THE EMERGENCE OF ANTIBODIES WITH EITHER IDENTICAL OR UNRELATED INDIVIDUAL ANTIGENIC SPECIFICITY DURING REPEATED IMMUNIZATIONS WITH STREPTOCOCCAL VACCINES*

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(Received for publication 14 January 1970)

In previous reports from this laboratory, evidence has been presented which indicates that rabbit antibodies to streptococcal carbohydrates have several of those characteristics of the myeloma proteins which are indicative of molecular uniformity. For example, these antibodies and their light chains exhibit marked restriction in electrophoretic mobility (1, 2). Quantitative precipitin studies with anti-allotypic sera indicate exclusion of allotypic markers (3). Analysis of the three N-terminal amino acid residues of the L chains has revealed a marked restriction in amino acid sequence heterogeneity when compared to normal light chains (4, 5). These antibodies also possess individual antigenic specificity (6); a characteristic first described by Kunkel and associates for the myeloma proteins and for several human antibodies to carbohydrate substances (7, 8). Detection of individual antigenic specificity employs anti-antibodies which are raised in a different species. The term idiotypy has been used to describe a similar, but not identical, antigenic individuality of antibodies to undetermined antigens of \textit{Salmonella} (9) and \textit{Proteus} (10). Recently, Daugharty et al. reported idiotypic antibodies to an azobenzoate antigen (11). Detection of idiotypy employs anti-antibodies which are raised within animals of the same species and of identical allotype.

The earlier studies on the uniform properties of streptococcal antibodies have been concerned primarily with antibodies to the carbohydrates of Groups A-variant and C streptococci (1, 2, 6, 12). Similar uniformity is reported here for antibodies to Group A streptococci. The three streptococcal carbohydrate antigens, Group A, Group A-variant, and Group C, possess a similar branched rhamnose polymer. The Group A carbohydrate specificity is dependent upon terminal N-acetylglucosaminide residues (13), while that of Group C is dependent upon terminal N-acetylgalactosaminide residues...
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residues (14). The antigenic specificity of the Group A-variant carbohydrate resides in the rhamnose polymer itself (15).

The studies reported here extend the earlier observations on the individual antigenic specificity of streptococcal antibodies which were produced after primary immunization (6). Antibodies with identical individual antigenic specificity may occur after both primary and secondary immunization. On the other hand, after secondary immunization a population of antibodies may occur with an antigenic individuality distinct from that of the antibodies which occurred after primary immunization.

Although it has been shown previously that the antibodies to streptococcal carbohydrates possess individual antigenic specificity, there is no quantitative information on the proportion of the molecules in a single preparation which bears this specificity. This question has been examined here.

Materials and Methods

Streptococcal Vaccines, Streptococcal Cell Walls, and Group-Specific Carbohydrates.—The preparation of these materials has been previously described (1, 14, 16).

Immunization Procedures.—The source of rabbits and the immunization schedule for preparing streptococcal group-specific antisera have been previously described (6, 17).

Serological and the Immunochemical Methods.—These methods have been previously described (5, 6, 17).

Individual Antigenic Specificity.—Detection of individual antigenic specificity employed goat antisera to rabbit antibody preparations. The immunization procedure has been described previously (6). Double diffusion in agar analysis was done using the gel chambers described by Wadsworth (18) and Holm (19).

The quantitative precipitin analysis, which employed streptococcal antibodies and their goat antisera, has been carried out as follows: 0.2 ml of goat antiserum was added to each tube of a series containing 10–100 μg of rabbit antibody or control γ-globulin preparations. Saline was added to each tube to a total volume of 1 ml. The reaction was carried out for 72 hr at 4°C. The protein in the precipitates was measured in 1 ml 0.1 M NaOH using a Technicon AutoAnalyzer which had been adapted to perform the Lowry determination (20).

Selective absorption of antibody components with individual antigenic specificity from whole rabbit antisera by immunosorbent columns containing solidified goat anti-antisera was performed as follows: 5 ml of goat antiserum were absorbed at equivalence with pooled rabbit fraction II. The absorbed goat antisera were solidified by addition of glutaraldehyde, according to the method of Avrameas and Ternynck (21). The solidified sera were homogenized, washed, and packed into 1 X 10 cm columns. The columns were loaded with 0.05 ml portions of the homologous rabbit antisera. The eluted fractions containing the unabsorbed serum protein were pooled and concentrated to the original volume. The selectively absorbed antibody components were determined by comparing the densitometric tracings developed from the electrophoretic patterns of the antisera before and after column absorption.

