IMMUNOLOGIC STUDY OF STRAINS OF BACILLUS PFEIFFERI ISOLATED FROM A CASE OF MENINGITIS.

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The discovery of *Bacillus pfeifferi* by Pfeiffer in 1892, although made after the pandemic of 1899–90 had spent its force, was followed by rather general acceptance of the etiological relation of the organism to the disease. In the two succeeding decades it was found to have a widespread distribution in the upper air passages of normal individuals. In the next decade there were a number of minor and mild epidemics, in several larger American cities, of a disease clinically like influenza in which, not the influenza bacillus, but the streptococcus appeared to be the causal agent. These epidemics, however, were not associated with the dangerous complications nor did they have the high mortality of true epidemic influenza. With the passage of time since the 1899–90 pandemic the position of *Bacillus pfeifferi* as a pathogenic microorganism had become one of minor importance when the last pandemic made its appearance in 1918. The large amount of bacteriologic work done throughout the world in this epidemic still leaves the part played by *Bacillus pfeifferi* as the causative agent of pandemic influenza in doubt. Immunologic investigations have not helped to clear up this doubt.

That *Bacillus pfeifferi* may have well marked invasive and pathogenic properties is established by its occurrence as the only microorganism in a small proportion of cases of secondary bronchopneumonia and especially by its relation to sporadic cases of acute purulent meningitis.

Kuskow,[1] in 1895, estimated suppurative meningitis in the 1899–90 epidemic at 2.5 to 5 per cent of the fatal cases. That the reported cases from which he de-

[1] Kuskow, N., *Virchows Arch. path. Anat.*, 1895, cxxxix, 406.

rived these figures were due to *B. pfeifferi* was not established by bacteriologic examination, and his figures cannot be accepted as giving the true incidence of meningitis in influenza. In 1904 Jundell[2] reported two cases and found in the literature twelve cases from which pure cultures of the bacillus were obtained. These cases dated from 1895. In those previous to this date the organism was not cultivated or the cultures were impure. In 1911 Wollstein[3] reported 49 cases, eight being cases studied by herself. There was a further group of nine reported cases in which the cultures were contaminated, and a group of eight which she considered doubtful. Torrey[4] reported two cases in 1916, making a total of 82 certain cases recorded up to that time; of these cases eight recovered. Lacy[5] in 1918 reported one case in which the organism was cultivated from the meninges at autopsy but not from the spinal fluid during life, and another case in which *B. pfeifferi* was grown in pure culture from the spinal fluid. Additional instances are reported by Nyberg,[6] Brown,[7] and Moody,[8] each of whom reported two cases; and by Aaser,[9] Packard,[10] Bhat,[11] and Hills,[12] each of whom reported one case.

The important point to be noted in connection with these 94 cases is that they occurred during the period between the two pandemics of 1899–90 and 1918–19. The small number of cases reported since the last epidemic began and experience in civil and military hospitals during the epidemic indicate that meningitis due to *Bacillus pfeifferi* is a sporadic infection of not very great frequency. While there seems to have been some increase in meningitis due to *Bacillus pfeifferi* since the height of the epidemic, in view of the widespread distribution of this organism the increase would appear not so great as might be expected if *Bacillus pfeifferi* were the cause of epidemic clinical influenza. Perhaps this fact may have some bearing upon any attempt to determine the part played by *Bacillus pfeifferi* in epidemic clinical influenza. Other points of interest which appear from the

[2] Jundell, I., *Jahrb. Kinderheilk.*, 1904, lix, 777.
[3] Wollstein, M., *Am. J. Dis. Child.*, 1911, i, 42.
[4] Torrey, R. G., *Am. J. Med. Sc.*, 1916, clii, 403.
[5] Lacy, G. R., *J. Lab. and Clin. Med.*, 1918–19, iv, 55.
[6] Nyberg, C., *Finska läk.-sällsk. handl.*, 1915, lvii, 1369.
[7] Brown, A., *Canad. Med. Assn. J.*, 1915, v, 1076.
[8] Moody, E. E., *J. Missouri Med. Assn.*, 1916, xiii, 328.
[9] Aaser, E., *Tidsskr. norske Lægefor.*, 1916, xxxvi, 393.
[10] Packard, F. R., *Ann. Otol., Rhinol. and Laryngol.*, 1916, xxv, 706.
[11] Bhat, K. S., *Lancet*, 1917, ii, 384.
[12] Hills, R., *New York Med. J.*, 1918, cvii, 345.

reported cases are the low age incidence, most of the cases occurring in very young infants or in children and only four of 94 in adults, and the high mortality, only nine of 94 cases having recovered.

