CHAPTER IV.

THE BACTERIOLOGY OF INFLUENZA.

BY

SIR FREDERICK W. ANDREWES, M.D., F.R.S.

In the previous chapters of this report the general features of the epidemics of influenza have been described. In discussing those features which are conventionally termed epidemiological, the assumption has been implicit that some living organism is the essential " materies morbi " of influenza, and we have not attempted to define its precise nature. In the present chapter this question must be explicitly raised, and a review will be attempted of the bacteriological findings in the recent epidemic.

Nearly 80 years have elapsed since the last world-wide outbreak of the disease, and during this period notable advances have been made, not only in the technique of bacteriology, but in the interpretation of the data which it reveals. It will be convenient, in the first place, to note certain of the factors which have contributed to widen the outlook of the pathologist in face of the problems offered by epidemic influenza, and especially those which are 'relevant to the discussion which will follow.

In 1890, the bacteriologist who sought to establish the connection between a given microbe and a given disease was restricted to the fulfilment of what were traditionally known as Koch's four postulates. These conditions, laid down at a time when the "germ theory" of disease was on its trial and was the subject of hostile criticism, were designed to remove all possibility of doubt as to the causal relationship of a microbe to an infective disease. They have lost none of their force with the advance in our knowledge; indeed the germ theory of disease is now so firmly established that we are in danger of too readily accepting a microbe as the causal agent of a disease, and of neglecting the rigid proofs which should be demanded. The conditions laid down by Koch are to-day easier of fulfilment than they were in 1890 : our means of cultivation have been greatly improved, and our range of experimental animals has been extended not merely to monkeys and anthropoid apes, but, with increasing daring, to man himself where the risk could be, justified. We are, further, able to employ lines of evidence unrecognised when the postulates were laid down and lying beyond their scope. Since 1890, the whole science of " immunology " has been developed ; all the data which arise from the study of immunity reactions, from the results of specific serum treatment and of prophylactic inoculation, may

now justly be used in support of the causal relation of a microbe to a disease.

A second direction in which the horizon of the bacteriologist has widened lies in the fact that he is no longer restricted to the bacteria proper in his search for a pathogenic microbe. Many infective diseases have been found due to animal micro-parasites, while in not a few of our commonest fevers the causal agent is, on good evidence, though usually without the strict fulfilment of Koch's postulates, referred to the so-called " filter-passing" group of microbes; that is to say, to organisms so small that they are invisible, or barely visible, with the highest powers of the microscope, and are capable of passing through the pores of a filter which will keep back most, if not all, known bacteria. It need scarcely be said that evidence as to such invisible agents of disease must be even more jealously scrutinised than where a visible and readily-cultivable virus is in question.

There is a third direction in which much has been learned, and it is one of particular importance in relation to influenza. Even when the primary microbic cause of a disease has been established beyond question, it by no means follows that all the customary clinical phenomena of the disease are to be traced to this cause. On the contrary, they are often due to what are termed "secondary infections" by microbes other than that which is the primary cause, and such secondary infections may be fraught with greater danger to life than the primary infection itself. It is now well known that the healthy human body harbours on its cutaneous and especially on its mucous surfaces, a teeming and variable bacterial flora, many members of which are potential causes of disease. That they habitually produce no such effect depends upon two inversely-related factors—the resistance of the healthy body and the virulence of the microbes. Equilibrium of this nature is, however, readily upset : the resistance of the body may become lowered to a point at which even feebly-virulent organisms can invade it, or the virulence of the saprophytic flora may be raised to a point at which even the healthy body may be invaded. We know, too, that this saprophytic flora, and notably that of the respiratory tract, is in a constant state of flux. In crowded centres of population, individuals are constantly exchanging bacteria by a mechanism identical with that by which ordinary infections spread—in the main, so far as respiratory diseases are concerned, by the channel of "mouth-spray." If proof of this be needed, it is furnished by modern investigations into the prevalence of meningococcus carriers when cerebro-spinal fever is rife. The importance of secondary infections is well exemplified in such diseases as scarlet fever and measles. We do not, it is true, certainly know the primary cause of either of these fevers, though there is strong evidence in each case that it is a filter passer. But we have good reason for believing that neither of the

diseases, in an uncomplicated form, is a very serious affection, at least in this country. Their mortality is due to their complications, which depend upon secondary infections ; streptococcal and pneumococcal invasions are the usual direct causes of death. The effect of the primary virus is so to depress the resistance of the body as to enable these almost ubiquitous saprophytes to gain a foothold. Their virulence is enhanced by successful invasion of the tissues, and when such fevers are epidemic there is little reason for doubting that the very means by which the primary virus spreads from case to case serves, at the same time, to convey the dangerous agents of secondary complications in a state of increased potency for harm.