The proportion of antibody molecules with individual antigenic specificity within an isolated antibody component was determined as follows: the antibody preparations were labeled with 125I according to the method of McFarlane (22). A 20 μg portion of the labeled antibody was precipitated with an excess of the homologous goat anti-antisera which had been absorbed at equivalence with pooled rabbit fraction II. The radioactivity in the precipitates
and in the supernatants was measured and calculated as per cent of the total radioactivity used in the test. Controls included a similar protocol with heterologous goat anti-antisera.

**Electrophoretic Methods.**—Zone electrophoresis was carried out on cellulose acetate membranes as previously described (1, 2).

Preparative electrophoresis employed 0.5% Seakem Agarose as the supporting medium. This method has been reported in detail elsewhere (5, 17).

Polyacrylamide gel disc electrophoresis of reduced and alkylated γ-globulin was performed according to the method of Reisfeld and Small (23). Densitometric tracings of the gels were obtained with a Gilford linear transport as previously described (5).

**Purification of Group A Specific Antibody.**—γ-globulin fractions obtained by preparative electrophoresis were absorbed onto Group A streptococcal cell walls (6). The suspension was allowed to stand for 1 hr at room temperature. For each 5 mg of antibody, 0.1 ml of tightly packed trypsinized cell walls were used. This enzyme treatment removes the outer protein layer and exposes the carbohydrate so that it can function as an immunoadsorbent surface. The cell walls were centrifuged and the supernatant which contained nonantibody γ-globulin was saved for subsequent analysis. The cell wall pellet was washed 3 times with cold saline. Dissociation of the group-specific antibody from the cell walls was carried out by the addition of 5% N-acetylglucosamine in 0.5 M NaCl, 0.1 M Tris (hydroxymethyl)aminomethane, pH 7.5, for 24 hr at room temperature. Several subsequent dissociation steps employed the same eluent. The antibody adherent to the cell walls after this treatment was eluted with either 0.5 M NaCl, adjusted to pH 2.5 with HCl (6), or with 7 M guanidine HCl in 1 M Tris, pH 8.2.

**RESULTS**

Illustrated in Fig. 1 are the microzone electrophoretic patterns of two antisera from rabbits immunized with Group A streptococci. These antisera were collected after the second course of immunization. Approximately 10% of all immunized rabbits developed at least one prominent electrophoretically monodisperse antibody component. Both of the antisera in Fig. 1 have two distinct antibody components. In each case, the slow component has the greater restriction in electrophoretic mobility. Although the bulk of the antibody in antiserum R23-61 was not precipitable with soluble carbohydrate, absorption studies with homologous cell walls removed both components, whereas absorption with heterologous cell walls did not do so. All of the antibody in antiserum R23-65 was precipitated with the soluble Group A carbohydrate. Previous studies have clearly indicated that the γ-globulin absorbed by homologous cell walls is antibody to the group-specific carbohydrate (17). Thus, antiserum R23-61 contained nonprecipitating antibody to Group A carbohydrate, and antiserum R23-65 contained precipitating antibody. Calculation of the γ-globulin in the antisera before and after absorption affords an estimate of the antibody content. Antiserum R23-61 contained 18.6 mg/ml of γ-globulin; 16.7 mg/ml were specifically absorbed. Antiserum R23-65 contained 16.8 mg/ml of γ-globulin; 15.1 mg/ml were specifically absorbed.

The electrophoretically distinct antibody components in antisera R23-61 and R23-65 have been isolated by preparative agarose electrophoresis, and employed in subsequent studies to identify other properties which indicate uni-
formity. Slow and fast migrating γ-globulin components thus obtained are referred to as slow fraction and fast fraction, and are indicated as slow Fx and fast Fx in the figure. It is recognized that an antibody component isolated by preparative electrophoresis contains nonspecific γ-globulin in addition to the specific antibody. In most instances, however, specific antibody accounts for 85–95% of the protein in these isolated components.