OBSERVATIONS.

The isolation of five strains of *Bacillus pfeifferi* from a case of meningitis admitted to the Sarah Morris Hospital for Children gave an opportunity for studying the immunologic reactions of the microorganism as obtained from a single case and for comparing definitely invasive strains with others, perhaps saprophytic, from the upper air passages. The results, together with the recently reported immunologic studies of others, appear to be of fundamental importance for an understanding of the biology of the organism and may have some bearing upon the possibility of developing a therapeutic antiserum.

Isolation of Strains.

The patient, a white, male infant age 17 months, on admission showed vomiting, twitching all over the body, fever, rigidity of the body, discharging right ear, and strabismus. The illness had begun with fever and vomiting 4 days before admission; muscular twitching appeared 2 days and rigidity 1 day before admission; strabismus developed on the day of admission. Death occurred on the 7th day in the hospital. The first culture of *Bacillus pfeifferi* was obtained from spinal fluid removed on the day of admission. This fluid had a cell count of 3,500 and all the characteristics of a fluid from acute purulent meningitis. On the following day cultures were made from the nose, throat, nasopharynx, ear, and blood.

A direct film of the spinal fluid stained by Gram's method showed a Gram-negative pleomorphic organism. There was variation in both size and morphology from coccus-like forms to rather long, slender bacilli. Direct films were not received in the laboratory from the nose, throat, nasopharynx, or ear.

Dewdrop colonies with the typical appearance of *Bacillus pfeifferi* appeared on blood agar cultures from the nose, throat, nasopharynx, blood, and spinal fluid, but not from the ear. Films made from

these colonies appeared, in the case of the spinal fluid culture, as Gram-negative pleomorphic organisms resembling those seen in the direct film. Films made from colonies of the other cultures showed typical, very small, Gram-negative bacilli fairly uniform in size. Subcultures were made on plain agar, Avery's[13] dextrose blood broth, and brown agar. No growth was obtained from the plain agar subcultures. In the Avery broth there was a rather slight growth showing some pleomorphism. In films from the spinal fluid colonies on the brown agar plates, the morphology was more typical than on whole blood agar. Colonies from the other sources tended to become more pleomorphic on brown agar than on whole blood agar. The brown agar was made by adding 2 per cent of defibrinated human blood to plain agar and heating to 80°C. long enough to produce a brown color. The growth on this medium was luxuriant, becoming more so after cultivation, which was in decided contrast to the very fine growth on blood agar. The ear cultures gave only a pure growth of *Staphylococcus albus*. From spinal fluid removed on the day after admission, when the cell count had increased to 10,000, *Bacillus pfeifferi* was again obtained in pure culture. Subcultures from selected colonies of the first spinal fluid culture and of the nose, throat, nasopharyngeal, and blood cultures on brown blood agar and on unheated whole blood agar were used for study.

Pathogenicity.

Determination of the pathogenicity of the strains was outside the scope of the investigation. The effect of an early generation of the spinal fluid culture was tried on a rabbit and a guinea pig, and some additional information upon the relative pathogenicity of the recently isolated strains from the other sources was obtained from the immunization of rabbits. A rabbit, two-thirds grown, received an intravenous injection of a salt solution suspension of the entire growth of a 24 hour brown blood agar culture of the second generation; the inoculation was without effect. Instillation of a heavy suspension of the spinal fluid strain into the nose of a guinea pig was also without effect. Since the invasive spinal fluid strain had proved to be

[13] Avery, O. T., *J. Am. Med. Assn.*, 1918, lxx, 17.

non-pathogenic for the first rabbit used, suspensions of living cultures of the other strains were used for the immunization of rabbits. The rabbits injected with the strains isolated from the blood, throat, and nasopharynx died after one injection. In all three instances, *Bacillus pfeifferi* of typical morphology and cultural characteristics was isolated from the heart's blood.

Immunization.

For the production of immune serums, rabbits one-half to two-thirds grown were used. The two animals which had withstood the effect of the first intravenous injection of living suspensions of the spinal fluid and nose strains were subjected to further injections. Since the strains isolated from the blood, throat, and nasopharynx had proved fatal to rabbits injected intravenously with suspensions of living organisms, the immunization of other rabbits was begun with suspensions of these strains killed by heating to 80°C., living suspensions being used later. An additional rabbit was immunized with the spinal fluid strain. The intravenous injections were repeated every other day. When trial bleedings from the ear vein gave satisfactory agglutination, the animals were bled from the heart[14] and the serums were separated. In this way there were prepared two serums against the spinal fluid organism, and one serum against each of the other strains. These serums were used for a study of agglutination, complement fixation, and phagocytic activity.

