# THE SEPARATION OF TYPES AMONG THE PNEUMOCOCCI HITHERTO CALLED GROUP IV AND THE DEVELOPMENT OF THERAPEUTIC ANTISERUMS FOR THESE TYPES.*

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In connection with the study of the use of concentrated refined antibody preparations in the treatment of pneumonia due to Types I, II and III it seemed desirable to study the possibility of treating pneumonia cases due to pneumococci of the miscellaneous types hitherto classified as Group IV. In general, clinical results have indicated that these cases are benefited very little by treatment with the therapeutic antibody preparations now in use. Furthermore, since some cases due to these organisms are severe and a considerable percentage develop septicemia it seems worth while to try to find a suitable serum therapy for them.

The classification of pneumococci into Types I, II and III and Group IV is generally known and accepted. Other types have been differentiated, but the grouping has not been generally utilized, probably due to the absence of diagnostic or therapeutic serum suitable for use with the new types and to the belief that the types did not occur in lobar pneumonia with sufficient frequency to warrant attention. Lister (1) found another type more prevalent than Types I, II and III in South Africa. Olmstead (2) in a study of 213 strains by direct agglutination found that the miscellaneous strains which she studied could be classified in twelve groups. Clough (3) classified 94 strains in twelve groups. Strains giving delayed or incomplete but marked agglutination with Type II serum were classified by Avery (4) as Subgroup II strains and separated by agglu-

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tination and protection tests into Subgroup II A and Subgroup II B. Stillman (5) described further Subgroup II strains on the basis of agglutination tests. Among 55 strains isolated during the influenza epidemic, we (6) differentiated nineteen types which represented four small groups and thirteen unrelated strains. Griffith, (7) studying forty-nine strains not Types I, II or III from respiratory diseases, thirty-four of which were from lobar pneumonia, distinguished twelve serological types. He found that strains corresponding to the Sub II A and Sub II B of Avery predominated. Robinson (8) found eight groups among 65 strains of the so called Group IV. One group was identified as the cause of an epidemic. Sugg, Gaspari, Fleming and Neill, (9) and Harris, Sugg and Neill (10) described a group of strains which were immunologically related to Type III.

In the present investigation it was necessary, preliminary to the selection of suitable strains for the production of therapeutic serum, again to find into what types the miscellaneous strains were divisible and which types were most prevalent. The majority of the strains[1] were isolated at the Harlem Hospital; a smaller number[2] from the Bellevue Hospital. 120 strains from lobar pneumonia cases which did not agglutinate or reacted atypically with diagnostic antiserums for Types I, II and III, were studied. Strains of Subgroups II A and II B were obtained from Dr. Avery at the beginning of the investigation; and later, in order to learn something concerning the distribution of the types, strains were obtained from Dr. Robinson representative of the types found in Pittsburg, also a strain of a non-typical Type III from Dr. Sugg of Nashville, Tennessee. Antiserums for five of the types found in England were sent us by Dr. Griffith. Monovalent antiserums were prepared and agglutination tests carried out. By direct agglutination tests, the 120 case strains studied were divided into ten groups containing four or more strains each, one group of only two strains, and two strains differing from each other and from the preceding types, and a miscellaneous group differing from all the preceding types and among which further types were not separable with the antiserums thus far prepared. It is apparent that additional agglutinating antiserums might divide the group of undifferentiated strains thus adding to the number of types. The search for new types is to be continued. We are designating the ten types having

[1] We are indebted to Miss Dedrick, Miss Brown and Miss Gideon for these cultures.

[2] We are indebted to Dr. Sutliff for these cultures.

four or more strains by Roman numerals from Type IV to Type XIII. The agglutination results with representative strains of each type and antiserums prepared for each type are given in Table I. Only a small amount of cross-reaction occurred among the types. When rabbit antiserums are employed this is usually the case; generally there is more cross-reaction when horse antiserums are used.

Each group of strains was studied in regard to the ability of representative strains to stimulate the production of protective antibodies.