Specific antibody, devoid of other serum γ-globulin, can be recovered from the isolated components by the use of homologous cell walls as immunoabsorbsents. For example, the slow electrophoretic fractions, depicted in Fig. 1, were absorbed onto Group A cell walls and the antibodies directed against the immunodominant β-N-acetylglucosaminide residues of the Group A carbohydrate were eluted with N-acetylglucosamine. A portion of the antibodies are not eluted by this procedure, an indication that they have specificity for additional antigenic determinants of the carbohydrate. Such antibodies were subsequently eluted with guanidine HCl or with 0.5 M NaCl, adjusted to pH 2.5. Disc electrophoresis of the reduced and alkylated antibodies obtained by these elution procedures from R23-61 are depicted in Fig. 2. The L chains of the whole IgG normally resolve into 8 or 10 bands. This is not readily apparent in this figure because the individual bands are not resolved by the photograph due to their density. These gels have been overloaded with equal amounts of 400 μg of reduced and alkylated γ-globulin per gel in order to insure that minor components are visible. The light chains of the total slow fraction migrate in five
bands. The specific antibody in the slow fraction which was absorbed onto cell walls and eluted with N-acetylglucosamine has L chains which migrate in one heavy band on the gel. L chains of the antibody which was subsequently eluted from the cell walls with guanadine migrate in five bands and this distribution resembles that observed for the L chains of the total slow fraction.

Although the use of cell walls as an immunoabsorbent for large batch preparations of antibody has not been successful for technical reasons, the results of these pilot studies suggest that specific immunoabsorbents will enhance the prospect for isolating a single antibody population from an antiserum. Preliminary studies are now under way to develop immunoabsorbents for this purpose.

In earlier reports, it was shown that those antibodies to Group A-variant and Group C carbohydrates which exhibit physicochemical evidence for uniformity, also possess individual antigenic specificity (6). A similar property has been described here for the Group A antibodies. Antisera have been raised in goats against selected antibody components of the two antisera depicted in Fig. 1. For antiserum R23-61, the slow fraction obtained by preparative electro-
phoresis was employed as antigen. In the case of antiserum R23-65, only the specific antibody recovered from the slow fraction was used as antigen. It was obtained by precipitation at equivalence with Group A carbohydrate. The precipitate, dissociated with 0.5 M NaCl and adjusted to pH 2.5, was used for immunization. Immunoelectrophoretic data indicated that all goat antisera to these preparations were specific for rabbit γ-globulin. Use of these anti-antisera in double diffusion in agar experiments revealed that both of the antibody preparations possessed individual antigenic specificity. Certain features of this specificity for the slow fraction in antiserum R23-61 were examined in greater detail.

The specific antibody was selectively recovered from the slow fraction of antiserum R23-61. This was achieved by elution of the antibody with N-acetylglucosamine from an antibody–cell wall agglutination reaction. The nonantibody γ-globulin which was not absorbed onto the cell walls was saved for the

![Fig. 3. Upper frame: densitometric tracing developed from the γ-globulin domain of the microzone electrophoretic pattern of antiserum R23-61. The cross-hatching indicates the specific antibody which is specifically absorbed out of the antiserum by homologous cell walls. Lower frame: immunodiffusion analysis. The specific antibody and the nonantibody γ-globulin of the slow fraction were applied as indicated by the arrows. The γ-globulin solutions contained 1 mg protein per ml. The lower wells contained goat anti-2' slow fraction.](image)
subsequent experiment. In the double diffusion analysis, depicted in Fig. 3, the reaction between the selectively eluted antibody and its own anti-antiserum spurs over the reaction between the nonantibody γ-globulin of the slow fraction which was not absorbed onto the cell walls and this same anti-antiserum. Such a result indicates that individual antigenic specificity is confined to the specific antibody in these electrophoretic components and is absent in the nonantibody γ-globulin.

