STUDIES ON IMMUNOLOGICAL RELATIONSHIPS AMONG THE PNEUMOCOCCI.

I. A Virulent Strain of Pneumococcus Which Is Immunologically Related to, but Not Identical with Typical Strains of Type III Pneumococci.

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INTRODUCTION.

The present paper deals with a strain of Pneumococcus which is related to, but not identical with typical strains of the Type III group. The general interest in strains of bacteria that possess a specific immunological relationship to one of the "fixed" types (1) of any important pathogenic group acquires special interest in the case of pneumococci in view of the fact that the specificity of Pneumococcus Types I, II and III furnishes the most clear-cut example in all bacteriology of the dependence of exquisite biological specificity upon the chemical constitution of the bacteria themselves (2).

In order to avoid any confusion that might arise from either the term "subgroup" or the term "atypical," the terms "Thomas strain" and "anti-Thomas serum" are used throughout the present paper to designate the "non-typical" strain and its antiserum.

It is important to point out at the beginning, that the Thomas strain is virulent, killing mice within 36 hours in doses of 1×10^{-8} cc. of plain broth culture. As shown by Tillett (3), the type-specific agglutination of the "S" forms of Type III pneumococci involves an anti-S antibody in contrast to the anti-P antibody

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which agglutinates the "R" or degraded forms of all types of pneumococci. The characteristics which distinguish the Thomas strain from the typical Type III pneumococcus are recognized by S-anti-S reactions, and have nothing to do with those which distinguish the "S" forms (S-producing) of pneumococci from the avirulent, degraded or "R" forms (non-S-producing). Although not described in detail, the controls included in all experiments were sufficient to eliminate any complications arising from failure to differentiate the anti-S reactions from anti-P reactions. The high degree of virulence of the strain, the characteristic anti-S character of the agglutination of the bacteria and of the precipitation of young broth culture filtrates by the homologous antiserum, as well as actual tests of the non-type-specific protein-precipitating (anti-P) capacity of the antiserum, are in themselves convincing evidence that the Thomas strain is not a "degraded" form of typical Type III pneumococcus.

The Thomas strain exhibits no immunological relationship to Type I or II pneumococci with the exception of the species-specific P-anti-P relationship which is common to all pneumococci: the Thomas pneumococci are not agglutinated by Type I or II antiserum nor do these antisera confer passive protection; anti-Thomas immune serum is likewise non-reactive against Types I and II pneumococci; similarly, absorption of Type I or II antiserum with Thomas bacteria and absorption of anti-Thomas serum with Type I or II bacteria have no effect upon the type-specific antibodies.

EXPERIMENTAL

Methods.

In this investigation, five different methods have been used in testing the immune sera for the presence of the antibody specifically reactive with Type III pneumococci. These methods can be arranged in the following order of delicacy: (1) specific, passive protection of mice against virulent Type III bacteria; (2) agglutination of concentrated suspensions of heated Type III bacterial cells in salt solution; (3) agglutination of heated broth culture; (4) agglutination of unheated broth culture; (5) precipitation of solutions of the Type III specific S substance. The different procedures simply represent different methods of detecting the same anti-S antibody, and the positive results obtained when an immune serum is tested by one method, and the negative results obtained when the same serum is tested by another method, are due to differences in delicacy of the method of test.

The Relationship of the Thomas Strain to Typical Type III Strain of Pneumococci as Evidenced in Tests with Potent Anti-Type III Serum and Potent Anti-Thomas Serum.

The comparison in Table I of the immunological properties of the Thomas pneumococci with those of typical Type III pneumococci, is

TABLE I.

The Relationship of the Thomas Strain to Typical Type III Pneumococci as Evidenced in Tests with Potent Anti-Type III Horse Serum and Potent Anti-Thomas Rabbit Serum.