TABLE I.

*The Agglutination Titer of the Antiserums for Representative Strains of Each of the Recently Separated Types with the Homologous Strains and Strains of Each of the Other Types.*

| Type strains | Type antiserums | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | IV | V | VI | VII | VIII | IX | X | XI | XII | XIII |
| IV | 1–640 | 1–5 | —* | 1–20 | — | 1–20 | — | — | — | — |
| V | 1–10 | 1–320 | — | 1–20 | — | — | 1–5 | — | — | — |
| VI | —* | 1–40 | 1–160 | — | — | — | — | — | — | — |
| VII | — | 1–20 | — | 1–640 | — | 1–5 | 1–20 | — | — | — |
| VIII | — | — | — | 1–40 | 1–640 | 1–20 | — | — | — | — |
| IX | — | 1–5 | 1–5 | 1–20 | — | 1–640 | 1–5 | — | — | — |
| X | — | — | — | 1–20 | — | — | 1–640 | — | — | — |
| XI | — | — | — | — | 1–40 | 1–20 | — | 1–320 | — | — |
| XII | — | — | — | — | — | 1–5 | — | — | 1–160 | — |
| XIII | — | — | — | — | — | 1–5 | 1–5 | — | — | 1–160 |

* Indicates that no agglutination was found in dilutions as low as 1–5.

The power of antiserums produced by injection of a single strain to protect against freshly isolated as well as old strains belonging to the same type, was determined. The amount of cross-protection of antiserums for one type against strains of other types was studied also. Antiserums for at least two strains of each type were prepared. Cultural or morphological peculiarities of the strains were noted. The special data are given in detail in a discussion of each group of strains.

*Type IV.*—Twenty-one strains are of this type, nineteen were from recent cases and two were stock strains. One of the stock strains is an "antitoxin" strain from the Lilly Laboratories, the other a repre-

sentative of one of the groups, "Group IV B," obtained from Robinson. Of the nineteen cases due to this type, sixteen were rated[3] as severe and seven of these died; three were rated as mild. Four of the patients who died and one who recovered had positive blood cultures.

The strains of Type IV had a peculiarly high virulence for mice, all being fully virulent when first tested and remaining so throughout the

TABLE II.

*Protection Tests Showing the Comparative Protection of a Polyvalent Type I, II and III Concentrated Antibody Preparation and of an Antiserum for Type IV against a Type IV Culture.*

| Polyvalent Types I, II and III antibody preparation | | Monovalent Type IV antiserum (rabbit) | |
|---|---|---|---|
| Antiserum amounts | Results in mice | Antiserum amounts | Results in mice |
| *cc.* | | *cc.* | |
| 0.1 | +48 hrs. | 0.001 | Survived |
| 0.05 | +24 hrs. | 0.0005 | Survived |
| 0.02 | +48 hrs. | 0.0002 | Survived |
| 0.01 | +24 hrs. | 0.0001 | +48 hrs. |
| 0.005 | +48 hrs. | 0.00005 | +48 hrs. |
| | | 0.00002 | +30 hrs. |
| | Amount | Colony count | Results in mice |
| | *cc.* | | |
| Control | 0.000001 | 153 | +48 hrs. |
| of virulence | 0.0000001 | 16 | +48 hrs. |
| of culture | 0.00000001 | 2 | +48 hrs. |

*Method:* 0.001 cc. of culture (0.5 cc. of dilution 1–500 in broth) and the desired amount of serum diluted with saline (0.5 cc. of required dilution) was drawn into a syringe and injected intraperitoneally into mice.

*Control:* 0.5 cc. of the dilution made in broth containing the required amount of culture and 0.5 cc. broth was drawn into a syringe and injected into mice.