**Emergence of Antibodies with Identical Antigenic Specificity after Repeated Immunizations.**—In all of the work reported so far on the individual antigenic specificity of antibodies to the streptococcal carbohydrates, the antibodies were isolated from a single antiserum collected either after primary or secondary im-

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**Fig. 4.** Microzone electrophoretic patterns of two antisera collected from rabbit R23-61 after primary (1') and secondary (2') immunization, and of the γ-globulin fractions of each which were isolated by preparative electrophoresis.
munization. The following studies were devised to show that antibodies with identical antigenic specificity may occur with repeated courses of immunizations. Furthermore, antibody with this particular antigenic specificity was not detectable by the means employed here in the interval between immunizations when group-specific antibody was absent.

Antibody components of identical electrophoretic mobility were isolated by preparative electrophoresis from antisera collected from the same rabbit after primary and secondary immunization. Microzone electrophoresis of the purified
fractions from the antisera of rabbit R23-61 is shown in Fig. 4. Slow and fast migrating antibody components from primary and secondary immunizations are referred to as 1' slow fraction, 1' fast fraction, 2' slow fraction and 2' fast fraction, respectively. The corresponding goat antisera are designated with the prefix anti-; for example, anti-2' slow fraction. Illustrated in Fig. 5 is a double diffusion analysis of the antibody fractions obtained from rabbit R23-61. In

![Diagram showing antibody fractions](image)

**Fig. 6.** Quantitative precipitin reactions between goat anti-2' slow fraction and 1' and 2' slow fractions of rabbit R23-61, and pooled fraction II.

the experiment depicted in the upper frame, unabsorbed anti-2' slow fraction was applied to the lower wells. The reaction of 1' slow fraction with anti-2' slow fraction spurs over the reactions of fraction II and 1' fast fraction with this anti-antiserum. In the experiment depicted in the lower frame, anti-2' slow fraction was absorbed with pooled fraction II. As to be expected, there is no detectable reaction with fraction II. The reactions with both 1' slow fraction and 2' slow fraction form a line of identity.

Depicted in Fig. 6 are quantitative precipitin tests performed with 1' slow fraction and 2' slow fraction of rabbit R23-61. Unabsorbed anti-2' slow fraction
was used as the antiserum. Pooled fraction II is included as a control. The quantitative precipitin curves of both 1' slow fraction and 2' slow fraction are nearly identical. Both antibody preparations are approximately twice as reactive with the anti-antiserum as is fraction II. Thus, the 1' slow fraction contained antibody with an individual antigenic specificity identical to that of the antibody in the 2' slow fraction. This is the case, even though the 1' slow fraction was only an obscure minor component in the electrophoretic pattern of the whole serum. Presumably, 1' slow fraction was the product of a minor population of plasma cells, which become prominent with secondary immunization.

The following experiment was designed to determine the point in time that antibodies with individual antigenic specificity were first detectable during each immunization period. The rabbits in these studies are immunized intravenously 3 times a week for 3-4 wk. The same schedule, after a rest period of 3-6 months, is used for each successive immunization. Each antiserum collected from rabbits R23-61 and R23-65 at weekly intervals throughout two or three immunization periods was precipitated at equivalence with the corresponding anti-2' slow fraction. The concentration of each antiserum was adjusted with saline so that the sample added to each tube in the precipitin test contained 100 μg of γ-globulin. Table I gives the amounts of protein precipitated at equivalence from each antiserum collected from these rabbits. Pooled rabbit γ-globulin and heterologous rabbit sera were used to establish the reactivity of the goat antisera with γ-globulin other than the homologous antibody. Precipitates in excess of that obtained with the control sera or preimmune sera are indicative of the specific antibody identical to that used to generate the anti-antiserum in the goat.

The specific antibody population became clearly detectable during the 3rd wk of primary immunization, and during the 2nd wk of second or third immunizations. With successive immunizations, this antibody population was detectable in higher concentrations than in the first. No γ-globulin with this individual antigenic specificity was detected in the interval between immunizations at those times when the antisera contained no antibody to Group A carbohydrate.

Emergence of Antibodies with Unrelated Individual Antigenic Specificity upon Repeated Immunizations.—In occasional rabbits a major antibody component may emerge during second immunization which has an electrophoretic mobility different from the major antibody component initially present during primary immunization. The following experiment was devised to show that a new electrophoretically monodisperse antibody component after second immunization does, in fact, possess an antigenic individuality which is unlike the individuality of the major antibody in the primary immunization antiserum.

