CHARACTERISTICS OF STREPTOCOCCAL GROUP-SPECIFIC ANTIBODY ISOLATED FROM HYPERIMMUNE RABBITS*

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A previous report described the development of the immune response in rabbits to streptococcal group-specific carbohydrates following intravenous immunization with streptococcal vaccines (1). In a continuation of these studies, certain rabbits were noted to develop an unusually high level of streptococcal group-specific antibodies. Examination of the zone electrophoresis patterns of these immune sera revealed a remarkably sharp and narrow band within the γ-globulin region. This report is concerned with the immunologic, chemical, and physical properties of the specific γ_G-globulin isolated from these sera. The findings suggest a selective manufacture in certain rabbits of specific immune γ_G-globulin which exhibits a restricted range of electrophoretic properties compared to that of the normal complement of γ-globulin.

Materials and Methods

Streptococci.—Group A-variant strain A486 was obtained from Dr. R. C. Lancefield of the Rockefeller University.

Rabbits.—7-lb. New Zealand red rabbits were supplied by Baumgartel Bunny Farm, Marion, Iowa.

Streptococcal Vaccine.—The vaccine was prepared from Group A-variant streptococci, strain A486. The bacteria were grown in 1000 ml of Difco Todd-Hewitt broth, collected by centrifugation, and washed three times with saline. The collected bacteria were resuspended in 10 ml of saline, adjusted to pH 2.0, which contained 1 mg/ml of pepsin, and were incubated for 2 hr at 37°C. The treated bacteria were washed two times with saline and finally resuspended in 50 ml of formalized saline. The vaccine was stored at 4°C.

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Immunization.—All immunizations were by the intravenous route and followed the protocol listed in Table I.

Streptococcal Group-Specific Carbohydrate.—Groups A, A-variant, and C streptococcal carbohydrates were isolated from hot formamide digests of cell walls by previously described methods (2).

Quantitative Precipitin Analysis.—Quantitative precipitin tests were performed by a previously described method (3).

TABLE I

Immunization Schedule with Group A-Variant Streptococcal Vaccine and Immune Globulin Response

Day	Vaccine	Rabbit No. 8-0				Rabbit No. 8-1			
		Serum No.	Total serum protein	γ-Globulin	A-var. agg. titer	Serum No.	Total serum protein	γ-Globulin	A-var. agg. titer
	ml		*g %*	*g %*			*g %*	*g %*	
1	0.25	1	6.48	0.68	20	1	6.66	0.50	20
2	0.25								
3	0.25								
8	0.50	2	4.85	0.71	160	2	—	—	—
9	0.50								
10	0.50								
15	0.75	3	6.59	1.34	320	3	6.63	0.81	1280
16	0.75								
17	0.75								
22	1.00	4	—	—	—	4	—	—	—
23	1.00								
24	1.00								
29		5	10.79	5.57	40,000	5	8.14	2.33	640
39		6	8.89	2.94	20,000	6	—	—	—
44		7	8.23	1.91	640	7	—	—	—
71		8	7.06	1.15	80	8	—	—	—

Group-Specific Streptococcal Agglutinins.—Group-specific streptococcal agglutinins were detected by a previously described technique which employs streptococcal cell walls (1).

Zone Electrophoresis.—Zone electrophoresis of serum proteins employed a Beckman model R101 Microzone cell. Serum samples supported in a cellulose acetate medium were run in veronal buffer, pH 8.6 for 20 min at 250 v (2 to 8 ma). The acetate strips were then stained with ponceau S stain, cleared, and recorded in the Analytrol equipped with the model R-102 Microzone scanning attachment.

Protein Determination.—Total protein in the serum was determined by the biuret method (4). The γ-globulin was calculated from the densitometric pattern of the zone electrophoresis and the total protein value of the serum.

Starch Gel-, Acid Urea Starch Gel-, and Immunoelectrophoresis.—Starch gel electrophoresis was performed by the method of Smithies (5), acid urea starch gel electrophoresis by the method of Poulik (6), and immunoelectrophoresis by the method of Scheidegger (7).

EXPERIMENTAL

The protocol for the immunization of rabbits 8-0 and 8-1 with Group A-variant streptococcal vaccine is presented in Table I. Also recorded are the values for the serum total protein, γ-globulin, and Group A-variant agglutination titers before, during, and at the end of immunization. It will be noted that

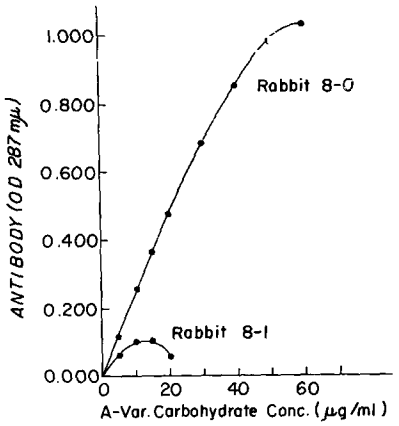

FIG. 1. Quantitative precipitin reaction between Group A-variant carbohydrate and Group A-variant antisera. $\frac{1}{10}$ ml of serum was employed in each tube and the final volume was 1 ml.