The approximate number of diplococci injected were estimated by colony count of blood agar poured plates.

period of the investigation. By fully virulent we mean that ten or fewer diplococci killed mice in less than 48 hours. This dose was usually contained in 0.00000001 cc. of 18 hour broth culture as shown by colony count in poured blood agar plates. In order to test the

[3] We are indebted to Dr. Bullowa and Dr. Rosenbluth for the ratings made in the clinical investigation of pneumonia at the Harlem Hospital and to Dr. Sutliff for those at the Bellevue Hospital.

potency of antibody stimulated by injection of this type, an antiserum from a rabbit inoculated for 5 weeks with a representative strain was tested to determine its protective value against the strain injected and fifteen other strains classified in this group. The results indicated 100 to 500 protective units[4] against each strain. The remaining strains were not tested.

The protective value of a trivalent Type I, II and III concentrated antibody preparation having 1500 protective units against Type I, 1000 against Type II and 20 against Type III was tested against Type IV. This preparation had less than one unit per cc. of protection for Type IV, whereas the control homologous antiserum (rabbit) had 500 units of protection per cc. The results of these tests are shown in Table II. Monovalent antibody preparations for Type I, for Type II and for Type III and other trivalent antibody preparations than the one mentioned above were tried with similar results. It is apparent that the antibody preparations for Types I, II and III are practically useless for treatment of Type IV infections and that antibody preparations to be efficient must be homologous to this type. With this in view the immunization of horses with a Type IV strain was begun. After 21 weeks of injections the antiserum from a horse had 100 protective units per cc. being suitable for diagnostic and experimental therapeutic use.

It is interesting that our Type IV corresponds with one of the types, "Pn. 10," found by Griffith in England. His strains were isolated from three cases of lobar pneumonia, one of bronchopneumonia and one of acute bronchitis. Our Type IV also corresponds with the "Group IV B" differentiated by Robinson in Pittsburg which included six strains from lobar pneumonia with a case mortality of 33 per cent. Considering the limited number of cases in both series the mortality figure closely approaches ours of 37 per cent. Because of its apparent wide distribution and its virulence it is expected that this type will prove to be of especial importance.

*Type V.*—Six strains from cases are included in this group and two stock strains, the Subgroup II A strain of Avery and a strain of Group IV E of Robinson. All the pneumonia cases due to this type were

[4] A unit is ten times the smallest amount of serum which protects mice against 100,000 fatal doses of a fully virulent culture inoculated intraperitoneally.

especially severe. Five out of six of the patients were shown to have positive blood cultures and these cases died.

All the strains examined of this group when cultivated in blood broth media showed a tendency to hemolyze blood cells; with some strains the tendency was very much more marked than with others

TABLE III.

*Protection Tests Showing the Comparative Degree of Protection of a Type II Concentrated Antibody Preparation and of a Type V Antiserum against a Type V and a Type II Strain.*

| Protection against Type V | | | | Protection against Type II | | | |
|---|---|---|---|---|---|---|---|
| Type II antibody | | Monovolent Type V antiserum (rabbit) | | Type II antibody | | Monovalent Type V antiserum (rabbit) | |
| Antiserum amounts* | Results in mice | Antiserum amounts | Results in mice | Antiserum amounts | Results in mice | Antiserum amounts | Results in mice |
| *cc.* | | *cc.* | | *cc.* | | *cc.* | |
| 0.02 | Survived | 0.002 | Survived | 0.0002 | Survived | 0.1 | +22 hrs. |
| 0.01 | Survived | 0.001 | Survived | 0.0001 | Survived | 0.05 | +46 hrs. |
| 0.005 | +96 hrs. | 0.0005 | +48 hrs. | 0.00005 | Survived | 0.02 | +46 hrs. |
| 0.002 | +30 hrs. | 0.0002 | +48 hrs. | 0.00002 | +72 hrs. | 0.01 | +52 hrs. |
| 0.001 | +48 hrs. | 0.0001 | +48 hrs. | 0.00001 | +48 hrs. | 0.005 | +46 hrs. |
| 0.0005 | +48 hrs. | | | | | 0.002 | +22 hrs. |
| | Amount | Colony count | Results in mice | | Amount | Colony count | Results in mice |
| | *cc.* | | | | *cc.* | | |
| Control | 0.000001 | 144 | +48 hrs. | Control | 0.000001 | 120 | +48 hrs. |
| of virulence | 0.0000001 | 12 | +48 hrs. | of virulence | 0.0000001 | 12 | +48 hrs. |
| of culture | 0.00000001 | 2 | +48 hrs. | of culture | 0.00000001 | 3 | +48 hrs. |

* 0.001 cc. of 18 hour broth culture was injected with the different amounts of antiserum.