Dilution of serum	1. Agglutination of broth culture				2. Precipitation of S substance contained in filtrate of young broth culture				3. Type-specific passive protection				
	Typical Type III strain		Thomas strain		Typical Type III strain		Thomas strain		Dose of culture	Typical Type III strain		Thomas strain	
	Type III serum	Anti-Thomas serum	Type III serum	Anti-Thomas serum	Type III serum	Anti-Thomas serum	Type III serum	Anti-Thomas serum		Type III serum	Anti-Thomas serum	Type III serum	Anti-Thomas serum
									cc.				
Undiluted	+++	+++	+++	+++	+++	+++	+++	+++	0.01	D	D	D	S
1/5	+++	++	+++	+++	+++	+	±	+++	0.001	D-S	D-S	D-S	S
1/20	++	0	0	+++	+++	0	0	+++	0.0001	S	S	S	S
1/40	±	0	0	+++									
1/320	0	0	0	++									
1/640	0	0	0	±									

0 = indistinguishable from controls; ± = slight granulation; + = granular sediment with definite particles when thoroughly shaken; ++ = bacteria sedimented in disc form but supernatant not entirely clear and disc easily broken when shaken; +++ = compact disc with supernatant entirely cleared.

D = mice died within 24 to 72 hours in all tests; D-S = protection irregular, mice surviving in some experiments and dying in others; S = mice survived in all tests. Virulence controls (without serum or with heterologous serum) dying within 72 hours when injected with 1×10^{-8} cc. of culture.

based upon tests made with the most potent anti-Thomas and the most potent anti-Type III serum which we have obtained. While there are wide variations in the individual potencies of the anti-Thomas and anti-Type III serum obtained from different animals, it is desirable to base the first comparison upon the immunological properties exhibited in immune sera containing the complete expression of the antigenic properties of the two related but different kinds of pneumococci.

As shown in Table I, the Thomas strain of pneumococci reacts with anti-Type III serum, although its agglutination, filtrate precipitation and passive protection are not as pronounced as are the corresponding reactions of typical Type III pneumococci in anti-Type III serum. Thus, in respect to the reactions in potent samples of Type III immune serum the Thomas strain appears to be related to typical Type III pneumococci in about the same way as Avery's (1) Subgroup *IIa* strains are related to typical Type II pneumococci.

However, the comparison of the two strains in the anti-Thomas serum gives a more clear-cut differentiation between the Thomas and the typical Type III strains. In the first place, the anti-Thomas serum reacts not only with the Thomas pneumococci but also with typical strains of Type III pneumococci. In the second place, the potency of the anti-Thomas serum against the Thomas bacteria is much greater than the potency of anti-Type III serum against Type III pneumococci themselves; the invariable protection of mice against 0.01 cc. of broth culture (1 million lethal doses) and the definite agglutination of broth cultures by 1/320 dilution of serum being of a much higher order of potency than that obtained with typical Type III pneumococci in anti-Type III serum whether from rabbits or hyperimmunized horses. This high degree of homologous potency of the antiserum produced by immunization with the atypical strain was a frequent occurrence in Avery's (1) work with the different Subgroup Type II pneumococci, but the reactivity of typical Type III strains in the anti-Thomas serum is in direct contrast to the lack of any reactivity of typical Type II strains in any of the different Subgroup Type II antisera.

Variations in the Relative Potencies of Different Type III Immune Sera in Respect to Antibodies Reactive with a Typical Type III Strain and Antibodies Reactive with the Thomas Strain.

The comparison in Table I of the Thomas strain with a typical Type III strain was based upon their reactions in immune sera chosen for their potency against both the Thomas strain and typical Type III strains. During the investigation, many tests have been made with a number of different Type III immune sera. The results of these tests showed a wide range of differences in the relation between the anti-Type III and the anti-Thomas potency in different anti-Type III sera. The reactivity of the serum against typical Type III pneumococci had no regular relation to its reactivity against the Thomas pneumococci. For example, one of the anti-Type III sera that was most reactive against typical Type III pneumococci did not agglutinate the Thomas strain at all after 2 hours incubation at 37°C. and only occasionally agglutinated it after storage in the ice box overnight. Similarly, one of the anti-Type III sera that was weakly reactive against typical Type III strains was as reactive against the Thomas strain as any of the sera that we tested. This lack of any relation between the anti-Thomas potency and the anti-Type III potency of the individual sera is important if it represents a difference in the relative proportion of two slightly different antibodies in the serum obtained from different horses after immunization with Type III pneumococci.

