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A preliminary report on the classification of Pneumococcus IV.

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A study of the miscellaneous group of Pneumococci, called type IV by the workers of the Rockefeller Institute, was undertaken at the Presbyterian Hospital in connection with the investigation of post-operative pneumonia instigated by Dr. Brewer and reported by Dr. Whipple before the Surgical Section of the Academy. Merely a beginning of the study has been made and the results given here are based on agglutination reactions only, which have been so clear cut as to warrant some conclusions.

Most of the strains examined have been obtained, by mouse passage, from sputum or saliva of surgical cases before operation and may be considered normal mouth inhabitants. The others have been recovered from the sputum of post-operative pneumonia cases, of pneumonia cases in the medical wards of the hospital, of bronchitis cases, from lung cultures at autopsy, and from abscess cultures. All the strains have failed to react with serum of types I and II, for which we are indebted to the Rockefeller Institute.

Immune serum has been obtained by successive inoculations of rabbits. Only sera agglutinating their homologous strains through at least a 1 in 80 dilution have been used, most of the sera agglutinate through 1 in 160.

Strains have been tested as soon as isolated against all immune sera on hand, in equal parts of serum and culture. Readings have been recorded at the end of two hours' incubation and on the following day. Positive reactions have been confirmed by tests in dilutions of 1 in 10 to 1 in 80.

Two hundred and thirteen cultures have been tested with from 1 to 15 different sera. Owing to lack of serum comparatively few cultures have been tested with all sera. This short series of agglutination tests indicates a differentiation of pneumococcus IV strains (some parasitic, some saprophytic), into more than 12 groups, some of which have subgroups. The groups are made

up of strains that have cross-agglutinated and no interaction between the different groups has been observed. Cross-agglutination tests have been incomplete, only one immune serum being used in some groups. When other immune sera have been used, as in group *A*, the results have been the same with all, and the strains have agglutinated each other in about the same dilutions.

All two hundred and thirteen cultures were tested with group *A*, which consists of 12 strains that have been agglutinated by one or more of the three immune sera of this group. None of these strains have been agglutinated by serum of group *B*, or by serum of any other group with which they have been tested. Four of them were recovered from the sputum of post-operative pneumonia cases, but as they were not agglutinated by the patients' serum the significance of their presence in the sputum is uncertain. The other eight members of the group were from normal mouths.

Two hundred and eight strains have been tested with serum of group *B* (one strain), and ten have been agglutinated. One strain was recovered from the sputum of a post-operative pneumonia case and was agglutinated by the patient's serum, one was from a bronchitis case, the other eight were from normal mouths. Another strain from a post-operative pneumonia case, agglutinated by the patient's serum and originally agglutinated by serum of group *B* in a 1 in 40 dilution later lost its agglutinability for this group. Its immune serum has no effect on cultures of group *B* and a strain agglutinated by its serum is not agglutinated by serum of group *B*. Two other instances of this sort suggest the possibility of a change in agglutinative properties, though other explanations may be offered.

Of one hundred and six cultures tested against serum of group *C*, seven have been agglutinated, one from a post-operative pneumonia case, one from a thyroid abscess, the other five from normal mouths.

Ninety-seven cultures have been tested with serum of group *D*, and five agglutinated, two from post-operative pneumonia, one from pneumonia, and two from normal mouths.

One hundred and seventeen cultures have been tested with serum of group *E*, and four agglutinated, one from post-operative pneumonia not agglutinated by the patient's serum, and three from normal mouths.

With one serum of group *F*, a strain from a pneumonia case, forty-nine cultures have been tested and only the homologous strain has been agglutinated in a high dilution, that is, through 1 in 160. Three other strains agglutinated by this serum (two from normal mouths, and one from a lung culture of a lobular pneumonia case at autopsy) formed a subgroup, agglutinated through 1 in 20 only by this serum, through 1 in 160 by immune serum of one member of the subgroup. The latter has no agglutinative action on the type cultures even 1 in 2.

Of one hundred and five cultures tested against one serum of group *G*, three have been agglutinated, two of them from post-operative pneumonia cases, and one from a normal mouth. One of the post-operative pneumonia strains was agglutinated by the patient's serum.

Immune sera of groups *H*, *I*, *J*, and *K*, have been on hand only a short time, but although few tests have thus far been performed with these strains, two members of each group have been found.

L represents a single strain, recovered from the sputum of a post-operative pneumonia case and agglutinated by the patient's serum. Immune serum of this strain has been tested with one hundred and fifty-three different cultures and has agglutinated the homologous strain only. This is apparently not a common mouth inhabitant.

CONCLUSIONS.

Pneumococcus IV strains isolated from pneumonia sputum and normal mouths seem to fall into a large number of groups. However, a considerable proportion of them are classifiable. Twelve fall into one group, ten into another, seven into another, five into another. Several other groups of from two to four have appeared and it is probable that further tests will result in an enlargement of these groups.

It seems possible that the agglutinative properties of strains may change.