*Method:* See foot-notes to Table II.

but in general it was greater than that of the other pneumococcus cultures studied. This ability to produce hemolytic substances may account for the severity of the septicemic cases. The strains when first isolated from patients were all highly virulent for mice, but did not hold their virulence as well as the strains of Type IV. After the virulence had fallen attempts to raise the virulence sufficiently high to carry out accurate protection tests were usually unsuccessful.

As noted above the Subgroup II A of Avery is of this type. Avery found that the strains were agglutinated to a considerable degree in Type II serum although the agglutination was usually delayed and often incomplete. He also found that Type II antiserum protected to some extent against Subgroup II A cultures while antiserum for Subgroup II A failed to protect against Type II cultures. Our results have been similar to his.

In order to find whether the therapeutic preparations, now available, were potent against infection with this type, protection tests were carried out using polyvalent antibody preparations for Types I, II and III, and monovalent antibody preparations for Type I, for Type II and for Type III. The Type II antibody preparation having 2000 units against Type II had only 10 to 20 units per cc. against the Type V strain. The polyvalent antibody preparations also had 10 to 20 protective units per cc. against Type V strains. Type I and Type III antibody had less than 1 unit per cc. against Type V. Comparative tests with the serum of a rabbit immunized against a Type V strain and with Type II antibody are shown in Table III. The rabbit serum for the Type V strain had 100 units of protection per cc. It is evident that a preparation containing 10 to 20 units per cc. would be of little value as compared with one containing 100 units per cc. or more. Neither the polyvalent antibody preparations for Types I, II and III nor the Type II antiserum is suitable for therapeutic use against infections with this type. Since diagnosis and treatment must be carried out independently of Type II we have thought it better to give the type an entirely distinct designation to avoid confusion. Potent antiserum for determining the type and for therapeutic use has been obtained by inoculation of horses.

Griffith found this type second in prevalence among the types into which he separated Group IV. Robinson found this type, termed by him "Group IV E," most numerous during the winter of 1928 in Pittsburg.[5] On account of its prevalence, virulence and special tendency to infect the blood stream, this type should be of especial interest for serum treatment.

*Type VI.*—Seven strains from pneumonia cases are included in this

[5] Personal communication.

group, also one stock strain, the Subgroup II B strain of Avery. Of the seven patients two had positive blood cultures and died. Three of the cases were rated as moderate in severity and two as mild. The case strains of this type were generally of somewhat lower virulence for mice than those of Types IV and V. Those which were virulent when isolated had a tendency to lose their virulence very rapidly. Type VI strains hemolyzed blood less actively than Type V but more actively than the majority of the other pneumococcus cultures studied. The strains of Type VI are similar to these termed Sub II B by Avery. He found the relationship of this type similar to that of the Subgroup II A strains to Type II. He found that Subgroup II B was immunologically distinct from Subgroup II A. Our results were similar. We found that monovalent or polyvalent preparations which were highly protective against Type II would protect against Type VI to a slightly greater degree than against Type V. However, protection to a high degree was obtained with antiserums prepared by injections of Type VI strains. The largest group separated by Griffith from the so called Group IV corresponded with our Type VI. He found it next in prevalence to Types I and II.

The data available indicate that infections with this type are milder than with Types IV or V and it may be expected that cases will respond more readily to treatment.

*Type VII.*—Eleven strains from cases of pneumonia were of this type. Seven cases were rated as severe and two of these died. Two cases were moderate in severity. No data were available in regard to the ratings of the other two cases. One of the fatal cases and one case which recovered had positive blood cultures.

