RABBIT ANTIBODIES TO STREPTOCOCCAL CARBOHYDRATES

Influence of Primary and Secondary Immunization and of Possible Genetic Factors on the Antibody Response*

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A number of variables are known to influence the magnitude of the immune response including the chemical and physical nature of the antigen, the method of immunization, prior sensitization to the same or a similar antigen, and the genetic background of the animal. Furthermore, these factors may either amplify or limit the wide variability in the characteristics of the immune globulins which are produced.

The amount of precipitins to streptococcal carbohydrates has varied between 1 and 30 mg/ml of antiserum following the primary intravenous immunization of rabbits with bacterial vaccines (1–3). Although the γ-globulin in most of these antisera is polydisperse when examined by zone electrophoresis, not infrequently the bulk of the γ-globulin is monodisperse and consists predominantly of precipitins to the streptococcal cell wall carbohydrate. From this point of view, these antibodies bear a resemblance to the myeloma proteins. Perhaps more striking, however, is the fact that they share with myeloma proteins the property of individual antigenic specificity (4). This term has been employed for the antigenic individuality which appears to be a characteristic feature of each myeloma protein as well as of human antibodies to several different carbohydrate substances (5, 6).

Previous studies suggest that prior exposure to the same or a closely related antigen may have an influence on the occurrence in rabbits of antibodies with uniform characteristics. For example, rabbits which had heterogenous, electrophoretically polydisperse precipitins in either low or high concentration

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after primary immunization may exhibit a strikingly high concentration of monodisperse antibodies after a second series of intravenous immunizations (7). For these reasons, the efficacy of secondary immunization in eliciting a monodisperse antibody response has been examined in greater detail here.

It is now abundantly clear that genetic factors have a control over the immune response, and some of the evidence to support this will be reviewed in the discussion. The marked differences in the concentrations of antibody to streptococcal carbohydrates which have been observed among more than 100 rabbits immunized with streptococcal vaccines, and the emergence in certain rabbits during the course of immunization of high concentrations of a uniform population of antibodies has raised the possibility that genetic factors influence both the magnitude and the uniform quality of the immune response to these specific haptenic determinants. In studies reported here, the evidence suggests that the immune responses of the parental generation and F_1 generation conform to a similar pattern.

Materials and Methods

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

Streptococcal Vaccines.—Vaccines were prepared from all three strains as previously described (1, 4).

Streptococcal Cell Walls.—Streptococcal cell walls were prepared by previously described methods (8).

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 (9). In addition to the formamide-extracted Group A-variant carbohydrate, a preparation of this antigen was used which was obtained by digestion of cell walls with *Streptomyces albus* enzyme (10).

Rabbits.—Random bred but impure line of New Zealand Red rabbits were obtained from the Carver's Rabbitry, Somerville, N. J.

Immunization.—The immunization procedure has been described previously (1, 4).

Qualitative and Quantitative Precipitin Analysis.—Qualitative and quantitative precipitin analyses were performed as previously described (1). The amount of antibody in 1 ml of serum was calculated from quantitative precipitin data as described earlier (4, 7).

Zone Electrophoresis.—Zone electrophoresis of serum proteins employed a Beckman model R101 microzone cell as was previously described (4).

Preparative Zone Electrophoresis of Serum.—Preparative zone electrophoresis was performed in Seakem® agarose as the supporting medium, as previously described (4). With certain batches of agarose, separation of the monodisperse globulin components can only be achieved if the agar gel, after it has been added to the mold, is stored at 4°C for 24 hr before use. When used in this fashion, agarose has proved preferable to Difco Ion agar (Difco Laboratories Detroit, Mich.).

Protein Determination.—Total protein in rabbit antisera was determined by the biuret method and the γ-globulin calculated from the densitometric scan of the zone electrophoretic patterns and the total protein value of the serum. Protein concentrations of the preparative agarose block electrophoresis fractions were determined by the modified Folin-Ciocalteu method using Cohn's Fr II of rabbit serum as a standard (11).

Slide Agglutinin Test.—Agglutinating antibodies were determined by the slide agglutination test, employing Group A-variant cell walls (12).

Immunoelectrophoretic Analysis.—Immunoelectrophoretic analysis was done by the micromethod of Scheidegger (13). Anti-rabbit serum and anti-rabbit γ-globulin were purchased from Hyland Laboratories, Los Angeles, Calif.

RESULTS

In these studies attention will be directed to three aspects of the immune response in rabbits following primary and secondary immunization with streptococcal vaccines: variability in the concentrations of precipitating and nonprecipitating antibodies to the group carbohydrates; the frequency of the occurrence of electrophoretically monodisperse antibodies; and, a comparison between the immune response of the parental generation to that of the F_1 generation.

The vaccines employed in all of these experiments consisted of whole heat-killed Groups A, A-variant, and C streptococci which had been treated with pepsin at pH 2 to remove the bulk of the surface proteins. This treatment exposes the group carbohydrate as the most superficial antigen. This exterior location of the antigen is probably responsible for the fact that the antibodies to the group carbohydrate are frequently the predominant component of the immune response. As a general rule, maximum antibody levels were achieved in the case of primary immunization after 4 wk of three intravenous injections weekly. Secondary immunizations were begun after a rest period of 4–6 months. Peak levels of secondary response antibody were usually achieved after 3 wk of three intravenous injections weekly. Careful evaluation of the antibody response at frequent intervals during the course of immunization does reveal, however, that maximum primary and secondary responses may occur at 3 wk and 2 wk, respectively. Prolonging the immunization of those rabbits which respond poorly has not been helpful in forcing production of higher antibody concentrations. For these reasons, and to maintain consistent treatment of the data, only antisera collected after 4 wk of the first series of injections were used to measure primary responses. Only antisera collected after 3 wk of the second series of injections were used to measure secondary responses.

Immune Response Following Primary and Secondary Immunization.—Three groups of rabbits were immunized separately with Groups A, A-variant, and C vaccines.

36 out of 40 rabbits survived primary immunization with Group A vaccine. 17 of these survivors were started on second immunization and 13 survived the full course of injections. 41 out of 45 rabbits survived primary immunization with Group A-variant vaccine. 23 of these survivors were started on second immunization and 13 survived the full course of injections. 39 out of 43 rabbits survived primary immunization with Group C vaccine. 33 of these survivors were started on second immunization, and 29 survived the second course of injections.

