ON MENINGITIS DUE TO HÆMOPHILIC ORGANISMS —SO-CALLED INFLUENZAL MENINGITIS.¹

By HERBERT HENRY, M.D., B.S., *Demonstrator of Pathology in the University of Sheffield; Assistant Physician to the Sheffield Royal Hospital.*

From the Pathological Laboratory, University of Sheffield.

(PLATES XXI.—XXIII.)

FROM time to time there have been recorded cases of meningitis, occurring either sporadically or during epidemics of influenza, which have been attributed to the *Bacillus influenzae* of Pfeiffer. In the diagnosis of any form of meningitis the clinician is faced with numerous difficulties, and even when the diagnosis of the disease is definitely established, in any given case he can often but hazard a guess as to the nature of the infecting organism. It is for this reason that the description of a considerable number of so-called influenzal cases is quite valueless, for the diagnosis has been based on clinical evidence alone with no bacteriological examination.

It is probable that cases of meningeal infection due to an influenza-like bacillus are by no means rare, for during a period of two years' observation nine cases of this nature have been met with, whereas within the same period there have occurred only four cases of meningococcal meningitis. It is certain, too, that some at least of the cases described in the past as being due to the meningococcus are really influenzal in character. This is particularly true of those cases in which diplococcal forms have been found in films made from exudate, but in which cultures have remained sterile, for the influenza-like organisms in question, under certain conditions, exhibit marked bipolar staining, so as to resemble a diplococcus, and cultures are possible only in media containing hæmoglobin.

There is no reason to look on the disease as one which has recently increased in frequency, but rather as one which has hitherto eluded observation; for, as in other diseases, with a more wide-

¹ Received June 24, 1912.

spread knowledge of its existence comes an apparent increase in its incidence.

As far as British observers are concerned, the disease would appear to be one that has been detected only at intervals. The first recorded British case in which the organism was recovered during the life of the patient is that described by Douglas (1907¹⁰). This was followed by the case of Dudgeon and Adams (1907¹¹), in which meningitis and arthritis were part of a pyæmic process. In both these cases the organism from its morphology and its cultural appearances is described as being the influenza bacillus, but in neither case were any inoculations carried out. In the investigation of an epidemic of meningococcal meningitis, occurring in and around Edinburgh, M'Donald (1908¹⁶) found in the cerebro-spinal fluid certain "leptothrix organisms." There were five cases in all of this nature out of a series of over fifty cases. Three of these would appear to have been cases of mixed infection; in another, the leptothrix occurred along with the meningococcus; and in the fifth, in addition to the leptothrix, there occurred a coccus which may have been *Diplococcus crassus*. In only one of these cases were cultures successful, and there is reason for assuming that these growths afterwards became contaminated. The cerebro-spinal fluid obtained from this case and the impure cultures proved to be non-pathogenic. In the other four cases no inoculations were carried out. I am inclined to believe, for reasons stated later, that some at least of M'Donald's leptothrix forms are identical with, or closely related to, the organisms I am about to describe herein. On the other hand, the case described by Ritchie and M'Donald (1909¹⁹) would seem to be quite different, for in this the leptothrix forms grew readily on ordinary laboratory media even at room temperature and were not apparently hæmophilic.

Again, Batten (1910¹) has described the occurrence of five cases of meningitis from the wards of Great Ormond Street, which were said to be definitely influenzal in character, the diagnosis in each case being made from the morphology of the organism and its cultural features.

The best discussion on the subject is certainly that by Ritchie (1910²⁰), who describes certain cases of meningitis as being due to influenza-like bacilli. He would thus take up a position intermediate between those who on insufficient grounds proclaim such cases to be influenzal and those who describe the occurrence of leptothrix forms.

In the present communication I have sought to show that there exists a whole group of hæmophilic organisms giving rise to meningitis, the individual members of which group range from the *B. influenzae* of Pfeiffer, which, when it does kill, usually kills by toxæmia, to the organism described by Cohen (1909⁶), which is of very high virulence and kills laboratory animals regularly by septicæmia. It is for this reason and in an endeavour to be less committal than previous observers that I have chosen the title "On Meningitis due to Hæmophilic Organisms—so-called Influenzal Meningitis."

I propose first to give a brief description of the clinical features of several cases of meningitis due to hæmophilic organisms, together with a description of the pathological appearances presented by these cases after death. I shall also describe the organisms isolated from these cases as regards their morphology and cultural features. An attempt was made during the latter part of the work to compare these organisms with a strain of real *B. influenzae* originally obtained from

Pfeiffer's laboratory, but unfortunately the investigation had to cease abruptly and the results are therefore incomplete.

RECORD OF CASES.

CASE 1.—Donohoe, male, æt. 4 years.

April 1909.—Measles, followed by otorrhœa.

June 14, 1909.—Acute mastoiditis; temperature, 103°; operation.

June 25, 1909.—Operation wound healed; temperature normal.

July 4, 1909.—Vomiting; head retraction; Kernig's sign; temperature, 102°·8. The fluid obtained by lumbar puncture showed a thick purulent deposit, composed of polymorphs with a few mononuclears and red cells. Inside the polymorphs were found small, badly staining diplococci. Cultures on plain agar showed a scanty growth of a small, Gram-negative cocco-bacillus. Subcultures on ordinary agar were negative. No animal inoculations were done.

