of these antigenic differences are not known. The Fu antigenic site detected with this monkey antiserum may include part of both the heavy and the light polypeptide chains in a region of the molecule where the chains are in close apposition because of an interchain disulfide bridge. Alternatively, the antigenic site might be present on only one of the chains (presumably the heavy chain) but require stabilization by the other polypeptide chain through a disulfide bond.

Other evidence indicating multiple antigenic forms of IgA-globulin has recently been presented (*4*). It will be of interest to determine whether the subclasses of IgA described by these investigators correspond to the ones presented in this report (*5*).

WILLIAM D. TERRY
MARJORIE S. ROBERTS
Immunology Branch, National Cancer Institute, Bethesda, Maryland 20014

References and Notes

1. H. M. Grey and H. G. Kunkel, *J. Exp. Med.* **120**, 253 (1964); W. D. Terry and J. L. Fahey, *Science* **146**, 400 (1964).
2. M. Harboe, J. Deverill, H. C. Godal, *Scand. J. Haemat.* **2**, 137 (1965).
3. J. L. Fahey and C. McLaughlin, *J. Immunol.* **91**, 484 (1963).
4. J. P. Vaerman and J. F. Heremans, *Science* **153**, 647 (1966); R. E. Ballieux, personal communication; H. G. Kunkel and R. A. Prendergast, *Proc. Soc. Exp. Biol. Med.* **122**, 910 (1966).
5. We thank Dr. J. L. Fahey for support and encouragement in this work.

3 June 1966

Vitamin C–Induced Increase of Dermatan Sulfate in Cultured Hurler's Fibroblasts

Abstract. *In fibroblasts taken from patients with Hurler's syndrome and grown in culture, dermatan sulfate constituted a larger percentage of the total sulfated glycosaminoglycans than it did in cultured fibroblasts from unaffected individuals. Moreover, the addition of ascorbic acid (vitamin C) to the culture medium markedly increased the concentration of dermatan sulfate in the Hurler's fibroblasts but not in the normal fibroblasts. The biochemical phenotype of the Hurler's cells persisted during 28 weeks of serial culture.*

Hurler's syndrome (gargoylism) is an inhibited disorder in the metabolism of acidic glycosaminoglycans (mucopolysaccharides), characterized by a massive deposition in tissues and an increased excretion in the urine of dermatan sulfate with or without heparan sulfate. Accumulation of glycosaminoglycans in the tissues is associated with dwarf-