Presented in Figs. 1, 2, and 3 are the antiserum concentrations of group-

specific precipitins and γ-globulin following primary and secondary immunizations. These figures are histiogram plots of antiserum concentrations of both the group-specific precipitins and total γ-globulin for each of the rabbits in

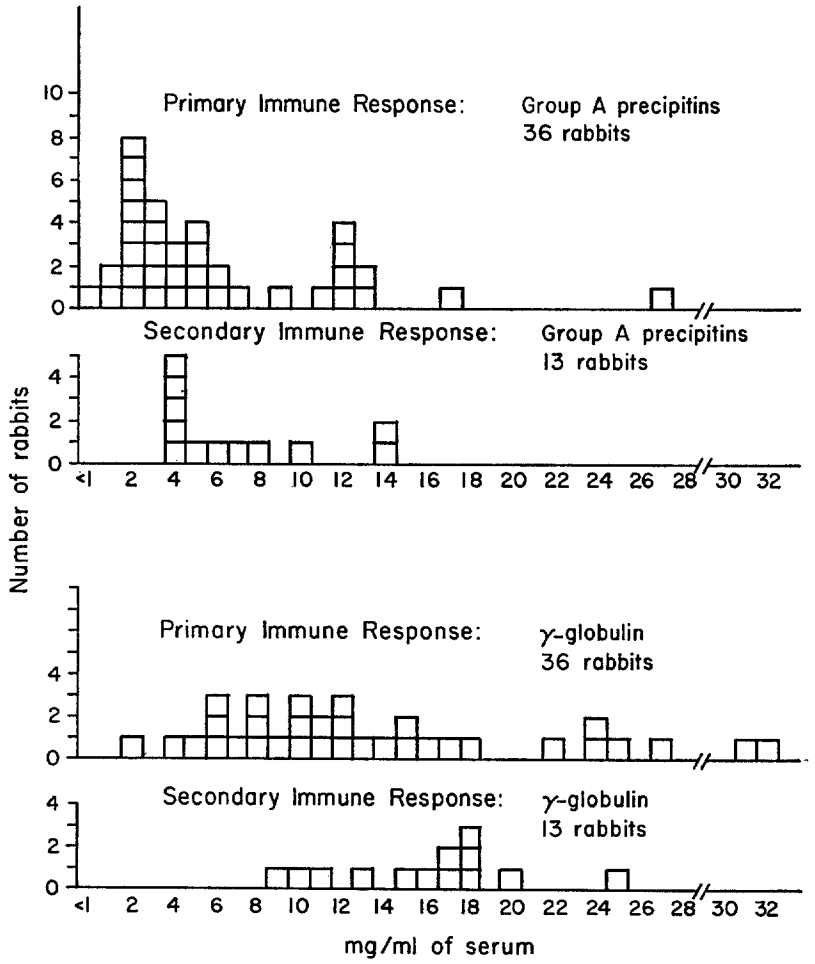

FIG. 1. Serum concentrations of Group A precipitins and γ-globulin for 36 rabbits after primary and secondary intravenous immunizations with Group A streptococcal vaccine.

each of the three groups. At first glance, the wide range of antibody concentrations after both primary and secondary immunization does not appear particularly surprising. Closer inspection of the data, however, reveals several features of these immune responses which deserve special emphasis.

Attention will first be directed to the data on the antiserum levels of the

group-specific precipitins. A somewhat higher concentration of precipitins was achieved after either the primary or secondary immunization with Group C vaccine than with Group A vaccine. In the case of Group C, the precipitin

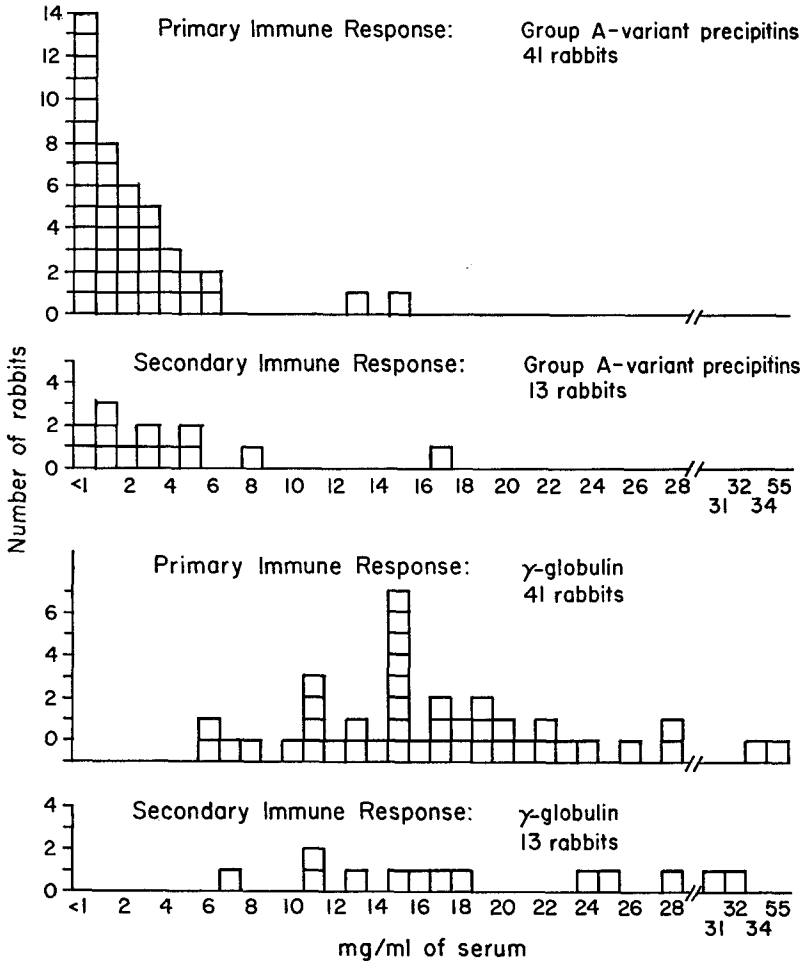

FIG. 2. Serum concentrations of Group A-variant precipitins and γ-globulin in 41 rabbits after primary and secondary intravenous immunizations with Group A-variant streptococcal vaccines.

levels varied between 1 and 19 mg/ml. After secondary immunization the values ranged between 2–31 mg/ml. The concentration of Group A-variant precipitins was much below that of Group C and Group A. For example, one-half of the rabbits after primary immunization with Group A-variant had precipitin con-

centrations of 1 mg/ml or less. Nearly all of the rabbits receiving secondary immunization with Groups A and C vaccines had a higher concentration of precipitins than after primary immunization. This was not nearly as striking, however, in the case of the rabbits immunized with Group A-variant vaccine.

