RELATIONSHIPS BETWEEN RELATIVE BINDING AFFINITY AND ELECTROPHORETIC BEHAVIOR OF RABBIT ANTIBODIES TO STREPTOCOCCAL CARBOHYDRATES*

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Rabbit antibodies to the carbohydrates of streptococci and other bacteria may have several properties of the myeloma proteins which are indicative of molecular uniformity (1-3). Studies to demonstrate this uniformity have primarily employed rabbit antisera which resemble myeloma sera because they contain a single monoclonal antibody peak (4-7). But antisera containing a single monoclonal peak occur in the minority of rabbits immunized with bacterial vaccines. The majority of the rabbits respond with either multiple discrete antibody components, each possessing electrophoretic homogeneity, or with heterogeneous antibodies (2, 3).

Described in this report is the isolation of such multiple antibodies by means of affinity-immunoabsorbent chromatography. The evidence suggests that the net electrical charge of an antibody bears a reciprocal relationship to its relative binding affinity for the immunoabsorbent column. In general, the greater the electrophoretic mobility of an antibody component, the lower the relative affinity for the immunoabsorbent column. Furthermore, the relative binding affinity of an antibody for the immunoabsorbent has provided another means for assessing the uniformity of rabbit antibodies to streptococcal carbohydrates.

Materials and Methods

Immunization, Immunological, and Immunochemical Procedures.—The source of rabbits, the preparation of streptococcal vaccines, the immunization schedule, and the immunological and immunochemical procedures have been described previously (1, 2, 5).

Electrophoretic Procedures.—Microzone electrophoresis was performed on cellulose acetate membranes as previously described (1, 2). Polyacrylamide gel disc electrophoresis in urea was performed according to the method of Reisfeld and Small (8). Densitometric tracings of the stained gels employed a Gilford linear transport attached to a Gilford spectrophotometer.* This work was supported by National Institutes of Health Grants AI 08429 and AI 09217, and by a Grant-in-Aid from the American Heart Association. The work was also sponsored by the Commission on Streptococcal and Staphylococcal Diseases of the Armed Forces Epidemiological Board, and was supported in part by the U.S. Army Medical Research and Development Command, under research contract No. DADA 17-67-7043.

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Preparative zone electrophoresis was performed in 0.5% Seakem Agarose as previously described (2, 5).

Immunoabsorbents.—Immunoabsorbents with the group-specific carbohydrate of Group C streptococci were prepared by methods analogous to those employed by Kristiansen et al. for the blood Group A substance (9). Preparation of the Group-specific carbohydrate by extraction from streptococcal cell walls employed the procedures described by Krause and McCarty (10). Sepharose 4B was used as the insoluble matrix (11-13).

Method 1.—100 mg of Group C carbohydrate was partially deacetylated in 100 ml of 20% p-toluene sulfonic acid for 6 hr at 50°C with gentle shaking. The partially deacetylated carbohydrate was then extensively dialyzed against water for 48 hr, and lyophilized. Quantitative precipitin tests which employed the carbohydrate antigen before and after partial deacetylation suggest that 40–60% of the antigenic sites were lost by the deacetylation process. The appearance of free amino groups was determined qualitatively by the ninhydrin reaction (14). The lyophilized and partially deacetylated carbohydrate was then dissolved in 50 ml of 0.5 M NaHCO3, pH 8.5.

100 ml of swollen Sepharose 4B (4g) was activated with 4g of cyanogen bromide. Activation was achieved by stirring at pH 11. The pH was kept constant with an automatic titrator loaded with 4 N NaOH. Completion of the reaction was indicated by stabilization of the pH, which was usually achieved after 4–6 min. The activated Sepharose was then immediately washed on a coarse sintered glass funnel with ice-cold water, followed by ice-cold 0.5 M NaHCO3. It was then quickly added to the antigen solution. The reaction was allowed to take place for 24 hr at room temperature with gentle stirring. The Sepharose–carbohydrate complex was then washed sequentially with: 0.1 M of NaHCO3, 0.1 M of Na-acetate, pH 4.5, H2O, and 0.1 M PO4-buffered saline, pH 7.2.