Depicted in Fig. 7 are the zone electrophoretic patterns of two antisera collected from rabbit R22-79 after the first and second courses of immunizations.
with Group C streptococcal vaccine. A monodisperse component in the primary response antiserum exhibited a slow electrophoretic migration. After the second immunization, a somewhat less restricted component of similar mobility appeared. At the same time, there emerged a second distinct antibody component

TABLE I
Quantitative Precipitation of Equivalence of Rabbit γ-Globulin in Streptococcal Antisera Collected throughout Several Periods of Immunizations by Unabsorbed Goat Anti-Antibody

| Immunization period | Days from onset of primary immunization | Rabbit 23-65 precipitate with anti-2 slow Fx§ | Rabbit 23-61 precipitate with anti-2 slow Fx§ |
|---------------------|----------------------------------------|---------------------------------------------|---------------------------------------------|
| Primary:            |                                        |                                             |                                             |
| 4 wk of i.v. immunization | 0 282§ 246§ |                                             |                                             |
|                     | 7 281 242 |                                             |                                             |
|                     | 14 296 250 |                                             |                                             |
|                     | 21 326 356 |                                             |                                             |
|                     | 28 342 375 |                                             |                                             |
| Secondary:          |                                        |                                             |                                             |
| 3 wk of i.v. immunization | 213 274§ 242§ |                                             |                                             |
|                     | 228 392 410 |                                             |                                             |
|                     | 235 398 512 |                                             |                                             |
| Tertiary            |                                        |                                             |                                             |
| 3 wk of i.v. immunization | 498 276§ |                                             |                                             |
|                     | 512 351 |                                             |                                             |
|                     | 519 389 |                                             |                                             |
| Means of control sera | -- 278 ± 1.8% | 245 ± 2%                                         |                                             |

* The volume of all antisera was adjusted with saline so that each sample of antisera employed in the test contained 100 μg of γ-globulin.
‡ The goat anti-antisera were prepared against the slow antibody components isolated from the rabbit antisera collected on day 235.
Mean of control sera: to obtain the mean of the controls, the two unabsorbed anti-antisera were tested with two normal rabbit sera, one heterologous Group A antiserum, one heterologous Group C antiserum, and pooled rabbit fraction II.
§ Rabbit sera for these tests were collected prior to the first intravenous injection of vaccine for each of the immunization series.

which migrated more rapidly than the first. Microzone electrophoresis of these components, isolated by preparative electrophoresis, is shown in the same figure. Depicted in Fig. 8 are the densitometric tracings developed from the polyacrylamide gel disc electrophoretic patterns of these reduced and alkylated antibody fractions. 1' slow fraction and 2' fast fraction have a similar degree of restriction. There is one predominant light chain in each case. 2' slow fraction has four light chain bands and is more heterogeneous than 1' slow fraction. The
mobilities of the light chains correspond to the mobilities on microzone electrophoresis of the whole antibody from which they were derived.

The uniform properties, including individual antigenic specificity, of the slow fraction from the first immunization antiserum of rabbit R22-79, have been described in detail in a previous report (6). The secondary response antiserum requires additional comment. It contained 42 mg γ-globulin/ml of serum; 22 mg/ml were precipitated by Group C carbohydrate. Absorption studies, employing Group C carbohydrate, as well as homologous cell walls, revealed that

the slow fraction has antibody activity to the Group C carbohydrate, whereas the fast fraction has not. The fast fraction also failed to show antibody activity in homologous passive cutaneous anaphylaxis (PCA) experiments. Although emergence and subsequent disappearance was clearly connected with secondary immunization, the antigenic component in the vaccine which stimulated this event has not yet been identified. Further studies are underway to determine the specificity of this antibody.