July 13, 1909.—Death. At *autopsy* there was found extensive cerebro-spinal meningitis, the other viscera being apparently healthy. Cultures on ordinary agar from brain and cord gave a few colonies of a streptococcus. On blood agar there grew in addition to the streptococcus very numerous colonies of an influenza-like organism. The organism died out after three subcultures at three-day intervals. No animals were inoculated.

CASE 2.—Alderson, male, æt. 3½ years.

June 30, 1909.—Acute mastoiditis; temperature, 104°; operation.

July 1, 1909.—Temperature normal.

July 3, 1909.—Temperature, 102°; vomiting.

July 5, 1909.—Drowsiness, vomiting, cerebral breathing, head retraction. The cerebro-spinal fluid showed thick purulent deposit composed entirely of polymorphs. A few small cocco-bacilli were found inside the leucocytes, also a few groups of similar organisms lying free in the fluid. Cultures in broth, on agar, and on blood serum gave negative results. Here again, as in the previous case, one failed to recognise that the organism was essentially hæmophilic.

July 10, 1909.—The cerebro-spinal fluid showed much thicker purulent deposit with large numbers of free cocco-bacilli and a few Gram-positive streptococci. On blood agar mixed growths were obtained, but on media without hæmoglobin only a few streptococcal colonies were found. Subcultures of the organism were made every two days, but after the eighth day no further growths could be obtained. No animal inoculations were done.

July 13, 1909.—Death. At *post-mortem* there was found septic cerebro-spinal meningitis. The thoracic and abdominal viscera appeared quite healthy.

CASE 3.—Machin, female, æt. 5 months.

January 8, 1910.—Vomited twice; stiffness of neck.

January 13, 1910.—Twitching of right arm and leg.

January 14, 1910.—Brought to hospital; temperature, 102°; pulse, 160; Kernig. Admitted with tentative diagnosis of meningitis.

January 22, 1910.—Marked Kernig, head retraction, occasional vomiting, much wasting. The cerebro-spinal fluid was markedly purulent, and showed cocco-bacilli crowded in large numbers inside the polymorphs, together with large clumps of organisms lying free in the fluid. A plentiful growth was obtained on blood agar tubes, but the organism died at the end of ten days after two day replants.

January 23, 1910.—Unconsciousness, with Cheyne-Stokes' breathing.

January 27, 1910.—Death. *Post-mortem*.—No naked-eye lesions were found in the thoracic and abdominal viscera. There was no evidence of

middle ear or sinus infection. Growths on blood agar were obtained from the cerebro-spinal fluid, while cultures made from the heart blood, liver, spleen, and lung remained quite sterile. The animal inoculations are detailed in the last section of this paper.

CASE 4.—Brook, female, æt. 5 years.

April 18, 1910.—Admitted to hospital after being in bed for three days with restlessness and temperature 100°. Had had discharge from left ear for two weeks. The child was very dull and apathetic, but screamed loudly on being disturbed. There was a small perforation of the left membrana tympani and a moderate amount of thin purulent discharge. There was no swelling or tenderness over the mastoid, no optic neuritis, no squint, no paresis. At operation on the same day the left mastoid was found full of granulation tissue, with a small amount of pus. The left lateral sinus and the dura of the middle fossa were exposed, but found to be normal. The cerebro-spinal fluid removed the same day was said to be quite clear and was not sent for examination. Pieces of bone and granulation tissue removed at operation gave copious pure growth of an influenza-like bacillus on blood media only.

April 19, 1910.—Death. *Post-mortem.*—Meningitis about base and over lateral aspect of left hemisphere. Animal inoculations detailed below.

CASE 5.—Turner, female, æt. 10 months.

July 5, 1910.—Was brought to hospital because of vomiting and wasting; diagnosed as a case of severe marasmus.

July 15, 1910.—Admitted to hospital; showed great wasting with marked bulging of the anterior fontanelle.

July 20, 1910.—Vomiting, head retraction, Kernig's sign, temperature 101°. The swelling over the anterior fontanelle was much more prominent.

July 26, 1910.—The skin over the anterior fontanelle ruptured, and there was discharged a large amount of yellowish watery pus.

August 2, 1910.—The cerebro-spinal fluid showed large numbers of an influenza-like bacillus both inside the polymorphs and lying free in the fluid. The organism grew only on blood-containing media.

August 3, 1910.—Death. *Post-mortem.*—The thoracic and abdominal viscera showed no gross lesions, except for swelling of the liver, spleen, and kidneys, and intense congestion of both lungs. There was widespread purulent cerebro-spinal meningitis. Cultures were made from the vertex of the brain, from the base, from the lateral ventricles, from the heart blood, lung, liver, and spleen, and in each case pure growths were obtained with the exception of those taken from the vertex, where, in addition to the Pfeiffer-like organism, there occurred a streptococcus, probably from contamination through the fistulous opening at the anterior fontanelle. The organism was found too in both middle ears, but films taken from the roof of the nose showed a pneumococcus only. Inoculation results are detailed below.

CASE 6.—Marsden, male, æt. 16 months.

December 2, 1910.—Noticed by mother to be tired and sleepy.

December 8, 1910.—Convulsions and screaming began. The knees and ankles became swollen, red, and painful. No vomiting.