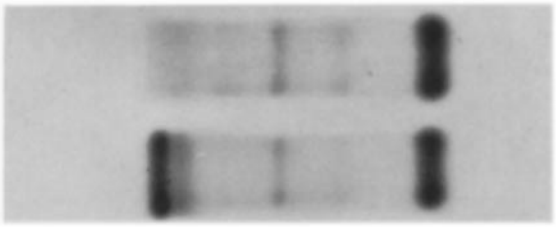

FIG. 2. Zone electrophoresis of sera collected from rabbit 8-0 which had been immunized with Group A-variant streptococcal vaccine. The pattern for serum 1 obtained prior to immunization is at the top, and the pattern for serum 5 obtained at the end of immunization is at the bottom.

serum 5 of rabbit 8-0 had an A-variant cell wall agglutination titer of 40,000, whereas serum 5 of rabbit 8-1 had a titer of 640. Group A-variant quantitative precipitin curves for serum 5 for both rabbits are presented in Fig. 1. $\frac{1}{10}$ ml of heat-inactivated serum was employed for each tube in the test. Rabbit 8-0 serum was sufficiently potent to precipitate 60 μg of carbohydrate at equivalence, whereas only 10 μg were precipitated by rabbit 8-1 serum. No significant cross-reactions were noted for either serum when tested with Groups A and C carbohydrates.

Depicted in Fig. 2 are the zone electrophoresis patterns for sera 1 and 5 of rabbit 8-0. A sharp narrow band in the γ-globulin region is clearly visible in the immune serum pattern. The densitometric scan of the zone electrophoresis patterns for selected sera of rabbit 8-0 is depicted in Fig. 3. A sharp narrow peak is noted within the γ-globulin region of serum 5 of rabbit 8-0. This is in contrast to the usual immune response which is characterized by a diffuse elevation

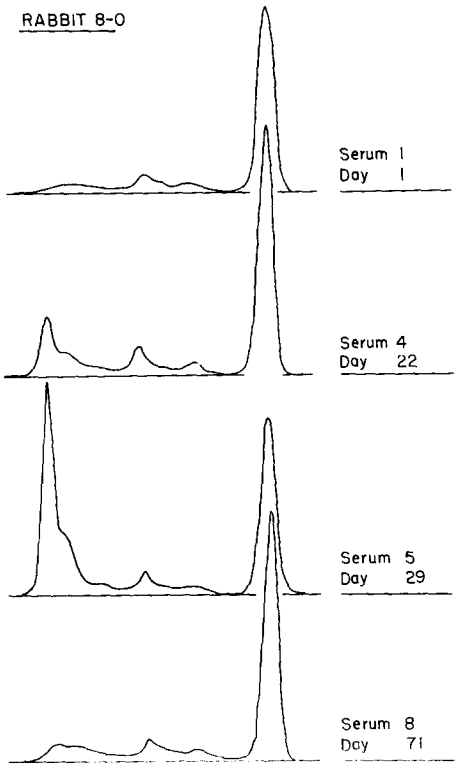

FIG. 3. Tracings of the densitometric scan of zone electrophoretic patterns for the sera of rabbit 8-0. Intravenous immunization with Group A-variant streptococcal vaccine was continued through the 24th day.

of the γ-globulins in the electrophoretic pattern. In the case of rabbit 8-0, the first serum which exhibited a detectable narrow peak in the γ-globulin region was collected on the 22nd day of immunization. With the termination of immunization after the 24th day, the peak gradually diminished so that by day 71 it was essentially absent.

Immunoelectrophoresis was performed on sera 1 and 5 of rabbit 8-0, and precipitin lines were developed with goat anti-γ_G-globulin. Serum 1 developed a line which is typical for γ_G-globulin. In the case of serum 5, the precipitin line

was distorted by the development of a widened segment in the mid-γ-region. This distortion of the pattern reflects a localized high concentration of γ-globulin and is consistent with the finding by zone electrophoresis of a markedly elevated narrow peak of γ-globulin. In the pattern illustrated in Fig. 4, serum

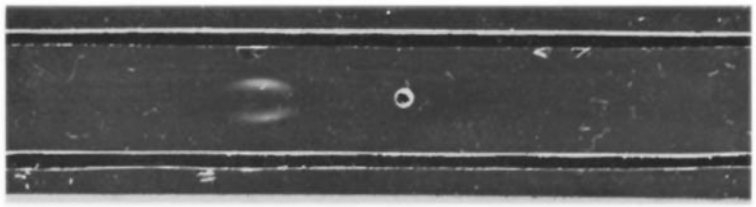

FIG. 4. Immunoelectrophoresis of Group A-variant antiserum 5 of rabbit 8-0. Central origin, serum 5 of rabbit 8-0; lateral troughs Group A-variant carbohydrate.