Method 2.—This method employed the dibasic amino acid lysine as a link between the Sepharose and the carbohydrate antigen. 100 ml of swollen Sepharose 4B was activated with 100 mg of cyanogen bromide for 6 min at pH 11, in a total volume of 20 ml of H2O. The pH was then quickly brought down to 8.5 with 1 N HCl, and the solution immediately added to the Sepharose-lysine complex in 100 ml of 0.5 M NaHCO3, pH 8.5. After 24 hr of reaction time at room temperature, the gel was washed as described above. Columns were packed with these immunoabsorbents and equilibrated with 0.1 M of PO4-buffered saline, pH 7.2.

Affinity Chromatography.—The antiserum was added to the column and unabsorbed protein was rinsed through with 0.1 M PO4-buffered saline. This effluent, containing the unabsorbed serum components, was collected and concentrated to the original serum volume. Comparison of the microzone electrophoresis patterns of the antiserum before and after column absorption allowed an estimation of the absorbed antibodies, and of the capacity of the immunoabsorbent. In most cases, the capacity was between 2 and 4 mg of antibody per 1 ml of packed Sepharose.

Two-thirds of the column volume contained the packed Sepharose. The remaining column volume was left to receive the starting buffer of the gradient, which was employed to elute the absorbed antibodies. The volumes for each of the three buffers apply to a column containing 100 ml of packed Sepharose. 50 ml of 0.1 M PO4-buffered saline, pH 7.2, was added to the top of the column. The mixing chamber contained 200 ml of 0.01 M Na-acetate–buffered saline, pH 5.0, and the reservoir contained 200 ml of 0.5 M acetic acid, 1 M NaCl, pH 2.5. This sequence of buffers permits a sigmoidal decrease of the pH. The salt concentration increases in a linear fashion.

Absorption and elution were performed at 4°C. The eluted antibody was collected in 60-
80 fractions and the protein concentration in each fraction was determined using a Technicon AutoAnalyzer (Technicon Corp., Ardsley, N.Y.) which was adapted to perform the Lowry determination (15).

**Individual Antigenic Specificity.**—Anti-antisera to isolated antibody components were prepared in guinea pigs. The guinea pigs received a total of 100 μg of antibody in Freund's adjuvant (200 μg mycobacteria/ml) in the footpads. At the same time, 5 mg of pooled rabbit IgG was injected intravenously in order to induce immune tolerance to common IgG determinants (16). 4 wk later the guinea pigs were bled out. Approximately one out of three antisera prepared in this fashion is monospecific for the antibody population used as antigen. Such antisera were employed to demonstrate differences in individual antigenic specificity of multiple antibody components.

**RESULTS**

42 New Zealand red rabbits, randomly selected from the stock of the supplier, received two courses of intravenous injections of Group C streptococcal vaccine. The interval between the two courses was 4 months. Depicted in Fig. 1 are the microzone electrophoretic patterns of representative examples of the antisera collected after the second course of immunization. The amounts of precipitating antibodies to Group C carbohydrate in each of the 12 antisera shown in the figure are recorded in the legend. It has been reported previously that 1 in 10-12 rabbits responds with a high level of antibody, the bulk of which is essentially confined to a single electrophoretically monodisperse component (2). Pattern 12 is such an antiserum and the antibody in this case
has a molecular uniformity similar to that of the myeloma proteins. Previous studies on “restricted” antibodies have focused primarily on antisera such as No. 12(1, 5–7).

Fig. 2. Upper frame, microzone electrophoretic patterns, Group C antiserum R 24–36. Abs (absorbed) antiserum after passage through the immunoabsorbent column. Pool I, antibody recovered in the first peak by gradient elution from the immunoabsorbent column. Pool II, antibody recovered in the second peak. Lower frame, gradient elution from the immunoabsorbent column of the antibody absorbed from antiserum R 24–36. Dotted line, pH gradient; solid line, protein curve. The two major eluted antibody components were designated as Pool I and Pool II.