Agglutination.

The macroscopic method was used. Each immune serum was used with its homologous strain and with the four heterologous strains. The serum was used in dilutions of 1:4, 1:8, 1:16, and so on by geometrical progression to 1:4,096. To each dilution of serum an equal quantity of *Bacillus pfeifferi* suspension was added, the total volume of fluid being 0.5 cc. This suspension was obtained by washing down twenty-four cultures with normal salt solution. The growths, since they were quite cohesive, were well shaken and then centrifuged for a few minutes to throw down the larger particles, and the supernatant fluid was used. A test was incubated at 56°C. for

[14] This was always done under anesthesia.

4 hours and placed in the ice box over night; another was incubated at 53°C. for 20 hours. Neither of these, however, proved so satisfactory as material incubated at 37°C. for 2 hours and placed in the ice box over night. Since it was feared that the strains might die out during subsequent work, cultures were made on brown blood agar slants in Blake flasks. From the luxuriant growths thus obtained large amounts of suspension were made, the organisms being killed by heating to 80°C. It was hoped that the use of such stock suspensions throughout all the work might give more satisfactory results. It was soon found, however, that agglutinations with these older suspensions were unsatisfactory. A slightly turbid fresh suspension of a 24 hour living culture gave better results than a more turbid suspension. The results of the agglutination tests are given in Table I,

TABLE I.

Agglutination.

Suspensions of *B. pfeifferi.*	Immune serums.						Normal rabbit serum.
	Spinal Fluid 1.	Spinal Fluid 2.	Blood.	Nose.	Throat.	Naso-pharynx.	
Spinal fluid.....	1:4,096	1:1,024	1:64	1:64	1:64	1:16	0
Blood.....	1:8	1:32	1:128	1:4	1:4	1:4	0
Nose.....	1:64	1:128	1:32	1:64	1:32	1:8	0
Throat.....	0	0	0	0	1:1,024	0	0
Nasopharynx.....	1:64	1:64	1:16	1:256	1:8	1:512	0

the figures being the highest dilution of serum at which clumping was apparent to the naked eye.

The results of the agglutination experiments show a wide variation in the titer of the serums for their homologous strains. Whether this is due to differences in agglutinability of the strains, to variations in agglutinogen content of the strains, or to variations in the response of the animals used it is impossible to decide. With the exception of the serum against the strain from the nose each serum agglutinates best its homologous strain. Each serum except that against the throat strain gives also cross-agglutination with the heterologous strains in low dilutions of serum. The serums against the spinal fluid organism have the highest titer, that against the strain from the throat is most specific, whereas the serums against the strains from

the nose and nasopharynx are least specific. It was originally intended to perform absorption agglutination experiments, but the exhaustion of the supply of serums and the later loss of some of the strains prevented this.

Complement Fixation.

In the complement fixation experiments two series of tests were set up. In the first, each immune serum was used with the spinal fluid strain. This exhausted the supply of serums, so that cross-fixation tests with the remaining strains as antigens could not be done. There was left just enough immune serum from the rabbits injected with the nose and throat strains to set up these serums with their homologous strains. After the original supply of serums was exhausted, an attempt was made to obtain a fresh supply by reimmunizing the original animals with stock killed suspensions of the strains which had been used before. This second lot of serums was not so satisfactory as the first but was used in a second series of experiments in which each serum was set up with all the strains. Because of the loss of several of the strains it was necessary to use as antigens the stock killed suspensions which had been prepared at the beginning of the work.

Undiluted serums heated at 56°C. for 30 minutes were used. In the first series the antigens were made of unheated bacterial suspensions such as were described under Agglutination. The system used was the anti-sheep rabbit in one-tenth the volume of the original Wassermann test. The serum, antigen, and complement, which consisted of two units of fresh guinea pig serum, were incubated at 37°C. for 1 hour. Two units of previously titrated anti-sheep amboceptor and a 5 per cent suspension of sheep corpuscles were then added and the whole was incubated for 1 hour. Antigen, serum, and hemolytic control tests were made each time. Serum and complement were kept constant; the antigens were varied, the figures given in Tables II and III being the fractions of the anticomplementary unit which gave complete inhibition of hemolysis.