The strains of Type VII were generally of low virulence for mice. None were suitable as test strains for carrying out protection tests as the virulence could not be raised. Therefore the protective value of a therapeutic antiserum (horse) prepared for this type could not be accurately determined by the mouse protection method. Possibly another method may be devised. This antiserum is suitable, however, for determining the presence of this type.

*Type VIII.*—Four strains from cases and two stock strains are of this type. Of the stock strains one is a representative of the "Group IV A" of Robinson and the other is the "Thomas" strain of Sugg.

Of the four cases three were rated as severe and one as moderate. One patient who had a positive blood culture died.

The Type VIII strains were found to agglutinate in Type III antiserum to a marked degree but less than typical Type III strains,

TABLE IV.

*Protection Tests Showing the Comparative Degree of Protection of Monovalent Antiserums for Type III and Type VIII against a Type III and a Type VIII Strain.*

| Protection against Type VIII | | | | Protection against Type III | | | |
|---|---|---|---|---|---|---|---|
| Type III antiserum (rabbit) | | Type VIII antiserum (rabbit) | | Type III antiserum (rabbit) | | Type VIII antiserum (rabbit) | |
| Antiserum amounts* | Results in mice | Antiserum amounts | Results in mice | Antiserum amounts | Results in mice | Antiserum amounts | Results in mice |
| *cc.* | | *cc.* | | *cc.* | | *cc.* | |
| 0.1 | +27 hrs. | 0.001 | Survived | 0.002 | Survived | 0.1 | Survived |
| 0.05 | +22 hrs. | 0.0005 | Survived | 0.001 | Survived | 0.05 | Survived |
| 0.02 | +22 hrs. | 0.0002 | +78 hrs. | 0.0005 | +45 hrs. | 0.02 | Survived |
| 0.01 | +27 hrs. | 0.0001 | +45 hrs. | 0.0002 | +45 hrs. | 0.01 | +45 hrs. |
| 0.005 | +45 hrs. | 0.00005 | +22 hrs. | 0.0001 | +27 hrs. | 0.005 | +45 hrs. |
| 0.002 | +27 hrs. | | | | | 0.002 | +23 hrs. |
| | Amount | Colony count | Results in mice | | Amount | Colony count | Results in mice |
| | *cc.* | | | | *cc.* | | |
| Control of virulence of culture | 0.000001 | 214 | +27 hrs. | Control of virulence of culture | 0.000001 | 163 | +48 hrs. |
| | 0.0000001 | 34 | +45 hrs. | | 0.0000001 | 24 | +48 hrs. |
| | 0.00000001 | 1 | +72 hrs. | | 0.00000001 | 1 | Survived |

* 0.001 cc. of 18 hour broth culture was injected with the different amounts of antiserum.

*Method:* See foot-notes to Table II.

this would lead to confusion of the more reactive of these strains with Type III in routine "typing" tests. Two strains of Type VIII were sent us from a hospital in response to our request for freshly isolated Type III cultures. It is impossible, at present, to judge the prevalence of this type, as other strains belonging to it may have been classified as Type III in the routine hospital tests. We were receiving the

strains which apparently did not fall with Types I, II and III. We are planning to study all the presumed Type III strains isolated during the coming season and will then have more definite information on this point.

Protection tests were carried out with monovalent rabbit antiserum for Type VIII and for Type III. The results with the monovalent rabbit antiserums are given in Table IV. The antiserum for the Type VIII strain had 500 protective units per cc. against Type VIII strains (four strains tested), but only 20 units per cc. against a stock Type III strain and 1 unit against a freshly isolated Type III strain. An antiserum for a stock Type III strain had 100 protective units per cc. against the stock Type III strain, 25 units against a freshly isolated Type III strain and less than 1 unit per cc. against Type VIII strains. The most potent Type III concentrated antibody available had only 1 to 2 protective units or less against Type VIII strains. Polyvalent antibody for Types I, II and III failed to protect.