December 9, 1910.—Admitted to hospital. The joint swellings had disappeared. There was left internal strabismus, head retraction, Kernig's sign, characteristic sharp cry, slight retraction of the abdomen, no *tache cérébrale*. Temperature was continuous, 103° to 105°; pulse rate, 148 to 160; respiration rate, 50 to 60. Lumbar puncture was performed three times, on the first two occasions no fluid was obtained, on the third puncture a very small amount of turbid fluid. This is said to have shown badly staining intracellular diplococci, which were taken to be meningococci. Accordingly the case was removed to the City Fever Hospital, 12th December.

December 15, 1910.—Death. *Post-mortem.*—There was found extensive purulent cerebro-spinal meningitis. Scattered throughout the lungs were

small areas of broncho-pneumonic consolidation. The kidneys were much swollen, and showed numerous fine cortical hæmorrhages. The organism was obtained in pure culture from brain, cord, heart blood, liver, and kidney. In the lungs there occurred in addition a streptococcus, a staphylococcus, and a pneumococcus, while cultures taken from the spleen remained quite sterile. Inoculation results are detailed below.

CASE 7.—*November 8, 1910.*—A specimen of cerebro-spinal fluid taken from a boy was sent to the laboratory for examination. This showed an enormous number of influenza-like organisms. Cultures were sterile, probably because the fluid was a day old and had been exposed to low temperature during the night. Attempts to get further material from, or information about, the case proved fruitless.

CASES 8 and 9.—These cases occurred in the Sheffield Royal Infirmary, and from each cerebro-spinal fluid was sent to the University for report, but here again attempts to obtain further material or information were without result.

MORBID ANATOMY.

The meningitis found post-mortem in these so-called influenzal cases varies much both in distribution and in intensity. In the case of the child Brook the inflammatory process appeared to be localised to one hemisphere. A similar observation is reported by Ghon (1902¹⁴). In one of Fraenkel's (1898¹²) cases, too, the posterior extremity of the right cerebral hemisphere was affected, and in the case recorded by Haedke (1897¹⁵) the inferior surface of the frontal lobe suffered most.

In the remaining five cases described in this communication the brain and cord showed a very intense and widespread purulent meningitis. I can do no better perhaps than give a description of the post-mortem findings in the child Turner, for the picture is one that is more or less typical of the rest. "On removing the skull-cap and dura there is found a dense yellow fibrino-purulent exudate, which has obliterated all the convolutions over the vertex. This has extended down the inner aspect of the hemispheres on to the corpus callosum, which is quite hidden from view. Posteriorly, the exudate has spread on to the occipital lobes, but here it is more aggregated along the lines of the sulci and vessels, from which it has crept in a thin layer on to the convolutions, the summits of which remain visible. The lateral aspect of the hemispheres shows dilated and engorged veins lying in the fissures and embedded in the same thick yellow exudate. The structures about the base of the brain are buried in viscid pus which has extended backwards over the pons and medulla, and from these structures to the under surface of the cerebellum. The spinal cord, too, particularly in its dorsal aspect, is surrounded by the same thick material." In addition, the ventricles are found to be distended with purulent fluid, and there is often much flattening of the cerebral convolutions.

The picture, therefore, presented by these cases is nothing more than that of a diffuse septic meningitis, and resembles very closely

the appearances produced in pneumococcal infection. This resemblance is still stronger in formalin hardened specimens, for the exudate may be stripped off with arachnoid and pia in a dense layer just as in pneumococcal meningitis.

In many of the recorded cases the portal of entry of the infection has been obvious, for the meningitis has been preceded by infection in some other part of the body. Of these initial lesions, by far the most frequent is suppurative otitis media. In this connection it is interesting to note that Ritchie (1910²⁰) has quoted Kossel as having found in 108 post-mortems in children of 1 year and under, eighty-five cases of otitis media, in half of which an organism considered to be the influenza bacillus was present. Cohoe (1909⁷), too, mentions that at the Otological Congress in Bonn, in 1894, Hartman and Konel stated that they had found the influenza bacillus in 90 per cent. of the cases of otitis media of influenzal origin in infants. The meningeal infection in such cases may result by direct extension through carious bone, or, as Cheate (1907⁴) has suggested, it may pass by way of the blood vessels which run through the remains of the petroso-squamal suture from the antrum to the middle fossa without any direct extension of caries. Of other local lesions which by direct extension may give rise to meningitis may be mentioned frontal sinus and maxillary antral suppuration, such as occurred in one of Ghon's cases (1902¹⁴).

Again, inasmuch as the respiratory form of influenza is that most commonly met with clinically, Fraenkel (1898¹²) has suggested the naso-pharynx as a possible portal of entry, the bacilli being conveyed by the nasal lymphatics to the anterior fossa of the skull through the cribriform plate of the ethmoid. The occurrence of broncho-pneumonia, pleurisy, and pericarditis has been found in many influenzal cases complicated with meningitis, in which event one may assume that the bacilli are borne to the cerebral meninges by the blood stream, or pass to the cervical meninges by way of the thoracic lymphatics, as has been demonstrated in some cases of tuberculous meningitis.

But in such cases, unless there be clear clinical evidence, it must often be difficult to say from the post-mortem findings whether the respiratory lesions have preceded the meningitis, or have developed after symptoms of meningitis have manifested themselves.

Bertini (1904²), for instance, believed that the meningitis should be regarded as a multiple localisation of the infection, for he was unable definitely to determine in his case whether the meningitis or the pneumonia was the primary lesion. In Case 6, herein recorded, the child exhibited meningitic symptoms to start with, and the patches of broncho-pneumonia found at autopsy may be looked upon as secondary.