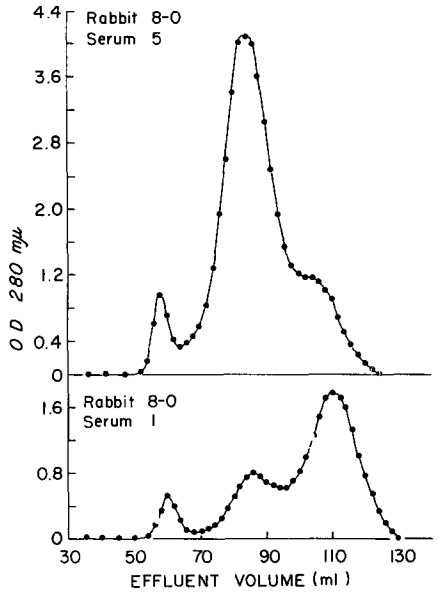

FIG. 5. Gel filtration on Sephadex G-200 of serum 1 and 5 of rabbit 8-0. Column dimensions, 40 × 2 cm. Protein concentration in the fractions was determined by measuring absorbency at 280 mμ.

5 was added to the origin and, after electrophoresis, Group A-variant antigen was added to the parallel troughs. A single short precipitin band developed in the central domain of the γ_G-globulin. With most antisera the line of antigen-antibody precipitation is continuous throughout most of the γ-globulin region.

In view of the fact that both 7S and 19S globulins have been reported as features of the immune response to bacterial antigens, the proteins in sera 1

and 5 of rabbit 8-0 were separated on Sephadex G-200. The elution pattern for serum 1, as depicted in Fig. 5, consists of three peaks which correspond to 19S globulin, 7S globulin, and albumin, in proportions consistent with that observed in normal rabbit sera (8). The pattern for serum 5 has a very large peak in the 7S region followed by a shoulder in the albumin region and a distinct initial peak in the 19S region. The Group A-variant–precipitating antibody activity was identified in the 7S peak.

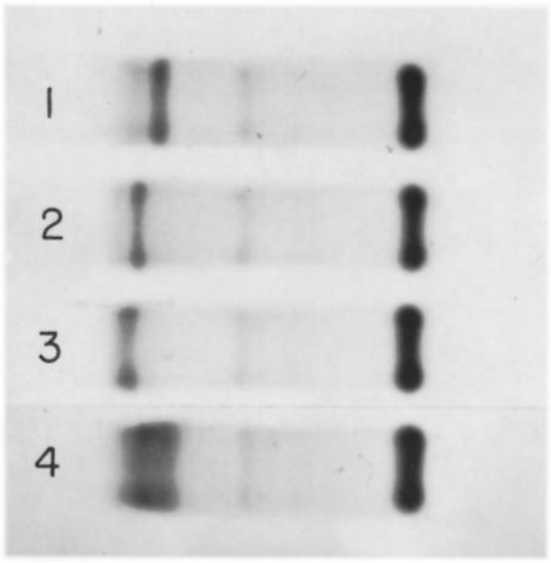

FIG. 6. Zone electrophoretic patterns for antisera of streptococcal Groups A-variant, C, and H: 1, Group A-variant serum; 2 and 3, Group C sera; and 4, Group H serum.

Examination of Other Streptococcal Antisera.—A survey was conducted on streptococcal-grouping antisera, obtained from Dr. Rebecca C. Lancefield, Rockefeller University, to identify by zone electrophoresis the occurrence of similar electrophoretically homogeneous peaks in the γ-globulin region. The zone electrophoresis patterns for several interesting sera are shown in Fig. 6. One out of 5 Group A-variant sera and 2 out of 4 Group C sera exhibited a narrow peak in the γ-globulin region of the patterns. Fourteen Group A sera did not reveal any unusual elevation of the γ-globulin, and thus far efforts in this laboratory to produce a Group A serum which exhibits a finding similar to that of rabbit 8-0 serum have met with failure. The electrophoretic pattern of the Group H serum depicted in Fig. 7 reveals a striking elevation of the γ-globulin with a concentration of 48.2 mg/ml. Four out of 5 Group H sera examined have somewhat similar patterns.

Absorption of Serum with Group A-Variant Carbohydrate.—The following experiments identify the Group A-variant carbohydrate antibodies as the major protein component of the electrophoretically homogeneous peak in serum 5 of rabbit 8-0.

Serum 5 was absorbed at equivalence with Group A-variant carbohydrate. Following absorption no Group A-variant antibody was detectable in the

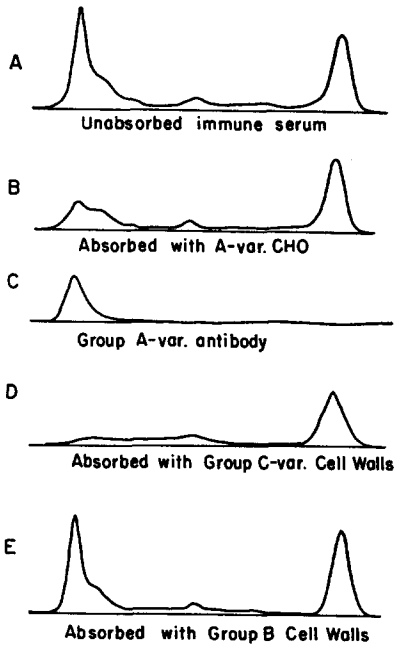

FIG. 7. Adsorption of serum 5 of rabbit 8-0 with Group A-variant carbohydrate, Group C-variant cell walls, and heterologous Group B cell walls. A, tracing of the densitometric scan of the zone electrophoresis pattern for unabsorbed serum 5; B, tracing for serum 5 absorbed with A-variant carbohydrate; C, tracing for Group A-variant antibody isolated from the specific immune precipitate; D, tracing for serum 5 absorbed with Group C-variant cell walls; and E, tracing for serum 5 absorbed with Group B cell walls.

