# IMMUNOLOGICAL RELATIONSHIPS OF CELL CONSTITUENTS OF PNEUMOCOCCUS.

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In the preceding paper (1) observations on the nature of the soluble specific substance of pneumococcus have been recorded. The present work concerns itself with the facts thus far ascertained in a comparative study of the chemical and immunological reactions of the protein[1] of pneumococcus. It seemed of interest to study the immunological relationship of the bacterial protein to the soluble specific substance of pneumococcus, and in the present paper certain differences in the serological specificity of the two classes of substances are brought out and related to their chemical nature. It would be beyond the scope of the present paper to attempt a review of the extensive literature on the subject of bacterial nucleoproteins in general, and this has already been done in reference books (2).

This work is presented, despite its incompleteness, because it points the way to a comparative study of the immunochemical relations existing between two different cellular constituents of the same organism. The first of these components, the so called soluble specific substance, has been discussed in the preceding paper; it need only be pointed out here that this reactive substance possesses none of the chemical properties of protein, that although antigenically it appears capable of stimulating little or no antibody response, serologically it exhibits to an extraordinary degree the reactions of type specificity in antipneumococcus sera. In other words, this non-protein constituent in isolated form is relatively and perhaps

[1] The word protein as used in this paper refers only to that portion of the dissolved pneumococcus cell precipitable in the cold by acetic acid and consisting mainly of nucleoprotein and mucoid.

absolutely inert as antigen, although it is highly reactive in the antibacterial serum of the homologous type of pneumococcus. On the other hand, by the methods described it is possible to separate from the bacterial cell another substance which is protein in nature and is also distinctive in its serological behavior from the soluble specific substance previously discussed.

Although the investigation of the relationship of chemical constitution to biologic specificity of pneumococcus is still in progress, the results thus far obtained are sufficiently interesting and perhaps significant enough to justify the presentation of facts already available.

## EXPERIMENTAL.

*Method.*—The bacteria from actively growing broth cultures of pneumococci are recovered by centrifugation. The amount of material that can conveniently be worked up at any one time is, therefore, dependent on facilities for rapid centrifuging. In the present study broth cultures in units of from 6 to 12 liters have been treated in the following manner. The bacterial sediment, after removal of the culture fluid, is resuspended in about one-tenth volume of 0.85 per cent salt solution. To this suspension is added the minimum amount of bile necessary to effect solution of the bacterial cells. This procedure is carried out at ice box temperature (4°C.) in order to inhibit the possible proteolytic action of the intracellular enzymes which are released by lysis of the cells. Undissolved material is removed from the bile solution by centrifugation; the clear supernatant fluid containing the dissolved constituents of the cells is then treated by the method ordinarily employed for the isolation of the so called nucleoproteins from alkaline extracts of bacteria. The protein is precipitated from solution by the addition of dilute acetic acid (10 per cent). The acid is added slowly and the mixture carefully shaken; flocculation occurs promptly and is complete at a reaction faintly acid to litmus.

The precipitate obtained in this manner is separated by centrifugation and thoroughly washed two or three times in equal volumes of distilled water. The washed precipitate is redissolved in water by adding 0.1 N NaOH until the solution is faintly alkaline to litmus. This process of precipitation and washing is repeated at least three times. Before the last precipitation, the solution is passed through a Berkefeld filter V. The final precipitate is washed rapidly twice with acetone and once with dry ether and dried in a vacuum desiccator. The preparation obtained in this manner and referred to in the text as "protein" is a whitish, dry powder, readily soluble in faintly alkaline solutions.

Solutions of this substance give the usual qualitative color reactions characteristic of proteins of this nature: positive biuret, Hopkins-

Cole, Millon, xanthoproteic, and Molisch reactions. The hydrolyzed protein gives the purine reaction with Fehling's solution. The nitrogen content of a representative specimen of the protein was 16.0 per cent, the phosphorus content 0.5 per cent.

TABLE I.