In some instances, as in the case recorded by Dudgeon and Adams (1907¹¹), the meningitis seems to have been part of a pyæmic process,

for the primary lesion was an epiphysitis of the upper extremity of the radius. Another possible source of infection is mentioned by Davis (1909⁸), who has described two cases in twin brothers characterised by enteritis and mild peritonitis.

MORPHOLOGY OF ORGANISMS ISOLATED.

In films made during life from the cerebro-spinal fluid or taken after death from brain and cord, the organism appears as a minute cocco-bacillus which stains with some difficulty. The use of thionin, methylene-blue, and the usual stains is not followed by satisfactory results. The best preparations are to be obtained by placing flame-fixed or alcohol-fixed films in a weak solution of carbol fuchsin or carbol gentian-violet for from twenty to sixty minutes. This difficulty in staining, coupled with the fact that the organism is very small and may, moreover, occur but scantily in the cerebro-spinal fluid, may readily lead to its presence being overlooked. The very short bacillary forms frequently show bipolar staining, and this adds a still further difficulty in diagnosis, for the bipolar forms may be taken for diplococci. This actually happened in Case 6, which was notified as one of meningo-coccal meningitis.

In fresh cerebro-spinal fluid, and in the earlier phases of the disease, the organism occurs inside the polymorphs, at first in very small numbers, so that they may be found only after much searching, but eventually in large numbers crowded inside the leucocytes. In the later stages of the disease numerous organisms are to be found lying free in the fluid, and in some instances there seems to be but little attempt at phagocytosis. Often one comes across longer, slightly bent, bacillary forms with solid staining or with an irregularly beaded appearance. At times, too, long curved filaments may be met with, forms which have been previously described as "leptothrix." The frequency of these filamentous forms varies considerably, however. In some cases they are to be found only after prolonged search, whereas in others they occur in such numbers that their demonstration in microscopic preparations is easy.

This feature, namely the presence of the filamentous forms, is still much more marked when one comes to examine cultures. In some cases the cocco-bacillary form predominates throughout, and only on the third or fourth day are filaments in evidence; whereas in other cases, particularly in Cases 5 and 6, the septicæmic cases, filament formation in cultures occurred very early indeed. In the Turner organism numerous filaments were found after twenty-four hours' growth. In the Marsden organism they were much in evidence in eighteen-hour old cultures, while in cultures twenty-four to thirty hours old there appeared such a tangled mass of wavy filaments that

it was difficult at first to believe the organism to be identical with the small cocco-bacillus found in the tissues.

Dr. Cohen of the Pasteur Institute in Brussels was kind enough to send me two strains of organisms he had isolated from cases of septicæmic meningitis, together with a strain of *B. influenza* originally obtained from Pfeiffer's laboratory. These three organisms were morphologically identical with those I have already described. Both Cohen's organisms showed bacillary forms very early in cultures, with numerous wavy filamentous forms in older cultures, just as in the case of the Turner and Marsden organisms. The Pfeiffer organism showed long bacillary forms in older cultures, but the formation of filaments was never so marked as in the case of the organisms above mentioned.

In this connection it is perhaps interesting to quote Pfeiffer (1903¹⁷), who says in describing a pseudo-influenza bacillus from three cases of broncho-pneumonia: "Diese Stäbchen waren durch Form und Tinktion von den Influenzabazillen kaum zu unterscheiden, nur sahen sie im Durchschnitt etwas grösser aus. Auch in Kulturen zeigten sie das Verhalten der Grippeerreger. Sie wuchsen ausschliesslich auf Blutagar und bildeten Kolonien, die bis ins kleinste Detail den Influenzokolonien glichen. Trotzdem fanden sich bei näherer Untersuchung typische Wachstumsdifferenzen, welche eine Trennung der echten und der Pseudoinfluenzabazillen ermöglichten. Diese Unterschiede treten am besten hervor in 24 Stunden alten Kulturen auf menschlichem Blutagar. In Deckglaspräparaten, welche von derartigen unter möglichst identischen Bedingungen gewachsenen Kulturen hergestellt werden, erscheinen die Pseudoinfluenzastäbchen nach allen Dimensionen erheblich grösser als die echten Grippemikroorganismen. Dabei zeigen sie eine ausgesprochene Neigung zur Bildung längerer Scheinfäden, während solche in gleich alten Kulturen der echten Bazillen entweder ganz fehlen oder in sehr vereinzelt Exemplaren vorkommen. Diese Formdifferenzen sind so konstant und so erheblich, dass sie selbst dem ungeübten Beobachter auffallen müssen."

CULTURAL FEATURES OF ORGANISMS.

The various strains of organism isolated from these cases of meningitis grow only at body temperature and are essentially hæmophilic. It is possible to get scanty growths on plain agar from blood agar, but only, I believe, in circumstances where there is carried over a small amount of hæmoglobin from the older culture. I have never succeeded in growing any of the strains on plain agar even after they have been isolated from the body for some time. Good growths can be obtained on blood agar prepared either by admixture of melted agar with fresh rabbit blood, or by smearing the surface of ordinary agar with human, rabbit, or guinea-pig blood.