Such considerations as these must be kept clearly in view in a discussion of the primary cause of influenza, in which it is also true that it is the complications which kill—complications, too, which are largely identical with, and due to the same secondary infecting agents as, those of such a disease as measles. For where the primary virus is obscure and difficult of detection, it may easily happen that a secondary invader masquerades as a primary cause. A well-known example of such an error is offered by swine fever. Salmon and Smith described in this disease an organism which they termed the " hog-cholera bacillus," now known as B. suipestifer or B. choleræ suis. This organism, undoubtedly pathogenic for the pig, long passed as the cause of swine fever, till Dorset and Bolton demonstrated that the true virus was a filter-passer and B. suipestifer a secondary invader from the intestine. The latter organism will produce in the pig certain of the phenomena of swine fever, but the disease so caused does not spread to other pigs after the fashion of the genuine disease.

The preceding remarks have not been made at random, but to illustrate certain of the advances in knowledge, gained during the past few decades, which have deeply influenced the mental attitude of pathologists and bacteriologists in face of such a problem as that presented by epidemic influenza.

The History of Pfeiffer's Bacillus.

From a bacteriological point of view the outstanding result of the previous great epidemic was R. Pfeiffer's discovery of the organism associated with his name and which he called the "influenza bacillus." This discovery was not made at the time of the primary outbreak, but during a secondary epidemic wave in 1892 ; it was published in that year, but a more complete account was furnished by Pfeiffer in a paper entitled " The Aetiology of Influenza " in the *Zeitschrift fur Hygiene* in 1893. In this admirably lucid and well-written paper he describes how he found, in the sputum and pulmonary lesions of the cases which he studied, a minute Gram-negative bacillus in prodigious numbers. The difficulties attending the cultivation

of the organism were successfully overcome, and it was shown to require haemoglobin for its artificial growth. Pfeiffer failed to cultivate it from the blood of human cases, and he discredits Canon's observations to the contrary. He demonstrated the toxic effect of cultures upon the rabbit; guinea pigs and mice were found more resistant to the toxin : he endeavoured, though with indifferent success, to reproduce the disease in monkeys. He was unable to satisfy himself of any multiplication of the bacillus in his experimental animals; when death occurred it seemed due to a purely toxic effect.

Pfeiffer's observations aroused wide-spread interest, and his results were confirmed by others, but opportunity to put the causal relationship of his bacillus to the disease to more crucial tests disappeared, for the epidemic was at an end. The matter was therefore left in this position :—a new bacillus had been discovered, in connection with a disease not previously studied by bacteriologists, though only in a secondary wave of the epidemic. It had been shown to be toxic for laboratory animals, but the disease had not truly been reproduced in them : Koch's postulates had not been fulfilled. Nevertheless the close association of the organism with the lesions of the respiratory tract in human influenza and the profusion with which it was found in the sputum were so suggestive as to make out a *prima facie* case for Pfeiffer's bacillus as the cause of the disease. This conclusion was widely accepted and the "bacillus influenzae " took its place in bacteriological text books, perhaps with an admission that full proof of its relation to the malady was still wanting.

As commonly happens after a severe visitation of influenza, localised outbreaks of influenza-like disease, varying in extent and severity, have recurred at irregular intervals since the pandemic of 1890. Pfeiffer's bacillus was naturally sought for in such outbreaks, and not infrequently found. But often it was not found, and other pathogenic bacteria became recognised as the apparent causal agents in many of the minor epidemics, the evidence being of much the same order of validity as that attaching to Pfeiffer's bacillus in 1892. One has only to look at a list of the microbes which it is now deemed right to include in a " polyvalent catarrh vaccine " to realise how many are the organisms which now appear to be associated, and commonly on fair circumstantial evidence, though without the strict fulfilment of Koch's postulates, with febrile catarrhs of a more or less "influenzal" nature. Amongst the ingredients of such a vaccine we find not only Pfeiffer's bacillus, but streptococci, pneumococci, micrococcus catarrhalis and its allies, staphylococci, Friedlander's bacillus and B. septus. And we may well begin to wonder whether an affection, which has some claim to be regarded as a clinical entity, can possibly own such a variety of causal agents; for it is clearly possible that they may be of only secondary importance, the primary cause of febrile catarrh

being still unknown. The latter view is supported by Kruse's experiments on the transmission of " common cold" by material which has passed through a Berkefeld filter; similar experiments have more recently been carried out by Foster, and with even greater success. Should febrile catarrhs be proved primarily due to a filter-passing virus, the importance of the bacteria just enumerated would not be disproved, though they would be relegated to a secondary position. The prophylactic value of polyvalent anti-catarrh vaccines is thought by many to be not inconsiderable, though no trustworthy statistics on the subject are forthcoming.