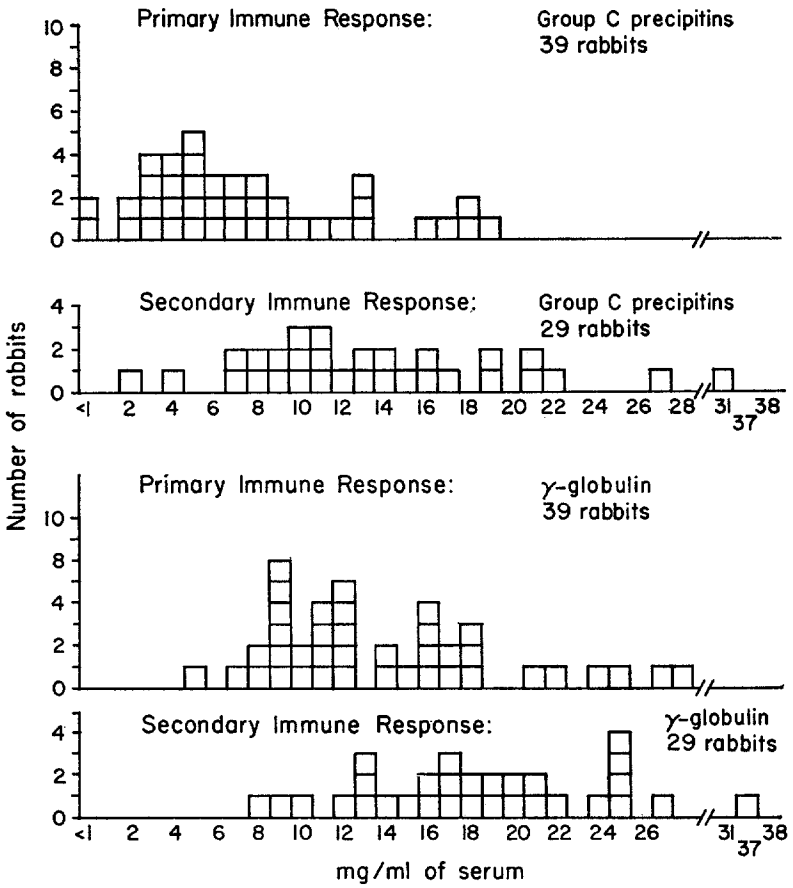

FIG. 3. Serum concentrations of Group C precipitins and γ-globulin in 39 rabbits after primary and secondary intravenous immunizations with Group C streptococcal vaccines.

The average increase in antibody concentration after secondary immunization over that observed after primary immunization, was 5 mg/ml for the Group A rabbits and 10 mg/ml for the Group C rabbits.

Illustrated in Fig. 4 are examples of the wide diversity between the antibody levels after primary immunization and after secondary immunization. Plotted here in the top half of the figure are the quantitative precipitin curves for three

rabbits which had pronounced increases in precipitin concentrations following secondary immunization over that observed after primary immunization. As these examples demonstrate, in some cases the concentration of precipitins after secondary immunization may be four to six times that observed after

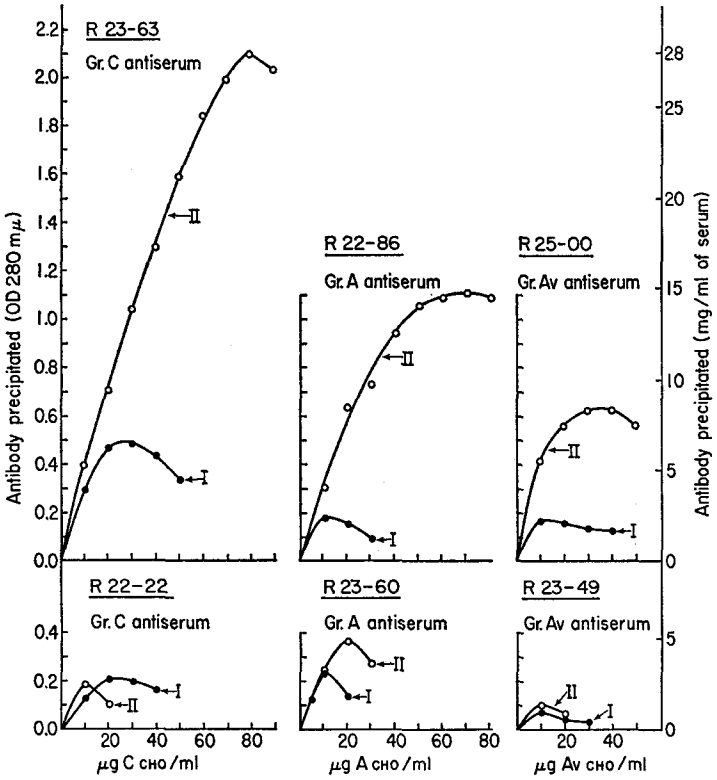

FIG. 4. Concentrations of group-specific precipitins following primary and secondary immunization with Groups C, A, and A-variant vaccines. The secondary responses for each of the three rabbits in the top frames were markedly higher than the primary responses. Both primary and secondary responses were low for the rabbits in the bottom frames.

The antibody content in 0.1 ml of serum, which was recovered from the precipitate, was redissolved in 0.1 N NaOH and measured spectrophotometrically. The amount of antibody precipitated at each antigen concentration is given both in terms of optical density of the redissolved precipitates (left scale) and antibody content of the whole serum as calculated from the optical density data (right scale). CHO, carbohydrate.

primary immunization. For comparison, in the bottom half of the figure, the data are also given for three other rabbits which had low levels of precipitins after both primary and secondary immunization.

The most prominent feature of the primary precipitin response, as depicted

in Figs. 1, 2, and 3, is the skew distortion of the histiogram plots toward the range of higher antibody concentrations. Such a finding suggests that there is a marked quantitative difference between the magnitude of the immune response in a minority of the rabbits with high precipitin concentrations and a majority with lower antibody levels. One possible explanation of this finding is that the magnitude of the immune response is under some form of genetic control. This possibility will be explored in a later section.