The colonies are distinctly visible on examination with a lens after

six to eight hours' incubation. At first they are very small, quite transparent, discrete, circular in outline, and with smooth margins. After eighteen hours the colonies are just visible individually to the naked eye. At twenty-four hours they are larger and begin to show faintly granular centres. At forty-eight hours isolated colonies may measure 2 mm. in diameter. They are then distinctly opaque and have slightly irregular margins. On ordinary blood agar adjacent colonies show little or no tendency to coalesce. However thick the culture may be, it is always possible to see that the growth is made up of individual colonies, their outlines much deformed from pressure of neighbouring colonies, tightly crowded and with but little tendency to run together, the whole field giving the appearance of a minute mosaic.

By using Cohen's cooked blood agar (1910⁵) it is possible after several subcultures to obtain a growth appearing as a continuous surface film, isolated colonies being found only along the margin. After thirty-six to forty-eight hours' incubation one finds in such cultures a thick grey layer of surface growth, just as profuse as the growth of *B. coli* on ordinary agar.

Good growths can be obtained, too, in blood broth, particularly if the tubes be incubated in a sloping position so as to get as large an air surface as possible.

The organism is one of feeble vitality, and is very susceptible to changes in temperature. It is killed by heating to 60° C. for five minutes, and an accidental exposure overnight to a temperature of 41° C. resulted in the death and loss of all my cultures of both the Turner and Marsden organisms. It has also been found impossible to get subcultures from blood agar growths left for six hours in front of an open window in winter.

When freshly isolated, subcultures have to be made every second, third, or fourth day, but gradually the interval can be prolonged, and it becomes possible to get growths in tubes inoculated from cultures fourteen to eighteen days old. It is preferable, however, to make replants every week or ten days. It is best, too, to use tubes with a large amount of condensation water, for the presence of moisture is essential and in dry tubes the organism rapidly dies out.

COMPARISON OF THE ORGANISMS ISOLATED FROM CASES OF MENINGITIS WITH THE *B. INFLUENZÆ* OF PFEIFFER.

Of the organisms responsible for suppurative meningitis, by far the most important is the meningococcus of Weichselbaum, the discovery of which, in 1887, gave an immense stimulus to the bacteriological investigation of all forms of meningitis, both epidemic and sporadic.

With the advent of Quincke's lumbar puncture, in 1895, the value of such investigations increased enormously, for the method enabled the pathologist to carry out observations while the patient was still alive, whereas previously his efforts had been confined to post-mortem material. Again, lumbar puncture afforded the ready means, not only of confirming Weichselbaum's findings, but of discovering that a large number of other organisms was responsible for suppurative meningitis. Of these latter, perhaps the most important are the bacilli which both morphologically and culturally resemble the *B. influenzae* of Pfeiffer. The occurrence of meningitis during epidemics of influenza was an established fact early in the history of the disease, and that such cases of meningitis were influenzal in origin was considered probable with the recognition of influenza as a distinct clinical entity, while with the discovery of Pfeiffer's bacillus the relationship between the epidemic form of influenza and meningitis could no longer be disputed. But the cases of meningitis described in the present communication—like others which have been described from time to time—did not occur in the course of an epidemic of influenza, and must therefore be looked upon as sporadic. This is a fact of very considerable importance, for it introduces the question as to how far the organisms isolated from such sporadic cases of meningitis agree in their features with the bacillus of Pfeiffer. In attempting to solve such a problem one is met at the outset by numerous difficulties. In the first place, although Pfeiffer's discovery was confirmed by competent observers, it was met with stubborn opposition, for cases which were clinically influenza were described in which the pneumococcus, the *Micrococcus catarrhalis* and other organisms, were found to be the infective agents. Moreover, the Pfeiffer bacillus was found to occur in a variety of conditions other than real influenza, as, for example, in scarlet fever and in measles; and, to still further increase the confusion, many investigators, including Pfeiffer himself, described the existence of pseudo-influenza bacilli. I have already quoted Pfeiffer's description of these bacilli, in which he refers to the presence of filamentous forms in cultures such as characterise the meningitis organisms. But perhaps the greatest difficulty one is met with in attempting to differentiate from Pfeiffer's bacillus a series of closely allied organisms, lies in the fact that the position of Pfeiffer's organism as regards its pathogenicity is by no means clearly established. Pfeiffer (1893¹⁸) originally maintained that his organism did not appear in the blood stream in cases of influenza, and that its constitutional effects were due to toxic action; but he used very small amounts of blood, and there can be no doubt that the blood stream is at times invaded, for Ghedini (1907¹⁹) and others, by using very much larger amounts of blood, have demonstrated this beyond dispute. Such a septicæmic condition, however, is the exception rather than the rule in human influenza. On the other hand, so called influenzal meningitis would appear

to be often but part of a septicæmic process. In the cases of Turner and Marsden, the only two of my series in which I made a complete bacteriological examination, the organism was obtained, not only from brain and cord, but also in the heart blood and from the various viscera, the growths obtained in each case being luxuriant and copious. Cohen and others have had exactly the same experience.