In an earlier report attention was directed to the frequent occurrence of two or more electrophoretically distinct antibody components (5). The serum concentrations of such components do not nearly approach, however, the serum concentration of the antibody in antiserum 12 or that of the human myeloma
proteins. Electrophoresis patterns 2-11 are examples of such multiple component antisera. Each component is precipitating antibody to the soluble Group C carbohydrate. The amounts of precipitating antibody in these sera range from 7 to 22 mg/ml. In all of the immunization studies performed thus far, it is uncommon for the electrophoretic analysis to reveal complete absence of restriction in the electrophoretic distribution of the antibody within the γ-globulin domain. Antiserum 1 is such a case, however, and 2 out of 42 rabbits had such a pattern.

Multiple antibody components, produced by a single individual in response to a single antigen, could be a rich source of information on the relationships between structural and functional properties of antibodies. Therefore, antibodies from such antisera have been isolated and some of their properties investigated.

Illustrated in Fig. 2 are the results obtained when antiserum R 24-36 was fractionated by affinity chromatography. As shown in the upper microzone electrophoretic pattern, this antiserum contained two electrophoretically distinct major antibody components. The second pattern shows the antiserum after passage through the immunoabsorbent column. The bulk of the antibody has been removed. Depicted in the lower frame of the figure is the protein pro-
file obtained when the absorbed antibody was eluted with the pH and salt gradient. The solid line is the protein curve, the dotted line the pH gradient. The antibody was eluted within two major peaks which are referred to as pools I and II, respectively. Pool I was eluted at a relatively low hydrogen ion and salt concentration and corresponds to the faster migrating antibody component. Pool II was eluted at higher hydrogen ion and salt concentrations and corresponds to the slower migrating antibody component. Similar results have been achieved with 11 other Group C antisera which contained two or
more distinct antibody components. Without exception, fast migrating antibody components were eluted at the beginning and slower migrating antibody components were eluted toward the end of the gradient.

As can be seen from the microzone electrophoretic patterns in the upper frame of Fig. 2, pools I and II recovered from the column correspond to the fast and slow antibody components in the original serum, but separation of the two components by the column procedure is not complete. For this reason, preparative agarose electrophoresis was employed as the second step in the purification process. The superimposed protein profiles of pools I and II from separate electrophoresis blocks are shown in Fig. 3. The relative electrophoretic distribution of both pools is similar to that observed on microzone electrophoresis. The peak components in each pool were collected and concentrated and used for the subsequent experiments. The overlapping material was discarded.

Depicted in the left frame of Fig. 4 are the microzone electrophoretic patterns of the two antibody preparations obtained from this antiserum after each purification step. Pools I and II were obtained from the immunoabsorbent column. After preparative electrophoresis of pools I and II, the two antibody preparations are referred to as fast fraction and slow fraction, respectively. It is clear that complete separation of the two fractions is achieved by the additional electrophoretic procedure. Depicted in the right frame of Fig. 4 are the densitometric tracings of the polyacrylamide gel disc electrophoretic patterns of each of these antibody fractions after reduction and alkylation. For comparison there is shown also the light-chain pattern of all of the antibody in the antiserum obtained by precipitation with soluble antigen at equivalence. The light chains resolve into a complex multibanded pattern. There are, however, a prominent fast migrating band and two prominent slow migrating bands. The light chains of pools I and II are more restricted. The predominant light-chain band of pool I corresponds to the fast band, and the predominant light-chain bands in pool II correspond to the slow bands in the pattern of the total antibody. A significant further restriction in heterogeneity has been achieved by preparative electrophoresis. The slow migrating band in pool I is eliminated in the fast fraction and the fast migrating bands of pool II are eliminated in the slow fraction. Overlap in the light-chain patterns of these two purified antibody components is no longer observed. These results indicate that complete separation of these two antibody components has been achieved by application of both immunoabsorbent chromatography and preparative electrophoresis. Furthermore, the fast fraction revealed a highly restricted light-chain pattern which is indicative of uniformity. N-terminal sequence analysis of the light chains, published elsewhere (17), suggest that the fast fraction is, in fact, a uniform antibody. The
two light-chain bands of the slow antibody component reflect two populations of molecules, a finding also consistent with the amino acid sequence data (17).

