

OCCURRENCE OF 19S AND 7S ANTI-IgGs DURING HYPER- IMMUNIZATION OF RABBITS WITH STREPTOCOCCI*

BY VIKTOR A. BOKISCH,† DAVID BERNSTEIN, AND RICHARD M. KRAUSE

(From The Rockefeller University, New York 10021)

(Received for publication 23 May 1972)

The occurrence of 19S anti-IgG has been frequently observed during hyperimmunization of rabbits with various antigens including bacteria (1, 2), soluble proteins (3), autologous IgG (4), and *Trypanosoma* (5). There are only a few reports which mention the occurrence of 7S anti-IgG. Williams and Kunkel (6), for example, described both 7S anti-IgG and 19S anti-IgG in some rabbits immunized with autologous IgG.

Rabbit streptococcal group-specific antisera provided a unique opportunity to reexamine the nature of the 19S and 7S anti-IgGs because they occur commonly during intravenous hyperimmunization with whole streptococcal vaccines. From preliminary studies in collaboration with Dr. Charles Christian, it was learned that concentrations of 19S anti-IgG were much greater in streptococcal antisera than those in antisera from rabbits immunized with Gram-negative organisms. Furthermore, it has been observed that occasional hyperimmune antistreptococcal sera were viscous, and in some instances, a gel or cryoprecipitate formed in the cold. Analytical ultracentrifugation of these sera revealed the presence of intermediate complexes which were indicative of 7S anti-IgG. One cryoglobulin was described in an earlier report (7), but the anti-IgG activity was not observed at the time.

The antisera from 88 rabbits hyperimmunized with Groups A and C streptococci have been examined for the occurrence of 19S and 7S anti-IgGs. These antibodies are much less common and occur in lower concentrations in the antisera of rabbits hyperimmunized with pneumococci, than in streptococcal sera. This finding suggests a specific role for antigen in the induction of anti-IgGs. Studies on inbred rabbit families suggest further that the ability to produce 7S anti-IgG after immunization with streptococci may be an inherited genetic trait.

Materials and Methods

Streptococci.—Streptococci Group A, strain J 17A4; Group A-variant, strain A486; and Group C, strain C74 were obtained from Dr. R. C. Lancefield, The Rockefeller University.

* This work was supported by the National Institutes of Health Grant AI 08429 and by a Grant-in-Aid from the American Heart Association.

† Fellow, Helen Hay Whitney Foundation.

Streptococcal Vaccines.—Vaccines were prepared from all strains as previously described (8).

Streptococcal Group-Specific Carbohydrate.—Group A, A-variant, and C streptococcal carbohydrates were isolated from hot formamide extracts of streptococci according to described methods (9).

Rabbits.—The rabbits were a random-bred but impure New Zealand Red line from the Carver Rabbitry, Somerville, N. J. Rabbits were used also from families obtained through selective breeding. The principles of selection and mating have been described previously (10).

Immunization.—The rabbits received three injections per week of 0.1 ml of vaccine for 4 wk. After a 3 month rest, they were again immunized with three injections per week for 3 wk. 40-ml blood samples were drawn before each immunization period and at weekly intervals during immunization. Frequent blood samples were taken throughout the week after the first and second immunization periods.

Pneumococcal Antisera.—Pneumococcal antisera have kindly been supplied by Dr. Charles Todd, City of Hope National Medical Center, Duarte, Calif.; Dr. Jack Pincus, National Institutes of Health, Bethesda, Md.; and Dr. Peter Strasbourg, Massachusetts General Hospital, Boston, Mass.

Quantitation of IgM and IgG.—The radial diffusion technique described by Mancini et al. (11) was used for the quantitative measurement of IgM and IgG in serum. Monospecific antisera were prepared in goats. Rabbit IgG and IgM were isolated from serum according to described methods (12) and used as standards in the test. Since most of the sera to be tested had 19S and 7S anti-IgGs, the influence of these anti-IgGs on the quantitative results was investigated. For this purpose known amounts of IgM and IgG were mixed with known amounts of either 19S or 7S anti-IgGs. The concentration of IgM and IgG before and after addition of anti-IgGs was determined by radial diffusion. The results showed that 19S anti-IgG at the usual serum concentration had no effect on either the IgM or IgG determination, nor was there any influence of 7S anti-IgG on the IgM determination. High concentrations of 7S anti-IgG did, however, markedly reduce the IgG value. The IgG concentration in sera containing more than 5 mg of 7S anti-IgG/ml was calculated, therefore, as previously described, from the densitometric scan of the zone electrophoretic pattern and the total protein value of the antiserum (13).

Hemagglutination Test to Measure 19S Anti-IgG.—This test was based on the agglutination of IgG-coated cells. A suspension of 2% washed rabbit (F) erythrocytes in phosphate-buffered saline was incubated for 1 hr at 37°C with an equal volume of 1:160 diluted anti-rabbit blood group substance F antiserum, kindly supplied by Dr. Charles Christian, Cornell Medical Center, New York. The cells were washed three times and adjusted to a 0.5% suspension. Agglutination experiments were carried out in microtiter plates. Uncoated rabbit cells were used as a control. In order to compare agglutination titers obtained with different batches of cells, a standard serum was included in every experiment. The same IgG coat was used throughout the study.

Measurement of 7S Anti-IgG.—An assay was designed to measure the concentration of 7S anti-IgG in rabbit antisera. The test is based on the ability of 7S anti-IgG to coprecipitate with heterologous antigen-antibody complexes. The first step in the development of the test was to determine the capacity of heterologous antigen-antibody complexes to absorb 7S anti-IgG. This was performed with an anti-Group C serum, R3387, which was known to have a high content of 7S anti-IgG. It was important that the serum containing the 7S anti-IgG is present as the complexes are formed, rather than added to preformed complexes. Therefore, to each tube in the test was added 0.1 ml of a 1:10 dilution of antiserum R3387, to be tested for 7S anti-IgG. Increasing amounts of Group A streptococcal carbohydrate antigen and anti-Group A antibody at ratios of equivalence were then added to the series of tubes. One set of tubes was incubated for 18 hr at 4°C and the other at 37°C. The two temperatures were chosen in order to assess the effect of possible cryoprecipitation which occurs in undiluted serum after

prolonged incubation at 4°C. In general, however, cryoprecipitation is minimal or absent in antisera diluted 1:10. Precipitates were washed in cold phosphate-buffered saline, dissolved in 0.1 N NaOH, and the total protein (complexes and the 7S anti-IgG) was measured by the Folin method.