Attention will be directed next to the concentration of total γ-globulin in the

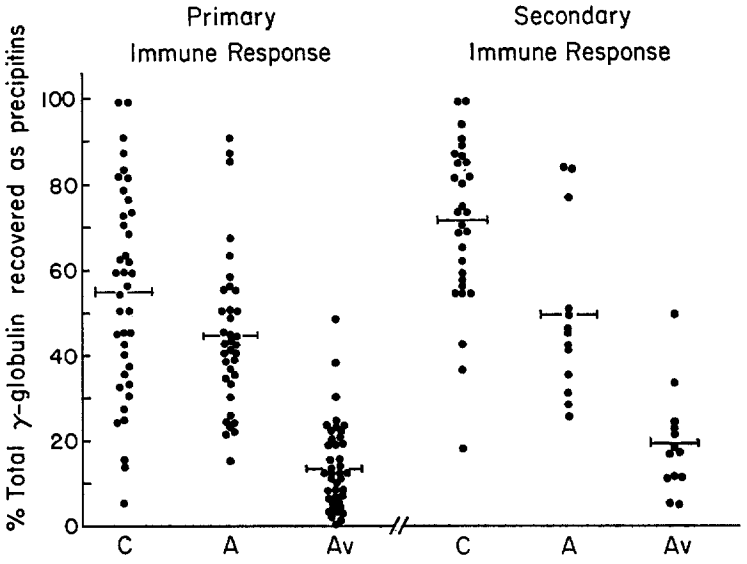

FIG. 5. Per cent of the total γ-globulin recovered as precipitins in the three groups of rabbits presented in Figs. 1, 2, and 3, which had received primary and secondary immunizations with streptococcal vaccines. Rabbits which had received Groups C, A, and A-variant vaccines are designed C, A, and Av, respectively.

antisera of rabbits after primary and secondary immunization with Groups A, A-variant, and C vaccines. These data are plotted in the lower portions of Figs. 1, 2, and 3. It is evident that there are not major differences among the three groups of rabbits. The average γ-globulin concentration is somewhat higher than the concentration of precipitins for the Group A and C rabbits, but the difference is most striking for rabbits immunized with Group A-variant vaccine. Presented in Fig. 5 is the percentage of the total γ-globulin which was recovered as precipitins for each rabbit in all three groups. In general, a greater proportion of the total γ-globulin was precipitated with group carbohydrate from the antisera of the Group C rabbits than from antisera of the Group A. The Group A-variant rabbits had the least proportion of total γ-globulin which

was precipitable with antigen. The wide variation among the Group C sera is striking. In the case of three rabbits, 90% or more of the total γ-globulin was recovered as precipitable antibody, whereas in three others, recovery was less than 20%.

For over one-half of the Group A-variant antisera, less than 15% of the total γ-globulin was precipitated by group carbohydrate. In the case of some antisera, there was essentially no detectable precipitating antibody. To be certain that this absence of re-activity was not due to partial denaturation of the antigen during the vigorous hot formamide extraction process, antigen was pre-

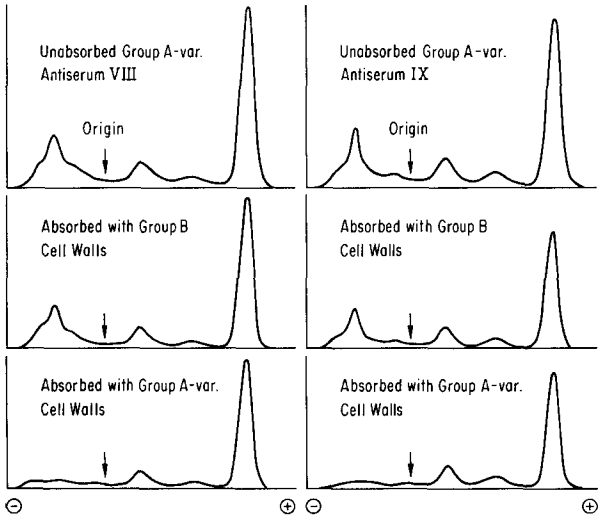

FIG. 6. Densitometric tracings of the microzone electrophoretic patterns of Group A-variant antisera before and after absorption with heterologous Group B cell walls and homologous Group A-variant cell walls.

pared from cell walls by enzymatic means, a procedure less likely to alter the natural state of the antigen (10). This enzymatically prepared antigen was no more reactive with the antisera than was the formamide-extracted antigen. The large discrepancy between the amounts of total γ-globulin and the concentrations of precipitins in these A-variant antisera raises a question about the antibody specificity of that portion of the total γ-globulin which is not precipitable with group carbohydrate. The most obvious explanations are that the nonprecipitable γ-globulin is antibody produced to other cellular constituents in the vaccine, or is a nonspecific γ-globulin produced in response to the overall stimulus of the immunization procedure. In a number of instances, however, neither of these alternatives proved to be the explanation.

From the studies which follow, it is clear that for many Group A-variant

antisera, the bulk of the γ-globulin is nonprecipitating antibody which has specificity for the specific determinants of this group carbohydrate. The results of an experiment which illustrate this for two of the Group A-variant antisera are depicted in Fig. 6. In this figure are the densitometric tracings of the zone electrophoretic patterns of antisera VIII and IX before and after absorption with homologous and heterologous streptococcus cell walls. The bulk of the γ-globulin was absorbed by homologous Group A-variant cell walls but not by the heterologous Group B cell walls, and is probably nonprecipitating antibody to the Group A-variant carbohydrate. Because the cell walls used for absorption contain mucopeptide in addition to the group carbohydrate and undoubtedly traces of other cellular material, additional studies were undertaken to assure that the nonprecipitating antibody had Group A-variant specificity.

TABLE I

Streptococcal Cell Wall Agglutination Reactions with Nonprecipitating Group A-Variant Antibodies Recovered from Antiserum VIII

Inhibitor	Antibody, μg/ml						
	200	100	50	25	12.5	6.25	0
1 mg/ml							
Saline control	++++	+++	++	++	+	+	0
A-variant CHO	+	+	0	0	0	0	0
Group A CHO	++++	++	++	++	+	+	0
Group C CHO	++++	++	++	++	+	+	0

The strength of the agglutination reaction was graded + to ++++. Zero is no agglutination.

See text for method of recovery of nonprecipitating antibodies from the antiserum.

CHO, carbohydrate.

