STUDIES ON HUMAN ANTIBODIES

I. Starch Gel Electrophoresis of the Dissociated Polypeptide Chains*

By G. M. EDELMAN, Ph.D., and ELVIN A. KABAT, Ph.D.

(From The Rockefeller Institute, the Departments of Microbiology and Neurology, College of Physicians and Surgeons, Columbia University, and the Neurological Institute, Presbyterian Hospital, New York)

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Considerable evidence indicates that antibodies to single well defined antigens consist of populations of molecules varying in physical, chemical, and immunologic behavior (1). This heterogeneity is not limited to the occurrence of antibodies in each of the three classes of γ-globulins (γ, γ_{1A}, and γ_{1M}), but is manifested by the same apparent continuum of physico-chemical properties as is found for the normal γ-globulins within each class (2, 3). In contrast to normal γ-globulins, the myeloma globulins and pathological macroglobulins belonging to each class are relatively homogeneous (4, 5).

Are specifically purified antibodies of an individual, like the pathological proteins, discernibly less heterogeneous than his total γ-globulin? One technique for studying this problem is starch gel electrophoresis in 8 M urea of the dissociated polypeptide chains of γ-globulin molecules (6). Under these conditions, normal 7S γ-globulin shows a more slowly migrating band corresponding to H polypeptide chains and a more rapidly migrating diffuse zone corresponding to L polypeptide chains. Individual myeloma globulins yield one or a few sharply resolved L bands, which differ in each case, and usually correspond to the Bence-Jones protein from the same patient (6, 7). The H chains of γ-globulins of different classes migrate with characteristic relative mobilities (8–10). Purified guinea pig antihapten antibodies of the 7S γ class showed a series of sharp L bands which differed for antibodies of different specificities, suggesting that their heterogeneity was more limited than that of normal 7S γ-globulin from the same animals (11).

The present study was undertaken to determine whether human antibodies would show similar behavior. Subject 1 had been immunized to four different antigens, and his serum also contained antibodies to a fifth antigen. Four of the antibodies were directed against the polysaccharides dextran, levan, blood

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group A substance (12), and teichoic acid of *Staphylococcus aureus* (13). The other antibodies were directed against tetanus toxoid. A number of other individuals previously immunized with dextran (14), and whose serum contained antibodies to teichoic acid as well, were also studied.

As with human myeloma globulins and purified guinea pig antibodies, a series of sharp L chain bands was obtained for the dissociated human antibodies. These bands differed in number and distribution for the human antibodies of different specificities. Within a particular specificity, certain differences were also found among different individuals.

EXPERIMENTAL

Antisera.—The human antisera from individuals immunized with dextran were as follows: 1, $20D_{10}$ and D_{11}, $30D_4$, $38D_2$, $49D_2$, $54D_1$, $98D_1 + D_2$, $173D_1 + D_4$, $176D_2$, Ca. D_1, and Fo. D_1.

Sera from these individuals also gave precipitin reactions (13) with two samples of teichoic acid from *S. aureus* (15, 16). In addition, subject 1 had been immunized with hog blood group A substance, dextran, and levan (*cf.* 14). This subject was also immunized with alum-precipitated tetanus toxoid at the end of 1962 and early 1963; his serum also reacted with teichoic acid. Studies on the antidextran of most of these individuals have been reported (14, 17); antidextran in these sera was found to be specific for terminal non-reducing chains of α 1,6 linkages.

Preparation of Antibody for Starch Gel Electrophoresis.—Antibodies for starch gel electrophoresis were obtained as thoroughly washed specific precipitates prepared by addition of specific antigen in the region of maximum precipitation. The same antisera were absorbed sequentially with dextran and with teichoic acid. With antiserum 1, samples were successively absorbed with several antigens. In addition, a number of washed dextran-antidextran specific precipitates were digested with dextranase (18, *cf.* 1), the small quantity of insoluble precipitate removed, and the soluble antibody examined by starch gel electrophoresis. The insoluble residues from the dextranase treatment were examined as well. From the antibody N contents of the sera an amount of specific precipitate was taken to contain about 1 mg of antibody protein, but several of the teichoic acid-antiteichoic acid specific precipitates from subjects 1, 54, and 216 probably contained less than this quantity of antibody. In addition, we examined the two fractions of antidextran from subject 20 obtained by absorption of the antibody on sephadex and elution first with isomaltotriose and then with isomaltohexaose (19). In studies on the tetanus antitoxin from subject 1, the same amount of purified tetanus toxoid (Lederle Laboratories, Pearl River, New York) that was used to precipitate the antibody was examined along with the specific precipitate.

