THE IMMUNOLOGICAL SPECIFICITY OF STAPHYLOCOCCI

III. Interrelationships of Cell Constituents*

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That staphylococci may be classified into at least two immunological types has been reported in a previous communication (1). The separation into distinct types is determined by the elaboration of a specific carbohydrate which for strains derived from pathogenic conditions is chemically different from that produced by organisms isolated from saprophytic sources (2). While, therefore, precipitation of the polysaccharide extracted from different cultures exhibits a striking type specificity, agglutination of the bacteria, themselves, on the other hand, reveals a much broader or species specific reactivity. It was consequently suspected that the species specificity observed in the agglutination reaction might be governed by a common reactive protein constituent of the cell irrespective of type distinction. Since a similar condition has already been demonstrated in the case of Pneumococcus (3), and later Friedländer's bacillus (4) and Streptococcus (5), this possibility seemed sufficiently plausible to merit investigation. Accordingly a study has been undertaken of the interrelationships of the protein and carbohydrate derivatives of Staphylococcus, and the results of this study are reported at the present time.

Methods

The Specific Carbohydrates.—Purified specific carbohydrates were prepared by the method described in a preceding communication (2). The technique of precipitation has also been outlined (1).

The Protein Solutions.—Protein solutions of representative strains of Type A and Type B Staphylococcus were prepared from young broth cultures (16 to 20 hours). The sedimented bacteria from 6 to 8 liters of culture were spread with a

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spatula in a thin layer in a Petri dish. The organisms were then placed in the incubator (37°C.) for 30 to 60 minutes to allow more or less thorough drying. After desiccation, the organisms formed a somewhat sticky, gummy mass which made grinding difficult and lengthy so that the expedient of washing the dried bacteria first with alcohol and then with ether was resorted to, apparently without affecting the antigenicity or reactivity of the protein. The extracted and dried bacteria were then ground in a special grinding apparatus devised for the purpose in this laboratory (6). Gram stains of the bacterial mass were made from time to time to ascertain the degree of disintegration which, as a rule, was complete within 6 hours or less. The finely ground powder was then taken up in N/100 NaOH to effect solution of the protein. After centrifugation, the protein was precipitated with a minimum amount of normal acetic acid. The precipitate was collected and redissolved in alkali. Acid precipitation and solution with alkali were usually repeated two or three times and the final product was made up in saline made slightly alkaline to litmus. Solutions used for immunization were filtered through a Berkefeld V filter.

The method of immunization employed was that described in an earlier report (1).

Properties of the Specific Carbohydrate

Antigenic Properties.—That chemically purified carbohydrates derived from bacteria exert no antigenic effect was originally believed by a number of investigators. More recently, scattered reports (7) indicated, however, that bacterial carbohydrates particularly in the case of Pneumococcus Type I, may serve as antigens under conditions which were not clearly understood. The confusion into which the subject had been thrown was eventually clarified by the observations of Avery and Goebel (8), who showed that in the case of Pneumococcus Type I at least, the polysaccharide exists as an acetylated carbohydrate which is antigenic, but that in the process of purification usually employed deactylation occurs with a concomitant loss of antigenicity.

A study of the antigenicity of the polysaccharides of Staphylococcus indicated that in the condition originally used, they possessed no antigenic properties if the failure to stimulate agglutinins or precipitins in rabbits be accepted as a measure of antigenicity. Further attempts to determine whether the carbohydrates are true antigens were made by acetylating the polysaccharides. But this artificial alteration of the carbohydrates did not modify the original lack of antigenicity. It should be pointed out that even in the case of Pneumococcus the acetyl polysaccharide does not elicit antibody formation in rabbits.

The antigenicity mentioned above refers only to the ability to stimulate in white mice active immunity to infection.

Since the ability of the whole organism to induce type specific precipitins in rabbits is not great (1), an effort was made to ameliorate the conditions necessary for antibody formation. In one experiment, the intact bacteria were acetylated and then injected repeatedly intravenously but without inciting specific precipitin improvement in any of the rabbits studied. Adsorption of the soluble specific substance on collodion particles as described by Zozaya (9) was next investigated. By this method, also, the specific carbohydrates of Staphylococcus revealed no measurable antigenic effect.