The antibody employed for these studies was recovered in the following way. 1 ml of A-variant antiserum VIII depicted in Fig. 6 was mixed with 0.5 ml of packed A-variant cell walls. After incubation at 37°C for 2 hr, followed by storage for 24 hr at 4°C, the mixture was centrifuged and the supernatant discarded. The cell walls were rinsed several times with cold saline. The antibody was eluted at 0°C with acidified saline adjusted to pH 2 with HCl. The mixture was centrifuged and the supernatant combined with several subsequent acidified saline washings of the cell walls. The protein solution was concentrated and dialyzed against tris buffer. 80% of the total γ-globulin present in the initial serum was successfully recovered by this procedure of absorption onto and elution from the cell walls. The eluted antibody was identified as γG-globulin by immunoelectrophoresis and the preparation was essentially devoid of other serum proteins. Microzone electrophoresis revealed that this antibody had a migration similar to the γ-globulin in the initial serum.

The specificity for the A-variant determinants of this nonprecipitating γ-globulin eluted from the Group A-variant cell walls is borne out by cell wall agglutination inhibition studies. The methods have been reported in detail elsewhere (12). Briefly, drops of twofold serial dilutions of antibody are added to the depressed wells of a glass plate. A drop of saline is added to each well of a series containing the antibody dilutions. The glass plate is gently rotated to mix the reactants. A drop of A-variant cell wall suspension is added to each well. The agglutination is recorded after 5 min. For the demonstration of haptenic inhibition, a drop of Group A-variant carbohydrate solution, 1 mg/ml of saline, is added instead of a drop of saline to each well of another series containing the antibody dilutions.

Presented in Table I are the results of A-variant cell wall agglutinations with decreasing concentrations of nonprecipitating antibody recovered from anti-

TABLE II

Occurrence of Electrophoretically Monodisperse Antibodies to Group Carbohydrates following Primary and Secondary Immunization of Rabbits with Streptococcal Vaccines

Vaccine	Primary immunization		Secondary immunization	
	No. rabbits	No. rabbits with monodisperse antibodies	No. rabbits	No. rabbits with monodisperse antibodies
Group A	36	0	13	3
Group A-variant	41	4	13	3
Group C	39	4	29	3

serum VIII by the method described above. As little as 6.25 μg/ml of the antibody caused brisk agglutination. Addition of 1 mg/ml of A-variant carbohydrate markedly inhibited this agglutination reaction. Such inhibition of agglutination is specific because no appreciable inhibition was achieved with Group A carbohydrate or Group C carbohydrate. Similar data have been obtained for 14 other Group A-variant sera which had high levels of γ-globulin but essentially no detectable precipitins. It is concluded that nearly all of the γ-globulin in Group A-variant antisera is antibody to the group carbohydrate, which may be either precipitable or nonprecipitable in character. In some sera, the predominant portion of the antibody is precipitable with soluble antigen, but in the majority little or no antibody is precipitable. It is not uncommon for sera to contain both precipitating and nonprecipitating antibody. Group A and Group C antisera may also possess nonprecipitating antibodies, and, indeed, several sera have antibody which is predominantly of this nature. Such an event is obviously less common than in the case of the Group A-variant antisera.

The picture which emerges from these immunization studies is that the bulk

of the total γ-globulin is antibody to the carbohydrate, whereas a far smaller proportion represents antibodies to all the other cellular components of the bacterial cell. This is not invariably the case, however. Certainly other antigens in the vaccine may give rise on occasion to significant levels of antibody. For example, an appreciable amount of the total γ-globulin in certain immunized rabbits was identified as antibody to the mucopeptide of the cell walls (14), and to the cytoplasmic polyglycerophosphate (15).

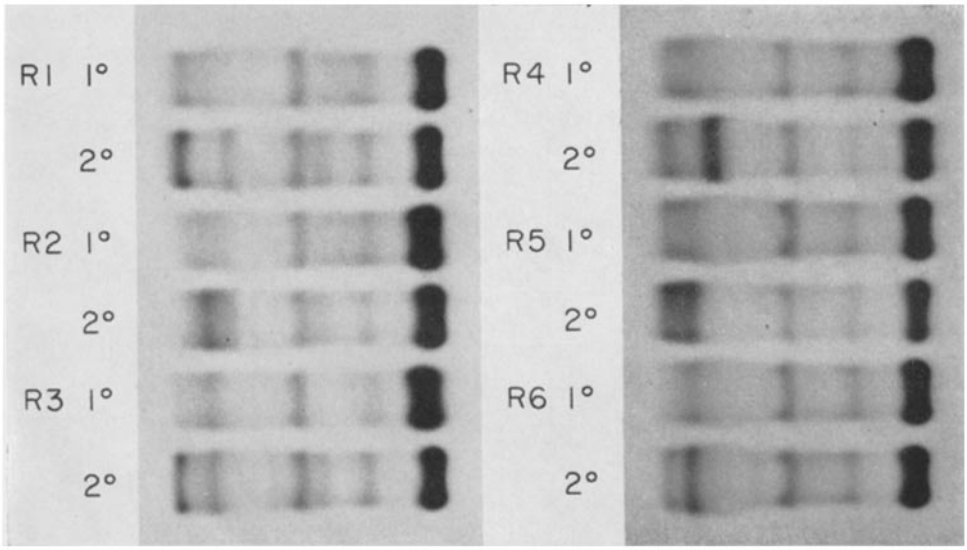

FIG. 7. Microzone electrophoretic patterns of paired antisera from six rabbits collected after primary and secondary immunization. In each instance secondary response sera contain one or two electrophoretically monodisperse components of γ-globulin which are not observed in the primary response sera. Rabbits R1, R2, and R3; immunized with Group A streptococcus. Rabbit R4; immunized with Group A-variant vaccine. Rabbit RS and R6; immunized with Group C vaccine.

1°, primary immunization antiserum; and 2°, secondary immunization antiserum

Occurrence of Uniform Antibody Populations following Primary and Secondary Immunization.—One of the unique features of the immune response to the streptococcal vaccines is the occurrence of a high concentration of electrophoretically monodisperse antibodies to the group-specific carbohydrates. It has been reported that rabbits which do not respond in this fashion after primary immunization may do so after secondary immunization (7). Previous estimates of the frequency of the occurrence of monodisperse antibodies after either primary or secondary immunization have necessarily been based on data from a limited number of rabbits. Presented in Table II is the number of rabbits

with monodisperse antibodies following primary and secondary immunization. The rabbits tabulated here are the same as those presented in Figs. 1, 2, and 3. Rabbits which exhibited monodisperse antibodies after primary immunization were not immunized a second time. Therefore, the rabbits which exhibit such a response after secondary immunization are in addition to those identified with monodisperse antibodies after primary immunization. Except in the case of rabbits immunized with Group C, monodisperse antibodies were observed in a larger proportion of the rabbits after secondary immunization than after primary immunization. Electrophoretic patterns of the primary and secondary antisera of representative rabbits which exhibit monodisperse antibodies after secondary immunization are illustrated in Fig. 7. In some instances the secondary response antisera possess one prominent monodisperse antibody component, whereas others have more than one. It has been feasible, however, to isolate a monodisperse component from antisera of either sort by preparative electrophoresis. Isolation of the precipitins from such a component is achieved by methods previously employed (4). For several selected examples, individual antigenic specificity has been demonstrated, and disc electrophoretic patterns of the light chains revealed one or two major bands.