Experiments were performed to show that the differential dissociation from immunoabsorbent columns of multiple antibody components is due to differences in relative binding affinities. This was done by adjusting the conditions so that two or more antibody components compete for the binding sites on the column. Before performing such an experiment the antibody-binding capacity

![Diagram of antibody elution](image)

**Fig. 5.** Differences in relative binding affinity between electrophoretically slow and fast antibody components as shown by the elution of antibody from immunoabsorbent columns which have been loaded below and over capacity. Densitometric tracings of microzone electrophoretic patterns. Top frame, Group C antiserum with two antibody components. Frames marked effluent: left, antiserum after passage through the column when it was overloaded; right, antiserum after passage through the column when it was not overloaded. Frames marked eluted antibody I and II: left, antibody components sequentially recovered by gradient elution from the overloaded column; right, antibody components recovered from the column not overloaded.

of immunoabsorbent columns was determined as follows:

Columns containing 10 ml of packed immunoabsorbent were loaded with small portions of antisera until the first traces of antibody were detected in the effluent. The absorbed antibody was recovered and measured. This gave an indication of the capacity of the column. Antisera were then selected with a known precipitating antibody content and with two electrophoretically distinct antibody peaks. A standardized column was loaded with such an antiserum either within the range of column capacity or overloaded with an amount of antiserum which contained antibody in 100% excess. When the column is overloaded, there is competition between antibody components for a limited number of antigenic sites. Under these conditions, antibodies with the greatest affinity for the column are absorbed, and those with the least affinity are not absorbed.

Depicted in Fig. 5 are the results of such an experiment. The top frame shows
the densitometric scan of the electrophoretic pattern of this antiserum. Two antibody peaks are clearly visible. The left part of the figure illustrates the results obtained when the column was overloaded with 100% excess of antibody. The effluent contained, in addition to all of the other serum proteins, most of the antibody in the faster migrating antibody peak, whereas the slow peak was absorbed to the column. The absorbed antibody, recovered by subsequent gradient elution, consisted primarily of the slow component and

![Graph](image)

**Fig. 6**. A comparison between the elution conditions required to recover seven antibodies from an immunoabsorbent column, and their electrophoretic mobilities. These seven antibodies were isolated from six Group C antisera. Abscissa, centimeters of electrophoretic migration measured after 24 hr of agarose electrophoresis (0 is the origin). The electrophoretic distribution for each antibody component is indicated by the peaks. Dotted line, electrophoretic distribution of IgG in preimmune serum by the same procedure. Open circles, pH, and closed circles, salt concentration, measured in the peak column elution fraction for each antibody component. The length of the vertical lines from each open and closed circle indicates the range in pH and salt concentrations from the beginning to the completion of the elution for each antibody component.

contained only a small portion of the fast component. Depicted in the right part of the figure is the control experiment. When the column was loaded within its capacity, all of the antibody was absorbed to it. By gradient elution, both the fast and the slow antibody components could be sequentially recovered.

The interpretation of these experiments is that competition between fast and slow migrating components for a limited number of antigenic sites was decided in favor of the slow migrating antibody component. Similar results have been achieved with 11 other Group C antisera which contained two or more distinct antibody components. These data suggest that in a single antiserum, antibodies of slow electrophoretic mobility have a higher relative bind-
ing affinity for the streptococcal Group C carbohydrate than have fast migrating antibodies. Because the hydrogen ion and salt concentrations required to elute an antibody from the immunoabsorbent have a reciprocal relationship to its electrophoretic mobility, the elution conditions can be used as a relative measure for the binding affinity of an antibody to the column.

Fig. 6 summarizes the elution conditions which were required to recover seven different antibody components, each with a distinct electrophoretic mobility. Each of these isolated components, except the one with the most rapid mobility, precipitated with soluble Group C carbohydrate. These antibodies were isolated from six different Group C antisera. The abscissa measures the distance the antibodies migrated during 24 hr of preparative agarose electrophoresis performed under standard conditions. The peaks indicate the electrophoretic distribution of each component. The dotted line shows the electrophoretic distribution by the same procedure of IgG of normal rabbit serum. The open circles indicate the pH and the closed circles the salt concentration which were required to dissociate the antibody from the immunoabsorbent column. As is evident from these data, hydrogen ion and salt concentration decreases continuously with increasing electrophoretic mobility. It should be pointed out that the antibody peak with the greatest mobility to the anode did not bind to the immunoabsorbent column at pH 7.2 and a salt concentration of 0.25 moles/liter. In this case, the pH and molarity of the starting buffer were taken as elution conditions. Thus certain antibody components which emerged during immunization with the streptococcal vaccine possess a high anionic charge and do not react with the carbohydrate antigen. It has yet to be learned if a high anionic charge determines an extremely low binding affinity for antibodies to streptococcal carbohydrates.