Serum Reactive Properties.—That the soluble specific substances of Staphylococcus react to high titre in homologous immune serum, has already been reported (1). It is therefore obvious that the specific polysaccharides are true haptenes as originally defined by Landsteiner (10).

Skin Reactive Properties.—Since the study on the skin reactive properties of the carbohydrates of Staphylococcus is still under way, it is not desirable to make a complete report of cutaneous reactions at the present time. It will suffice, therefore, merely to point out that the polysaccharides may elicit skin reactions in patients recovering from Staphylococcus infection. As small quantities as 0.2 cc. of a 1:200,000 dilution may be sufficient to cause a type specific, wheal and erythema reaction. Thus far all the reactions have been to Type A carbohydrate only. Since, however, Type A strains alone appear to be pathogenic (1), it is to be expected on the basis of type specificity that Type B reactions will occur rarely, if at all. Experiments conducted in this laboratory (11) on the induction of skin reactivity in rabbits and monkeys indicate that skin reactions to the carbohydrates are elicited only rarely in these animals, but when they do occur they present the exquisite specificity usually observed in carbohydrate reactions. Thus, skin reactions to the polysaccharides may be observed under experimental conditions as well as during spontaneous infection in man.

Properties of the Protein Constituent

Antigenic Properties.—In contrast to the lack of antigenicity witnessed in the specific polysaccharides, the protein constituent of

Staphylococcus evokes an antibody response in rabbits. Antibodies are stimulated readily following intravenous or intracutaneous injections of the protein. The antibodies are demonstrable by precipitation. By the precipitation test, it may be observed that precipitins for the specific carbohydrates are not present in antiprotein sera. The precipitins for the protein, however, are present in relatively high titre. An examination of the data presented in Table I reveals in this connection that antiprotein sera precipitate protein derived not only from homologous strains, but protein derived from strains of heterologous type. In other words, unlike the carbohydrates, the protein constituent of Staphylococcus is an antigen devoid of type

TABLE I

Precipitation of Staphylococcus Protein in Antiprotein Sera

Antiprotein serum	Protein*	Dilution of protein					
		1:2400	4800	9600	19,200	38,400	76,800
B_2A (Type A)	13 (Type A)	+++	+++±	++	+	±	−
	Mx3 (" B)	++++	+++	+++	++	+	−
Mx3 (Type B)	13 (" A)	+++	++	++	+	−	−
	Mx3 (" B)	+++	+++	++	+	±	−

* Staphylococcus protein does not precipitate in normal rabbit serum.

specificity and it is shared in common by all staphylococci irrespective of type distinction.

Serum Reactive Properties.—It has already been made obvious that the proteins derived from Staphylococcus as indicated react in antiprotein sera. That precipitation of the proteins occurs with equal readiness in antibacterial sera is brought out by the data given in Table II. It will be seen that proteins prepared from four different strains (two, Type A, and two, Type B) precipitate in both Type A and Type B antibacterial sera. While minor variations in titre are apparent, nevertheless the species specificity of the proteins is definitely manifested. The higher titre of precipitation in homologous immune sera may be explained on the basis that the proteins were not chemically pure and that they actually contained a certain amount of soluble specific substance which was also precipitated in the type

specific sera. This, then, may account for the apparently greater activity of the protein in homologous antisera.