Attention is especially directed to the electrophoretic patterns of secondary response antisera of rabbits R1 and R3 depicted in Fig. 7. It is to be noted that each antiserum has a distinct discreet antibody component which has a slow migration. Such slow components can be isolated by preparative electrophoresis with particular ease because there is little other γ-globulin with similar slow mobility in the antiserum.

Genetic Influence on the Primary Immune Response.—The data on the primary immune responses, tabulated in Figs. 1, 2, and 3, raises the possibility that the antibody response to group carbohydrate is under some form of genetic control. This is suggested by the disparity between those few animals with high responses and the majority with less remarkable antibody levels. In the following studies a comparison of the magnitude of the primary immune response has been made between the parental generation and the F_1 generation.

Although the subsequent genetic studies are based on an analysis of primary responses of parental and F_1 generation, under ideal circumstances, any effort to examine the effects of genetic control on the immune response should employ the responses to secondary immunization. It is always uncertain if the occasional exceptionally high response after first immunization, is, in fact, primary and not secondary. It is conceivable that in some cases there has been inadvertent prior exposure of the rabbit to the same or a cross-reactive bacterial antigen. Certainly the isolation of Group C streptococci from rabbits has been reported (16). It might be expected that natural infection with Group C streptococci would potentiate the antibody response to any subsequent immunization with vaccine. Although prior infection with streptococci of the rabbits

employed here is not known to have occurred, such an event would have escaped detection.

The examples of the marked differences between antibody concentrations after primary and secondary immunizations presented in Fig. 4 clearly indicate that the capacity to respond maximally is often not evident until the second immunization. Likewise without data on the capacity to respond to second immunization, there is no certainty that a rabbit is, in fact, a low responder. After having taken into account these reservations about basing a genetic analysis on primary immune responses, such data are presented here for selected breeding pairs of rabbits and their offspring because they do sug-

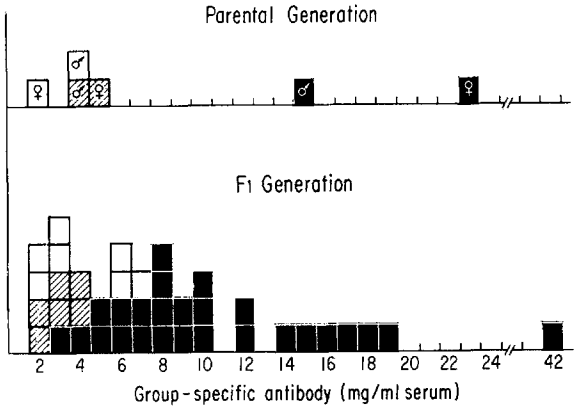

FIG. 8. Serum concentrations of Group C precipitins for three pairs of rabbits and their F_1 generations following primary immunization with Group C vaccine. Open squares, one low response pair and their offspring; shaded squares, another low response pair and their offspring; and black squares, high response pair and their offspring.

gest genetic control of the immune response. In a subsequent report, the analysis will be extended to secondary response data.

Presented in Fig. 8 are the concentrations of precipitins to Group C carbohydrate in the antisera of three breeding pairs of rabbits and their offspring following primary immunization with Group C streptococcal vaccine. There were two breeding pairs with low primary responses from which were derived 13 F_1 generation rabbits, and one breeding pair with high primary responses from which were derived 26 F_1 generation rabbits. It is obvious that the majority of the F_1 generation of the high response parents have higher antibody levels than the F_1 generation of the low response parents. Partial overlap in the antibody levels between the two groups of offspring, as shown in Fig. 8, is to be anticipated because these rabbits do not yet represent genetically established lines. Nevertheless, the data in Fig. 8 which show a similarity between parents and offspring are statistically significant with an χ^2 value of 15.91 and a P value

of < 0.001. Breeding studies are currently underway to backcross F_1 generation rabbits with parents and to inbreed the F_1 generation. Finally, other breeding pairs with high antibody responses have been identified so that their F_1 generation can be crossed with those reported here.

In addition to the achievement of high antibody responses to bacterial antigens through selective breeding, there may also be genetic control of the immune responses which exhibit electrophoretically monodisperse antibody.

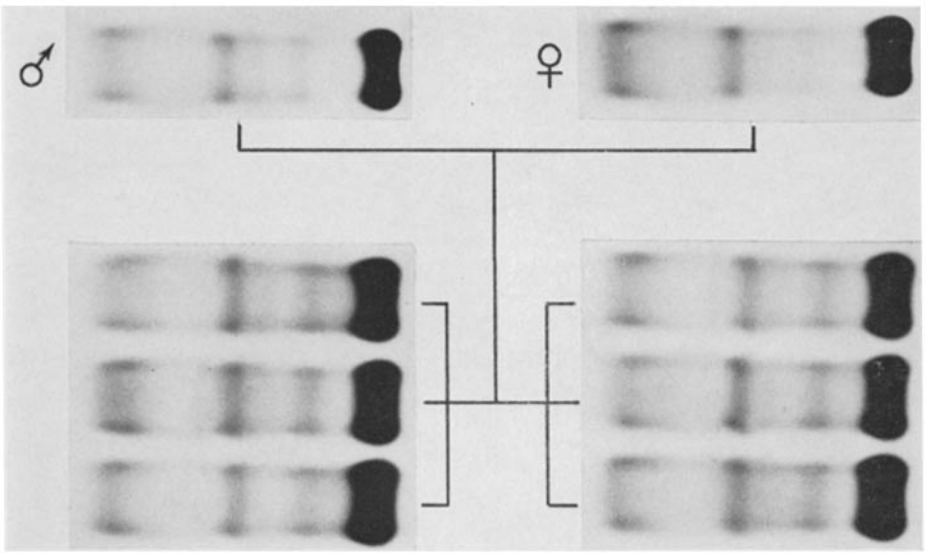

FIG. 9. Microzone electrophoretic patterns of primary immune response antisera from one of the low response pairs and their offspring depicted in Fig. 8.