The picture which emerges from these studies is that the net electrical charge of antibodies to Group C carbohydrate bears a direct relationship to the relative binding affinity. Such appears to be the case for multiple components isolated from one rabbit and for antibodies isolated from a number of different rabbits. But, as will be noted below, there are some exceptions to this generalization.

Binding Affinity to the Immunoabsorbent as an Additional Criterion for Homogeneity.—Evidence for the molecular uniformity of rabbit antibodies to streptococcal carbohydrates has been documented in previous reports. For example, the antibodies possess individual antigenic specificity (5, 18), and the light chains, monodisperse by disc electrophoresis (5, 6), possess a single amino acid sequence through the first 30 residues from the N-terminus (17). The fast component in antiserum R 24–36, depicted in Figs. 2 and 3, is such an antibody with these uniform properties (17). The elution of this antibody component from the immunoabsorbent column as a symmetrical peak and within a narrow pH range is a further indication of functional homogeneity. It is now clear
that this property is an additional useful characteristic for assessing the uniformity of an antibody preparation. This is illustrated by the behavior of the antibodies in antiserum R 24-61.

The electrophoretic pattern of the antiserum R 24-61 is depicted in Fig. 7. It is clear that the electrophoretic pattern reveals two major antibody components. It was anticipated that the fast component in this serum would be a single uniform antibody. The light chains, for example, were distributed in a single band by disc electrophoresis. But this prediction did not prove to be the case. By use of affinity chromatography, the electrophoretically fast antibody component was resolved into two distinct fractions.

Illustrated in the upper frame of Fig. 8 is the gradient elution profile obtained by affinity chromatography of antiserum R 24-61. Two distinct and narrow peaks are followed by a more broad biphasic peak. The eluted antibody was accordingly divided into four pools which are referred to as pools I through IV, respectively. Pools I and II were further purified by recycling on the same column and the elution profiles obtained are shown in the second and third frames of Fig. 8. Pools III and IV were further purified by preparative agarose electrophoresis.

Microzone electrophoresis of the four purified antibody fractions are shown in the left frame of Fig. 7. They are referred to as fractions I to IV and correspond to pools I to IV designated in Fig. 8. Fractions I and II have iden-
Fig. 8. Upper frame, gradient elution profile of the absorbed antibody from antiserum R 24-61. Dotted line, pH gradient, solid line, protein curve. Pools I to IV were made as indicated. Middle frame, gradient elution profile obtained by repetition of the immunoabsorbent column procedure with pool I. Lower frame, gradient elution profile obtained by repetition of the immunoabsorbent column procedure with pool II.
tical electrophoretic mobilities and correspond to the fast migrating antibody component in the antiserum. Fractions III and IV had slightly different electrophoretic mobilities but correspond to the slow migrating antibody component.

Disc electrophoresis of these reduced and alkylated antibody fractions are shown in the right frame of Fig. 7. The light chains of all the antibody recovered from the whole antiserum resolve into four major and several minor bands. The light chains of fractions III and IV have one major band and two minor bands. The light chains of fraction III had somewhat greater mobility than those of fraction IV, and this difference is consistent with the corresponding mobilities of the two antibody fractions. In contrast, the light chains of fractions I and II, distributed primarily in one band, had identical mobilities. Thus two distinct antibodies are present in the fast serum component and are separated from each other because each had a different relative binding affinity for the immunoabsorbent column, even though they possess identical net charge properties. Subsequent individual antigenic specificity studies indicated that each of these two electrophoretically identical antibody components possesses its own antigenic individuality.