Variations in the Relative Potencies of Anti-Thomas Serum from Different Rabbits in Respect to Antibodies Reactive with Typical Type III Strains and Antibodies Reactive with the Thomas Strain.

During the investigation, ten different rabbits were immunized with heat-killed suspensions of the Thomas pneumococci. A summary of the results of the tests of the potencies of these anti-Thomas rabbit sera is presented in Table II.

An analysis of Table II reveals two important facts: (1) the anti-Thomas sera obtained from ten rabbits showed no great difference in their respective potencies against the homologous strain; (2) in spite

of the uniformly good anti-Thomas immunity response, the sera of the same rabbits showed great differences in their respective potencies against the typical Type III strain.

If the homologous potency be dismissed as uniformly good in all of the anti-Thomas sera, it is possible to arrange the sera from the ten rabbits into three groups in respect to their potency against typical

TABLE II.

Variations in Relative Potencies of Anti-Thomas Immune Serum from Different Rabbits in Respect to Antibodies Reactive with Typical Type III Pneumococci and Antibodies Reactive with the Thomas (Homologous) Strain.

Anti-Thomas sera	Antibodies reactive with typical Type III pneumococci					Antibodies reactive with Thomas (homologous) strain		
	Agglutination			Precipitation of S substance contained in culture filtrate	Passive protection of mice against 1×10^{-4} or 1×10^{-5} cc. of culture	Agglutination of unheated culture by 1/160 or 1/320 dilution of serum	Precipitation of S substance contained in culture filtrate in high dilution of serum or of antigen	Passive protection of mice against at least 1×10^{-2} cc. of culture
	Unheated culture	Heated culture	Concentrated suspension of heated bacterial cells					
Serum from 2 rabbits	+	+	+	+	+	+	+	+
Serum from 7 rabbits	0	±	±	0	+	+	+	+
Serum from 1 rabbit	0	0	0	0	0	+	+	+

0 = negative results in all tests with all sera; ± = definitely positive results with 5 of the sera, but equivocal results with 2 sera of this group; + = definitely positive results in all tests with all sera.

Type III pneumococci. The first group includes the sera from two rabbits: these anti-Thomas sera agglutinated unheated broth cultures of Type III pneumococci almost as well as the best Type III immune horse sera and were much more reactive than some of the diagnostic sera supplied by biological houses for routine typing. The second group includes the sera from seven rabbits: they failed to agglutinate unheated cultures of Type III strains, some of them agglu-

tinated heated cultures, most of them agglutinated concentrated suspensions of the bacterial cells and all of them passively protected mice against typical Type III pneumococci. The third group includes the serum from one rabbit which, although highly potent against the Thomas strain, failed entirely to show any type-specific immunity against typical Type III strains; the lack of protection against minimal doses, which is the most delicate criterion, being accepted as evidence of the complete lack of anti-Type III antibodies. From the standpoint of the probability of the presence of two different anti-S antibodies in the same antiserum, it is important to note that these marked variations in anti-Type III potency occurred in anti-Thomas sera which showed no significant differences in their potency against Thomas pneumococci.