It was necessary to measure independently all the protein in the antigen-antibody complexes in order to calculate the amount of coprecipitated 7S anti-IgG. This was done by measuring the protein content in a series of tubes containing 0.1 ml of a 1:10 dilution of a preimmune serum (devoid of 7S anti-IgG) and Group A antigen and anti-Group A antibody in the same increasing concentrations as were employed in the experimental tubes. The difference in protein content between the corresponding precipitates formed with the preimmune serum

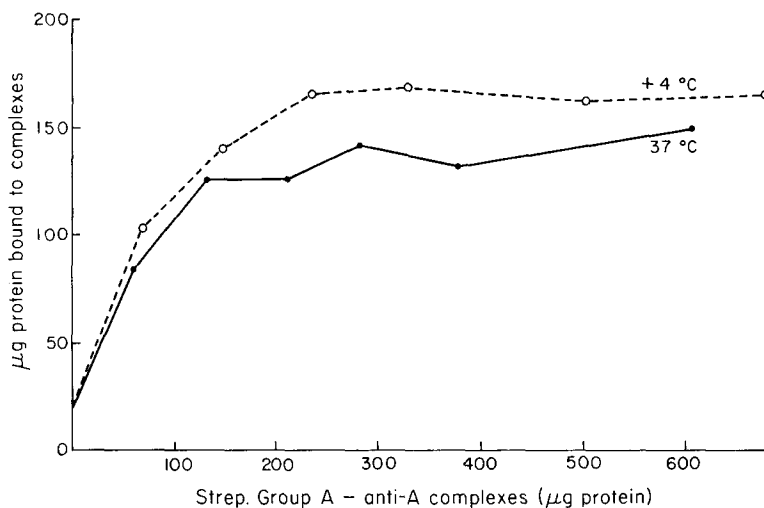


FIG. 1. Coprecipitation reaction of serum R3387 at 4°C and 37°C. The values indicate the amount of IgG which coprecipitates with antigen-antibody complexes. As will be shown in Fig. 6, the bulk of this IgG is 7S anti-IgG. The remainder is normal IgG bound to the 7S anti-IgG.

and the immune serum R3387 was taken as the amount of 7S anti-IgG which was brought down by coprecipitation. In a control series of tubes, the heterologous group-specific carbohydrate alone without anti-Group A antibody was added to the immune serum R3387. No measurable precipitate was formed in these tubes.

Depicted in Fig. 1 are the precipitin curves obtained with this quantitative coprecipitation test for Group C antiserum R3387. Values are given for 4°C and 37°C. At 4°C, 160 µg of 7S anti-IgG coprecipitated with 300 µg of antigen-antibody complexes. Addition of more complexes did not result in further increase of coprecipitation. The same type of curve was obtained when the experiment was carried out at 37°C, but at this higher temperature somewhat less anti-IgG was coprecipitated. The small difference of 20 µg between the coprecipitate formed at 4°C and 37°C is due to a higher binding efficiency of anti-IgG at low temperatures. No 7S anti-IgG was detected in the supernatants of the tubes which had received 300 µg and more complexes. This indicates that the test is a measure of all the 7S anti-IgG which is reactive with immune complexes.

Based on these results, the concentration of 7S anti-IgG in antisera was measured by adding an excess amount of antigen and antibody at ratios of equivalence to the serum. In a typical

experiment, 300 μ g of antibody, 10 μ g of carbohydrate antigen, and 0.1 ml of a 1:10 diluted test antiserum were allowed to react for 18 hr at 4°C in a total volume of 0.3 ml. The protein content of the precipitate was measured and the amount of 7S anti-IgG calculated as described above.

In these experiments it was important that the antigen-antibody complexes were formed in the presence of the 7S anti-IgG. When the test was carried out by using preformed washed complexes, the amount of 7S anti-IgG recovered by coprecipitation was only 10% of that obtained when the reactants were added so that complex formation and coprecipitation occurred simultaneously. By using radiolabeled purified 7S anti-IgG, it was shown that 7S anti-IgG was absorbed onto the preformed complexes but was readily eluted by washing with cold phosphate-buffered saline. With simultaneous complex formation and coprecipitation, 7S anti-IgG was tightly bound to the complexes. It was, presumably, trapped in the antigen-antibody lattice.

Quantitative Precipitin Analysis for Antistreptococcal Carbohydrate.—Quantitative precipitin analysis with the group-specific carbohydrates was performed as previously described (8). The amount of streptococcal group-specific antibody in 1 ml of serum was calculated from the quantitative precipitin data as previously described (8, 14).

Immunoabsorbents.—Immunoabsorbents for the purification of antibodies to Group A carbohydrate were prepared as previously described (15).