A very interesting and detailed study of this type has been published recently by Sugg, Gaspari, Fleming and Neill, and by Harris, Sugg and Neill. Our findings agree in general with theirs. The "Group IV A" of Robinson corresponding to this type, was one of the three types of miscellaneous pneumococci found most prevalent in Pittsburg during the winter of 1927. The mortality among his cases was 14 per cent.

The differentiation of this type from Type III is very important in the investigation of the use of antibody preparations in Type III cases. At least some part, even if small, of the failure with Type III antibody treatment is probably due to the failure to exclude cases due to this type. We hope that in the future this type may be identified and we are preparing a specific antiserum for therapy.

*Type IX.*—Six strains from pneumonia cases are of this type. One of the cases was rated as severe, four as moderate, one as mild. One patient who had a positive blood culture died. All strains were of comparatively low virulence for mice when tested. Perhaps this was because the strains had been grown for some time on artificial media before the classification was made and virulence tests were carried out. Now that an antiserum for identification of this type is available it is hoped that more virulent strains suitable for protection tests will be

encountered. An antiserum has been secured for this type by the inoculation of a horse.

*Type X.*—Five strains from cases of pneumonia are of this type. All were from severe cases, three were fatal, two of which were shown to have positive blood cultures. The cultures were of moderate or low virulence for mice. An homologous rabbit antiserum had 500 protective units per cc. while a polyvalent antibody preparation for

TABLE V.

*Grouping of Pneumococci of So Called Group IV.*

| Types | Total strains including stock strains* | No. of strains from cases 1927–1928 | Percentage of case strains in each type | No. of deaths | Percentage of deaths in each type | No. of cases having positive blood cultures |
|---|---|---|---|---|---|---|
| Type IV | 21 | 19 | 15.8 | 7 | 36.8 | 5 |
| Type V | 8 | 6 | 5.0 | 5 | 83.3 | 5 |
| Type VI | 8 | 7 | 5.8 | 2 | 28.6 | 2 |
| Type VII | 11 | 11 | 9.2 | 2 | 18.2 | 2 |
| Type VIII | 6 | 4 | 3.3 | 1 | 25.0 | 1 |
| Type IX | 6 | 6 | 5.0 | 1 | 16.7 | 1 |
| Type X | 5 | 5 | 4.2 | 3 | 60.0 | 2 |
| Type XI | 4 | 4 | 3.3 | 1 | 25.0 | 1 |
| Type XII | 4 | 4 | 3.3 | 2 | 50.0 | 2 |
| Type XIII | 4 | 4 | 3.3 | 0 | 0 | 0 |
| Total strains typed | 77 | 70 | 58.3 | 24 | 34.3 | 21 |
| Strains not typed | 50 | 50 | 41.7 | 11 | 22.0 | 15 |
| Total strains | 127 | 120 | 100.0 | 35 | 29.2 | 36 |

* Stock strains obtained from Dr. Avery, of The Rockefeller Institute for Medical Research, Dr. Robinson, of the William H. Singer Memorial Research Laboratory, Pittsburg, and Dr. John Y. Sugg, of the Vanderbilt University, Nashville, Tennessee.

Types I, II and III had only 1 unit against Type X. The results indicate that in therapy this type also must be considered as an entity.

*Type XI.*—Four strains from cases of pneumonia are of this type. One patient died. Three cases were rated as moderate in severity and one of these had a positive blood culture. Type XI strains have a moderate tendency to hemolyze blood. They were of moderate to high virulence for mice when first isolated and lost their virulence rapidly.

A rabbit immunized with a representative of the type had 200 to 500 protective units per cc. against the different strains classified as this type. A polyvalent antibody preparation for Types I, II and III had 1 to 5 protective units or less against Type XI strains.

*Type XII.*—Four strains from cases of pneumonia are of this type. Three of the four cases were rated as severe and were shown to have positive blood cultures. Two of the cases were fatal. The fourth case was moderately ill. Type XII strains had moderate virulence for mice.