The distinctive antigenic specificities of the 1' slow fraction and 2' slow and fast fractions were determined in double diffusion experiments which employed goat anti-1' slow fraction and anti-2' fast fraction. The 1' slow fraction and the 2' slow fraction showed a line of identity when tested with anti-1' slow fraction. Both slow fractions spurred over 2' fast fraction and over pooled fraction II.
Fig. 8. Densitometric tracings of the 9.4 M urea polyacrylamide gel disc electrophoretic patterns of the reduced and alkylated γ-globulin fractions of rabbit R22-79 which are depicted in Fig. 7. Top frame: 1' slow fraction; middle frame: 2' slow fraction; lower frame: 2' fast fraction. The direction of migration is from left to the right.
When anti-2' fast fraction was used, 2' fast fraction spurred over both 1' and 2' slow fractions and over pooled fraction II. Quantitative precipitin tests on these antibody fractions with anti-1' slow fraction and anti-2' fast fraction are illustrated in Fig. 9. Anti-1' slow fraction shows the strongest precipitin reaction with its homologous antigen, 1' slow fraction. Although the reaction with 2' slow fraction is somewhat less, it is still significantly stronger than with pooled fraction II and 2' fast fraction. This diminished reactivity of 2' slow fraction is a reflection of the more pronounced heterogeneity of 2' slow fraction than that exhibited by 1' slow fraction.

Anti-2' fast fraction has a greater precipitin reaction with 2' fast fraction than that observed with 1' and 2' slow fractions. Furthermore, the reactivity with these components is comparable to that observed with fraction II. Such results indicate that the antigenic specificity of 2' fast fraction is distinct from that shared by 1' and 2' slow fractions. Clearly, the second immunization has stimulated the emergence of a major new population of antibodies, with electrophoretic properties unlike the first population and with a distinct antigenic individuality, which was not present as a major component during primary
immunization. Furthermore, each of the two electrophoretically distinct antibody components in the second immunization antiserum possessed unrelated individual antigenic specificities.

The Proportion of an Antibody Preparation Bearing Individual Antigenic Specificity.—Recently Daugherty et al. (11) have shown that only a portion of each purified population of rabbit anti-azobenzoate antibodies in a preparation were reactive with the homologous anti-antiserum. Similar studies were done here to determine the proportion of molecules in each streptococcal antibody preparation which possesses individual antigenic specificity.

| Goat antiantibody absorbed with pooled rabbit fraction II | Per cent antibody recovered in precipitate and supernatant |
|----------------------------------------------------------|----------------------------------------------------------|
|                    | Antiserum R 22-79 | Antiserum R 23-61 | Antiserum R 27-11 |
|                    | Fast Fx | Slow Fx | Fast Fx | Slow Fx | Fast Fx | Slow Fx |
| R 22-79 Anti fast Fx | 91     | 8       | 97     | -       | -       | -       |
| R 22-79 Anti slow Fx | 4      | 98      | 70     | 31      | -       | -       |
| R 23-61 Anti slow Fx | -      | -       | 3      | 95      | 80      | 21      |
| R 27-11 Anti fast Fx | 2      | 100     | -      | -       | 1       | 99      | 89      | 8       |

Fig. 10. The proportion of an antibody preparation precipitated by homologous and heterologous anti-antisera. All precipitin tests were done with an excess of anti-antibody. The per cent of rabbit antibody recovered in precipitate (ppt.) and supernatant (supt.) was calculated from the radioactivity. Each antibody preparation was labeled with $^{125}$I.

Samples of the antibody preparations, isolated by preparative electrophoresis, were labeled with $^{125}$I and precipitated with an excess amount of the homologous anti-antiserum which had been absorbed at equivalence with fraction II. Controls employed two absorbed heterologous anti-antisera. The proportions of the radioactive antibody in the precipitates and the supernatants were calculated as per cent of the total radioactive antibody used in the test. The results for antibody fractions from antisera R23-61, R22-79, and R27-11 are shown in Fig. 10. The antibody from antiserum R27-11 showed an exceptional degree of uniformity as judged by several criteria, including distribution of genetic markers (3) and N-terminal amino acid sequence of the light chain (5). 89% of this antibody preparation is precipitated by its individual anti-antiserum. A similar result is achieved with the 2' fast fraction of antiserum R22-79. Since
these antibody preparations were obtained by preparative electrophoresis alone, they contain approximately 10–15% nonspecific γ-globulin, which does not react with the anti-antibody. Only 70% of the 2′ slow fraction of antiserum R22-79 was precipitated by the antiserum against the 1′ slow fraction. This result is consistent with the other experimental data pointing to a certain degree of heterogeneity of this antibody. It is apparent that experiments such as this afford an additional means to estimate the uniformity of an antibody preparation.