Another difficulty in establishing the etiological rôle of the influenza bacillus is in that it has given extraordinarily divergent experimental results in the hands of different investigators. Pfeiffer (1893¹⁸) and his pupils found the bacillus of influenza pathogenic for rabbits and apes only, in the former by intravenous injection and in the latter by intratracheal inoculation, but they concluded that fatal results were produced by intoxication, not by septicæmia. Cantani (1896³) found it possible to infect guinea-pigs, producing pleurisy and peritonitis, but without any real septicæmia, for he says: "Im Blute, im Peritonealexsudate und in allen Organen habe ich nie, weder microscopisch, noch kulturell, Influenzabacillen nachweisen können." Delius and Kolle (1897⁹) produced septicæmia, but in only a few cases, and that too with the organism in a condition of high virulence. In striking contradistinction to the difficulty experienced in inducing septicæmia with Pfeiffer's bacillus is the comparative ease with which such a result has been attained by those who have used strains of an organism which has resembled Pfeiffer's bacillus and which has been isolated from cases of sporadic meningitis. For instance, Cohen (1909⁶) working with four strains of influenza bacillus taken from diverse sources, found that in the case of one strain only was he able to recover the bacillus in cultures from the heart blood, whereas he has been able to get constant results with similar organisms isolated from cases of meningitis. Intravenous inoculation always produced in rabbits an intense septicæmia, fatal in twelve hours, and subcutaneous inoculation resulted in pericarditis, pleurisy and peritonitis, which resulted in death after about six days. He found, too, that it was possible to immunise rabbits against the meningitis organism, that the serum of such animals contained agglutinins, and that in addition it possessed preventive and curative properties against the infection, whereas the serum of animals inoculated against Pfeiffer's bacillus possessed no such curative or preventive properties.

With the organisms isolated from two cases of so-called influenzal meningitis, Ritchie (1910²⁰) was able to produce in guinea-pigs a septicæmia which proved fatal in thirty-six hours, as the result of inoculating one and a half cultures. Intravenous and subcutaneous inoculations in rabbits were without effect.

My own inoculation experiments started with the Machin organism, and continued with the Brook, Turner, and Marsden organisms. Throughout I have used as a medium ordinary agar

smearcd with human blood, and have worked with culture tubes showing approximately the same surface area.¹

With the Machin bacillus three rabbits were inoculated, the first intravenously, the second intraperitoneally, and the third subcutaneously, each receiving a whole blood agar surface growth three days old and made from the material obtained at post-mortem. None of these animals showed any signs of illness, nor were any lesions evident after the animals were killed. In the case of the Brook bacillus a series of six rabbits was used, only one of which succumbed (see Table). The bacillus was not recovered from the heart blood or the viscera of this animal, and one may, in consequence, assume that death occurred by intoxication. In sharp contrast with this are the results obtained in the case of the Turner bacillus, which induced septicæmia both in rabbits and in guinea-pigs,—in the former after intravenous inoculation, in the latter after both subcutaneous and intraperitoneal inoculation. The Marsden bacillus also proved pathogenic to guinea-pigs when given intraperitoneally. The “van Hasselt” bacillus obtained from Cohen proved to be of great virulence to guinea-pigs, even in old cultures, although it had been isolated from the body for many months, and the same was true too of Cohen’s “van C.” bacillus. The real influenza bacillus, “Pfeiffer typique,” obtained from the same source, was at first innocuous to guinea-pigs, but after being subcultured for five months it was found to give septicæmia in guinea-pigs inoculated with one single blood agar growth intraperitoneally. As to why this organism should have become so intensely virulent I have no explanation to offer.

BACILLUS “MACHIN.”

Animal.	Inoculation.			Result.	Remarks.
	Date.	Method.	Amount.		
Rabbit 50 .	1910, Jan. 30	Intraperitoneally	One culture of 72 hours	...	Killed June 16, 1910 ; no lesions found.
„ 51 .	„ 30	Subcutaneously	„ „	...	„ „
„ 52 .	„ 30	Intravenously	„ „	...	Killed May 31, 1910 ; no lesions found.

¹ The surface of a well-sloped agar tube is an ellipse, and has therefore an area represented by πab , where a denotes the greatest length and b denotes the greatest breadth of the slope. With long tubes of 1·6 cm. in diameter it is possible to get a good slope 9 cms. in length ; in which case the area of the agar covered by growth (without any allowance for that part of the slope which is occupied by condensation water) is just over 11 sq. cms.

BACILLUS "BROOK."

Animal.	Inoculation.			Result.	Remarks.
	Date.	Method.	Amount.		
Rabbit 66 {	1910, June 4	Intravenously	$\frac{1}{2}$ culture of 72 hours	...	...
	" 8	"	1 " 24 "	...	...
" 67 {	" 4	"	$\frac{1}{2}$ " 72 "	...	...
	" 8	"	1 " 24 "	...	...
" 68 {	" 4	"	$\frac{1}{2}$ " 72 "	...	...
	" 8	"	1 " 24 "	Death, June 9, 1910	Cultures from heart blood sterile.
" 69 .	" 14	Intraperitone- ally	1 " 24 "	...	...
" 70 .	" 14	"	1 " 24 "	...	...
" 71 .	" 14	"	1 " 24 "	...	...

BACILLUS "TURNER."