TABLE I
Agglutination of IgG-Coated Cells by the IgM and IgG Fractions of Antisera

Serum	Agglutination titer			Concentration/ml serum		
	Serum	IgM Fx	IgG Fx	IgM	IgG	7S anti-IgG
3387 4b	1/10240	1/5120	1/80	1.0	34	15
3387 4a	1/10240	1/1280	0	1.3	33	14
3419	1/1280	1/256	1/16	0.6	40	5
2686	1/640	1/256	1/32	0.5	60	9
3439	1/2560	1/64	1/16	0.7	32	10
2684	1/160	1/64	1/128	0.4	43	5
3275	1/20	1/8	0	0.5	16	0

RESULTS

Measurement of 19S Anti-IgG.—19S anti-IgG in rabbit antisera to streptococcal carbohydrate was measured by agglutination of rabbit F red blood cells coated with rabbit anti-F isoantibody. It was first necessary to show that the 7S component of the antisera had little or no agglutinating activity, before this test could be used to measure levels of 19S anti-IgG. For this purpose, the 19S and 7S anti-IgGs in seven antisera were separated by gel filtration on Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). The fractions containing IgM and IgG were tested individually in the hemagglutination assay. The agglutination titers of the peak fractions of the IgM and IgG peaks are shown in Table I. In addition are listed the serum concentrations of IgM, IgG, and 7S anti-IgG. 7S anti-IgG was measured with the coprecipitation assay. Except for serum R2684, the agglutination titer of the

IgM fraction was between 4 and 500 times higher than that of the IgG fraction, even though these sera contained 5 or more mg of 7S anti-IgG/ml. Since it is uncommon for an antiserum to contain more than 5 mg of 7S anti-IgG/ml serum, it is concluded that the agglutination titer of whole serum is primarily a reflection of the 19S anti-IgG concentration.

Time-Course for the Appearance of 19S Anti-IgG.—The time-course for the appearance of 19S anti-IgG was studied with serial antisera drawn weekly

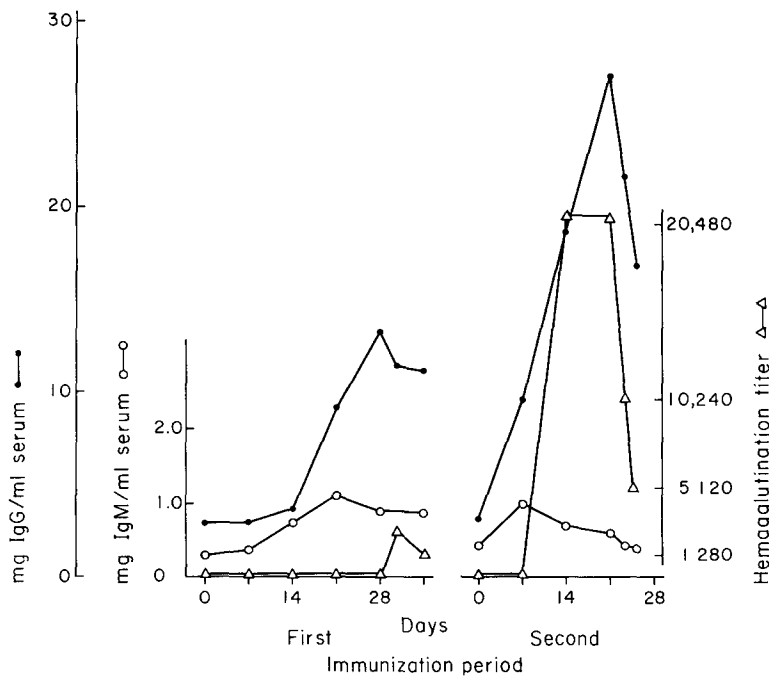


FIG. 2. Occurrence of 19S anti-IgG during immunization with streptococcal vaccine as detected by hemagglutination titer. Serum concentrations of IgG and IgM are also shown.

through the first and second series of immunizations. The first series of immunizations lasted 4 wk, and the second, after a rest period of 3 months, lasted 3 wk. Observations were made on 25 rabbits. Shown in Fig. 2 are the results from a single rabbit which are representative for the majority of the 25. 22 out of 25 rabbits had a hemagglutination titer greater than 1:640 during first immunization. A marked amnestic response of 19S anti-IgG was seen during the second immunization. One-half of the 25 rabbits had serum hemagglutination titers $\geq 1:10,000$. The lowest value was 1:640; the highest value, 1:160,000.

The IgM concentration was measured for all antisera. In general, the IgM level was as high during the first as the second immunization period. There

was no correlation between the concentration of this immunoglobulin and the agglutination titer.

Also depicted in Fig. 2 are the serum concentrations of IgG. Because the bulk of the antiserum IgG consists of precipitating and nonprecipitating antibodies to streptococcal carbohydrates, the antiserum IgG concentration was

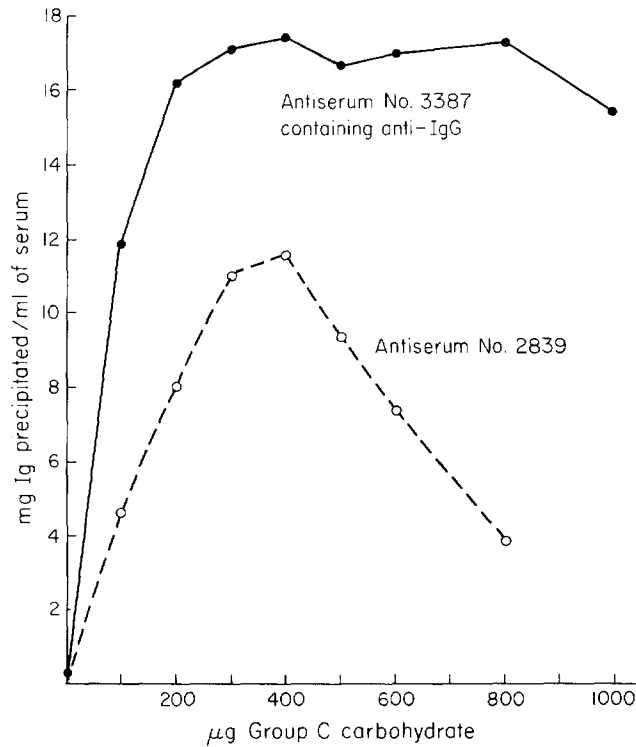


FIG. 3. Quantitative precipitin reactions between rabbit Group C antisera and streptococcal Group C carbohydrate. Antiserum R3387 contained 7S anti-IgG and serum R2839 had no detectable 7S anti-IgG.

employed as an index of the immune response. A survey of antisera from more than 100 rabbits immunized with either Groups A, A-variant, or C streptococci showed that all contained 19S anti-IgGs. There was, however, no correlation between the concentration of IgG and the hemagglutination titer.