The question, however, which is here at issue concerns the relation of Pfeiffer's bacillus to influenza and the light which has been shed on the subject since its discovery. A primary difficulty with which we are confronted at the outset lies in the difficulty of defining influenza. What is the relation between pandemic influenza and the minor epidemics of somewhat similar character, which occur irregularly during inter-pandemic periods, and between these, again, and febrile catarrhs ? It is probable that the most likely person to answer this question is the epidemiologist, for it is in the "natural history " of these respective visitations that their most conspicuous differences lie. The clinician is baffled by the fact that the affections shade off into one another in such fashion that he can draw no clear line between them. The bacteriologist, who ought to be able to answer, is on the horns of a dilemma : for until the clinician can tell him the difference between epidemic influenza and a febrile cold he cannot sit in judgment on Pfeiffer's bacillus ; and until he knows whether or not this organism is the primary cause of influenza, he cannot, by bacteriological means, decide between the two affections.

The following facts concerning Pfeiffer's bacillus may, however, be regarded as having been established in the course of the last five and twenty years :—

(1) It is not infrequently cultivated from swabbings taken from the pharynx of normal persons, living there as a temporary and apparently harmless saprophyte.

(2) It is still more commonly found in catarrhal conditions of the respiratory tract, and its presence, under such circumstances, may, or may not, be associated with fever and constitutional symptoms.

(3) It is familiar to laryngologists and rhinologists as the apparent causal agent of many chronic inflammatory conditions of the throat, nose and accessory sinuses.

(4) Certain cases of meningitis have been associated with the presence of this bacillus or of one very closely related to it.

(5) In rare instances it has been found to be the exciting cause of malignant vegetative endocarditis.

These facts at least show that Pfeiffer's organism is a not uncommon pathogenic agent in man, and one more especially related to catarrhal affections of the respiratory tract. They do not, on the other hand, disprove its causal relation to epidemic influenza. It might be argued that the original bacillus discovered by Pfeiffer was only one of a group of closely-related organisms, that it was the true cause of epidemic influenza and that the febrile catarrhs of inter-epidemic periods were due to other members of the group. Such an opinion, was, indeed, put forward by Pfeiffer himself, who found in certain catarrhs and broncho-pneumonias not associated with influenza, strains of bacilli related to B. influenzæ., but in his opinion separable from it by certain slight morphological and cultural differences : he called such strains " pseudo-influenza bacilli." Many bacteriologists would agree that we may be confounding under the common term, B. influenzas, a number of closely related species. Bordet's " whooping cough bacillus " would fall into such a group, and the bacillus described from meningitis cases is said to present certain differences from the classical B. influenzae. Very little serious systematic work has so far been attempted on this group of minute hæmophilous bacilli.

Or again, it might be argued that, even if all the forms of the bacillus encountered in non-epidemic times were referable to a single species, this might comprise a number of serological races, and that true epidemic influenza was due to the spread of some race of peculiar virulence. Such a hypothesis would be supported by the facts which have come to light concerning cerebro-spinal fever, in which epidemic outbreaks have been shown to be associated with the spread of certain types of the meningococcus (Gordon), or again by the relation of certain definite types of pneumococci with lobar pneumonia. It would, however, be pure hypothesis, supported only by such analogies as these, for the "influenza group" of bacilli does not readily lend itself to serological study: until the recent outbreak no facts were forthcoming as to the existence of well marked serological races amongst these organisms.

Looking back upon the experience gained between 1890 and 1918 it cannot be said that the status of Pfeiffer's bacillus as the cause of epidemic influenza had materially altered. No further confirmation had been obtained, nor had the observed facts disproved a possible relationship. Nevertheless the establishment of its widespread occurrence in non-epidemic times, often under conditions having no apparent relation to influenza, had rendered bacteriologists somewhat more critical of its claims—claims which, it was felt, could only be decided when a new pandemic arose.

Pfeiffer's Bacillus in the Outbreak of 1918.

It was therefore with considerable interest that the bacteriological results in the recent epidemic were awaited. They have

been conflicting, especially so during the summer outbreak, but it is now possible to take a general survey of the reports published from many parts of the world. There is a striking lack of unanimity in the opinions expressed by different workers, in different countries, and, it may be added, at different periods of the epidemic. Competent bacteriologists in France, America, Germany and a few in this country, either failed to find the bacillus or found it in a proportion of cases so small as seriously to damage its reputation. • This was particularly the case during the outbreak in the summer of 1918 : in the autumn outbreak it was more commonly found. On the other hand many British bacteriologists, working both in this country and with our forces in France, were able to find the bacillus in so many cases that they were convinced of its aetiological role in the disease.