sera by capillary precipitin tests, however, a minimal excess of carbohydrate was detectable. Electrophoretic patterns of unabsorbed and absorbed sera are depicted in tracings A and B of Fig. 7. The patterns reveal that absorption had removed the bulk of the narrow peak from the serum. The group-specific antibody was recovered from the precipitate by the method described below and subjected to electrophoresis. The pattern depicted in tracing C of Fig. 7 reveals a single component with a mobility similar to that of the tall γ-globulin peak in the unabsorbed serum.

Previous studies have detected nonprecipitating group-specific antibodies in

streptococcal antisera which can be absorbed with particulate cell walls but which are not removed by absorption with soluble antigen (1). It would be anticipated that the removal of these antibodies, as well as the precipitins, by cell wall absorption would yield a serum with a γ-globulin concentration which is less than that of the same serum following absorption with soluble antigen. That this proved to be the case is illustrated by the scan of the electrophoretic pattern of serum absorbed with C-variant cell walls which is depicted in tracing D of Fig. 7. C-variant cell walls were employed for absorption rather than A-variant cell walls, so that the procedure would remove all antibodies with variant specificity, but would not at the same time remove any traces of antibodies directed against the Group A determinant (9). It should be emphasized

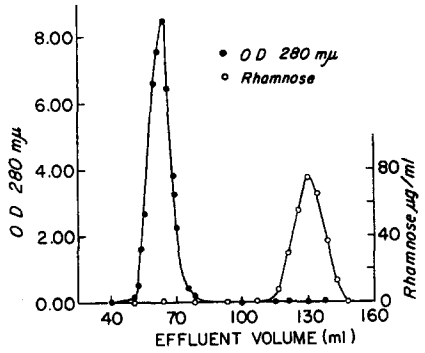

FIG. 8. Gel filtration on Sephadex G-100 in saline adjusted to pH 2.65 of solubilized specific Group A-variant immune precipitate collected from serum 5 of rabbit 8-0. Column dimension, 40 x 2 cm. Protein content of the effluent was determined by measuring absorbency at 280 mμ. Antigen was detected by analysis of the effluent for rhamnose.

that a control absorption of the serum with heterologous Group B cell walls did not remove the γ-globulin.

Isolation of Group-Specific Antibody.—Because the absorption results suggested that a major portion of the protein in the tall γ-globulin peak was group-specific antibody, methods were devised to recover the Group A-variant precipitins from a specific immune precipitate.

5 ml of serum 5 of rabbit 8-0 were heat inactivated at 56°C for 30 min and then mixed with 5 ml of saline containing 1 mg of Group C-variant carbohydrate, thus achieving a final antigen concentration of 100 μg/ml. The mixture was incubated for 2 hr at 37°C and for 4 days at 4°C. The precipitate was collected by centrifugation and washed three times in the cold with saline. The final product was dissolved in saline adjusted to pH 2.65 with HCl. The solution was clarified by centrifugation and passed through a Sephadex G-100 column, 2 × 40 cm. The protein was eluted from the column with saline acidified to pH 2.65 and collected in tubes containing sufficient phosphate or

tris buffer to neutralize the acid. Protein was detected in the fractions by measuring the optical density at 280 mμ, and antigen was detected in the fractions by testing for rhamnose (10).

The elution of the dissociated Group A-variant antibody and the antigen from the Sephadex G-100 column is depicted in Fig. 8. The antibody protein peak was eluted at an effluent volume of 60 ml, and the antigen, as detected by the rhamnose assay, at a volume of 130 ml. It is clear that there was complete