Animal	Inoculation.			Result.	Remarks.
	Date.	Method.	Amount.		
Guinea- pig 74	1910, Aug. 23	Intraperitone- ally	$\frac{1}{2}$ culture of 16 hours	Death, Aug. 24, 1910	Organism recover- ed from periton- eum and heart blood.
" 75	" 23	Subcutaneously	$\frac{1}{2}$ " 16 "	Death, Aug. 25, 1910	Organism recover- ed from heart blood.
" 76	" 23	Intraperitone- ally	1 " 16 "	...	...
" 77	" 23	Subcutaneously	1 " 16 "	...	...
Rabbit 78	" 23	Intraperitone- ally	$\frac{1}{2}$ " 16 "	...	...
" 79	" 23	Subcutaneously	$\frac{1}{2}$ " 16 "	...	...
Guinea- pig 82	" 25	Intraperitone- ally	$\frac{1}{2}$ " 16 " ex guinea-pig 74	...	...
" 83	" 25	Subcutaneously	$\frac{1}{2}$ culture of 16 hours ex guinea-pig 74	...	...
" 84	" 26	Intraperitone- ally	1 culture of 48 hours ex guinea-pig 74	Death, Aug. 28, 1910	Organism recover- ed from periton- eum and heart blood.
" 85	" 26	Subcutaneously	1 culture of 48 hours ex guinea-pig 74	...	...
" 86	" 26	Intraperitone- ally	$\frac{1}{2}$ culture of 24 hours ex guinea-pig 75	...	...
" 87	" 26	Subcutaneously	$\frac{1}{2}$ culture of 24 hours ex guinea-pig 75	...	...

[Table continued.]

MENINGITIS DUE TO HÆMOPHILIC ORGANISMS. 187

BACILLUS "TURNER"—continued.

Animal.	Inoculation.			Result.	Remarks.
	Date.	Method.	Amount.		
Rabbit 88	1910, Aug. 27	Intravenously	1 culture of 16 hours ex guinea-pig 75	Death, Aug. 28, 1910	Organism recovered from heart blood.
" 89	" 27	"	"	...	...
" 90	" 27	"	2 cultures of 16 hours ex guinea-pig 75	Death, Aug. 29, 1910	Organism recovered from heart blood.
" 91	" 27	"	"	...	...
Guinea-pig 92	" 28	Intraperitoneally	½ c.c. peritoneal fluid ex guinea-pig 84	...	...
" 93	" 28	"	"	...	...
" 101	1911, Jan. 10	"	2 cultures of 18 hours	Death, Jan. 11, 1911	Organism recovered from heart blood and from peritoneum.
" 102	" 10	Subcutaneously	" "	...	...

BACILLUS "MARSDEN."

Animal.	Inoculation.			Result.	Remarks.
	Date.	Method.	Amount.		
Guinea-pig 103	1911, Jan. 10	Intraperitoneally	2 cultures of 18 hours	Death, Jan. 11, 1911	Organism recovered from periton- eum and heart blood.
Guinea-pig 104	" 10	Subcutaneously	" "	...	...

BACILLUS "VAN HASSELT."

Animal.	Inoculation.			Result.	Remarks.
	Date.	Method.	Amount.		
Guinea-pig 109	1911, Feb. 5	Intraperitoneally	2 cultures of 24 hours	Death, Feb. 6, 1911	Organism recovered from periton- eum and heart blood.
" 110	" 5	"	1 culture of 9 days	"	"
" 138	July 2	"	2 cultures of 24 hours	Death, July 3, 1911	"
" 139	" 2	"	" "	"	"
" 140	" 2	"	1 culture of 24 hours	"	"
" 141	" 2	"	" "	"	"

BACILLUS "VAN C."

Animal.	Inoculation.			Result.	Remarks.
	Date.	Method.	Amount.		
Guinea-pig 111	1911, Feb. 5	Intraperitoneally	2 cultures of 24 hours	Death, Feb. 8, 1911	Organism recovered from peritoneum and heart blood.
" 112	" 5	"	1 culture of 9 days	...	...
" 142	July 2	"	2 cultures of 24 hours	Death, July 3, 1911	Organism recovered from peritoneum and heart blood.
" 143	" 2	"	" "	"	"
" 144	" 2	"	1 culture of 24 hours	...	...
" 145	" 2	"	" "	...	...

BACILLUS "PFEIFFER TYPEQUE."

Animal.	Inoculation.			Result.	Remarks.
	Date.	Method.	Amount.		
Guinea-pig 107	1911, Feb. 5	Intraperitoneally	2 cultures of 24 hours	...	...
" 108	March 5	"	1 culture of 9 days	...	...
" 134	July 2	"	2 cultures of 24 hours	Death, July 3, 1911	Organism recovered from peritoneum and heart blood.
" 135	" 2	"	" "	"	"
" 136	" 2	"	1 culture of 24 hours	"	"
" 137	" 2	"	" "	"	"

CONCLUSIONS.

There occur cases of meningitis, particularly in children under 2 years of age, due to a group of bacilli the individual members of which, in culture, present the common feature of being hæmophilic. The disease is one that occurs sporadically, and so far there is no evidence that it is capable of assuming epidemic proportions. The exact nature of the organisms isolated from such cases has been very variously interpreted. To those who have not carried the bacteriological examination sufficiently far, they are identical with the *B. influenzae* of Pfeiffer. By others the organisms are described as being influenza-like, and one observer has sought to establish a definite entity and has given the name "Septicæmic cerebro-spinal meningitis" to a disease caused by hæmophilic organisms of very high virulency. It is not improbable, too, that certain cases of so called "leptothrix" infection are really of this nature.

A hurried examination of the cerebro-spinal fluid taken from such cases may lead to the mistaken diagnosis of meningococcal infection.

The organisms in question tend to be slightly broader than the true influenza bacillus, but this is not a point on which much stress can be laid, for it is one that is difficult of determination. I have placed side by side in Plate XXI. Figs. 1 and 2, prints taken from the same negative but with different periods of exposure and development, which show this difficulty very well.