The most dramatic and visual documentation for the fact that all of the antibody to streptococcal carbohydrate in an electrophoretically monodisperse γ-globulin component is a single population, was derived from absorption studies. Specific immunoabsorbent columns were prepared from the goat anti-antisera which had been absorbed with fraction II and solidified with glutaraldehyde. In these experiments, depicted in Fig. 11, electrophoresis of absorbed

![Fig. 11. Microzone electrophoretic patterns of rabbit antisera before and after absorption onto and elution from immunoabsorbent columns constructed from the homologous goat anti-antisera, which had been absorbed at equivalence with pooled rabbit fraction II.](image-url)
and unabsorbed antiserum revealed that a specific immunoabsorbent removes the homologous antibody population from the antiserum, but heterologous antibody populations and nonspecific γ-globulin passed through the column. To be noted especially in this connection is the experiment with the secondary response antiserum of rabbit R22-79. The first immunization antiserum of this rabbit contained a slow antibody component, whereas the second immunization antiserum contained this slow component and an additional fast component. In this second response antiserum, the fast component was specifically absorbed by the homologous immunoabsorbent prepared with anti-2' fast fraction and unaffected by the heterologous immunoabsorbent prepared with anti-1' slow fraction. On the other hand, the anti-1' slow fraction immunoabsorbent removes most, but not all of the secondary slow fraction. The slowest portion of the slow fraction has been absorbed by the column, but the faster portion of the slow fraction was unabsorbed and is visible in the electrophoretic pattern. This finding indicates that a portion of the secondary slow fraction contains antibodies which are not identical to the antibodies in the primary slow fraction, and, therefore, consists of more than one antibody population. This is consistent with other evidence reported above which suggests that the secondary slow fraction of antiserum R22-79 is less uniform than the primary slow fraction.

**DISCUSSION**

Among the indirect criteria which have been used to judge the structural uniformity of antibodies, individual antigenic specificity is perhaps the one which is most indicative of a homogeneity similar to that observed in the myeloma proteins. Detection of individual antigenic specificity of these human immune globulins employs anti-antisera raised in rabbits which have been absorbed with fraction II (7, 24). Subsequently, this property was described for certain human antibodies such as those to blood group substances and other carbohydrate antigens (8). In some instances these antibodies were also monotypic with respect to light chains type, consisted of only one subgroup, and possessed but one set of genetic markers, even though they were derived from a heterozygous individual (25, 26).

The studies reported here reinforce the impression gained from earlier work on the utility of individual antigenic specificity for judging the uniformity of specific rabbit antibodies to streptococcal carbohydrates (6). In all of these studies, evidence for uniformity has been obtained by a combination of methods, including determination of allotypic markers, and N-terminal amino acid sequence of the light chains (1–6, 12). The picture which emerges from these studies is that antibodies which have a readily demonstrable individual antigenic specificity are homozygous at both the a and b locus, even though they are derived from a doubly heterozygous rabbit (3). The light chains in such an
instance are predominantly monodisperse by polyacrylamide gel disc electrophoresis, and possess only one major amino acid alternative at the first three N-terminal positions (4, 5). Anti-antibodies to such antibodies are readily raised in goats and are generally present in useful concentration 4 wk after administration of the rabbit antibody with Freund's adjuvant (6).

A much less restricted streptococcal antibody population than the ones described above may have light chains which are distributed in several bands upon disc electrophoresis and may have detectable heterogeneity in the N-terminal amino acid sequence of the light chain. Such antibody preparations are undoubtedly composed of several antibody populations and because of this heterogeneity, goat anti-antibodies do not commonly detect antigenic individuality by the precipitation method employed here. It is conceivable, however, that these streptococcal antibody preparations, composed, nevertheless, of a limited number of homogeneous antibody populations, do stimulate anti-antibodies which would detect individual antigenic specificity if more sensitive methods were employed. Kunkel and coworkers, for example, have examined quantitative aspects of individual antigenic specificity by means of a hemagglutination technique which employs red blood cells coated with the antibody preparation. Agglutination of these red cells by the anti-antibody is readily inhibited by the addition of the purified antibody in question (27).