*Type XIII.*—Four strains from cases of pneumonia are of this type. One case was rated as severe, three as moderate. None of the cases were fatal. These strains were of moderate virulence for mice when first isolated but rapidly lost their virulence.

The total number of strains studied, including the stock strains, the number from lobar pneumonia cases, the deaths with their percentages and the number of positive blood cultures are given in Table V. Since the number of cases studied is comparatively small, the figures may be deceptive as to the actual prevalence of the types and the mortality of the cases. This is especially true in regard to the mortality; as the infecting organism would be more easily isolated in pure culture from severe cases and would be grouped, while in the lightly infected cases it might not be recovered.

Monovalent antiserums for each of the types were tested to find whether there was any protective power against representative strains of each of the other groups where strains of sufficient virulence were available. These tests were made with the serum from immunized rabbits and very little protection was found, *i.e.*, less than 1 unit per cc. against heterologous strains. Concentrated Type II antibody preparations and concentrated polyvalent antibody preparations having a high potency against Type II were found to have 10 to 20 units against Types V and VI; an amount too low to ensure efficient treatment. These results indicate that to be effective a serum should contain antibodies stimulated by a type similar to the infecting type. Protection experiments have shown that monovalent antiserums of high protective value as determined by laboratory tests can be prepared for most of these types by inoculation of rabbits and horses and it is hoped that this will be true for the remaining types where at

present the work is not sufficiently advanced to make a definite statement. The final estimate of their value must be determined by the clinical use in the treatment of actual pneumonia cases.

In a recent paper Park, Bullowa and Rosenbluth (11) with reference to the unfavorable results of serum treatment of Type III cases, discussed the difference in the ability of Type III antiserums to protect against freshly isolated Type III strains as compared with old stock strains. The almost complete failure of the antiserums to successfully combat freshly isolated strains is probably due both to the low potency of the antiserum and to the remarkably large capsules which these organisms develop. The stock strains have smaller capsules, although they are "fully virulent" according to the definition which we have given above. The Type III stock strains had a much greater antibody stimulating power than the freshly isolated strains. Thus an antiserum prepared with a stock strain had 500 protective units against the stock Type III strains and 25 or less protective units against freshly isolated Type III strains, while an antiserum prepared with a freshly isolated strain had 5 units against the stock strain and considerably less than 1 unit against the freshly isolated strains. None of the freshly isolated strains of Types IV to XIII equalled Type III in the development of capsules, although there was considerable difference among representative strains in regard to this tendency. All surpassed freshly isolated Type III strains in antibody stimulating power. From these observations it is deduced that the new types are of a nature which should be susceptible to the action of suitable antiserums.

It is evident that the preparation of so many different type antiserums and the determination of the infecting type and the carrying out of specific treatment, while possible, will be a very laborious procedure. It would be very much simplified, if suitable polyvalent antiserums could be prepared, which would be effective against a large or considerable number of types and if a polyvalent antiserum could be used for identification of cases suitable for treatment. We are studying the possibility of preparing such a therapeutic antiserum. Certain difficulties have already been encountered, in as much as, the potency for the separate types is lowered somewhat in proportion to the number of strains injected. Possibly by combining the refined

and concentrated antiserums of horses immunized each against a few strains, a preparation can be obtained which will be sufficiently potent.

SUMMARY.

The pneumococci hitherto known as Group IV have been separated into ten types which have been designated by Roman numerals from IV to XIII. These have been correlated as far as possible with the types described by others. The prevalence and mortality of cases due to each type have been estimated in the limited number of cases studied.

Laboratory tests indicated that therapeutic antiserums for Types I, II and III have very little protective power against the recently separated types.

Monovalent antiserums of high agglutinative and protective power were prepared in rabbits for each type.

Several monovalent antiserums each specific for a type, which are suitable for agglutination and experimental therapeutic use, have been obtained by immunizing horses.

An antiserum prepared for one type had very little cross-protective power against other types.

A study of the possibility of preparing a suitable refined and concentrated polyvalent antiserum has been begun.

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