It seems unlikely that these differences in the amounts of anti-Type III antibody in anti-Thomas sera containing uniformly large amounts of the anti-Thomas antibody, are due to differences in the antigen injected. All of our ten rabbits were not immunized at the same time, and it happened that the first four rabbits (injected in June and July) gave better anti-Type III responses than two rabbits immunized later (injected in September and October). We thought at that time that the poorer response of the latter rabbits might have been due to a change in the antigenic properties of the Thomas strain. In order to rule out the possibility of the repeated mouse passage of the culture having changed the Thomas strain in the direction of loss of its Type III antigenic capacity, two rabbits were later (November and December) immunized with the mouse passage strain and two other rabbits with vaccine prepared from a culture which had been in the ice box for 3 months without animal passage. The sera of all four animals gave the usual strong anti-Thomas response; and the variations in the anti-Type III response were unrelated to the mouse passage of the culture.

Tests for the Presence of Type-Specific Anti-Type III Antibody in Anti-Thomas Sera by Precipitation of Solutions of the Purified Carbohydrate S Substance Derived from Typical Type III Pneumococci.

In preceding experiments, filtrates of young broth cultures of the Thomas strain and of typical Type III strains were employed as sources of the specific S substance elaborated by virulent type-specific pneumococci. It seemed important, however, to test the Thomas antisera against solutions of the purified carbohydrate S substance derived from typical Type III strains (4). A sample of the carbohydrate S substance furnished for this purpose by Dr. O. T. Avery

of the Hospital of The Rockefeller Institute, was tested against the anti-Thomas immune sera. The tests were made by adding 0.2 cc. of serum to 0.5 cc. of three different dilutions of the S substance (1/10,000, 1/50,000 and 1/100,000). The immune sera from four of the animals immunized earlier in the investigation were no longer available at the time the purified solution was obtained.

The results of the tests with the purified carbohydrate confirmed the results of the preceding tests with the filtrates of the Type III cultures, for the sera of some of the rabbits immunized with Thomas pneumococci precipitated the solutions of the highly specific and chemically purified carbohydrate substance prepared from typical Type III pneumococci. The prozone phenomenon was much more marked than in tests with anti-Type III horse serum. The two most reactive anti-Thomas sera precipitated the 1/50,000 solution better than the 1/10,000 solution, and gave no definite reaction at all in tests with 1/1,000 solution. This marked prozone made it seem inadvisable to attempt to increase the number of positively reacting anti-Thomas sera by repeating the tests with higher concentrations of antigen.

Absorption of Type III Immune Horse Sera with Suspensions of Typical Type III Pneumococci and with Suspensions of the Thomas Strain.

Anti-Type III serum was absorbed with suspensions of typical Type III pneumococci and with suspensions of Thomas pneumococci. In view of the factors that may influence the results of absorption tests, ten experiments were made with three different anti-Type III immune sera, under quantitatively different sets of conditions.

The results of these experiments were the same as those usually obtained in reciprocal absorption experiments with immunologically related, but different, kinds of bacteria. Absorption of the anti-Type III serum with the typical strain (homologous) completely exhausted it not only of antibodies reactive with typical Type III strains but also of those reactive with the Thomas strain. On the other hand, repeated absorption with the Thomas bacteria (heterologous) removed only the antibodies reactive with the Thomas strain and had little, if any, effect upon the potency of the serum when tested against the typical strain. The failure of repeated absorption with the Thomas bacteria to reduce the anti-Type III potency seems to us

to indicate the presence of at least two different type-specific (anti-S) antibodies in Type III immune horse serum, only one of which can be removed by the Thomas strain.

Absorption of Anti-Thomas Immune Sera with Suspensions of Typical Type III Pneumococci and with Suspensions of the Thomas Strain.

Anti-Thomas serum, potent against both typical Type III strains and the homologous Thomas strain, was absorbed with suspensions of the typical Type III bacteria and with suspensions of the Thomas bacteria under conditions analogous to those employed in the absorption of anti-Type III serum. The results of these experiments, which have been repeated many times with four different anti-Thomas immune sera, are summarized as follows:

Absorption of anti-Thomas serum with the homologous strain stripped the serum of antibodies reactive with typical Type III organisms as well as those reactive with the homologous (Thomas) organisms. Absorption with the typical Type III strain, on the other hand, removed only the antibodies reactive with typical Type III organisms and did not significantly diminish the anti-Thomas potency of the serum.