As has been found to be the case in comparative studies of agglutination and complement fixation with other species of bacteria, the complement fixation experiments with *Bacillus pfeifferi* show a lesser

degree of specificity than do the agglutination reactions. An unexpected result is the degree of fixation which normal rabbit serum gave with the different antigens. The serum of a number of different normal rabbits was used, but always with the same result. In the second series, in which the older stock suspensions were used as antigens, this phenomenon was even more marked. It would appear to be due to a property of the antigen rather than of the normal serum;

TABLE II.

Complement Fixation.

Antigens of *B. pfeifferi.*	Immune serums.						Normal rabbit serum.
	Spinal Fluid 1.	Spinal Fluid 2.	Blood.	Nose.	Throat.	Naso-pharynx.	
Spinal fluid.....	$\frac{1}{256}$	$\frac{1}{256}$	$\frac{1}{4}$	$\frac{1}{64}$	$\frac{1}{128}$	$\frac{1}{64}$	$\frac{1}{4}$
Nose.....				$\frac{1}{64}$			$\frac{1}{4}$
Throat.....					$\frac{1}{64}$		$\frac{1}{4}$

TABLE III.

Complement Fixation.

Antigens of *B. pfeifferi.*	Immune serums.						Normal rabbit serum.
	Spinal Fluid 1.	Spinal Fluid 2.	Blood.	Nose.	Throat.	Naso-pharynx.	
Spinal fluid.....	$\frac{1}{32}$	$\frac{1}{128}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{16}$	$\frac{1}{8}$
Blood.....	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$
Nose.....	$\frac{1}{32}$	$\frac{1}{32}$	$\frac{1}{16}$	$\frac{1}{64}$	$\frac{1}{64}$	$\frac{1}{16}$	$\frac{1}{8}$
Throat.....	$\frac{1}{32}$	$\frac{1}{32}$	$\frac{1}{8}$	$\frac{1}{32}$	$\frac{1}{32}$	$\frac{1}{32}$	$\frac{1}{8}$
Nasopharynx.....	$\frac{1}{32}$	$\frac{1}{32}$	$\frac{1}{32}$	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{16}$

the antigen appeared to be somewhat anticomplementary when used with normal rabbit serum, although it had no such action when added to the hemolytic system in the absence of rabbit serum.

Opsonins.

The opsonic index of each immune serum with its homologous influenzal strain was determined. Equal parts of suspensions of living *Bacillus pfeifferi*, normal rabbit leucocytes (collected in 0.2 per cent sodium citrate, washed, and suspended in normal saline

solution), and inactivated serum were mixed and incubated at 37°C. for 30 minutes. 50 polymorphonuclear leucocytes were counted and the number of cells taking part in phagocytosis was noted and compared with a similar series in which normal rabbit serum replaced the immune serum. The strain isolated from the blood was also set up with all the immune serums. The results of these experiments are given in Table IV. Because of the low opsonic indexes obtained in these experiments further work in this direction was not done.

With the exception of the first spinal fluid serum, all the serums used were of the second lot. The opsonin experiments show much the same kind and degree of variation as the agglutination tests. The spinal fluid serums, which had the highest agglutinin titers, have

TABLE IV.

Opsonins.

Suspensions of *B. pfeifferi.*	Immune serums.					
	Spinal Fluid 1.	Spinal Fluid 2.	Blood.	Nose.	Throat.	Naso-pharynx.
Spinal fluid	2.1	1.9				
Blood			1.4			
Nose				1.8		
Throat					1.5	
Nasopharynx						1.6
Blood	1.3	1.2	1.4	1.1	1.0	1.0

also the greatest opsonic activity. In the series in which the different serums were used with the same bacterial suspension, the organism being the strain from the blood, the action of the serum is best with the homologous organism, but the differences are not very marked.

DISCUSSION.