This is illustrated in Figs. 9 and 10. Depicted here are electrophoretic patterns of primary response antisera for one of the low response breeding pairs and the high response breeding pair which were presented in Fig. 8. Electrophoretic patterns are also given for all six offspring of this low response pair and for the first two litters of the high response pair. A number of the antisera of the high response offspring clearly exhibit one or two monodisperse components in the γ-globulin region of the electrophoretic pattern. In these rabbits, these components are precipitins to streptococcal carbohydrates. Such monodisperse components are not observed, however, in the offspring of the low response parents. It is, therefore, conceivable that not only the magnitude of the immune response, but the electrophoretically monodisperse quality of the antibodies is under genetic control. Continuation of breeding studies currently underway should clarify this question.

DISCUSSION

Although rabbit antisera to streptococcal group carbohydrates have been prepared by immunization with a vaccine of whole streptococci for more than 40 years (17), there has been no systematic attempt to examine the variety of

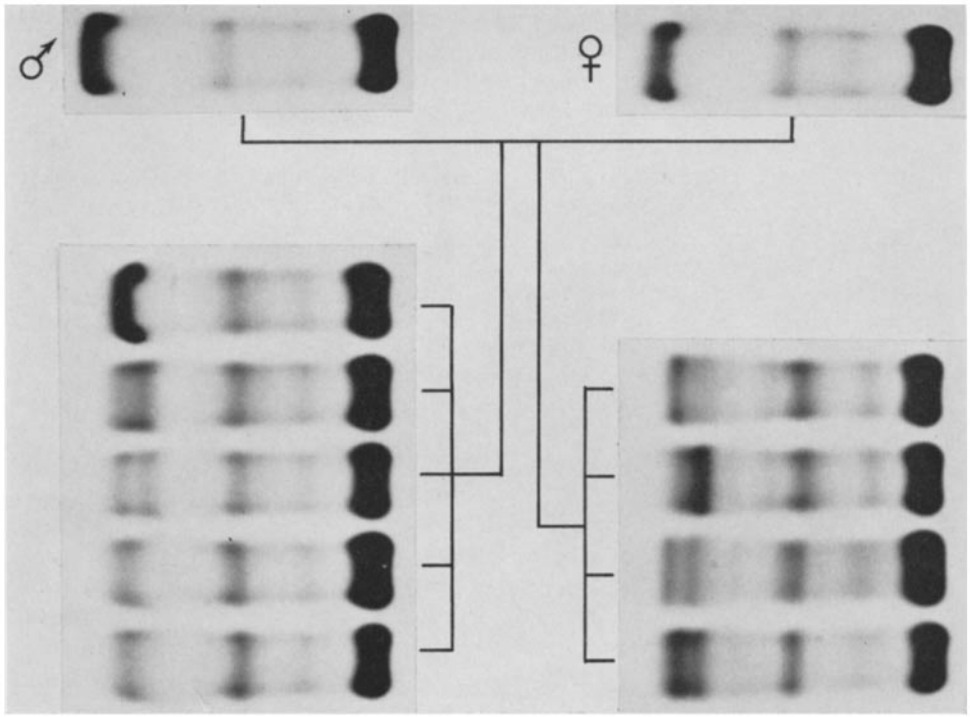

FIG. 10. Microzone electrophoretic patterns of primary immune response antisera from the high response pair and their first nine offspring depicted in Fig. 8. Note that most of the patterns reveal one or two electrophoretically monodisperse components, whereas the parents and offspring depicted in Fig. 9 do not exhibit this trait.

factors which influence the magnitude of the immune response. As long as the streptococcal grouping sera were primarily employed for the bacteriologic diagnosis of streptococci derived from clinical material and for immunochemical studies on the carbohydrate antigens, there was no major need to explore in detail the nature of the immune response in rabbits to these antigens. However, the unexpected discovery 3 yr ago that occasional rabbits produced high levels of exceptionally uniform antibodies to these carbohydrates has led to an assessment of the variable factors which may have an influence on immune responses of this sort (1). While there is no way to identify all of these variables, those

which might play a role include: the route of immunization; the physical state of the antigen; prior sensitization; and, the genetic background of the rabbit. In the studies reported here, prior exposure to the same antigen and the genetic background have been examined for their possible influence on the magnitude of the immune response and the occurrence of electrophoretically monodisperse antibodies.

There is little or no published information on the most effective composition of the vaccine for the production of potent streptococcal grouping antisera. Clearly the size of the particles in the vaccine may be important. Whole streptococci, with the carbohydrate as the outermost cell wall element, appear to stimulate a greater immune response than isolated cell walls which on a dry weight basis are $\frac{1}{5}$ the size of intact streptococci (M. McCarty, personal communication). Furthermore, the purified soluble carbohydrate, which has a molecular weight between 8000 and 10,000, is not antigenic in rabbits (R. C. Lancefield, personal communication).

The possible advantages and disadvantages in alternate routes or methods of immunization have not been reexamined in detail here for the streptococcal bacterial vaccines. The impressions acquired early on by Dr. Rebecca Lancefield were that alternate routes and the use of adjuvants were no more effective than intravenous immunization with vaccines composed of whole heat-killed streptococci. The studies of Humphrey and collaborators on intravenous immunization of rabbits with pneumococcal vaccines perhaps bear on this point. At the height of the immune response to the capsular antigen, the animals were killed and the organs removed to determine their in vitro capacity to produce antibody. By using an assay based on the incorporation of ^{14}C-labeled amino acids into γ-globulin, intravenous immunization led to antibody production primarily in bone marrow and lungs and in some cases predominantly in the lung itself (18–20).

In a previous report it was observed that rabbits which did not respond with a uniform population of antibodies after primary immunization might do so after secondary immunization (7). The predictable occurrence of such an event has now been observed in a far larger number of rabbits. While the explanation for the phenomenon is obscure, it is conceivable that intensive immunization over a prolonged period selects a restricted population of cells which undergoes proliferation. In this connection there is an intriguing parallel between these uniform populations of antibodies in rabbits after secondary immunization and the development of a myeloma-like condition in mink with Aleutian disease. It was observed that late in the course of Aleutian disease, some mink showed a transition from a heterogeneous hyper-γ-globulinemia to a homogeneous myeloma-like hyper-γ-globulinemia. Such a finding suggests the ascendency of a few predominant clones of plasma cells (21).