It was typical during first immunization for the 19S anti-IgG to occur after the rise of IgG antibody to streptococcal carbohydrates. This delay in occurrence may be an indication that the production of 19S anti-IgG is a consequence of the immune response to the streptococci.

Demonstration of 7S Anti-IgG in Antistreptococcal Serum.—The occurrence of 7S anti-IgG in certain antisera was suspected because of unusual solubility

properties of a serum Ig component. Such antisera became opaque and viscous when stored at 4°C. In a few cases a cryoprecipitate formed. Furthermore, some antisera gave abnormal quantitative precipitin reactions with streptococcal carbohydrates. Quantitative precipitin curves of these antisera with the carbohydrate antigen had a greater slope in the range of antibody excess than the typical antiserum and remained at a plateau in the range of antigen excess. This is shown in Fig. 3 which compares the precipitin curves of two antisera, one with and one without 7S anti-IgG. In the usual case, 11–12 mg of IgG is precipitated with 300–350 μ g of carbohydrate. With the abnormal serum, on the other hand, 17 mg of protein was precipitated with 350 μ g of antigen.

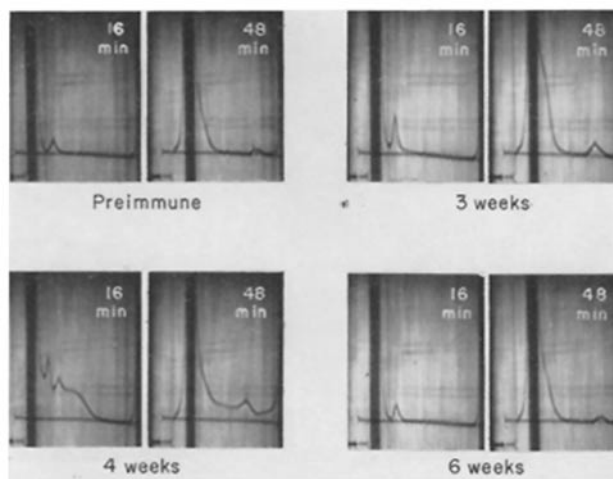


FIG. 4. Schlieren patterns of R3387 preimmune serum; two antisera were collected at 3 and 4 wk of immunization; and a third serum (6 wk) was collected after immunization. Temperature 20°C; speed 52,640 rpm; pictures taken after 16 and 48 min.

This excess of precipitating protein presumably was anti-IgG. Analysis of the washed and dissolved immune precipitate of the abnormal serum R3387 by immunoelectrophoresis showed predominantly IgG and only trace amounts of IgM.

The behavior of the abnormal sera upon ultracentrifugation was another indication for the occurrence of a 7S anti-IgG. Fig. 4 shows the sedimentation pattern of four serum samples of rabbit R3387 obtained before, during, and after immunization. The serum obtained 4 wk after the commencement of the immunization contained soluble protein complexes which extended from the area of 7S to and beyond the 19S region. The preimmune serum, as well as the 3- and 6-wk samples were devoid of these complexes. The 6 wk sample was obtained 2 wk after the immunization was discontinued.

Further ultracentrifugation studies were done on the protein collected from

the serum by cryoprecipitation. Fig. 5 shows a comparison of the sedimentation pattern obtained with the cryoprotein dissolved in phosphate-buffered saline at pH 7, and the same protein after dialysis against 0.1 M sodium acetate buffer, pH 4.1. The protein at pH 7 sedimented in a broad peak, indicating the formation of complexes, whereas the same protein at pH 4.1 sedimented in a sharp peak with an *s*-rate of 7S, an indication that the immune complexes which were dissolved at this low pH consisted of 7S material. The slow sedimenting shoulder in both panels represents albumin, a contaminant of the cryoprecipitate.

Evaluation of the Specificity of the Assay for 7S Anti-IgG.—The assay for the quantitation of 7S anti-IgG is based on the coprecipitation of 7S anti-IgG with complexes of streptococcal carbohydrates and anti-carbohydrate antibody. There were two questions about the specificity of the coprecipitation test for 7S anti-IgG which needed investigation before it could be used to measure the amount of 7S anti-IgG in streptococcal antisera. The first ques-

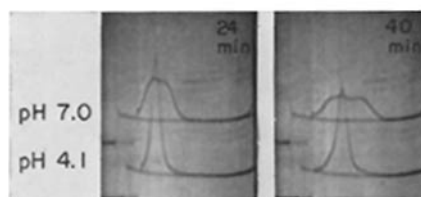


FIG. 5. Dissociation of 7S complexes at low pH. Solvent upper cell: 0.1 M PO_4 buffer, pH 7.0; lower cell: 0.1 M acetate buffer, pH 4.1. Temperature 20°C; speed 52,640 rpm; pictures taken after 24 and 40 min.

tion was concerned with the possibility that normal serum IgG was bound to the 7S anti-IgG, and thus coprecipitated with the immune complexes, a factor which would increase the apparent yield of 7S anti-IgG. The experiment which was designed to eliminate this possibility made use of 7S anti-IgG which appeared to be a single molecular species (16). Alkaline polyacrylamide disc electrophoresis of the purified material revealed that the light chains were distributed in one band. It can be assumed, therefore, that little or no other serum IgG is bound to this 7S anti-IgG preparation. This 7S anti-IgG was used in a quantitative coprecipitation test, into which ^{125}I -labeled normal IgG was also incorporated in order to determine the amount of normal IgG which would bind to the 7S anti-IgG and as a consequence coprecipitate.