Tests with Other Typical Strains of Type III Pneumococci.

In most of the previously described experiments, one strain (A 66, Hospital of The Rockefeller Institute) was utilized as the representative of the typical Type III group. In order to determine if the relations found between the Thomas strain and the representative typical strain would hold true for other "typical" strains of Type III pneumococci we have repeated most of the described experiments with three different Type III strains recently isolated from different patients at the Vanderbilt University Hospital.

The anti-Thomas serum agglutinated and protected against the recently isolated strains as well as in the previous tests with the Rockefeller laboratory strain. Similarly, the Type III immune horse sera were completely stripped of antibodies (both anti-Type III and anti-Thomas) by absorption with the Nashville strains; and absorption of the anti-Thomas sera removed the anti-Type III and not the anti-

Thomas antibodies just as had absorption with the previously used Rockefeller strain. The repetition of the preceding experiments with these different and recently isolated strains adds considerable strength to all of the results and makes the absorption experiments much more convincing.

While the three strains (isolated in Nashville) represent too small a number to argue for the immunological homogeneity of the Type III group, the experiments with the recently isolated strains do serve to rule out the possibility that the preceding absorption results were due simply to our having used the same strain as that commonly used in the production of the Type III diagnostic serum in the different laboratories, and show that the anti-Thomas immune serum contains antibodies reactive with more than one strain of "typical" Type III pneumococci.

The necessity of repeating the preceding work with those additional strains which were known not to be the same as those utilized in the production of the anti-Type III serum is especially evident in view of the possibility that all of the biological producing laboratories may use the same strain in the production of anti-Type III diagnostic serum. And, it seemed probable to us that perhaps this one strain, the original source having been the Hospital of The Rockefeller Institute, might be the same one as that which we have employed as the representative Type III strain in our preceding experiments.

The Protection Test as a Criterion of the "Type Purity" of Pneumococcus Cultures.

The Thomas strain has been plated out repeatedly and we are convinced that its serological relationship to Type III is real, and not an apparent relationship due to the use of a mixed culture containing a few Type III organisms together with an unrelated Group IV strain. The fact that Type III immune horse sera produced in four different laboratories (by immunization with presumably "pure" Type III organisms) possessed marked protective power against the Thomas strain, seems to us to be in itself convincing evidence of the "type purity" of the culture.

As pointed out by Avery (1) specific protection is the ultimate criterion of type specificity among the pneumococci. Since protection tests offer the most delicate index of the presence of pneumococcus type-specific antibodies and suffice to detect them in antisera when test-tube methods fail, they should likewise prove to be the most delicate criteria of the "type purity" of pneumococcus cultures. For example, agglutination with the usual type sera would probably fail to detect

the presence of virulent Group IV pneumococci if mixed in small amounts with a Type III culture, but if passive protection tests were made, the mice would be infected by the small numbers of virulent Group IV organisms in spite of the protection conferred against the Type III bacteria themselves.

While it seemed certain that this principle would always hold true with virulent cultures, we have made two different sorts of experiments in order to test it. First, experiments were made in which the protective power of Type III serum was tested against: (*a*) Type III bacteria by themselves; (*b*) mixed cultures containing Type III and Type II bacteria in proportion of 1,000 to 1; (*c*) mixed cultures containing Type III and Type II in proportion of 10,000 to 1. The results of these experiments were exactly what one would expect if the presence of sensitized Type III pneumococci did not affect the virulence of the heterologous organism. The Type III serum protected against Type III alone, and failed against mixtures of Type III bacteria with 0.1 and 0.01 per cent of the heterologous organisms. All of the mice were autopsied. It is obvious that the experiment was concerned only with the animals which were killed by the mixed culture and not by the same amount of "pure" Type III; and typing of the heart's blood culture from these mice indicated that the heterologous organism was the sole cause of death and that the sensitized Type III bacteria had failed to survive in the blood stream even in mice having a septicemia due to the heterologous type.