Reduction and Alkylation and Starch Gel Electrophoresis in 8 M Urea.—The procedure and conditions have been described (6). In most of the present experiments the antigen-antibody precipitate was dissolved in 0.1 to 0.2 ml of 8 M urea which was then made 0.1 M in 2-mercaptoethanol. After 2 hours at room temperature the solution was made 0.2 M in iodoacetamide and loaded directly into the origin of the gel.

RESULTS

A starch gel electrophoretic comparison of reduced alkylated antidextran antibodies is given in Fig. 1. In contrast to the diffuse L chain zone of dissociated normal human 7S γ-globulin (Fig. 1, origin 1), the antibodies showed sharp L

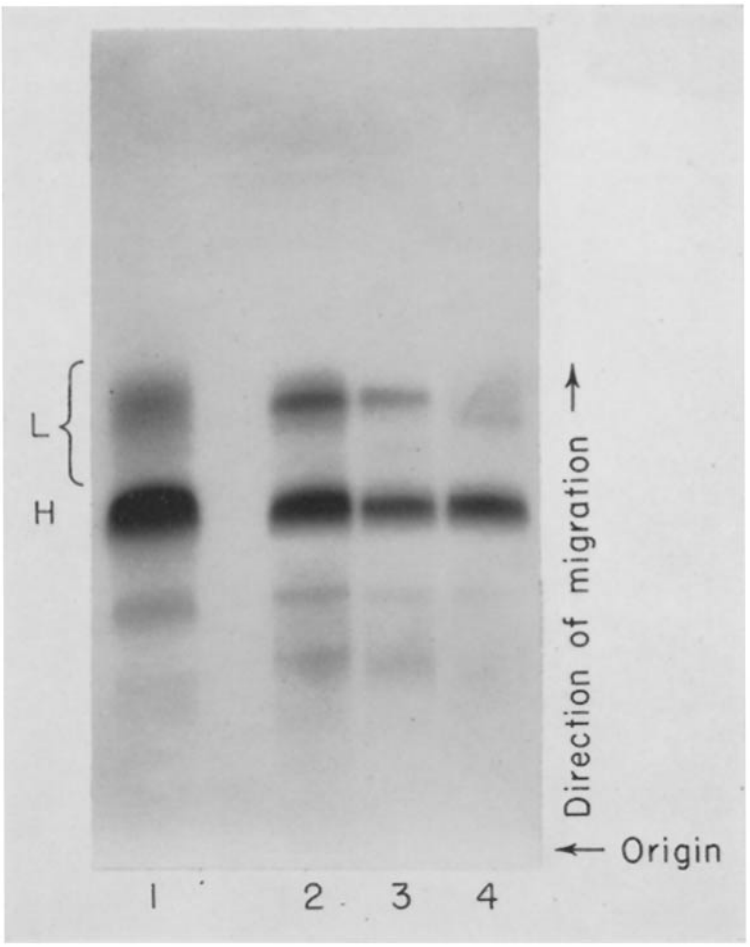

FIG. 1. Starch gel electrophoresis in 8 M urea-formate buffer of reduced alkylated purified human antidextran antibodies. Origin: 1, human 7S γ-globulin; 2, subject 20, antibodies isolated by extraction with isomaltotriose; 3, subject 20, antibodies isolated by subsequent extraction with isomaltohexaose; 4, antidextran antibodies from subject 1, recovered by treatment of specific precipitates with dextranase.

chain bands (Fig. 1, origins 2 to 4). The samples from subject 20 were separated to obtain antibodies with preferential affinity for smaller oligosaccharides (*e.g.* isomaltotriose; origin 2) or larger oligosaccharides (*e.g.* isomaltohexaose; origin 3). Both samples showed one predominant faster L chain band and one slightly slower L band. The sample from another individual (Fig. 1, origin 4; also Fig. 4 *b*, origins 1 and 2) showed equal staining of both bands. The bands migrating more slowly than the H and L bands are considered to represent incompletely

dissociated materials. In instances in which 19S and β_{2A} antibodies might have been present, they could also have contributed to the more slowly moving bands.