To a series of tubes were added 300 μg of anti-Group A antibody with the equivalent amount of carbohydrate, 120 μg of ^{125}I -labeled normal IgG, and increasing amounts of the homogeneous 7S anti-IgG. Coprecipitates which formed overnight at 4°C were washed, dissolved, and the protein content, as well as the radioactivity, were determined. A control tube contained the reactants without 7S anti-IgG. This precipitate was also washed and dis-

solved. Its protein content was measured and this value was subtracted from the experimental values to determine the protein in each tube due to 7S anti-IgG and any bound normal IgG.

Fig. 6 depicts the amount of coprecipitate due to 7S anti-IgG and ^{125}I -labeled normal IgG. Also shown are the calculated values for 7S anti-IgG alone. These were obtained by subtracting from the protein in each precipitin tube the amount of ^{125}I -labeled IgG as calculated from the radioactivity values. The experiment shows that at all of the 7S anti-IgG concentrations tested,

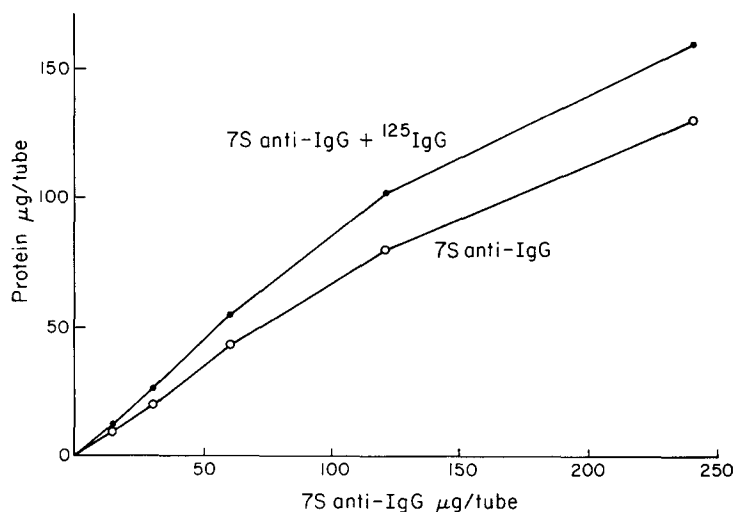


FIG. 6. Coprecipitation of normal IgG through binding to 7S anti-IgG. Closed circles (●) indicate the total amount of IgG, (7S anti-IgG plus bound normal IgG) which coprecipitated with immune complexes. The amount of 7S anti-IgG (open circles, ○) is calculated by subtracting the normal IgG bound to the coprecipitate as determined by the ^{125}I -radiolabeled IgG. See text for details.

approximately 20% of the coprecipitate consists of normal IgG which binds to 7S anti-IgG. The value of 7S anti-IgG as determined in the coprecipitation test must consequently be reduced by 20%. This has been done for all the values given below.

The second question in regard to the specificity of the test was concerned with the possibility that in the antiserum being tested 19S anti-IgG and its 22S complexes would bind to the coprecipitate. If this were the case, the calculated values for 7S anti-IgG concentrations would be too high. To investigate this possibility, the coprecipitation test was performed for four antisera in parallel, with and without the addition of purified IgM isolated from an antiserum (R2250) which had a titer of 19S anti-IgG of 1:40,000. The IgM of this serum was isolated by gel filtration on Sephadex G-200. Each strepto-

coccal antiserum was diluted such that a 1:10 dilution of the serum contained 1.3 mg IgM of serum R2250/ml. 0.1-ml aliquots were mixed with 300 μ g of anti-Group A antibody and 10 μ g of Group A carbohydrate. A 1:10 dilution of the same streptococcal serum without addition of isolated IgM was used as control.

Tabulated in Table II are the coprecipitation values of four different antisera with and without the addition of 19S anti-IgG. In the case of antiserum R3387, the addition of 19S anti-IgG had little influence on the amount of the protein in the coprecipitate. On the other hand, for antiserum R3525, there was an appreciable increase in the amount of protein in the coprecipitate with the addition of 19S anti-IgG. It should be kept in mind, however, that in these experiments 1.3 mg IgM had been added to 1 ml of a 1:10 dilution of the streptococcal antiserum. This corresponds to an actual serum concentration of IgM of 13 mg/ml. The average IgM concentration of the streptococcal antisera, however, is 0.7–1.5 mg/ml, approximately ten times lower than that

TABLE II
Influence of 19S Anti-IgG on Coprecipitation

Serum No.	IgG concentration mg/ml	μ g coprecipitate/0.1 ml		μ g coprecipitate due to 19S anti-IgG
		1:10 serum	1:10 serum + IgM	
3387	36	262	273	11
3439	38	146	218	72
3416	39	125	201	76
3525	50	43	128	85

used here. The protein in the coprecipitate due to 19S anti-IgG would therefore be only one-tenth of the value listed in the table for these four antisera. For antiserum R3525, for example, the value would be 8.5 μ g instead of 85 μ g. In none of the four sera tested would the coprecipitate due to 19S anti-IgG be greater than 10 μ g. From these considerations, only protein coprecipitates which were 10 μ g or greater were interpreted as indicative of 7S anti-IgG in the coprecipitation test.