Second, experiments were made to test the effect of the presence of small numbers of the Thomas bacteria upon the protective action of Type II serum; tests being made against: (*a*) Type II bacteria alone; (*b*) mixtures of Type II and Thomas bacteria in proportion of 10,000 to 1; (*c*) mixtures of Type II and Thomas bacteria in proportion of 100,000 to 1. The results of these experiments were analogous to the first ones; and in this case (due to the higher degree of homologous protection of the Type II serum), the protection test served to detect the presence of Thomas (heterologous) bacteria in the Type II culture even when present in the proportion of 1 to 100,000 of the homologous organisms.

DISCUSSION.

The preceding experiments dealt with the immunological properties of the Thomas strain of Pneumococcus which is related to but not identical with typical Type III strains. In respect to the reactions in potent anti-Type III immune horse serum, the relationship between the Thomas strain and typical Type III strains is about the same as that evidenced in anti-Type II serum between typical Type II strains and most of Avery's Subgroup II strains. But, when the comparison is made in anti-Thomas immune serum, it is evident that the relationship of the Thomas strain to typical Type III pneumococcus is different, for the anti-Thomas immune serum (from most rabbits) agglutinates and

protects against typical Type III, while none of the anti-Subgroup II immune sera were reactive against typical Type II pneumococci. The production of sera reactive against typical Type III pneumococci by injection of the Thomas strain is particularly interesting in view of the rarity of obtaining an effective anti-Type III immunity response in rabbits by the injection of typical Type III pneumococci. From these results it appears that antibodies reactive with typical Type III pneumococci can be produced more readily when rabbits are immunized with the Thomas bacteria than when immunized with typical Type III bacteria themselves. Whether the antigen in the Thomas bacterial cell which is responsible for the antibody reactive with the Type III bacteria is the same as the corresponding antigen in the Type III bacterial cell is another question.

The anti-Type III immune serum from different horses and the anti-Thomas serum from different rabbits, usually contained antibodies reactive both with the Thomas bacteria and with the typical Type III bacteria. It is particularly important that there was an entire lack of any regular relation between the relative anti-Thomas and anti-Type III potencies of the different individual antisera. The pronounced variations in the relative potencies of individual antisera in respect to antibodies reactive with the two kinds of pneumococci may represent differences in the relative proportion of two different anti-S antibodies in the antiserum from different individual animals. The variation in the anti-Type III potency of the different anti-Thomas sera is probably due to differences in the response of the individual rabbits to the particular Thomas antigen which gives rise to the anti-Type III antibody, for Tillett (3) found marked differences in the individual anti-Type III responses of rabbits when Type III bacteria themselves were injected. The possible variations in the antigenic character of the cultures (both of the Thomas and of the typical Type III strains) does not seem to be a likely explanation of the variations in the immune sera for we frequently obtained wide differences in the relative anti-Type III potency in the anti-Thomas sera of different rabbits immunized at the same time with equal amounts of the same Thomas vaccine.

The results of absorption experiments with both typical Type III antiserum and Thomas antiserum were the same as those usually ob-

tained in similar tests with immunologically related, but not identical bacteria. That is, homologous absorption removed all of the antibodies from each serum, and heterologous absorption removed only the antibodies reactive with the strain used in absorption and failed to exhaust the serum of antibodies reactive with the strain used in immunization. The failure of reciprocal absorption together with the variations in the relative potencies $\left(\text{ratio of } \frac{\text{Anti-Thomas potency}}{\text{Anti-Type III potency}}\right)$ of the antiserum from different individual animals could be presented as presumptive evidence that two different anti-S antibodies are contained in Type III immune horse serum. Although there is no evidence of complexity among the type-specific antigens of the "fixed" types of pneumococci, the same S substance united with slightly different protein constituents might give rise to related but slightly different type-specific antibodies.