Immunologic studies of *B. pfeifferi* have led to conflicting results. Park,[15] by agglutination methods, found that cultures from different cases, even in the same locality, differed essentially or completely from each other. Isolations from the same case were usually identical, but different strains could occasionally be obtained from the same case. The differences noted were stable and persisted after

[15] Park, W. H., *J. Am. Med. Assn.*, 1919, lxxiii, 318.

subculturing. Huntoon and Hannum[16] came to exactly opposite conclusions and claimed an intimate relation for different strains, even when these were isolated in widely separated regions. Their work was based upon a small number of strains. Their agglutinin titers with three monovalent serums and four strains were low, the maximum of 1:640 being reached only once. The absorption experiments gave a decrease in titer varying from 1:10 to 1:80. Small and Dickson[17] also found strain relations by agglutination methods and arranged their strains into four groups. The number of strains used by them was, however, only ten, of which three fell into one group, four into another, while the two remaining groups were each represented by only one strain; there was one additional strain which could not be placed in any of these groups because of its inagglutinability. The propriety of setting up groups with only single representatives must appear doubtful; we believe that the study of a larger number of strains might have increased the number of single strain groups indefinitely. Valentine and Cooper,[18] in agglutination and absorption experiments on 171 strains from autopsies and from the upper air passages of patients with influenza, found little or no evidence of identity and concluded that under *B. pfeifferi* is included a heterogeneous group of organisms, among which there may be small subgroups. Six strains isolated from as many members of a single family, all taken ill with influenza at about the same time, were all different. Utheim[19] isolated thirty strains from thirty patients and tested the agglutination of the patients' serums with the homologous strains. Eleven (36 per cent) gave a positive agglutination in dilutions which varied from 1:20 to 1:160. In cross-agglutination experiments only one strain was agglutinated by heterologous serum, giving a positive result with the serums of three patients in 1:40 dilution. Utheim concluded that "each strain seemed to be individual in its immunologic reaction." Bell[20] studied the agglutination reactions of thirty-six strains, isolated by pharyngeal culture, with twenty-seven monovalent serums. His results are particularly valuable because of the high titers which the serums developed. Cross-agglutination occurred frequently in low dilution, but in only a few instances in higher dilutions. Absorption experiments also showed such variation that grouping of strains was impossible; the organisms used for absorption might absorb all, part, or none of the agglutinin for other strains. Strains isolated from two brothers were identical; from one individual two strains were isolated which were identical with two isolated from another individual. Three strains isolated from a single individual were quite distinct from one another. Although identical strains may occur, Bell concluded that "the influenza bacillus represents a heterogeneous group of

[16] Huntoon, F. M., and Hannum, S., *J. Immunol.*, 1919, iv, 167.
[17] Small, J. C., and Dickson, G. K., *J. Infect. Dis.*, 1920, xxvi, 230.
[18] Valentine, E., and Cooper, G. M., *J. Immunol.*, 1919, iv, 359.
[19] Utheim, K., *J. Infect. Dis.*, 1920, xxvii, 460.
[20] Bell, H. H., *J. Infect. Dis.*, 1920, xxvii, 464.

organisms." Cooke[21] was likewise unable to find any antigenic relation when sixteen of the strains isolated by Bell were used as antigens in the complement fixation reaction with patients' serums.

The study of five strains isolated from the same case, of which strains two were definitely invasive, appeared to offer better possibilities of obtaining knowledge of the biology of *Bacillus pfeifferi* than the study of many strains from a large number of cases. Whether the primary invasion in the present case was one of the blood stream or of the meninges, it is impossible to say. We believe it more probable that the meninges were invaded first and directly from the upper air passages, and that the blood was invaded from the meninges. The five strains isolated show distinct immunologic differences. The strains isolated from the nose and the nasopharynx show a greater apparent relation than any other two strains, and there may be some slight relation between the spinal fluid and the nose strains. The relation in each instance, however, is less marked than that which occurs among certain members of the colon-typhoid intermediates which are considered to be distinct species. Unfortunately, absorption experiments could not be done, but it is believed that they would not have added much of importance since cross-agglutination was so slight. The opsonin studies, although phagocytosis was not strikingly increased by the immune serums, show much the same differences as those obtained by agglutination. Since the results given were obtained by comparing the action of immune serum on normal leucocytes with that of normal serum, the phenomenon of spontaneous phagocytosis in the absence of serum, noted by Davis,[22] may be excluded. The pathogenicity of the five strains for rabbits also varied, although no great importance may be attached to these results because of the small number of animals used. Suspensions of living cultures of the blood, throat, and nasopharynx strains caused death from bacteremia. The nose and the spinal fluid strains were non-pathogenic for rabbits in large doses.