Occurrence of 7S Anti-IgG in Antisera during the Response to Streptococcal Vaccine.—The coprecipitation test was employed to study the time-course of the occurrence of 7S anti-IgG during immunization with Group C vaccine. Eight rabbits which produced greater than 5 mg/ml of 7S anti-IgG were chosen for this study. The concentration of total IgG and of 7S anti-IgG was measured in weekly sera from the first and second immunization periods. Three out of eight rabbits produced measurable amounts of 7S anti-IgG in the first, whereas all eight rabbits manufactured 7S anti-IgG in the second immunization period. In Fig. 7 is shown the occurrence of 7S anti-IgG in two different rabbits during first and second immunization periods. The results

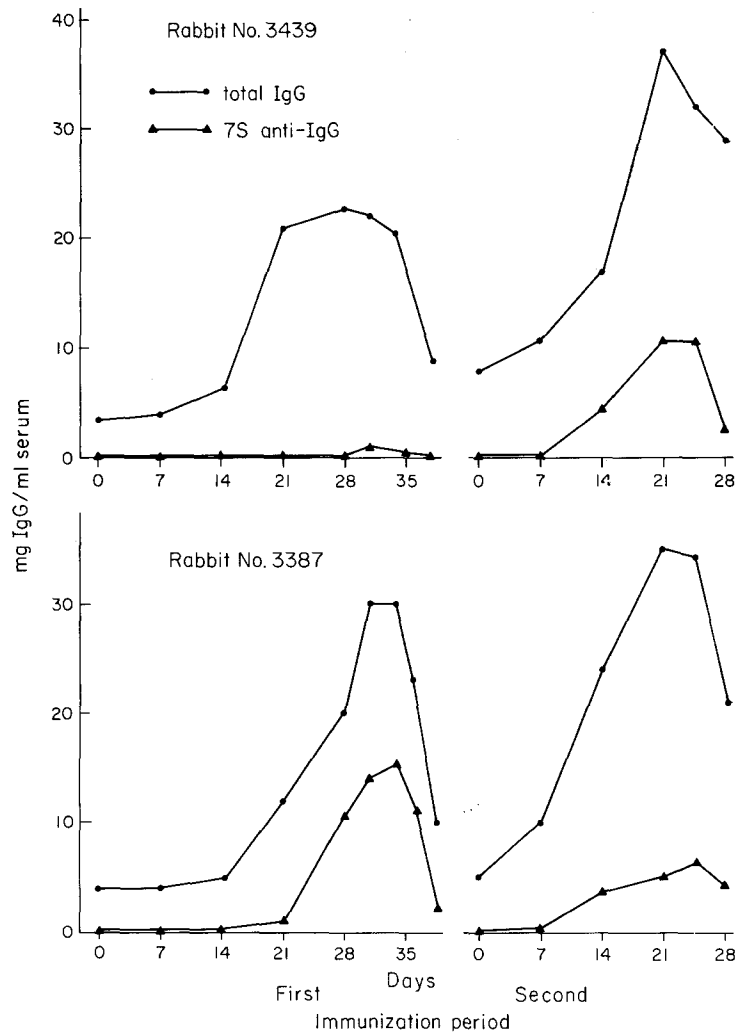


FIG. 7. Occurrence of 7S anti-IgG during immunization with streptococcal vaccine. Closed circles (●) indicate the serum concentration of IgG, closed triangles (▲) the concentration of 7S anti-IgG.

of rabbit R3439 are representative for seven of the eight rabbits tested in this fashion. In these animals, 7S anti-IgG occurred in the 2nd wk of the second immunization period, and reached its peak between the 3rd and 4th wk. Rabbit R3387 is an exception, inasmuch as it produced more 7S anti-IgG in the first immunization period than in the second, and because more than half of the total IgG in the first period consisted of 7S anti-IgG. As in the case of 19S anti-gG, it is to be noted that the concentration of serum IgG increases earlier

than the concentration of 7S anti-IgG. This may be an indication that the occurrence of anti-IgGs is a consequence of the hyperimmune response to the streptococcal immunization.

A survey was made of the 7S anti-IgG concentration in the antisera from 88 rabbits immunized with Group C streptococci. The 3rd wk bleeding of the second immunization period was used for the measurement of 7S anti-IgG, since at that time during immunization, the concentration of 7S anti-IgG was

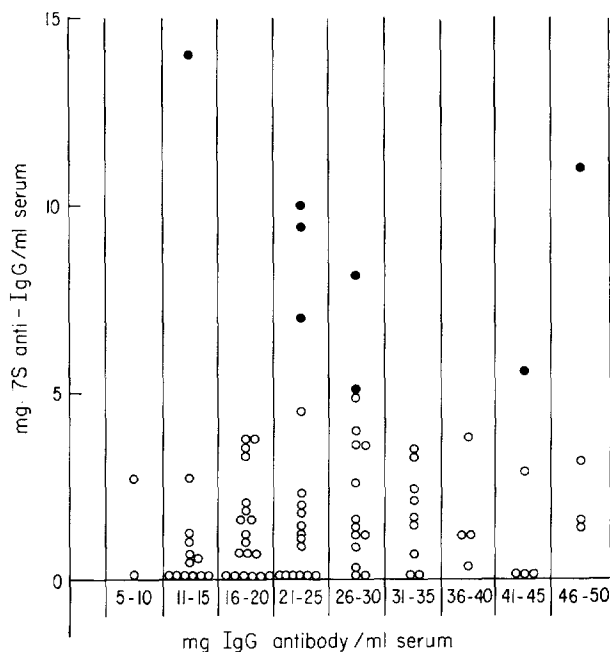


FIG. 8. Concentration of IgG antistreptococcal carbohydrate antibody and 7S anti-IgG in the antiserum of 88 rabbits immunized with streptococcus. Each circle represents the results on the serums of one rabbit. Closed circles (●) indicate rabbits which produce greater than 5 mg/ml of 7S anti-IgG.

at its highest levels. The data are shown in Fig. 8. 50 animals produced detectable amounts of 7S anti-IgG. In this figure the serum concentration of 7S anti-IgG is plotted against the concentration of anti-Group C antibody of the same sample. There is no strict correlation between the concentration of these two components.

There is some evidence from these studies that the occurrence of 7S anti-IgG is an inherited trait. This could be traced because most of the rabbits immunized here had inbred pedigrees. Serum samples were available from the initial breeding pairs and the corresponding F_1 and F_2 generations. The 88 rabbits tested for the occurrence of 7S anti-IgG were members of nine rabbit families. All

eight rabbits which produced greater than 5 mg of 7S anti-IgG/ml, indicated in Fig. 8 by closed circles, were members of three related families (34 rabbits). In six inbred families (45 rabbits) 70–100% of the family members had 7S anti-IgG, whereas in two families (30 rabbits) this antibody was detectable in only 30% of the family members. There was no difference in the antibody response to streptococcal carbohydrate between these high and low responder families.