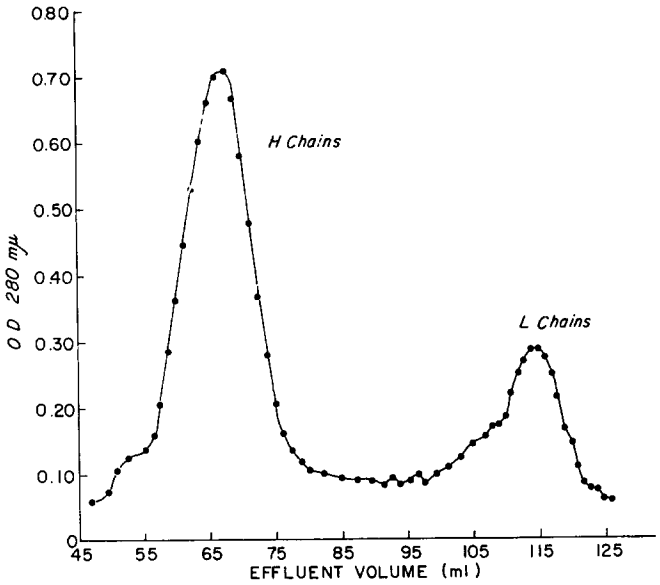

FIG. 9. Gel filtration on Sephadex G-100 in one normal propionic acid of reduced and alkylated Group A-variant antibody isolated from a specific immune precipitate of serum 5 of rabbit 8-0. Column dimension, 80 x 2 cm. Protein content of the effluent was determined by measuring absorbency by 280 mμ. The initial protein peak corresponds to the usual distribution for H chains and the second peak for that of L chains. The central fractions of each peak were pooled for further analysis by acid urea starch gel electrophoresis.

separation of antigen from antibody. The fractions containing the antibody were pooled and concentrated by vacuum dialysis.

The serologic activity of the recovered antibody which traveled as one peak in zone electrophoresis was found to be similar to that of the antibody in the initial serum. Thus, maximal precipitation of the purified antibody at a concentration of 11 mg/ml was achieved with 5 μg/ml of carbohydrate, whereas the initial serum with 55 mg/ml of γ-globulin gave maximal precipitation with 60 μg/ml of carbohydrate. Furthermore, at least 90% of the recovered antibody protein was precipitable upon the addition of sufficient antigen to establish equivalence. These data suggest that the exposure of this antibody to low pH

during the 2 hr period of the purification process did not adversely affect antibody activity.

Physical-Chemical Characteristics of the Purified Antibody.—Because of the remarkable electrophoretic homogeneity of the purified specific carbohydrate

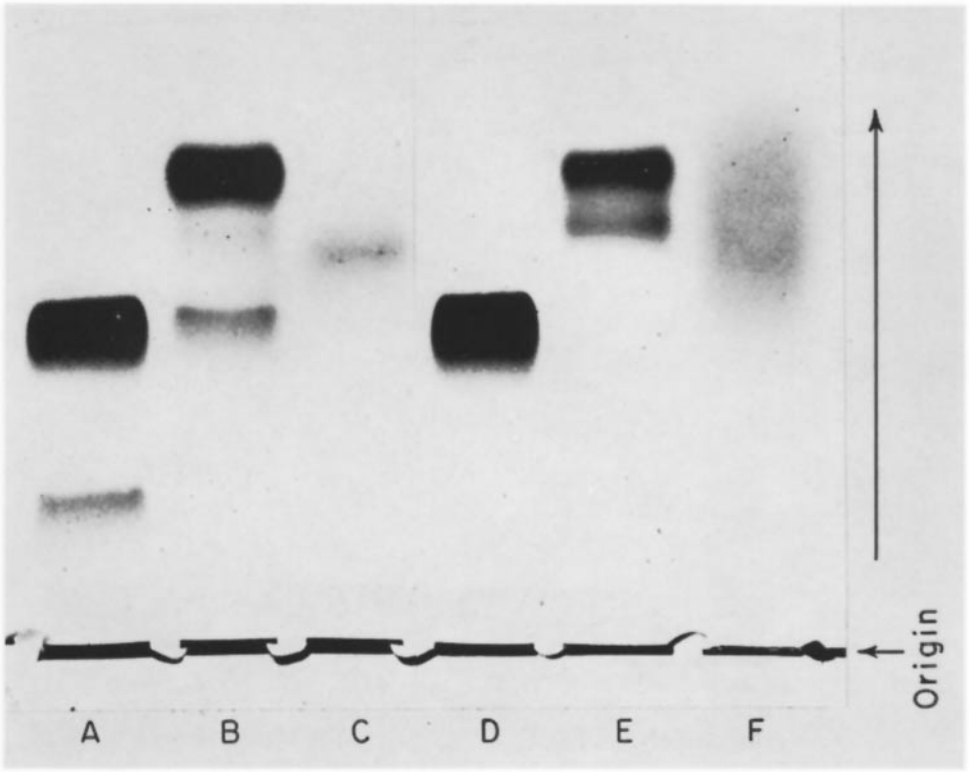

FIG. 10. Acid urea starch gel electrophoretic comparison between the H and L chains isolated from Group A-variant antibody obtained from a specific immune precipitate and the corresponding chains for normal rabbit γ-globulin. A, isolated specific antibody collected from serum 5 rabbit 8-0; B, pooled H chain fraction of specific antibody obtained by gel filtration as depicted in Fig. 9; C, pooled L chain fraction of specific antibody obtained by gel filtration as depicted in Fig. 9; D, normal rabbit γ_G-globulin; E, H chain fraction of normal γ_G-globulin; and F, L chain fraction of normal γ_G-globulin.

antibody, it was examined for some of the physical chemical characteristics and allotypic properties ascribed to other purified antibodies and to the multiple myeloma proteins.