More extensive comparisons of antidextran antibodies from six different individuals showed additional variation in band patterns (Figs. 2 *a* and 2 *b*). Two types of pattern were discerned. The first contained one or two fast moving

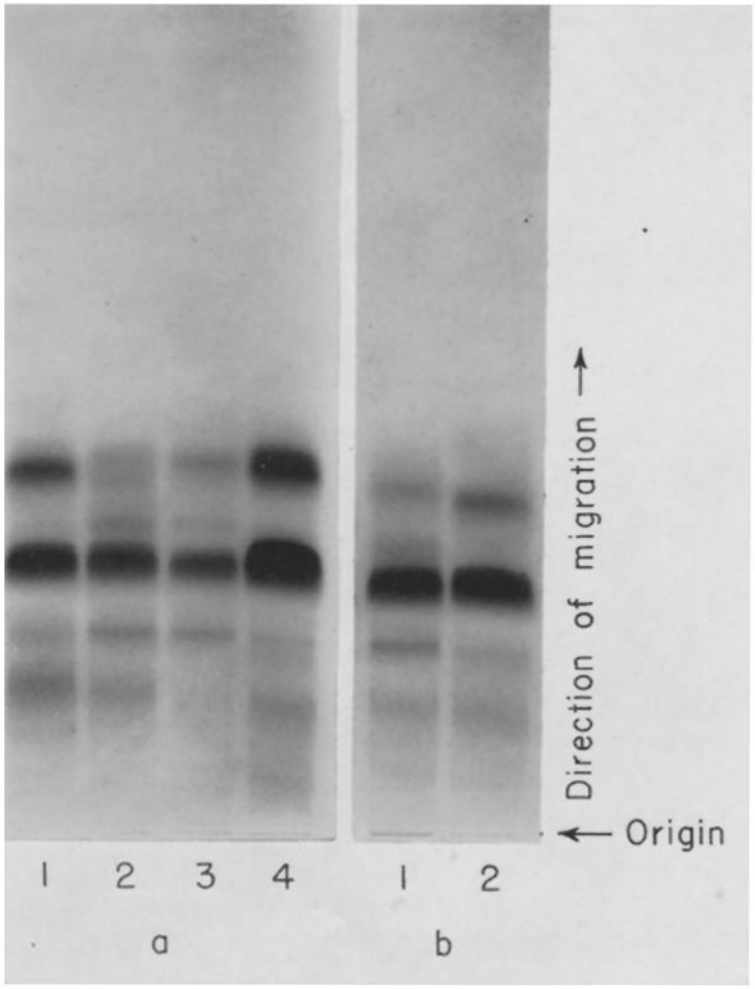

FIG. 2. Comparison of dissociated antidextran antibodies from six different individuals. *a* and *b* represent separate starch gel electrophoretic experiments performed after reduction and alkylation. *a*, antibodies purified with dextranase: 1, subject 20; 2, subject 176; 3, subject 49; 4, subject 30. *b*, antigen-antibody precipitates: 1, subject 98; 2, subject 38.

L bands resembling those shown in Fig. 1 (Fig. 2 *a*, origins 1 and 4). The second type of pattern contained an additional more slowly moving L chain band (Fig. 2 *a*, origins 2 and 3). Two distinct patterns were also seen in a separate experiment (Fig. 2 *b*). The small residues from antigen-antibody precipitates treated with dextranase showed patterns similar to those of the purified antibodies.

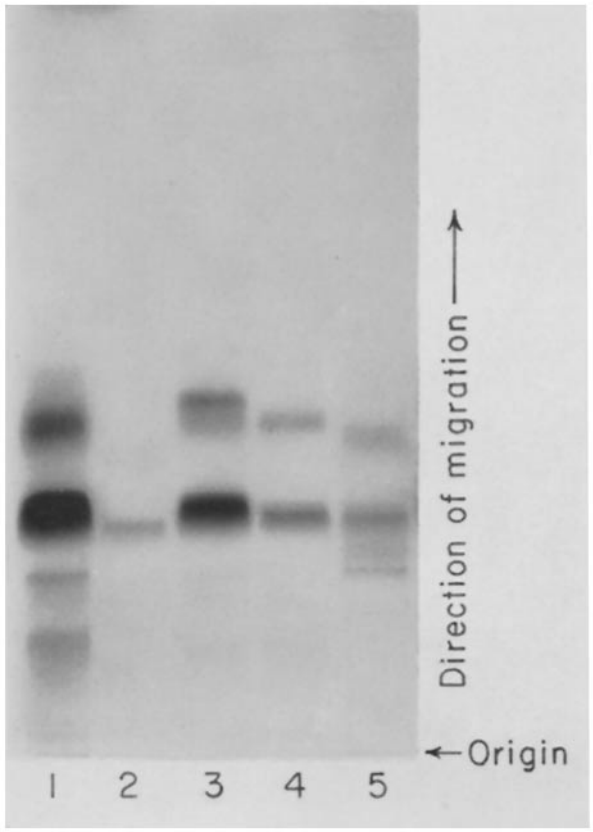

FIG. 3. Reduced alkylated antiteichoic acid antibodies. 1, subject Ca.; 2, subject 216; 3, subject Fo.; 4, subject 54; 5, subject 173. Material in origin 2 was insufficient in amount. All samples were reduced and alkylated in the form of antigen-antibody precipitates.