In his original paper on the Subgroup Type II pneumococci, Avery (1) pointed out that the serological relationship did not in itself indicate that the Subgroup Type II and typical Type II strains were related by the lineage of common descent. The later developments in knowledge (2) of the antigenic constituents of the pneumococcus cell show more clearly that a serological relationship like that between the Thomas strain and typical Type III strains is not always a true index of phylogenetic relationship. It is now well known that there are two sorts of antigen-antibody systems involved in the immunological reactions of pneumococci: the S-anti-S reactions of type specificity, and the P-anti-P reactions of species specificity. There is much evidence that the second of these reactions is the more likely to indicate phylogenetic or truly biological relationship. Since the S-anti-S reactions separate into distinct "types" the pneumococci which manifest group relationship by P-anti-P reactions, it is important to recognize the possibility of biologically fortuitous likenesses in the chemical structure of some one of the cell constituents of phylogenetically unrelated bacteria. This possibility is well illustrated by the similarity between the S substance of Type II pneumococci and the S substance of some strains of Friedländer's bacillus (5). The common sense of the biologist would preclude the assumption of a closer phylogenetic relationship between Type II pneumococcus and Friedländer's bacil-

lus than between Type II pneumococcus and Type I or III, simply because of a greater chemical likeness between the carbohydrates elaborated by the bacteria. But, in point of fact, as far as the S-anti-S reactions are concerned, the serological relationship of the Thomas bacteria to the typical Type III bacteria is no more pronounced than that between Friedländer bacilli and Type II pneumococci; and hence, there is no real reason to believe that the Thomas strain is any more closely related, in a truly biological sense, to Type III than to any other virulent Pneumococcus.

In the absence of any evidence of phylogenetic relationship, the Thomas strain can best be considered as a Pneumococcus which, in addition to distinct immunological properties of its own, possesses a partial antigenic relationship to the typical Type III pneumococcus. The degree of type specificity manifested by Types I, II and III pneumococci is of a higher order than that usually obtained between the "types" contained in most groups of bacteria. But, in view of the wide range of immunological possibilities that are presented by Group IV pneumococci, one can expect to find a certain number of pneumococci that are related to but not identical with one of the "fixed" types. While there have been few, if any reports of pneumococci related to Type III it is quite possible that the use of a more highly reactive Type III diagnostic antiserum would result in the detection of strains related to Type III which would be included within Group IV on the basis of typing tests with weak Type III antiserum. This possibility is mentioned because of our own experience with the Thomas strain. When first typed in our laboratory, it was considered a Group IV strain and its Type III relationship was not recognized until a subsequent typing test was made with a more potent Type III antiserum than that which we had been using for routine typing.

SUMMARY.

The paper reports a study of a virulent, S-producing strain of Pneumococcus which is immunologically related to, but not identical with typical strains of Type III pneumococcus. In a potent anti-Type III serum, the relationship of this strain to typical Type III strains appears to be about the same as the relationship of Avery's Subgroup Type II

strains to typical Type II. But a more pronounced distinction is evident in the antiserum produced by immunization with the strain related to Type III. This antiserum contained antibodies specifically reactive with typical Type III bacteria as well as antibodies reactive with the homologous strain, while anti-Subgroup Type II immune sera are devoid of antibodies reactive with typical Type II pneumococci.

The results of absorption experiments were the same as those usually obtained with immunologically related, but not identical bacteria. The failure of reciprocal absorption and the marked variations in the relative potencies of the antiserum from different individual animals might be presented as presumptive evidence that two different anti-S antibodies are contained in Type III immune horse serum.

The theoretical significance of virulent pneumococci which are related to but not identical with the "fixed" types, is discussed from the standpoint of their importance in the biological classification of the Pneumococcus group.

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