The first question which arises concerns the validity of the negative results recorded. Pfeiffer's bacillus is admittedly not an easy organism to cultivate, and its colonies on most media are small and readily overlooked ; but thanks to Pfeiffer's researches and to his discovery of its hæmophilic proclivities, the methods of successful culture had long been well known. Shortly before the summer outbreak, Levinthal had published an account of a new medium, made with boiled blood agar, which greatly facilitated the cultivation of the bacillus, while Matthews, a little later, also described a medium, made by the addition of trypsinised blood to agar, on which the bacillus grows with exceptional luxuriance. Though some workers may have used these media during the summer epidemic, it is probable that the majority employed ordinary human blood agar. Some of the failures to grow Pfeiffer's bacillus are perhaps due to this cause : it is certain that during the autumn outbreak, when the new media were more widely known and used, the proportion of cases in which the bacillus was found and of observers who found it, was greater than had been the case in the summer.

Such an explanation of the facts recorded is, however, unconvincing. Pfeiffer's bacillus can be cultivated by an experienced bacteriologist without the aid of the new media, useful as they have proved. Apart from cultivation, the bacillus, when present in influenza sputum or in the lung-juices, is usually there in immense numbers, and is so readily found in well stained films that a competent observer could hardly overlook it. Amongst these who have recorded their failure to find the organism are bacteriologists of the highest repute : the failures were perhaps more numerous in Germany than elsewhere : such men as Kolle, Gruber, Friedemann and many others failed to find it, while Uhlenhuth and Pfeiffer himself were puzzled at their want of regularity in finding it. It is expressly stated by Sobernheim that, using precisely the same technique, he uniformly failed to find it in the summer epidemic and almost uniformly succeeded in October,

But even in Germany some observers reported the common presence of Pfeiffer's bacillus, for instance, Simmonds at Hamburg, and Dietrich in the German Army, while in the autumn many found it. In England it was commonly found, even from the first: Matthews got it in every one of a dozen cases, and Mcintosh found it frequently. In the British Armies in France it was the organism most regularly and abundantly present, though the figures available refer more particularly to the autumn epidemic. Gibson and Bowman record it in 68 per cent, of the bronchial fluids examined, 32 per cent, of the lungs, 85 per cent, of the tracheal scrapings, and 20 per cent, of the pleural fluids. Tytler, Janes and Dobbin found it in 56 out of 67 cases examined, and Patterson, Little and Williams, in 46 cases, failed only once to find it in the bronchi and lungs. The French bacteriologists, in Paris and elsewhere, found it commonly in autumn, though by no means always in the summer. In the United States observers were divided in their opinions. The Danish observers, in a report published by the Serum Institute at Copenhagen sum up against Pfeiffer's bacillus as a cause of influenza. They found it in only 37 per cent of the cases. In Buda-Pesth it was found in abundance, and in Italy it was frequently met with. In the sudden outbreak at Johannesburg in South Africa, Lister found it in the lungs in 53 out of *56* fatal cases. It may be added that Pfeiffer's bacillus has repeatedly been cultivated from the blood, during the epidemic, but only in a small proportion of cases.

The foregoing is only a brief summary of some of the more important records. It makes no pretence at completeness, but it suffices to give a fair idea of the findings. Put still more shortly, it may- be said that during the earlier outbreak of the disease in the summer of 1918 Pfeiffer's bacillus was found in some countries in a large proportion of the cases, sometimes in nearly all. In other countries it was sometimes found and sometimes not; in one country—Germany—it was found only exceptionally during the summer. Our troops in Mesopotamia experienced both the summer and autumn epidemics in 1918, yet Dr. Ledingham, who was then acting as pathological consultant, informs us that Pfeiffer's bacillus was not found during the epidemic in that country. In the autumn epidemic it was found more regularly everywhere, even in Germany.

The evidence does not carry conviction as to the primary causal relationship of Pfeiffer's bacillus to epidemic influenza. The varying results cannot be correlated with the competence of the observers. The observed facts, notably the earlier negative findings in certain localities, and the more general positive results in the autumn outbreak, would be better explained on the assumption that the bacillus played a very important secondary role in the disease, and was not the primary infecting agent. So far as the evidence derived from association with influenza can take us, the case for Pfeiffer's bacillus must still

be deemed unproven as regards its primary causal agency, though, its intimate connection with the disease is even more firmly established than before.