Our results, as well as those of Park,[15] Valentine and Cooper,[18] Utheim,[19] Bell,[20] and Cooke,[21] are directly opposed to those of Hun-

[21] Cooke, J. V., *J. Infect. Dis.*, 1920, xxvii, 476.
[22] Davis, D. J., *J. Am. Med. Assn.*, 1907, xlviii, 1563.

toon and Hannum,[16] and of Small and Dickson.[17] The evidence obtained from strains isolated from the same case or from different cases against identity of strains, and even against any close immunologic relation of strains, appears overwhelming. What bearing these results have upon the question of the etiological relation of *Bacillus pfeifferi* to the epidemic disease, it is difficult to say. Park[15] maintains that epidemic strains must be biologically identical if they are to be considered to have any causal connection with the disease. For most species of bacteria this is undoubtedly true. With *Bacillus pfeifferi* the question arises whether it may be possible that the wide variations in strains indicate biological instability which, under proper conditions, becomes an important factor in the causation of epidemics. In spite of the fact that immunologic characters become fixed upon cultivation of *Bacillus pfeifferi* outside the body, it is difficult to exclude the possibility that the organism is so labile that it may undergo changes under natural conditions in the human body. Although different strains, even from the same case, may show wide variations in experimental immunologic reactions, we believe that such strains are not so distinct from each other as are the various members of the colon-typhoid intermediate group, for instance. While the present case yielded five strains which have little in common except cultural and morphologic characters, it is probable that the subculturing of more colonies from the original cultures would have yielded a still larger number of immunologically different strains. However great the differences between the strains which were isolated, it is difficult to believe that these strains are not genetically the same.

If, as appears to be the case with antimeningococcus serum, the agglutinin and opsonin titers of an immune serum are a measure of the therapeutic value of the serum, the multiplicity of strains or races of *Bacillus pfeifferi* would seem to render impossible the preparation of a serum which might be of value in influenzal meningitis. Such a serum was prepared by Wollstein[23] at The Rockefeller Institute and was found to have definite value in experimental influenzal meningitis of monkeys. The serum had a low agglutinin titer but

[23] Wollstein, M., *J. Exp. Med.*, 1911, xiv, 73.

markedly increased phagocytosis. The immunologic relation of the organisms used in the experimental infection to those used in the preparation of the immune serum is not apparent from her report. The use of this serum in one of the cases reported by Torrey[4] was followed by recovery. Packard's[10] case also received this serum and recovered. Hills'[12] patient who did not receive the serum until the 4th day of the disease died on this day. Although one hesitates to draw conclusions from such a small number of cases, recovery may have been due to the serum, since the usual mortality of influenzal meningitis is approximately 90 per cent. In view of later immunologic studies, there is no reason to suppose that the organisms from the recovered cases would have been found to be identical with the strains used in the preparation of the serum. The present opsonin experiments with monovalent serums indicate strain variations comparable with those obtained in the agglutination experiments. The opsonin titers of our serums were so low that we do not attach great importance to the results obtained; it is possible that serums with higher opsonin values might have shown less variation between strains and a greater degree of group reaction. It is also possible, if strains of *Bacillus pfeifferi* are genetically related but immunologically distinct, that the therapeutic value of a serum in meningitis may depend upon properties other than those which can be measured by agglutination or opsonin reactions.

SUMMARY.

Five strains of *Bacillus pfeifferi* were isolated from a case of meningitis. These strains came from the spinal fluid, blood, nose, throat, and nasopharynx.

Immunologic reactions show no definite relations between these strains, although those from the nose, throat, and nasopharynx might be presumed to be related to one another. It is also presumable that the spinal fluid strain was derived from the upper air passages, and that the blood was invaded from the meninges. In spite of immunologic differences, it is believed that the five strains were genetically related.

The variations in these five strains from a single case are as great as those which have been found by others for strains from different cases, or individuals.

As determined by immunologic reactions, the number of so called strains of *Bacillus pfeifferi* is apparently limited only by the number of cultures which have been or might be isolated. It is inconceivable that under the designation *Bacillus pfeifferi* is included a heterogeneous mixture of innumerable distinct races of bacteria; there must be some biological relation which cannot be established by agglutination, complement fixation, or opsonin reactions.

The variations which have been noted may be an indication of a degree of instability which may have some bearing upon questions relating to the epidemiology of *Bacillus pfeifferi* infections and to the serum therapy of influenzal meningitis.

The strain variations which have been shown to exist by immunologic methods do not support the theory of the etiological relation of *Bacillus pfeifferi* to epidemic influenza, unless it can be shown that such variations are due to instability of the organism. The fact that the incidence of meningeal involvement was only slightly increased during the last pandemic is also evidence against the causal relation of the organism to the pandemic disease.