The following experiment was devised to demonstrate the mobility and distribution pattern in acid urea gel electrophoresis of the L chains isolated

from the purified specific antibody by the method of Fleischman et al. (11). Reduction of purified antibody at a concentration of 10 mg/ml was accomplished by treatment with 0.2 M mercaptoethanol for 2 hr at room temperature. Subsequent alkylation was prepared in the cold using an excess of iodoacetamide. The reduced and alkylated antibody, after dialysis against 1 N propionic acid at 4°C for 48 hr, was filtered through a Sephadex G-100 column equilibrated with 1 N propionic acid. The protein concentration in the fractions of the effluent volume was measured at an OD of 280 mμ in a spectrophotometer.

The separation of the reduced and alkylated antibody into two protein components as a result of gel filtration is depicted in Fig. 9. The elution volumes for the two components and the relative protein concentrations in each indicate that the antibody had been separated into H and L chains by the reduction and alkylation procedure. Fractions from the first and second peaks were concentrated by vacuum dialysis and analyzed by acid urea starch gel electrophoresis. The results are depicted in Fig. 10. H chains and L chains, prepared from normal rabbit γ_G-globulin by a similar procedure were also subjected to this method of electrophoresis. It is to be noted that the L chain preparation of the specific antibody migrates as a narrow band in the gel, whereas the corresponding L chain preparation from normal γ_G-globulin migrates as a diffuse smear.

Starch gel electrophoresis of the normal γ_G-globulin and the purified group-specific γ_G-globulin was carried out by the usual procedure (5). The normal γ_G-globulin traveled as 2 distinct bands, whereas the immune γ_G-globulin revealed one distinct band. Investigation of the allotypy (12) of rabbit 8-0 serum and the purified specific antibody was performed by Dr. Charles Todd, University of Illinois, Urbana. The immune globulin in the preimmunized serum revealed that the rabbit was phenotypically a3b4, homozygous at both the *a* and *b* loci. The purified specific antibody also was typed as a3b4. While the physical-chemical studies suggest that this purified rabbit antibody has certain features in common with other antibodies isolated from specific immune precipitate and with the multiple myeloma proteins, it was not possible to determine an absence of one of the allotypes at either genetic locus.

DISCUSSION

Certain rabbits undergoing hyperimmunization with intravenous administration of streptococcal vaccines yielded sera which exhibited a prominent narrow peak in the γ-globulin region of the zone electrophoresis pattern. In one instance 55 mg/ml of γ-globulin was detected in the serum. Specific absorption studies identified streptococcal group-specific antibodies as the major component of the γ-globulin peak. The antibody, isolated from precipitating antigen-antibody complexes, was identified as a 7S protein and a γ_G-globulin. Further analysis suggested that the normal complement of γ-globulin had greater electrophoretic diversity than the specific immune γ-globulin.

Evidence for specific antibody with a restricted range of electrophoretic properties compared to that of normal γ-globulin was derived from several sources. The specific immune globulin exhibited only one band on alkaline starch gel electrophoresis whereas the γ-globulin from preimmunized serum was distributed in 2 bands. The mobility patterns in acid urea starch gel electrophoresis of L chains isolated from both specific immune γ-globulin and normal γ-globulin also support the views that the immune γ-globulin represents a restricted population of antibodies. It is to be noted that rabbit H chains exhibit greater electrophoretic mobility than the L chains on acid urea gels. This finding, which is unlike the patterns of human H and L chains, has been commented upon recently by Feinstein (13). The L chains isolated from the specific immune γ-globulin migrated as a single narrow band rather than the usual diffuse smear exhibited by normal L chains. This finding is consistent with the work of Edelman et al. (14, 15) on guinea pig immune γ-globulin, in which the L chains from normal γ-globulin were distributed as a diffuse smear in acid urea starch gel electrophoresis, whereas L chains isolated from several purified specific antibodies were distributed into a limited number of characteristic bands. Another example of a banding pattern for L chains in acid urea starch gel electrophoresis occurs in the case of the multiple myeloma proteins and certain other purified antibodies isolated from man (16). In the latter study the authors were able to demonstrate antigenic determinants which are specific for the isolated antibodies. Such antibodies exhibited a marked degree of electrophoretic homogeneity.

Analysis of the allotypic markers of the specific antibody did not provide additional evidence that an antigenically limited type of antibody molecule had been formed during immunization. It was perhaps unfortunate that the rabbit was genetically homozygous at both allotypic loci. Evidence for loss of or relative decrease in one of the alleles in specific antibody has always been obtained by testing serum from rabbits heterozygous at one or both loci (17–19). In human myeloma protein and specific antibodies it has been found that one of the alleles at the Gm or Inv locus may not be expressed on the purified protein (20, 21).

The physicochemical studies reported here on purified streptococcal group-specific antibody support the view that immune globulins developed against a specific hapten possess greater homogeneity of structure than that observed with normal γ-globulin. It should be emphasized, however, that a large body of evidence has established that antibodies produced against even the most simple haptenic determinants vary widely with respect to binding affinities (22). In previous studies both binding affinities and electrophoretic properties have indicated considerable heterogeneity of specific rabbit antibodies. Although the electrophoretic properties of the specific rabbit antibody reported here suggest homogeneity, its binding affinity would be of interest.

