OCCURRENCE AND CLASSIFICATION OF THE HAEMOGLOBINOPHILIC BACTERIA

EMIL JACOBSENS BOGTRYKKERI KBHVN.

INVESTIGATIONS INTO THE OCCURRENCE AND CLASSIFICATION OF THE HAEMOGLOBINOPHILIC BACTERIA

BY

MARTIN KRISTENSEN

CHIEF OF DEPARTMENT AT THE STATE SERUM INSTITUTE

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Immediately after the „Spanish illness“ had arrived in Denmark in the beginning of July 1918, an investigation was set on foot at the State Serum Institute into the bacteriology of the disease.

As a collaborator in this work I succeeded in demonstrating PFEIFFER'S „Influenza Bacillus“ , few days after the beginning of the investigation in 5 of the patients from whom samples were taken. This finding directed the bacteriological investigations, which were undertaken under the direction of the Chief of the Department at that time, Dr. OLUF THOMSEN, to a large extent towards demonstrating Pfeiffer's bacillus in influenza and other diseases, as well as in healthy people.

I continued these investigations in the following years, for the first few months in collaboration with Dr. THOMSEN and afterwards independently. The original object was only to obtain as comprehensive an idea as possible of the occurrence of the bacillus here in Denmark in the various phases of the pandemic and after it had ceased. But another question soon claimed my attention to a greater extent. It appeared that while the colonies of Pfeiffer's bacillus from influenza patients were always of very characteristic appearance in cultures and easy to distinguish from colonies of other species of bacteria and the microscopic picture did not usually display great differences, the conditions were more complex in the case of samples from healthy persons as colonies of haemoglobinophilic bacteria could vary considerably more, both in macroscopic and in microscopic appearance, making it difficult to judge where the boundary between Pfeiffer's bacillus and other bacteria ought to be placed.

At that time there was very scanty information in the literature bearing upon the differences between the individual strains of Pfeiffer's bacillus and upon possible connecting links between this and other kinds of bacteria. The task of obtaining some enlightenment concerning the classification of the haemoglobinophilic bacteria therefore immediately suggested itself. The following points came up for consideration:

1. How is Pfeiffer's bacillus to be distinguished from other kinds of bacteria?

2. Are well-characterised individual differences to be found between the various strains and if so what are they?

3. If the different strains of Pfeiffer's bacillus cannot be regarded as identical among themselves, can they then be divided into clearly defined groups or do all possible transitions exist?

4. Can any relation be demonstrated between the location of Pfeiffer's bacillus and its characters? For example are Pfeiffer's bacilli from influenza distinct from those found in healthy people or in whooping-cough?

Such an investigation was required not only as a link in the ordinary natural description of micro-organisms but, as will be further explained in this work, it as also of considerable importance as regards the question which has been so much discussed since the beginning of the last pandemic, namely the relation of Pfeiffer's bacillus to influenza.

In order to elaborate a classification which should be as natural, that is to say, unfortuitous as possible I have made a point of examining a considerable number of cultures of different origin and of investigating every culture in as many different ways as possible. This work is therefore mainly of a statistical nature. To make it practicable it was necessary to employ very elementary methods of investigation.

Concurrently with the carrying out of my investigations a rather extensive literature covering the same ground has appeared; and it appears that the various authors have largely arranged their investigations on similar lines to those I had in hand before I became acquainted with the works in question. On the whole my results agree with those recorded by others. I have therefore not been able to bring forward much which at the present moment can be regarded as new, but

claim in different ways to have carried out more thorough investigations than those which at present exist, and also to have suggested fresh points of view.

A very important point in all investigations relating to the occurrence of Pfeiffer's bacillus is the technique of cultivation particularly the constitution of the nutritive medium itself. As there have latterly been introduced a large number of new nutritive media for this microbe, media which to some extent constitute a considerable advance, and since no connected critical review has hitherto been attempted dealing with all these media and with the technique of cultivation in its entirety I have felt impelled to try to give such a review, although it is a little outside the scope of the principal theme of my work, but partly also in connection with my own experience in this field.

Perhaps I should expressly state that it has not been my purpose to undertake an investigation of the biology of the haemoglobinophilic bacteria in general and particular regard has therefore not been taken to such points as were not so suitable for the classification. Still less it has been my object to deal with the whole question of the bacteriology of influenza to say nothing of the pathological anatomy, clinical aspect, and epidemiology of the disease.

I must express my best thanks to my Chief, Dr. Th. Madsen, for the excellent facilities for working put at my disposal and for the help he has given me in various ways in my work. I am also indebted to Prof. Oluf Thomsen for urging me to take the subject up, for much good advice, and for the interest with which he has followed the work especially in the early and most difficult period.

Through the liberal and sympathetic attitude which the army medical corps has consistently displayed towards the Serum Institute it has been possible to undertake investigations of considerable extent in the army both among influenza patients and healthy soldiers. For permission to do this I must express my hearty thanks first and foremost to General Ammentorp of the army medical service, and also to the Chiefs of the garrison hospital, particularly to Dr. Knudsen (Staff-Doctor) and Dr. Schiødte (Senior-Doctor).