Differences in the individual antigenic specificity between antibody fractions I and II of antiserum R 24-61 were demonstrated with guinea pig antisera to each of these fractions. These antisera are referred to as anti-fraction I and anti-fraction II. In the immunodiffusion experiment depicted in Fig. 9, anti-fraction I reacts exclusively with fraction I. Anti-fraction II shows, in addition to the reaction with fraction II, a cross-reaction with fraction I. This one way cross-reaction may be due to the incomplete elimination of fraction I from the fraction II preparation employed to immunize the guinea pigs. In such an event the anti-fraction II would contain antibodies to fraction I.
Additional support for the distinctiveness of antibody fractions I and II stems from the current amino acid sequence analysis of their light chains. Both light chains belong to the variable region subclass V_{II} (17) but each has a unique amino acid sequence. Such studies, coupled with the individual antigenic specificity data and relative binding affinity characteristics, indicate that two antibodies have been isolated from a single antibody component which by all electrophoretic criteria was thought to have been homogeneous. It is therefore clear that the relative binding affinity of an antibody preparation provides an additional useful method for assessing the uniformity of an antibody preparation.

DISCUSSION

A large body of literature has stressed the heterogeneity of the immune response to even the most simple haptenic determinants. The isolation of multiple but discrete antibody components to streptococcal carbohydrates after immunization with streptococcal vaccines is a reflection of the complexity of the immune response to an antigenic stimulus. Previous reports from this laboratory have stressed the immune response to streptococcal carbohydrates in the occasional rabbit in which the bulk of the total antibody is representative of a single molecular species (1, 5–7). Such a response is an uncommon occurrence, however, in rabbits randomly selected from suppliers. Most antisera contain two or more antibody components as judged by electrophoretic criteria, and immunochemical studies indicate that each of these components possesses reactivity for the immunodominant site of the carbohydrate antigen (2).

The fractionation procedures used in the present experiments permit the isolation of multiple antibody components from a single Group C serum by exploiting the differences in relative binding affinities. The term relative binding affinity as used in this report refers to the relative affinity of an antibody component for an immunoabsorbent column. These studies were based on the initial observation of Parker and Briles that heterogeneous antibodies to Group A streptococcal carbohydrate in an antiserum could be separated by affinity chromatography into structurally and functionally restricted components (19). This procedure has an advantage in that it can be used for antibodies to many different kinds of carbohydrate antigens, provided the antigen can be converted into an insoluble immunoabsorbent. The demonstration that an electrophoretically homogeneous antibody was separated into two components which were distinct by binding affinity, individual antigenic specificity, and N-terminal light chain sequence, stresses the utility of affinity immunoabsorbent chromatography for isolating antibodies with minor structural and functional differences.

1 Hood, L. Personal communication.
In previous reports from this laboratory, it has been shown that an IgG antibody component with a distinct electrophoretic mobility has a characteristic individual antigenic specificity (5, 18). These observations have been augmented here by the demonstration that differences in the net charge of antibodies are accompanied by differences in their relative binding affinity for the immunoabsorbent column. Such a procedure has been employed for affinity studies because it has not yet been feasible to perform equilibrium dialysis experiments with the immunodominant sugar of Group C carbohydrate, N-acetylgalactosamine. Nevertheless, the relative binding affinity for the immunoabsorbent column provides additional evidence for the molecular uniformity of antibodies to the streptococcal carbohydrates.

Others have based the isolation of multiple antibody components from an antiserum on the fortuitous observation that one of the antibody components cross-reacted with another antigen. For example, Jaton et al. (20) isolated a uniform antibody from an antiserum to pneumococcal Type VIII polysaccharide which cross-reacted with Type III polysaccharide. Kindt et al. (7) exploited the cross-reaction of an antibody to Group C streptococcal carbohydrate with Sephadex. Such purification procedures, however, may not be generally applicable because the detection of a cross-reacting antigen remains a matter of chance.