Although the biological process which gives rise to exceptionally high levels of electrophoretically homogeneous antibody is obscure, it may be dependent upon several factors, such as the chemical nature of the antigen, the physical state of the antigen, the route of immunization, and the genetic background of the rabbits.

The streptococcal antigens, at least in the case of Groups A, A-variant, and C streptococci, are relatively simple chemical polymers, which contain only one or two major sugar constituents (23, 24). The Group A-variant carbohydrate is an unusual bacterial antigen in that it consists almost entirely of one sugar, L rhamnose. McCarty has identified a rhamnose disaccharide as the major feature of the antigenic determinant of Group A-variant specificity (25). The Group A carbohydrate has a similar rhamnose moiety as that of Group A-variant, but the specific determinant grouping is a terminal β-*N*-acetyl-glucosaminide residue on the rhamnose side chains, which at the same time mask the A-variant reactivity of the subterminal rhamnose disaccharide groups. In the case of the Group C carbohydrate, the rhamnose moiety possesses terminal *N*-acetyl-galactosaminide residues, which confer group specificity and at the same time mask the A-variant antigenic reactivity of the rhamnose moiety. Although the group-specific antigens of each of these three groups of streptococci are relatively simple compounds as compared to protein antigens, the Group A-variant antigen is the least complex structure and conceivably may give rise to antibodies with a restricted range of specificities and physicochemical and allotypic characteristics.

Although the vaccine was administered intravenously in the case reported here, there are no adequate studies which compare various routes of administration of the streptococcal vaccines. The physical state of the antigen in the vaccine deserves comment. The vaccine is prepared from streptococci which have been treated with pepsin at pH 2.0 to destroy streptococcal proteins including the M and T antigens, and formalin is subsequently added. It is thus likely that after such treatment the cell wall group carbohydrate, comprising approximately 15% of the dry weight of the vaccine, is the major active antigen at the surface of the bacteria. The particulate nature of the vaccine may also be an important factor in achieving high levels of antibody, but it is conceivable also that one or more of the other components of the streptococcus may provide an adjuvant effect. One of the major elements of the cell wall which is not antigenically altered by the preparative procedures is the mucopeptide matrix (26), and previous studies have demonstrated that mucopeptide has some of the properties of endotoxin (27). In view of the fact that endotoxin has been shown to have adjuvant properties, it is conceivable that mucopeptide possesses a similar capacity. Current studies are in progress to test the adjuvant quality of the streptococcal mucopeptide.

The studies conducted thus far indicate one in 5 to 10 New Zealand red

rabbits after immunization exhibit an electrophoretically homogeneous antibody peak which possesses Group A-variant antibodies. Although the findings at this point are preliminary, similar results have not been obtained with New Zealand white rabbits. It is thus conceivable that the breed of rabbits may be another important factor in the occurrence of this hyperimmune response. Studies are under way to select New Zealand red rabbits which exhibit hyperimmune responses and to follow the immune response in their offspring after similar immunization.

Other reports have directed attention to the occurrence of unusually high responses of γ-globulin in rabbits following the intravenous immunization with bacterial vaccines. Recently Askonas et al. (28) identified markedly elevated γ-globulins in rabbits following intravenous immunization with pneumococcal Type III vaccine, but the antibody isolated from specific immune precipitates was not characterized in a fashion similar to that reported here.

Horses immunized with vaccine prepared from various bacteria, including pneumococci, may show exceptionally elevated responses of specific antibody, and in certain instances free flow electrophoresis has revealed sharp γ-globulin peaks (29). In view of the current interest in highly purified specific antibodies, examination of sera from animals immunized with various bacteria may yield additional antibodies with physical-chemical properties and electrophoretic characteristics which are distinctive from that observed for the normal complement of γ-globulin.

SUMMARY

Intravenous immunization of New Zealand red rabbits with streptococcal group-specific bacterial vaccines yielded sera which possessed markedly elevated γ-globulin. The sera of one rabbit immunized with Group A-variant vaccine possessed 55 mg/ml of γ-globulin. The bulk of this γ-globulin, identified as γ_G-globulin, was homogeneous by zone electrophoresis and of specificity directed against the Group A-variant carbohydrate antigen. L chains isolated from specific antibody obtained from an immune precipitate were distributed as a single band on starch gel electrophoresis, whereas the normal γ-globulin traveled as a diffuse smear. These data suggest that the rabbit streptococcal Group A-variant antibodies possess a limited range of physicochemical properties and electrophoretic mobility compared to that generally observed for the normal complement of γ-globulin.

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BIBLIOGRAPHY

1. Karakawa, W. W., Lackland, H., and Krause, R. M., The production of streptococcal group-specific antibodies in rabbits. I. The identification of Groups A and A-variant agglutinins in Group A antisera, *J. Immunol.*, in press.