After dissociation, antibodies to teichoic acid also showed sharp L chain bands (Fig. 3). Three of the patterns (origins 1, 3, and 4) showed a band of similar mobility. In addition, the sample in origin 3 showed a sharp, faster moving band, and that in origin 5 showed a band not present in the samples in origins 3 and 4, but possibly present in that of origin 1.

Two experiments comparing five antibody preparations obtained from the

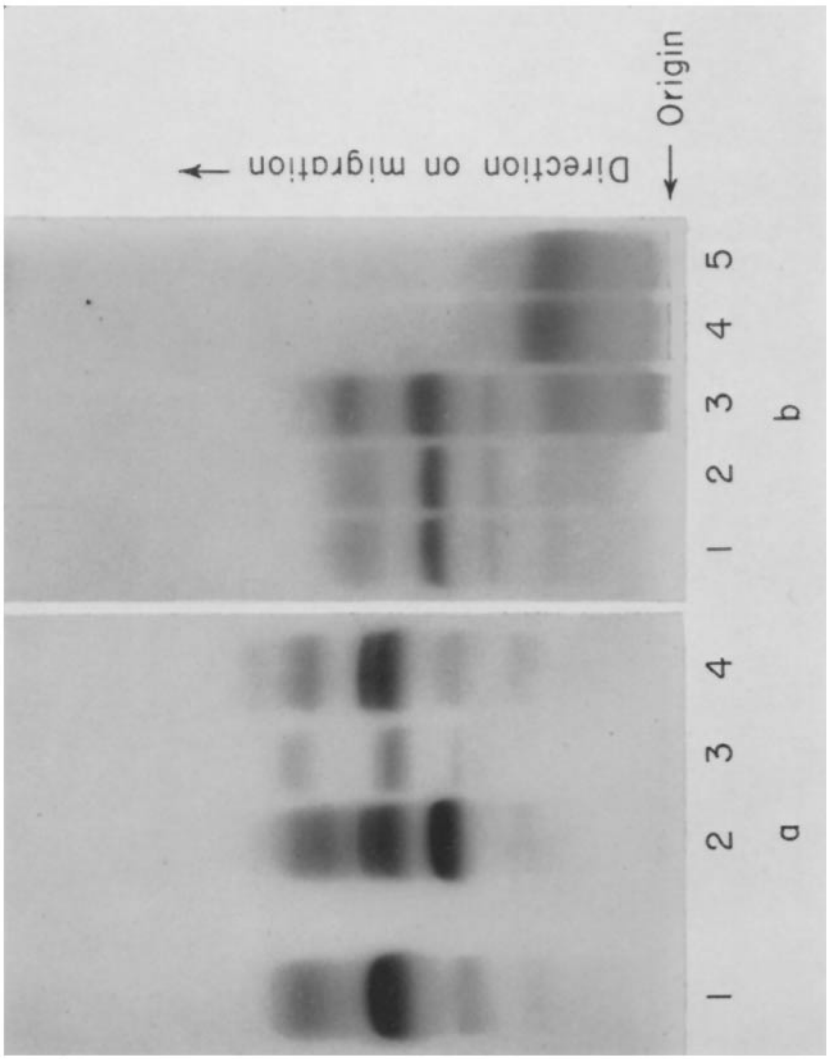

FIG. 4. Dissociated antibodies of five different specificities from subject 1. *a* and *b* represent two separate experiments. *a*: 1, reduced alkylated human 7S γ-globulin; 2, antiblood group A antibodies; 3, antiteichoic acid antibodies; 4, antilevan antibodies. *b*: 1, antidextran antibodies (reduced in form of antigen-antibody precipitate); 2, antidextran antibodies (reduced after purification using dextranase); 3, antitetanus toxoid; 4 and 5, tetanus toxoid.