Occurrence of 19S and 7S Anti-IgGs in Antipneumococcal Sera.—The infrequent occurrence of 19S and 7S anti-IgGs in anti-pneumococcal sera suggested that the production of these antibodies is dependent in part on the nature of

TABLE III
19S and 7S Anti-IgGs Produced by Rabbits Injected with Streptococcal and Subsequently Pneumococcal Vaccine

Rabbit No.	Sera	19S anti-IgG titers	7S anti-IgG mg/ml	IgG mg/ml
3357	Preimmune	0	0	7
	Antistreptococcal	1/20480	0	23
	Prepneumococcal	1/160	0	10
	Antipneumococcal	1/640	0	19
3522	Preimmune	0	0	6
	Antistreptococcal	1/20480	2.3	23
	Prepneumococcal	1/80	0	7
	Antipneumococcal	1/320	0	23
3460	Preimmune	0	0	8
	Antistreptococcal	1/320	1.8	26
	Prepneumococcal	0	0	9
	Antipneumococcal	0	0	21

the antigenic stimulus. Sera from 38 rabbits immunized with pneumococcal vaccines Types III or VIII were tested for 19S and 7S anti-IgG. Six rabbits were immunized according to the schedule used for the streptococcal vaccine and the other 32 animals were immunized according to the schedule of Kimball et al. (17) in which a first course of injections is given three times a week for 4 wk. This is followed without a rest period with one injection each week. The serum samples of this group were collected 6–10 wk after the start of the immunization period. Only eight of all 38 rabbits produced 19S anti-IgG with an average titer of 1:60 to 1:120. These titers are approximately 20 times lower than the average titer of antistreptococcal sera. None of the rabbits immunized with pneumococci produced 7S anti-IgG.

The role of the antigen for the induction of anti-IgGs is further demonstrated in an experiment in which three rabbits have first been injected in the usual

fashion with streptococcal vaccine and after a 4 month rest period for 4 wk, with pneumococcal vaccine.

The 19S anti-IgG titers and the 7S anti-IgG concentration in antisera after immunization with both bacterial vaccines are summarized in Table III. In all of these rabbits, the 19S anti-IgG titer was considerably higher in the anti-streptococcal sera than in the antipneumococcal sera. Two of the three rabbits produced 7S anti-IgG after streptococcal immunization, but not after immunization with pneumococci. The concentration of anti-capsular antibody after pneumococcal immunization was similar to that of anti-carbohydrate antibody after the prior immunization with streptococci.

DISCUSSION

Rabbits which have received prolonged immunizations with streptococcal vaccine produce high concentrations of precipitating and nonprecipitating antibodies to the streptococcal carbohydrate antigens. In the majority of the rabbits, such antibodies make up the bulk of the serum IgG. Not uncommonly, however, IgG components are present (5–20 mg/ml of serum) which have no detectable specificity for any known antigenic determinant in the streptococcal cells (18). It is now clear from the studies reported here that such IgG components are commonly 7S anti-IgG. Furthermore, as will be described in a later report, these 7S anti-IgG components have a remarkable degree of molecular uniformity (16).¹

A coprecipitation test was developed to measure the 7S anti-IgG concentration in the streptococcal antiserum. The principle is based on the coprecipitation of the 7S anti-IgG with complexes of streptococcal carbohydrate and antibody. A survey of 88 rabbit anti-Group C streptococcal antisera revealed that eight sera had 7S anti-IgG in concentrations greater than 5 mg/ml. 57% of all sera had detectable levels of 7S anti-IgG (greater than 1 mg/ml). It is possible that all of the sera do, in fact, possess 7S anti-IgG, but it escaped detection because of the limited sensitivity of the test system.

110 Group A, Group A-variant, and Group C streptococcal antisera were surveyed for the presence of 19S anti-IgG. This antibody was measured with an agglutination assay which employs rabbit F red blood cells coated with rabbit anti-F IgG (1, 2). All antisera had agglutination titers indicative of 19S anti-IgG. Hemagglutination titers as high as 1:160,000 were noted for several antisera. This was an unexpected finding because much lower titers have been seen with antisera from rabbits immunized with Gram-negative organisms (1, 2).

One possible explanation for the difference between the hemagglutination titers of the streptococcal antisera and *Escherichia coli* antisera is that the im-

¹ Bokisch, V. A., D. Bernstein, and J. W. Chiao. 1972. Isolation and physicochemical characterization of 7s anti-IgGs of rabbit anti-streptococcal antisera. Paper in preparation.

mune response after streptococcal vaccine is, in general, greater than that after immunization with Gram-negative organisms. This difference in part may be due to the fact that rabbits can tolerate a greater antigenic challenge of streptococcal vaccine than of a vaccine of Gram-negative bacteria. The lower toxicity of streptococci is probably due to the absence of endotoxin, a constituent of Gram-negative organisms. The issue, however, appears more complex than just the amount of antigenic stimulation. It was surprising for example, that rabbit antipneumococcal sera had no, or relatively low, titers of 19S anti-IgG, and no detectable 7S anti-IgG. These antipneumococcal sera, however, had total IgG serum levels which were as high as those of the streptococcal antisera. It seems possible therefore, that the nature of the antigen influences the amount of anti-IgGs which are produced.

Although streptococcal vaccine appears to be an especially efficient stimulus for the production of both 7S and 19S anti-IgG, it should be emphasized again that these antibodies also occur after immunization with other antigens. As was pointed out in the introduction, others have reported 19S anti-IgGs in sera of rabbits which have been immunized with a diverse group of substances including proteins, bacteria, and parasites. In one report 7S anti-IgG is described (6). In this case, the rabbits had been immunized with either horse spleen ferritin or autologous papain-digested IgG.