2. Krause, R. M., and McCarty, M., Studies on the chemical structure of the streptococcal cell wall. I. The identification of a mucopeptide in the cell walls of Groups A and A-variant streptococci, *J. Exp. Med.*, 1961, **114,** 127.
3. McCarty, M., and Lancefield, R. C., Variation in the group-specific carbohydrate of Group A streptococci. I. Immunochemical studies on the carbohydrates of variant strains, *J. Exp. Med.*, 1955, **102,** 11.
4. Gornall, A. G., Bardawill, C. J., and David, M. M., Determination of serum proteins by means of the biuret reaction, *J. Biol. Chem.*, 1949, **177,** 751.
5. Smithies, O., An improved procedure for starch gel electrophoresis: Further variations in the serum protein of normal individuals, *Biochem. J.*, 1959, **71,** 585.
6. Poulik, M. D., The use of urea-starch-gel electrophoresis in studies of reductive cleavage of an α_2-immunoglobulin, *Biochem. et Biophysica Acta*, 1950, **44,** 390.
7. Scheidegger, J. J., Une micro methode de l'immunoelectrophorese, *Internat. Arch. Allerg. and Appl. Immunol.*, 1955, **7,** 103.
8. Porath, J., and Flodin, P., Gel filtration: a method for desalting and group separation, *Nature*, 1959, **183,** 1657.
9. Karakawa, W. W., Osterland, C. K., and Krause, R., Detection of streptococcal group-specific antibodies in human sera, *J. Exp. Med.*, 1965, **122,** 195.
10. Dische, Z., and Shettles, L. B., A specific color reaction of methylpentoses and a spectrophotometric micromethod for their determination., *J. Biol. Chem.*, 1948, **175,** 595.
11. Fleischman, J. B., Pain, R. H., and Porter, R. R., Reduction of γ-globulin, *Arch. Biochem. and Biophysics, (Suppl.)*, 1960, **112,** 107.
12. Oudin, J., Allotypy of rabbit serum proteins. I. Immunochemical analysis leading to the individualization of 7 main allotypes, *J. Exp. Med.*, 1960, **112,** 107.
13. Feinstein, F. A., Electrophoretic behavior of rabbit gamma-globulin fragments and chains, *Nature*, 1964, **202,** 4939.
14. Edelman, G. M., Benacerraf, B., and Ovary, Z., Structure and specificity of Guinea Pig 7S antibodies, *J. Exp. Med.*, 1963, **118,** 229.
15. Edelman, G. M., and Poulik, M. D., Studies on the structural units of the γ-globulins, *J. Exp. Med.*, 1961, **113,** 861.
16. Kunkel, H. G., Mannik, M., and Williams, R. C., Individual antigenic specificity of isolated antibodies, *Science*, 1964, **140,** 1218.
17. Gell, P. G. H., and Kelus, A., Deletions of allotypic γ-globulins in antibodies, *Nature*, 1962, **195,** 4836.
18. Reider, R. F., and Oudin, J., Studies on the relationship of allotypic specificities to antibody specificities in the rabbit, *J. Exp. Med.*, 1963, **118,** 627.
19. Lark, C. A., Eisen, H. N., and Dray, S., Distribution of allelic allotype among γ_G-immunoglobulins and purified anti-hapten antibodies from the same rabbit, *J. Immunol.*, 1965, **95,** 404.
20. Harboe, M. H., Osterland, C. K., Mannik, M., and Kunkel, H. G., Genetic characters of human γ-globulins in myeloma protein, *J. Exp. Med.*, 1962, **116,** 719.
21. Allen, J. C., Kunkel, H. G., and Kabat, E. A., Studies on human antibodies. II. Distribution of genetic factors, *J. Exp. Med.*, 1964, **119,** 453.

22. Eisen, H. N., and Siskind, G. W., Variations in affinities of antibodies during the immune response, *Biochemistry*, 1964, **3,** 996.
23. McCarty, M., The streptococcal cell walls and its biological significance, *in* The Streptococci, Rheumatic Fever, and Glomerulonephritis (J. W. Uhr, editor), Baltimore, Williams and Wilkins, 1964.
24. Krause, R. M., Symposium on relationship of structure of micro-organisms to their immunological properties. IV. Antigenic and biochemical composition of hemolytic streptococcal cell walls, *Bact. Rev.*, 1963, **27,** 369.
25. McCarty, M., Variation in the group specific carbohydrate of Group A streptococci. II. Studies in the chemical basis for serological specificity of the carbohydrates, *J. Exp. Med.*, 1956, **104,** 629.
26. Karakawa, W. W., and Krause, R. M., unpublished observations.
27. Rotta, J., Prendergast, T. J., Karakawa, W. W., Harmon, C. K., and Krause, R. M., Enhanced resistance to streptococcal infection induced in mice by cell wall mucopeptide, *J. Exp. Med.*, 1965, **122,** 877.
28. Askonas, B. A., Farthing, C. P., and Humphrey, J. H., The significance of multiple antibody components in serum of immunized rabbits, *Immunology*, 1960, **3,** 336.
29. Van der Scheer, J., Wyckoff, R. W. G., and Clarke, F. H., The electrophoretic analysis of several hyperimmune horse sera, *J. Immunol.*, 1940, **39,** 65.