The organisms do show, however, bacillary and filamentous forms in culture more readily than does Pfeiffer's bacillus. They are also more readily recoverable from the heart blood and from the tissues than is the case with the organism of influenza. Moreover, they tend to kill laboratory animals by septicæmia rather than by toxæmia, and fatal results occur with much greater regularity than in the case of Pfeiffer's bacillus.

The investigation of such a series of hæmophilic organisms from the standpoint of their pathogenicity is not profitable, and it is probable, as Ritchie has suggested, that investigations into toxin production and into the serum reactions of immunised animals hold out a much better chance of differentiation.

I have to take this opportunity of expressing my indebtedness to Dr. Cohen of the Pasteur Institute in Brussels for sending me cultures of his organisms. I have also to thank Dr. Wilkinson for permission to make use of cases, for his kindness in providing me with material, and for the interest he has shown in the work throughout. For the use of Case 6 I have to express my heartiest thanks to Dr. Porter, to Dr. Yates for notes on the same case, and to Dr. Egerton Williams, through whose efforts I was enabled to carry out a full post-mortem.

REFERENCES.

1. BATTEN *Lancet*, London, 1910, vol. i. p. 1677.
2. BERTINI *Riv. di Clin. Pediatrica*, Firenze, 1904, vol. ii. p. 673 (Ref. given by Cohoe).
3. CANTANI *Ztschr. f. Hyg.*, Leipzig, 1896, Bd. xxiii. S. 265.
4. CHEATLE *Practitioner*, London, 1907, vol. lxxviii. p. 104.
5. COHEN *Centralbl. f. Bakteriöl. u. Parasitenk.*, Jena, 1910, Abth. I. Orig. Bd. lvi. S. 464.
6. „ *Ann. de l'Inst. Pasteur*, Paris, 1909, tome xxiii. p. 273.
7. COHOE *Am. Journ. Med. Sc.*, Philadelphia and New York, 1909, vol. cxxxvii. p. 74.
8. DAVIS *Arch. Int. Med.*, 1909, vol. iv. p. 323.
9. DELIUS UND KOLLE *Ztschr. f. Hyg.*, Leipzig, 1897, Bd. xxiv. S. 327.
10. DOUGLAS *Lancet*, London, 1907, vol. i. p. 86.
11. DUDGEON AND ADAMS *Ibid.*, 1907, vol. ii. p. 684.
12. FRAENKEL *Ztschr. f. Hyg.*, Leipzig, 1898, Bd. xxvii. S. 315.

190 MENINGITIS DUE TO HÆMOPHILIC ORGANISMS.

13. GHEDINI *Centralbl. f. Bakteriöl. u. Parasitenk.*, Jena, 1907, Abth. I. Orig. Bd. xliii. S. 407.
14. GHON *Wien. klin. Wchnschr.*, 1902, Bd. xxvi. S. 667.
15. HAEDKE *München. med. Wchnschr.*, 1897, Bd. xxix. S. 806.
16. M'DONALD *Journ. Path. and Bacteriol.*, Cambridge, 1908, vol. xii. p. 442.
17. PFEIFFER Vide Beck, "Influenza," in Kolle and Wassermann's "Handbuch der pathogenen Mikroorganismen," 1903, Bd. iii. S. 403.
18. „ *Ztschr. f. Hyg.*, Leipzig, 1893, Bd. xiii. S. 357.
19. RITCHIE AND M'DONALD *Journ. Path. and Bacteriol.*, Cambridge, 1909, vol. xiii. p. 119.
20. RITCHIE *Ibid.* 1910, vol. xiv. p. 615.

DESCRIPTION OF PLATES XXI.-XXIII.

PLATE XXI.

- FIG. 1.—Film preparation of culture, twenty-four hours old, taken from the case of Brook, and showing the coco-bacillary character of the growth.
- FIG. 2.—Print taken from the same negative as in Fig. 1, but with longer exposure and development. The organisms appear to be considerably stouter.
- FIG. 3.—Film preparation of culture, eighteen hours old, taken from the case of Machin. It presents much the same appearance as the Brook organism.
- FIG. 4.—Film preparation of culture, three days old, taken from the case of Machin. It shows the development of bacillary and filamentous forms.

PLATE XXII.

- FIG. 5.—Film preparation of culture, eight hours old, taken from the case of Turner, and showing more distinct bacillary development than do the Brook and Machin organisms.
- FIG. 6.—Film preparation of same culture as in Fig. 5, taken after twenty-four hours' growth and showing the early and marked development of filamentous forms.
- FIGS. 7. and 8.—Film preparation of culture, thirty-six hours old, taken from the case of Turner, and showing the formation of long wavy filaments.

PLATE XXIII.

- FIG. 9.—Film preparation of culture, twelve hours old, taken from the case of Marsden. It shows the formation of very long bacillary forms.
- FIG. 10.—Film preparation of culture, eighteen hours old, taken from the case of Marsden. Filaments are already much in evidence.
- FIG. 11.—Film preparation of the Marsden organism taken from culture twenty-four hours old, and showing long tangled wavy filaments.
- FIG. 12.—Film preparation of the Marsden organism taken from culture thirty hours old, and showing a tangled mass of filament.

(All the microphotographs are made from preparations taken from blood agar growths and stained with weak carbol fuchsin. The magnification in each case is 1500 diameters.)

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

FIG. 7.

FIG. 8.

FIG. 9.

FIG. 10.

FIG. 11.

FIG. 12.