*Precipitin Reactions of Pneumococcus Protein (Type II) and Soluble Specific Substance (Type II).*

| Protein from Pneumococcus Type II. | Antipneumococcus serum. | | | Antityphoid Serum "T — A — B." | Normal horse serum. |
|---|---|---|---|---|---|
| | Type I. | Type II. | Type III. | | |
| Lot 4, 1:1,000 | ++ | ++ | ++ | — | — |
| " 5, 1:1,000 | ++± | ++ | ++ | — | — |
| Soluble specific substance of Pneumococcus Type II, 1:50,000. | — | ++++ | — | — | — |

± indicates faint cloud; +, cloud; ++, marked cloud; +++, marked cloud with precipitation; and ++++, heavy precipitation, supernatant fluid clear.

TABLE II.

*Precipitin Reactions of Pneumococcus Protein (Type II).*

| Protein from Pneumococcus Type II. | Antipneumococcus serum. | | | Normal horse serum. |
|---|---|---|---|---|
| | Type I. | Type II. | Type III. | |
| Lot 7, 1:200 | ++ | ++ | ++ | — |
| 1:400 | ++ | ++ | ++ | — |
| 1:800 | ++± | ++ | ++ | — |
| 1:1,600 | ++± | + | + | — |
| 1:3,200 | ++ | + | + | — |
| 1:6,400 | + | ± | ± | — |

## *Precipitin Reactions of Protein from Pneumococcus Type II in Antipneumococcus Sera Types I, II, and III.*

Clear solutions of protein derived from Pneumococcus Type II were prepared by grinding weighed amounts of dry preparation with water, adding dropwise the minimum amount of 0.1 N sodium hydroxide to effect solution, and diluting to volume with salt solution. The antipneumococcus and antityphoid sera used were diag-

nostic sera prepared by immunization of horses with the respective bacteria. The reactions were carried out by adding increasing dilutions of protein solution in amounts of 0.5 cc. to an equal volume of serum so diluted that each 0.5 cc. contained 0.2 cc. of the original serum. All tests were incubated in the water bath at 37°C. and readings made at the end of the incubation period and after 18 hours at ice box temperature.

From Tables I and II it appears that solutions of protein prepared from one type of pneumococcus (Type II) react in about equal degree with all three types of antipneumococcus sera, and not with antityphoid or normal horse serum. The preparations of protein derived from Pneumococcus Type II by the method employed in the present investigation, therefore, seem to be species-specific rather than type-specific. This fact, if confirmed by subsequent investigation of the protein of pneumococci of other types, would indicate on the basis of specific precipitin reactions that all pneumococci possess in part a common basal specific protein. It must be borne in mind, however, that the immune sera in these tests were not prepared by immunization with the isolated protein of the cell but by inoculation of horses with the intact bacteria. Immune sera are now being prepared from animals injected with purified protein as free as possible from other cell constituents such as the non-protein soluble substance. Until this is completed, it would, of course, be premature to attempt any interpretation of the significance of these reactions in terms of biologic specificity.

## DISCUSSION.

It seems justifiable, then, on the grounds of the data presented in this and the preceding paper to conclude that the pneumococcus cell possesses at least two distinct substances which are intimately concerned with the biologic specificity of this organism. As a corollary of this, it would appear that pneumococcus immunity is related to two entirely distinct bacterial substances. One of these cellular constituents is the so called soluble specific substance, which as already pointed out is non-protein in nature and in its present state of purity is either carbohydrate or intimately associated with car-

bohydrate. This substance is chemically analogous to the bacterial gum which other investigators have isolated from various pathogenic and non-pathogenic encapsulated bacteria (3). However, previous studies have not related the chemical constitution of these polysaccharides to the serological or antigenic specificity of the organisms from which they were derived. The polysaccharide of pneumococcus, whether capsular substance or not, either is serologically reactive itself or is closely associated with some other substance which confers upon the organism the dominant character of type specificity. The protein fraction of the bacterial cell, on the other hand, is not type-specific but reacts in antipneumococcus serum regardless of type derivation. It is therefore species-specific, not type-specific.

The antigenic properties of these two substances; the relation of changes in specificity of pneumococcus to changes in virulence; and the relation of these substances to phenomena of infection and immunity are now being investigated.

## SUMMARY.

1. The protein precipitated by acetic acid from solutions of pneumococci shows chemical reactions characteristic of nucleoprotein and mucoid.

2. The protein of pneumococcus, as contrasted with the non-protein soluble specific substance, exhibits species specificity rather than the type specificity characteristic of the latter.

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