Skin Reactive Properties.—Without attempting to make a final report now of the skin reactive properties of Staphylococcus protein, it is nevertheless desirable to indicate the contrast in skin reactivity of the protein and carbohydrate. As the serum reactivity suggests, the skin reactivity of the protein is also species specific. In addition, the skin reaction is not the immediate, wheal and erythema variety elicited by the carbohydrate, but the delayed inflammatory reaction

TABLE II

Precipitation of Staphylococcus Protein in Antibacterial Rabbit Sera

Protein from strain	Serum	Dilution of protein*					
		1:10	20	40	80	160	320
13, Type A	Normal	−	−	−	−	−	−
	13, Type A	++++†	+++	++±	++	+	−
	B_2A " "	++++	+++	++	+	−	−
	Mx3 " B	++	++	+	−	−	−
	148 " "	+++	++	+	±	−	−
Mx3, Type B	Normal	−	−	−	−	−	−
	13, Type A	+++	++	+	−	−	−
	B_2A " "	++±	++	+	±	−	−
	Mx3 " B	+++†	+++	++	+	±	−
	148 " "	++++	++±	+	±	−	−

* The solutions of protein were used as made, without standardization, so that the dilutions are not necessarily comparable.

† Reactions in homologous sera were mixed precipitations of protein and carbohydrates.

frequently described as tuberculin-like. Experimentally, rabbits have been made regularly skin reactive to the protein of Staphylococcus, and it was observed that the reactions in these animals were species specific and delayed (11). The reactions were in every way similar to those occurring in man.

The skin reactive properties of both carbohydrates and proteins are still being studied in both normal individuals and in patients with Staphylococcus infection. The detailed experiments and statistical data, therefore, will necessarily await future work. The indications

are nevertheless definite on the nature of the specificity and the character of the reactions elicited by the two constituents. This will be recognized as paralleling the observations on the skin reactivity of the carbohydrates and nucleoprotein of Pneumococcus (12), which demonstrated for the first time the principles underlying skin reactions to these bacterial derivatives.

It may be of interest to report an additional experiment on the toxicity of the two products of Staphylococcus. Since toxigenic strains apparently fall in Type A, it was decided to determine the toxicity of the carbohydrates of each type. The results were definite in showing that neither Type A nor B carbohydrate is toxic for normal rabbits or white mice. Large quantities are tolerated with no evident effect even in the case of the Type A polysaccharide which was isolated from a known toxigenic strain. It may be concluded, therefore, that while the Type A carbohydrate may be present in toxigenic strains, it is not itself toxic. Similar experiments with the protein also indicate that this constituent possesses no toxic properties as determined in normal animals.

DISCUSSION

That the soluble specific substance of Staphylococcus determines the type specificity of the bacterial cell from which it is derived is a fact which has been confirmed by experiments described in this paper. In the form in which it exists in the cell, the specific carbohydrate is a poor antigen (1) as judged by its ability to stimulate specific antibody response in rabbits. When isolated in a state of high chemical purity, the soluble specific substances lose what weak antigenic properties they originally possess. Moreover, acetylation or adsorption of the polysaccharide on collodion particles does not affect measurably the lack of antigenicity. While the carbohydrate is highly reactive in sera, it precipitates only in antibacterial sera. In the skin of patients infected with Staphylococcus, the carbohydrate elicits an immediate wheal and erythema reaction which is specific to type.

The proteins, separated from the bacteria as described above, are species specific; that is, they are common to both types of Staphylococcus. They are antigenic in rabbits and they induce the formation of precipitins which react with protein solutions obtained from either

type. The proteins react both in antiprotein and antibacterial sera. In addition, they exhibit skin reactive properties which evoke the delayed, inflammatory reaction specific of the species rather than the type. That the protein of Staphylococcus is endowed with even a broader reactivity than that indicated in this report was pointed out by Lancefield (13) when she showed them to possess serological reactivities in common with Streptococcus and Pneumococcus.

CONCLUSIONS

1. The carbohydrates derived from Staphylococcus are type specific.
2. The specific carbohydrates fail to induce formation of antibodies in rabbits.
3. Acetylation or adsorption of the carbohydrates on collodion particles does not render them antigenic.
4. The specific carbohydrates may be employed to elicit immediate, type specific, skin reactions in patients with Staphylococcus infection.
5. The protein of Staphylococcus is species specific.
6. The protein is antigenic and stimulates in rabbits species specific antibodies.
7. The protein causes in hypersensitive individuals a species specific, delayed, inflammatory skin reaction.

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