Association with the disease is not, however, the only field in which evidence may be sought. Attempts have been made to support the ætiological role of Pfeiffer's bacillus by a study of the specific immunity reactions. It has been shown that specific antibodies arise in the blood during the course of influenza. The evidence is clear that, both by agglutination and complement fixation, such antibodies, specific for Pfeiffer's bacillus, are demonstrable in a considerable proportion of sufferers and convalescents from influenza. Thus Hartley examined 42 cases, and the serum of 27 of these agglutinated Pfeiffer's bacillus in a dilution of at least 1 in 50. Control normal sera failed to do this. Gibson and Bowman obtained very similar results ; several of their cases showed agglutination with a serum dilution of 1 in 640. What is true of agglutination is true also of complement fixation, in regard to which Wollstein's observations may be cited. In the case of many diseases such observations would, be of high significance, but we cannot attribute to them much importance as evidence of the primary role of Pfeiffer's organism in influenza. For it is of such common occurrence in the disease, as a definitely pathogenic invader, that it would be strange indeed if antibodies were not formed against it. As a matter of fact similar antibodies have been found in influenza, when looked for, against other bacteria, such as streptococci, which are admitted to be merely secondary infecting agents.

In another direction, however, these immunological studies in influenza have proved of interest, for they distinctly suggest a wide range of antigenic variation in the bacillus recovered from different cases in the same epidemic. Of Hartley's cases some agglutinated certain strains and not others. Valentine and Cooper, reporting on agglutination and absorption tests on 171 strains in New York, could hardly find two which were serologically alike a result which suggests that their methods were too delicate to be of practical value. The results of the complement fixation test have also indicated more than one race of the bacillus.

Further evidence may be sought in the effects of attempted immunisation against influenza by means of vaccines containing Pfeiffer's bacillus. The results of some of these efforts, on a sufficiently large scale, have now been published, *e.g.*, figures from Australia, from the Home Forces of the British Army, and from New Orleans. The true significance of the figures, which at first sight are striking, is a matter for expert statistical analysis, and will be discussed elsewhere in this report. The figures appear to show a considerable decrease in the attack rate amongst the inoculated, as compared with uninoculated controls, a still greater decrease in the liability to serious pulmonary complications, and an extraordinary diminution in the fatality of

the disease which, in the British Army, was only 0.12 per 1,000 in the inoculated as against 2.25 per 1,000 in the uninoculated. The death rate figures cannot be held relevant to the primary causal role of Pfeiffer's bacillus, which is admittedly responsible for a large proportion of the fatal pulmonary complications of influenza, even if it is held to be a secondary invader. If, however, a due statistical analysis of the figures for the attack rate shows them to be significant, they will constitute the strongest argument yet brought forward in support of the bacillus as the primary cause of influenza.*

Attempts to convey influenza to human volunteers by spraying the nose and pharynx with pure cultures of Pfeiffer's bacillus, recently isolated from fatal cases of the disease, have been carried out by Lister and Taylor in South Africa, and by Wahl, White, and Lyall in America. The experiments were on such a small scale that the negative results recorded can hardly be regarded as of serious significance, especially as the subjects of the experiments were, in both cases, for one reason or another, unsuitable ones.

Evidence as regards a Filter-passing Virus in Influenza.

When, as many believed, Pfeiffer's bacillus was discredited by the findings in the summer outbreak in 1918, the attention of numerous observers was directed to the possibility of a filter-passer as the primary cause of the disease. It was, indeed, difficult to think of any other alternative, for Pfeiffer's bacillus was the only known organism with any serious claim to this status. The characters of the so-called "Diplococcus epidemicus," described by some bacteriologists, can hardly be regarded as differentiating it from previously known inhabitants of the normal respiratory tract.

During the year 1918 a number of different observers recorded experiments with material from influenzal cases filtered free from ordinary bacteria. Nicolle and Lebailly first tested the unfiltered sputum from a case by introducing it into the conjunctiva and nostrils of two monkeys. After an incubation period of six days the animals developed fever lasting three days, after which they remained thin and depressed. The blood of the monkeys, injected into a man, produced no effect. The sputum from influenza cases, diluted with saline, centrifuged and passed through a Chamberland L 2 filter, was injected into one man subcutaneously and into another intravenously. No effect was produced by the intravenous injection, but the man subcutaneously inoculated, after an incubation period of six days, developed headache and an evening rise of temperature lasting 11 days. The blood of this case, injected into another man, produced no effect.

De la Riviere took blood from four influenza cases, proved its sterility on culture, and passed it through a Chamberland

* *Vide* pp. 64-5,149, 175-6, 194.