An antigenic individuality, similar, but not identical to, individual antigenic specificity has been termed idio typy (9). In this case, rabbits have been immunized with Salmonella or Proteus, and the bacterial antibodies, absorbed to the bacteria, have been injected into other rabbits of the same allotype. The anti-antibodies react specifically with the donor antibody and are unreactive with the bacterial antibodies raised in other rabbits (9, 10). Although these observations have been both provocative and intriguing, for the most part, clearly defined and isolated antigens and antibodies have not been employed in these experiments. Recently, however, idio typy has been described by Daugharty and coworkers for anti-benzoate antibodies in rabbits, and in this case, the phenomenon has been demonstrated with isolated and well defined antigens and antibodies (11). Use of benzoate antigens and purified antibodies has afforded a quantitative estimate of the proportion of these antibodies which possess the idio typic characteristics. Examination of a number of anti-benzoate antibodies from different rabbits indicates that the proportion of the antibodies reactive with the specific anti-antibody varies from 4 to 56% (11). This is in contrast to the studies reported here for the individual antigenic specificity of streptococcal antibody components. Quantitative techniques have demonstrated that the major portion of an antibody component isolated from streptococcal antisera by preparative electrophoresis is precipitated by the goat anti-antibody absorbed with fraction II. The interpretation of such data is that the antibody preparation is composed of one major antibody population, and that the γ-globulin which is nonreactive with the anti-antibody is nonspecific γ-globulin which has not been eliminated from the antibody preparation when electrophoresis alone is used as the method of purification.

Streptococcal antibodies produced by a rabbit during repeated courses of immunizations may elicit either identical or distinct individual antigenic specificities. First
and second immunization antibodies from a single rabbit which have identical individual antigenic specificities have an identical electrophoretic mobility. Furthermore, the same antiserum may possess two electrophoretically distinct antibody components, each one possessing its own distinct individual antigenic specificity. These findings are in agreement with the recent studies by Nisonoff et al. (28) and by Oudin et al. (29, 30). Furthermore, Oudin et al. observed that a rabbit may produce antibodies with two or more idiotypic specificities when it is immunized with several antigens. Each idiotypic specificity was confined to the antibody population directed against one of the immunizing antigens. On the other hand, antibodies produced in different rabbits, immunized with the same antigen, never shared the same idiotypic specificity (29, 30).

The recurrence during second immunization of antibodies with an individual antigenic specificity identical to that of antibodies produced during primary immunization suggests that the same cell population, present during primary immunization, has reemerged with reimmunization and, as a consequence, synthesis of identical antibody molecules occurs in both instances. Another line of evidence that this might be the case comes from recombination experiments performed on rabbit anti-benzoate antibodies (31). Recombinants of heavy and light chains from two different sera collected from the same rabbit at 6-month intervals in the course of immunization had 60-100% as much binding capacity as recombinants from a single serum, and were much more active than heterologous recombinants. In view of the fact that an antibody-producing cell does not survive longer than a few days (32, 33), recall of antibodies with a specificity similar to that present during the first immunization reinforces the suggestion that cells with a "memory" function must be involved in the recognition of the antigen and subsequent antibody synthesis (33, 34).

SUMMARY

Electrophoretically monodisperse antibody components in rabbit antisera to the carbohydrates of the Groups A and C streptococci have been examined for their individual antigenic specificity. In these antibody components which were isolated by preparative electrophoresis, individual antigenic specificity was confined to the specific antibody and was absent in the nonantibody \( \gamma \)-globulin. Radioprecipitation experiments and the use of immune absorbent columns constructed from goat anti-antisera, which had been absorbed with fraction II, revealed that all the specific antibody in an electrophoretically monodisperse component was reactive with the homologous anti-antibody.

Antibodies with either identical or distinct individual antigenic specificities may occur in the same rabbit with repeated immunizations. Antibodies with identical antigenic specificity had identical electrophoretic mobility, whereas antibodies with unrelated antigenic specificities had distinct electrophoretic mobilities. In the interval between immunizations, if antibody to the carbo-
hydrate antigen was absent, there was no detectable antibody with individual antigenic specificity.

The authors acknowledge with pleasure the participation of Mr. Henry Lackland, Mr. Jay Greenblatt, Miss Rochelle Seide, and Mr. David Bernstein in the conduct of this work.

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