Use of the dissociation conditions of an antibody from an immunoabsorbent column as a relative measure of its binding affinity has shown that there is an inverse relationship between the net charge of an antibody and its relative affinity to the carbohydrate antigen. It has been demonstrated in the experiments of Sela and associates (21, 22) and of Benacerraf et al. (23) that an antigen with a certain electrical charge preferentially selects antibodies with the opposite charge. Since there are no exact data on the net charge properties of the streptococcal carbohydrates as they exist in the bacterial vaccine, it is not clear if this is the case for the streptococcal antibodies described here. It is likely that the streptococcal group-specific antigens as well as the whole streptococcus which is used as the vaccine have a very low net charge density, similar to that described for *Staphylococcus aureus* (24, 25). Therefore, a single rabbit may respond with antibodies selected from a wide spectrum of available net charge qualities, as was shown to be the case for the immune response to well defined neutral antigens (21–23). However, it is conceivable that a low net electrical charge of an antibody facilitates interaction with a neutral antigen and thus contributes to a higher binding affinity. This is a possible explanation for the frequent occurrence of antibodies to streptococcal carbohydrates which have a slow electrophoretic mobility and belong to the light chain variable-region subclass V,III (17).

Hood et al. (17) have demonstrated the occurrence of three subclasses in the N-terminal sequences of κ-light chains from rabbit anti-streptococcal
antibodies. These were designated as V,I, V,II, and V,III. A correlation was also observed between the light chain subclass and the net charge of an antibody. Antibodies with V,I, V,II, and V,III light chains possessed fast, intermediate, and slow electrophoretic mobilities respectively. Summarized in Table I are the data on eight antibody components which illustrate the relationship between the net charge, the relative binding affinities, and the light chain subclass. One antibody with light chains of the V,I subclass had a fast electrophoretic mobility and a low relative binding affinity. Three antibodies with V,II light chains had intermediate electrophoretic mobilities and an intermediate relative binding affinity. Four antibodies with V,III light chains had a slow electrophoretic mobility and a relatively high binding affinity.

More extensive structural studies on multiple antibody components will reveal whether the N-terminal sequence of the light chain influences the binding affinity directly, or more likely, whether the binding site sequences in variable-region subclasses exhibit specific net charge properties which limit the affinities within one subclass to a certain range. The latter would be in agreement with the studies by Freedman et al. (26) who demonstrated the participation of amino groups in the combining site of certain antibodies directed against negatively charged antigens, and the absence of amino groups in the combining site of antibodies directed against positively charged antigens.

**TABLE I**

*Relationship between Net Charge, Relative Binding Affinity, and Light Chain Subclass of Group C Streptococcal Antibodies*

| Antibody   | Net charge† (cm el. migration) | Relative binding affinity (dissociation pH) | Light chain* subclass |
|------------|--------------------------------|---------------------------------------------|-----------------------|
| R 27-11    | high                           | low                                         | V,I                   |
|            | (+1)                           | (5.5)                                       |                       |
| R 24-61 I* | intermediate                   | intermediate                               | V,II                  |
| R 24-61 II*| (±0 to −1.5)                  | (5.2 to 4.0)                               | V,II                  |
| R 24–36 fast Fx* |                        |                                             |                       |
| R 24–61 III* | low                           | high                                       | V,III                 |
| R 24–61 IV* | (−2 to −4)                    | (3.8 to 2.7)                               | V,III                 |
| R 26–90    |                               |                                             | V,III                 |
| R 24–36 slow Fx* |                            |                                             |                       |

* Light chain subclass taken from Hood et al. (17). The nomenclature for the antibodies in this paper was as follows: R 24–61 I and II = R 24–61; R 24–61 III and IV = R 24–61S; R 24–36 fast Fx = R 24–36 I; R 24–36 slow Fx = R 24–36S.

† cm electrophoretic migration measured after 24 hr of agarose electrophoresis.
ANTIBODIES TO STREPTOCOCCAL CARBOHYDRATES

SUMMARY

After repeated intravenous injections with Group C streptococcal vaccine, most rabbit antisera were shown to contain one or more IgG antibody components, as revealed by microzone electrophoresis. A procedure for the fractionation of multiple IgG antibody components from such streptococcal antisera is described. Separation is achieved on the basis of differences in relative binding affinities of the antibody components to immunoabsorbent columns. The evidence suggests that the electrophoretic mobility, and thus the net charge of an antibody, bears a reciprocal relationship to its binding affinity for the streptococcal Group C antigens. Furthermore, the relative binding affinity affords another means to assess the functional homogeneity of streptococcal antibodies. A possible relationship between light chain variable-region subclasses and binding affinities of streptococcal antibodies is discussed.

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