A search among inbred animals with known pedigrees revealed no obvious genetic influence on the occurrence of high titers of 19S anti-IgG. There is, however, some data to suggest that there may be a genetic influence on the occurrence of the 7S anti-IgG. Eight rabbits that produced more than 5 mg of 7S anti-IgG/ml belonged to three related families. Furthermore, there were families in which nearly every member produced 7S anti-IgG, and other families in which only 25–30% of the members produced 7S anti-IgG. Breeding studies are underway to determine if the occurrence of this antibody in immunized rabbits is a genetic trait.

The variety of specificities of the human anti-IgGs for different subgroup and genetic determinants have been especially useful for serologic and genetic studies on human Ig. It will be of interest to learn if the rabbit anti-IgGs have specificities for similar antigenic determinants on rabbit Ig. Such studies are underway.

SUMMARY

All 110 rabbits immunized with Group A, A-variant, and C streptococcal vaccines produced 19S anti-IgG in addition to antibodies to the streptococcal carbohydrates. 19S anti-IgG was detected by hemagglutination of rabbit red blood cells coated with rabbit anti-blood group F antibody. Antisera of 88 of these animals were also tested for 7S anti-IgG with a coprecipitation assay. This assay is based on the coprecipitation of 7S anti-IgG with complexes of streptococcal carbohydrate and anti-carbohydrate antibody. 50 of the 88 anti-

Group C streptococcal antisera contained 7S anti-IgGs. In eight antisera the concentration was greater than 5 mg/ml.

The data suggest a genetic influence on the occurrence of 7S anti-IgG. The eight rabbits which produced more than 5 mg/ml of 7S anti-IgG belonged to three related families. Moreover, there were families in which almost every member produced 7S anti-IgG and other families in which only 30% of the members manufactured 7S anti-IgG.

The streptococcal vaccine was an especially efficient stimulus for the production of 19S anti-IgG, whereas the pneumococcal vaccine was much less effective in this respect. Furthermore, 7S anti-IgGs were not detected in antipneumococcal antisera, although the concentration of anti-capsular antibodies was similar to that of anti-carbohydrate antibodies in antistreptococcal antisera.

The authors are especially grateful for the advice and guidance of Dr. Henry G. Kunkel and Dr. Charles Christian.

During the course of this work it has been a pleasure to have the participation of Mr. Jay Greenblatt and Mr. Woodson Merrell.

REFERENCES

1. Abruzzo, J. L., and C. L. Christian. 1961. The induction of a rheumatoid factor-like substance in rabbits. *J. Exp. Med.* **114**:791.
2. Christian, C. L. 1963. Rheumatoid factor properties of hyperimmune rabbit sera. *J. Exp. Med.* **118**:827.
3. Aho, K., and O. Wager. 1961. Production of "anti-antibodies" in rabbits. *Ann. Med. Exp. Biol. Fenn.* **39**:79.
4. Milgrom, F., and E. Witebsky. 1960. Studies on the rheumatoid and related serum factors. *J. Am. Med. Assoc.* **174**:138.
5. Klein, F., P. Mattern, and H. J. Kornman-v.d. Bosch. 1970. Experimental induction of rheumatoid factor-like substances in animal trypanosomiasis. *Clin. Exp. Immunol.* **7**:851.
6. Williams, R. C., and H. G. Kunkel. 1963. Antibodies to rabbit gamma-globulin after immunizing with various preparations of autologous gamma-globulin. *Proc. Soc. Exp. Biol. Med.* **112**:554.
7. Davie, J. M., C. K. Osterland, E. J. Miller, and R. M. Krause. 1967. Immune cryoglobulins in rabbit streptococcal antiserum. *J. Immunol.* **100**:814.
8. Osterland, C. K., E. J. Miller, W. W. Karakawa, and R. M. Krause. 1966. Characteristics of streptococcal group-specific antibody isolated from hyperimmune rabbits. *J. Exp. Med.* **123**:599.
9. Krause, R. M., and M. McCarty. 1962. 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.* **114**:127.
10. Eichmann, K., and T. J. Kindt. 1971. The inheritance of individual antigenic specificities of rabbit antibodies to streptococcal carbohydrates. *J. Exp. Med.* **134**:532.
11. Mancini, G., A. O. Carbonara, and J. F. Heremans. 1965. Immunochemical

- quantitation of antigens by single radial immunodiffusion. *Immunochemistry*. **2**:235.
12. Fahey, J. L. 1967. In *Methods in Immunology and Immunochemistry*. C. A. Williams and M. W. Chase, editors. Academic Press, New York. 321.
 13. Braun, D. G., and R. M. Krause. 1968. The individual specificity of antibodies to streptococcal carbohydrates. *J. Exp. Med.* **128**:969.
 14. Fleischman, J. B., D. G. Braun, and R. M. Krause. 1968. Streptococcal group-specific antibodies. Occurrence of a restricted population following secondary immunization. *Proc. Natl. Acad. Sci. U.S.A.* **60**:134.
 15. Rotta, J., R. M. Krause, R. C. Lancefield, W. Everly, and H. Lackland. 1971. New approaches for the laboratory recognition of M-Types of Group A streptococci. *J. Exp. Med.* **134**:1298.
 16. Bokisch, V. A., and D. Bernstein. 1971. Homogeneous rabbit IgG cryoglobulin with rheumatoid factor activity. *Fed. Proc.* **30**:528. (Abstr.)
 17. Kimball, J. W., A. M. Pappenheimer, and J. C. Jaton. 1971. The response in rabbits to prolonged immunization with type III pneumococci. *J. Immunol.* **106**:1177.
 18. Eichmann, K., and J. Greenblatt. 1971. Relationships between relative binding affinity and electrophoretic behavior of rabbit antibodies to streptococcal carbohydrates. *J. Exp. Med.* **133**:424.