L 3 filter ; he injected 4 c.c. of the filtrate under his own skin, and after three days got headache, muscular pains, and slight fever lasting a few days ; later his pulse became irregular.

Selter sprayed his own throat, and that of another person, with a saline filtrate of material from, the throats of influenza cases ; both developed mild but typical influenza.

Leschke filtered sputum, lung juice, and similar material from influenza cases, diluted with saline, first through three layers of hardened filter paper and then through a Chamberland or Berkefeld filter. The experiments were controlled by cultures on ordinary media. He claimed to cultivate a filter-passer which grew as masses of small round bodies ; he could not grow it beyond the first generation. After many unsuccessful attempts, be finally succeeded in infecting human beings by spraying. Typical influenza was produced the same day or on the day following, with fever and tracheitis. On one occasion two people who nursed the experimental subject developed influenza.

Gibson, Bowman, and Connor carried out an elaborate series of experiments at Abbeville during the height of the epidemic amongst the British Expeditionary Force. They used animal experiment only, justly remarking that human experiments during the epidemic would have been of little value. As a criterion of a positive result they relied, on hæmorrhagic and inflammatory changes in the animals' lungs identical with those seen in early fatal cases of influenza in man. The animals employed were monkeys, baboons, rabbits. guinea-pigs, and mice, and, with all these, successful results were obtained. The technique of the filtration experiments was carefully controlled. A Chamberland L 1 bis or F was employed. In monkeys, the inoculations were carried out subconjunctivally or by nasal instillation, or both ; in rabbits, intravenously or subcutaneously; in guinea-pigs and mice, subcutaneously. With filtered material, free from ordinary bacteria, positive results were obtained with sputum from early cases, but less easily in later ones ; controls with sputum from ordinary acute bronchitis were negative. Filtered blood from influenza cases gave a positive result in a mouse and a doubtful one in a monkey ; unfiltered blood readily produced positive results on mice. Passage experiments from animal' to animal were successful; three rabbits in series yielded positive results, and an increase of virulence during passage was noted. The virus was also submitted to culture by Noguchi's method, and apparent growth of small coccoid bodies (from 0.1 to 0.2 in diameter) was obtained and carried on in subculture ; with these cultures positive results were obtained on a baboon, a monkey, rabbits, guinea-pigs, and mice, filtered and unfiltered extracts of the lungs of some of the animals thus affected again yielding positive results in further animals. The coccoid bodies were cultivated from the kidneys of infected

animals, filtrates of lung tissue, and filtered sputum from cases of influenza. In all successful animal inoculations the symptoms appeared on the fifth to the seventh day; the clinical symptoms were usually slight in comparison with the severity of the pulmonary lesions found after death; the main lesion was a patchy hæmorrhagic condition, with oedema. Broncho-pneumonia was never observed.

In Japan, Yamanouchi, Sakakimi, and Iwashima, made an emulsion of the sputum of 43 influenza cases in Ringer's solution. This was injected, unfiltered, into the throat and nose in 12 healthy persons, and a Berkefeld filtrate of the emulsion into 12 other healthy persons in a similar manner. All, except six who had recently suffered from influenza, developed the disease after an incubation period of two or three days, those receiving the filtered emulsion equally with the others. A filtrate of the blood of influenza cases, injected into the throat and nose of six healthy persons, produced influenza in all. Subcutaneous injection of filtrates of blood and sputum into eight persons equally produced the disease, except in one person who had recently had it. On the contrary emulsions of Pfeiffer's bacillus, alone and mixed with pneumococci, streptococci, &c, injected into the nose and throat of 14 normal persons, produced no effect at all.

Lister and Taylor experimented in South Africa, in February and March 1919, with filtered and unfiltered nasal washings and sputum from cases of influenza, and also with cultures of Pfeiffer's bacillus, using human volunteers and monkeys as the subjects of the experiments. The epidemic had already swept the country, and the 11 volunteers, who had escaped the disease, were presumably men of high resistance. With unfiltered sputum they succeeded in infecting two men out of five, the incubation being 36 hours, but neither men nor monkeys were affected when filtered material was used; nor did the application of living cultures of Pfeiffer's bacillus yield more than a doubtful indisposition in a single case only.

In Germany, v. Angerer claimed to find and grow a filter-passer in influenza, but he does not record experiment on man or animals. Binder and Prell also claim to have found and grown a filter-passer which they term "Aenigmo-plasma influenzae."