It remains to be clarified with certainty why some rabbits have a high im-

mune response following intravenous immunization with streptococcal vaccines, whereas others respond poorly. The data reported here on the primary immune responses of parents and offspring are consistent with the notion that the magnitude of the immune response is under some form of genetic control.

No attempt will be made here to give a complete review of the literature on the influence of genetic factors on the immune response. A number of recent studies with mice, rats, and guinea pigs have demonstrated beyond doubt that the capacity to respond to specific antigenic determinants is genetically controlled (22–25). Pinchuck and Maurer have shown that the ability of mice to form antibodies against the random terpolymer $glu_{57}lys_{38}ala_5$ is controlled by a codominant Mendelian factor (22). In guinea pigs it has been shown that the ability of poly-L-lysine to act as a haptenic carrier for the 2–4 dinitrophenyl group is transmitted as a simple Mendelian determinant (25). Humphrey noted a marked quantitative difference between the responses of Sandylop and Himalyean rabbits to immunization with branched multichained synthetic polypeptides (26). In an extension of these studies, McDevitt and Sela observed that CBA and C57 inbred mouse strains differed markedly in their response to synthetic polymers (23). Immunization of these mice with branched multichain-L-polypeptide, poly-(Tyr-Glu) poly-DL-ala-poly-L-lysine, resulted in a tenfold difference in the antibody produced by the two strains. The immune response of the F_1 generation confirmed the genetic transmission of the capacity to respond to this polymer. What is lacking thus far is compelling evidence of a similar sort which demonstrates genetic transmission of this kind in the rabbit.

There is some evidence that different strains of rabbits which were inbred for several generations exhibited wide variability in the antibody response to $glu_{56}lys_{38}tyr_6$ (27). In earlier studies Sang and Sobey noted wide variability in the immune response to tobacco mosaic virus, a finding consistent with some kind of genetic control (28). In none of these studies with rabbits, however, was there selective breeding of high response parents and the examination of their offspring for the immune response to the same antigen. It is conceivable that clearly defined genetic lines of rabbits with known blood group systems will be applicable to studies on the immune response to the bacterial antigens (29).

In many ways the magnitude of the immune response is not the most interesting biological question which is raised by these studies on the antibodies to streptococcal carbohydrates. What is more intriguing is the explanation for the occurrence of high concentrations of antibodies which have an unusual degree of homogeneity. This may be due, in part, to the uniform nature of the antigenic stimulus.

There have been several attempts to impose constraints on the diversity of the antibody population by devising haptens which have a reduction in the degree of chemical and structural heterogeneity. Success in this approach has been reported by Singer (30) with an antigen consisting of one DNPL haptenic group at the single SH group in the active site of papain, and by Haber et al. (31) with a synthetic polypeptide

of defined sequence carrying the DNP determinants at regular intervals. Unfortunately, the amount of antibody which is produced in response to these antigens has been low.

It is likely that the uniform quality of the antibodies observed in response to streptococcal carbohydrates, as well as to other carbohydrate substances, is due in part to the fact that they possess fewer immunodominant sites than do proteins. In this connection, it has been observed that human antibodies to several carbohydrates, including teichoic acid, dextran, and blood group substances, had several of the uniform characteristics which are typical of the myeloma protein (5, 6). Electrophoretic mobility of light chains, and the subgroup composition and genetic markers all point to a relative homogeneity of these and other human antibodies (32–34). One unusually homogeneous antibody to levan consisted exclusively of γG2-heavy chains and κ light chains (34). Recently, Pappenheimer et al. (35) have shown that rabbit antibodies to Type VIII pneumococcal polysaccharide have homogeneous binding affinity at all ligand concentrations. The conclusion which has been drawn from all of these observations of others, as well as the work reported formerly from this laboratory, is that carbohydrate antigens, and particularly bacterial carbohydrate antigens, may generate a population of antibodies with relatively uniform characteristics.

In addition to the influence of the antigenic stimulus on the occurrence of uniform antibodies, it is also possible that such an event is under some form of genetic control. Breeding studies are currently underway to answer this question. Whatever the mechanism which accounts for the occurrence in rabbits of hyper–γ-globulinemia with monoclonal characteristics, the transient nature of this immune response in rabbits stands in contrast to the persistence of monoclonal hyper–γ-globulinemias in man. Waldenström has stressed the persistent nature of these alterations despite the frequent absence of overt evidence for neoplasia (36). There is some evidence, however, that control mechanisms for the maintenance of these monoclonal proteins are genetically determined. It has been reported, for example, that immunoglobulins of a monoclonal nature occurred with greater frequency among the kindreds of propositi with monoclonal hyperglobulinemia than was expected from an analysis of kindreds of control propositi (37). Certainly families have been identified in which a large variety of γ-globulin disturbances occur in different members (38, 39). Except for the possibility that in a few instances the monoclonal component was a Waldenström macroglobulin with autoantibody activity (40, 41), there is no assurance that monoclonal globulins represent an immune response to a specific antigenic stimulus. From this point of view, these observations in man are not analogous to those in rabbits where there is an occurrence of specific antibodies which possess uniform characteristics. Nevertheless, it is conceivable that in these rabbits, as well as in the families cited above, there is an inheritance of control mechanisms which regulate the occurrence of a hyper–γ-globulinemia which in certain individuals is of a monoclonal type.

SUMMARY

In a search for possible genetic factors which may influence the immune response to the streptococcal carbohydrates, over 100 rabbits have been immunized with streptococcal vaccines, and representative examples of high and low response pairs mated. The concentration of precipitins to the group-specific carbohydrates has been measured in the antisera following primary intravenous immunization with heat-killed streptococcal vaccines, Group A, Group A-variant, and Group C. For the majority of rabbits, the concentration of precipitins varied between 1 and 10 mg/ml of antiserum; while in the minority, it was between 11 and 32 mg/ml. The offspring of rabbits with high antibody levels had a significantly higher concentration of antibody than was seen in the offspring of rabbits of low response parents. Such data suggest that the magnitude of the immune response to these carbohydrate antigens is under some form of genetic control.

Not uncommonly in rabbits with hyper-γ-globulinemia following primary immunization, the group-specific precipitins are the predominant component of the γ-globulin. An unusual feature of such components is that they are electrophoretically monodisperse, and possess individual antigenic specificity. In this respect they resemble the myeloma proteins. When a response of this sort is not seen after primary immunization, it may occur after secondary immunization. Therefore, prior exposure to the same or closely related antigen may also have an influence on the occurrence of high concentrations of such uniform antibodies.

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