same individual, each of different specificity, are illustrated in Figs. 4 *a* and 4 *b*. The antibodies directed against blood group A substance (Fig. 4 *a*, origin 2) yielded bands corresponding to H_γ chains and also slower bands corresponding to $H_{\gamma 1M}$ chains (8–10). The L chains of this preparation showed a complex pattern consisting of bands superimposed upon a diffuse zone. Antiteichoic acid antibodies showed a single fairly diffuse L band (Fig. 4 *a*, origin 3). The antibodies to levan showed two L bands, one deeply staining and the other staining faintly and of quite high mobility. A separate experiment using antidextran antibodies (Fig. 4 *b*, origins 1 and 2) revealed patterns similar to those of Fig. 1: two slightly separated L chain bands were found. In the same gel, the antibodies to tetanus toxoid showed a fast L chain band and a slower diffuse zone (Fig. 4 *b*, origin 3). Control comparisons of the toxoid that had been submitted to the same conditions of reduction and alkylation (Fig. 4 *b*, origins 4 and 5) allowed identification of the toxoid component in the antigen-antibody precipitate used in origin 3.

DISCUSSION

The starch gel electrophoretic behavior of purified human antibodies is similar to that of purified guinea pig (11, 20) and mouse antibodies (21). These studies on three different animal species suggest that the degree of heterogeneity of specifically purified antibodies is less than that of whole γ-globulin, at least with respect to the L chains. Recent experiments by Kunkel *et al.* (22) further substantiate this conclusion and suggest the possibility of a correlation between starch gel electrophoretic behavior and the antigenic uniqueness of purified human antibodies.

The present data indicate that antibodies of different specificity from a single individual show differences in pattern. Differences among five antibodies from a single individual were comparable to those reported earlier for different antibodies from a single guinea pig (11). Within a given specificity the human antibodies from different individuals showed variations in their band patterns, but an insufficient number of sera of a given specificity were studied to permit recognition of possible similarities such as those reported for guinea pig antibody (11, 20).

Purified human antibodies directed against polysaccharides composed of a single sugar with a predominant type of linkage have yielded relatively simple L chain patterns. The antidextran preparation from one individual, whether purified with respect to the size of the combining region (Fig. 1, origins 2 and 3) or examined as total antibody (Fig. 2, origin 1), showed one predominant L chain band. The patterns closely resembled those of highly purified γ myeloma proteins (6, 7). Studies on the Gm and Inv groups of purified human antibodies to the antigens studied here also show a behavior corresponding to that of the myeloma globulins (23).

This analogy with myeloma proteins and Bence-Jones proteins which are known to be relatively homogeneous may have useful applications in detailed chemical studies of antibodies. In particular, starch gel electrophoretic criteria of purity would be valuable in characterizing antibodies purified for the purpose of determining their amino acid sequence or the structures of their combining sites.

The finding that different antibodies give different patterns of L chains in starch gel electrophoresis and that apparently lesser differences occur among antibodies of the same specificity formed in different individuals may indicate that one or more types of cells, each with the capacity to synthesize a specific gamma globulin, has responded to contact with antigen and that each such cell type synthesizes a gamma globulin with a characteristic L chain, as seems to be the case in the myeloma globulins (6, 7). The data in humans are as yet too limited to establish how many such cell types can respond to a single antigen or whether a single cell type in forming antibodies to more than one antigen synthesizes the same L chain. It should be noted that compared to the hyperimmunization of the laboratory animals, the methods of immunization of human beings with antigen employ minimal stimulation, and therefore may involve a much higher selectivity in cellular response. Maximal stimulation may recruit a larger variety of cell types for antibody formation, and therefore give rise to a variety of L chains (20).

It has been suggested, among other possibilities (24), that L chains by interacting with H chains may form the specific combining region. Experimental evidence for this has been obtained for equine (25) and guinea pig (26) antibodies. Thus, although there are data to indicate that H chains retain some capacity to combine with antigen (27–31), this activity is substantially enhanced when H chains and L chains from the same antibody are admixed (26). Moreover, Roholt *et al.* (32) have identified peptides from L chains of rabbit anti-*p*-azobenzenearsonate which appear to have been derived from the binding region of the antibody. These findings are consistent with the results of starch gel electrophoresis of dissociated antibodies originally directed against well defined antigens.

SUMMARY

Specific precipitates and purified human antibodies were reduced and alkylated and subjected to starch gel electrophoresis in 8 M urea to dissociate and separate the L and H polypeptide chains. Dissociated antibodies to dextran, levan, teichoic acid, blood group A substance, and tetanus toxoid showed sharp bands corresponding to L polypeptide chains. The patterns differed for antibodies of different specificities. Some differences were also seen among antibodies of the same specificity from different individuals. Purified antidextran antibodies showed particularly simple patterns resembling those of purified human γ myeloma proteins. In some cases only one L chain band was present.

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