While it is apparent that some of the observations summarised above are suggestive of a filter-passer as the cause of influenza, it cannot be said that any of them offer conclusive proof of such a proposition. Human experiment, in presence of an epidemic is a somewhat dangerous guide, while animal experiments, however similar the appearances produced, leave us in some doubt as to whether the disease caused was truly epidemic influenza. If we compare the description of the pulmonary lesions upon which Gibson, Bowman, and Connor relied as evidence that they had reproduced the disease in

animals with material which had passed through a filter, with that given by Wollstein of the lesions caused in her experimental animals with filtrates of cultures of Pfeiffer's bacillus, it is noticeable that they are largely identical. True, there was no long incubation period in Wollstein's experiments, but' she injected large doses of her toxic filtrates intravenously. Oedema, and hemorrhagic patches in the lungs, and congested or hæmorrhagic trachea and bronchi, were present in the one set of experiments as in the other. Indeed, all who have experimented with the toxin of Pfeiffer's bacillus, which appears to be a soluble one, bear witness to its selective hæmorrhagic action upon the lungs.

The strength of the case argued by Gibson and his colleagues lies in the facts that there was a definite incubation period in their experiments, that they succeeded in carrying on the effects through more than one generation of animals, and that they produced them with cultures. The weakness lies in the fact that the lesions in their animals do not afford convincing proof of influenza. The incubation period in their experiments was longer than in the naturally occurring human disease, and this is true also of the experiments of Nicolle and Lebailly, though not in those of de la Riviere, of Leschke, and of the Japanese observers.

The cultivation of filter-passing organisms is notoriously a difficult thing, and beset with many fallacies, as Arkwright has well pointed out in his recent criticism of the subject. The results so far recorded in influenza must be received with caution.

It is plain that far more data are required before we can fully accept a filter-passing virus as the primary cause of influenza. The most that can be said at the present moment is that a fair case has been made out for granting the possibility, some would even say the probability, of such a cause.

Secondary Infections in Influenza.

Whatever opinion may be held as to the primary cause of influenza, the organisms responsible for the infections of the respiratory tract, to which its chief terrors are due, are well known. They are Pfeiffer's bacillus, the pneumococcus, streptococci, and especially streptococci of hemolytic type, and more occasionally staphylococci, and other organisms to be mentioned in due course. Pfeiffer's bacillus may be placed in this category without prejudice to its possible claims to be regarded also as the primary cause of the disease.

Uncomplicated influenza, *i.e.,* fever and toxic constitutional symptoms without serious involvement of the lower respiratory tract, is not uncommonly seen in individual cases during an epidemic, though some degree of catarrh is the rule, so that

. bronchitis would be included in any account of the symptomatology of the affection. The uncomplicated disease, though annoying and painful, and often attended by prostration and followed by prolonged cardiac debility, is rarely of itself fatal. Exactly the same is true of measles and scarlet fever ; death from these affections is almost invariably due to complications
1 set up by secondary infections with microbes which are well known, and are certainly not the primary infecting agents. In all cases, including influenza, the secondarily infecting bacteria are species commonly found as saprophytes of the respiratory tract or fauces in normal persons. The normal resistance of the healthy body prevents any harmful invasion. But in the presence of acute infection of the respiratory tract by the specific microbes of the zymotic diseases in question the lowered resistance of the body is apt to permit of local, or even general invasion by these saprophytes, which are potentially pathogenic ; and the invading bacteria will be those which chance to be present, or predominant, in the affected individual. That is to say, the secondary invasion may be a different one in two separate cases of the same disease, different in two different localities during the same epidemic, different, on the whole, in different epidemics. Moreover, when once such a secondary invader has achieved success, it tends to become exalted in virulence by the ordinary mechanism of animal passage and, by repetition of the process, still further exaltation of virulence may be brought about till it can attack healthy persons. For the secondary agents which lead to the respiratory complications of influenza are present in the bronchial secretions and have the same opportunities for spreading from case to case as the primary causal agent. There is even reason for the belief that the secondary infecting agents may, under favourable conditions, go on spreading in epidemic fashion, after the primary infecting cause has disappeared. In the opinion of Macallum, the pneumonias which devastated some of the military camps in the United States began as complications of epidemic measles, but later spread as epidemic pneumonias after the measles had ceased. It is thus clear that the phenomena presented by the recent pandemic of influenza should be regarded from a double point of view; the outbreak of influenza, *per se*, is not necessarily the same thing as the outbreaks of respiratory complications which accompanied it. There were, so to speak, epidemics within the epidemic. It is precisely such considerations as these that render it so extremely difficult to determine the position of Pfeiffer's bacillus in relation to the outbreak as a whole.

Whether or not we assign to Pfeiffer's bacillus the primary or secondary role, there can be no doubt that it played a part of vast importance in the recent epidemic of influenza. Though we know it, at normal times, as a mere agent of catarrh, the more virulent types which prevail in epidemic times are more

highly toxic and seem especially to lead to escape of blood from the smaller vessels in the lungs and bronchi. It seems probable that we must regard Pfeiffer's bacillus as the chief cause of the hæmorrhagic oedema and the localised hæmorrhagic areas in the lungs which have been so conspicuous a feature of the late epidemic.

Next in frequency and importance, as secondary infections in influenza, come the *streptococci* and here all are agreed that at least two types are concerned. If, as most bacteriologists have done, we group them according to Schottmuller's classification by their characters on blood agar, we find that his *streptococcus viridans*, representing the types of feeble virulence habitually present in the respiratory tract, has been very prominent, often exceeding in abundance any other organism in the pulmonary secretions. It is unlikely that streptococci of this type have played a part of much importance in the phenomena of the epidemic ; they are notoriously of low pathogenic power, and their abundance merely reflects their normal frequency in the bronchial secretions. It is otherwise with the *streptococcus hoemolyticus*, an organism which probably corresponds, in great part, with that more usually known as streptococcus pyogenes. The presence of this streptococcus in the pulmonary lesions has been a distinguishing feature of the epidemic, especially in the autumn when the disease exhibited its highest fatality, and it was found in the worst cases. It was responsible, in conjunction with Pfeiffer's bacillus, for the hemorrhagic lesions in the lungs, and it was more frequently found than Pfeiffer's bacillus in the hæmorrhagic pleurisies and empyemata which were of common occurrence.

The *pneumoeoccus* does not appear, from most reports, to have played such an important part, as a secondary infection, as either of the preceding, though it was not infrequently found in some countries. Although pneumonias, catarrhal, interstitial and sometimes lobar, were not uncommon, the pulmonary lesion regarded by most histologists as characteristic of the epidemic was a hemorrhagic oedema rather than a pneumonia. Considerable variation occurred, however, at different centres, in the frequency with which pneumococci were found.

Staphylococci were noted by Tytler and his colleagues at No. 3 Canadian General Hospital in France, as the most abundant organisms in the bronchi after Pfeiffer's bacillus. This experience does not seem to have been a general one, though these common organisms were naturally found at times elsewhere. The same observers note the infrequency of hemolytic streptococci in their findings.

The *meningococcus* was noted in a certain number of cases in this country, and notably in a group of cases in Scotland and in cases landed from American transports at Southampton. It is probable that no great significance attaches to this finding;

when influenza attacks a person who happens to be harbouring the meningococcus in his nasopharynx, it is not unnatural that the organism should multiply in the respiratory tract, but there is no evidence that it played any important part in the pathological processes set up.

This list of secondary infecting agents does not, of course, represent all the organisms isolated from the lungs during the epidemic, but it includes all those which can be regarded as of serious import in the respiratory complications of the disease. Our information about the bacteriology of other complications is too fragmentary to be summarised here, though it is probable that the toxins of Pfeiffer's bacillus exercised an important influence on certain organs such as the heart.

Before leaving the subject of secondary infections in influenza, mention must be made of the terminal septicaemias which were not infrequently observed. Blood cultures were carried out by large numbers of observers. The majority of results were by all accounts negative, but the larger amounts of blood now taken, as compared with the practice in 1890, yielded a certain harvest of positive results. The organisms usually found in the blood were streptococci, both of the viridans and hæmolytic type, pneumococci and Pfeiffer's bacillus. Several writers explicitly state that their positive results were most frequent in advanced and severe cases, and it is a fair conclusion that they represent an overflow into the blood from the pulmonary lesions rather than a primary septicæmia. It is perhaps, too, not without significance that Pfeiffer's bacillus was not more often found in the blood than other organisms admitted to be secondary invaders.

Summary.

A survey of the evidence obtained during the epidemic of 1918 as to the bacteriology of influenza appears to lead to the following conclusions:—

(1) The position of Pfeiffer's bacillus as the primary cause of the disease has been in no way strengthened. Its case remains unproven, and the crucial tests to which it has been submitted seem to indicate it rather as a secondary infection of the highest importance and significance than as the primary " materies morbi." At the same time it cannot be asserted that, as a primary cause, it is wholly out of court.

(2) The evidence for a filter-passing virus as the primary cause of the disease is suggestive, but at present a final verdict cannot be given.

(3) The complications to which the epidemic has owed its abnormal fatality have been due to secondary infections, in which Pfeiffer's bacillus and the haemolytic streptococcus have played a predominant part.

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