I have also been in a position to carry out extensive inve-

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I am further much indebted to the Senior Physician, Dr. TOBIESEN, Prosector R. FOGH, Prof. BIE, Assistant Physician, Dr. M. CHRISTIANSEN, and Dr. ADOLPH MEYER for permission to use material from the Øresundshospital, the Blegdamshospital and Dr. MEYER's Clinic as well as for the help both they and the nursing staffs of the hospitals have extended to me in the collection of sputum and other material. Finally I thank Prof. CARL HANSEN for allowing me to carry out investigations at the canine clinic of the Veterinary Institute.

A small part of the contents of this paper has already been published in the following contributions:

OLUF THOMSEN, MARTIN KRISTENSEN, & FR. THORBORG: Undersøgelser over Influenza'ens („den spanske Syge"s) Aarsagsforhold. Copenhagen 1918.

MARTIN KRISTENSEN: Undersøgelser over Forekomsten af Pfeiffers Influenzabacil. Hospitalstidende 1919, 777 and 801.

OLUF THOMSEN & MARTIN KRISTENSEN: Recherches sur la bactériologie et l'épidémiologie de l'influenza de 1918—1919 („la grippe espagnole"). Acta medica scandinavica 53, 421 (1919).

MAX CHRISTIANSEN & MARTIN KRISTENSEN: 1) Tre Tilfælde af Meningitis, fremkaldt af Pfeiffers Bacil. Ugeskrift for Læger 1921, 551.

2) Quatre cas de méningite provoqués par le bacille de Pfeiffer. Acta medica scandinavica 55, 297 (1921).

MARTIN KRISTENSEN: Sur l'apparition du bacille de Pfeiffer dans une épidémie de grippe à Copenhague, Janvier 1922. Comptes rendus de la Société de Biologie, 86, 464 (1922).

Historical Review.

Introduction.

The most important advance in our ideas of the causation of influenza was made in connection with the pandemic 1889—92 when a number of different „miasmatic" theories had to give way to the view that influenza was spread exclusively by infection from man to man, and principally by direct transmission from a patient to a healthy person.

A singular and discouraging fate has however involved our knowledge of the microbe of the disease. The influenza pandemic referred to was the first which fell within the bacteriological era and it was a tempting task for medical bacteriology, still in its infancy, to discover the specific microbe of the disease. R. PFEIFFER (1,2) also apparently succeeded in completely raising the hitherto impenetrable veil which had obscured the solution of the problem for numerous other observers, by immediately finding in the first cases of influenza he investigated during a fresh outburst of the pandemic at the end of November 1891, a small Gram-negative rod, constantly present in large numbers, which on closer study proved to have the previously unknown characteristic of only growing on blood-containing or haemoglobin-containing media. The proofs he advanced that this organism was really the virus of influenza satisfied the requirements which were thought necessary at that time, as many of the pitfalls of bacteriological research which have later become evident were at that date still unknown.

Nevertheless the innumerable investigations on the bacteriology of influenza which, especially during the last pandemic

(1918—20), were carried out on a very large scale, have in so far lead to a negative result, that now in 1922 we are very far from being so well acquainted with the virus of influenza as they thought they were 30 years ago.

As is well known influenza is one of the diseases which are most difficult to define from the purely clinical standpoint (as well as from the pathological). Apart from the bacteriological finding we have only a single reliable sign of true influenza as opposed to other catarrhal infections with similar symptoms, — namely its occurrence in the form of pandemics, which by reason of the great infectivity of the disease, are characterised by a more intense, rapid, and extensive dissemination than any other epidemic disease. During the whole of the period intervening between 1892 and 1918 the view has been held that, as regards all the catarrhal infections, which in daily language are called „influenza" (and which will be referred to henceforward as „influenza" in inverted commas) and which it is impossible clinically to sharply define, we ought to consider the bacteriological factor as decisive and call the disease produced by PFEIFFER's bacillus true influenza, while all cases where this organism is not found should not be included under the term influenza although epidemiologically, clinically, and pathologically it is impossible to demonstrate any difference.

This view rests mainly upon PFEIFFER's work and partly on the succeeding investigations on „influenza" which took place in the next few years the results of which agreed on the whole with those of PFEIFFER.

If however we had not had this knowledge and not discovered the „specific" bacillus until 1898 for example we should hardly have had any ground during the following 20 years for regarding it as the virus of influenza or indeed for specially connecting it with this malady. It must certainly also be stated that the „influenza" bacillus, in contradistinction to the microbes of other infectious diseases, has had but slight importance for the clinicians. It has scarcely been usual for doctors to place much importance on getting their „influenza"" cases verified bacteriologically. We have seen it urged that although the presence of the influenza bacillus might be the criterion whether a given epidemic was influenza, the bacillus

had no great importance in the diagnosis of individual cases. The theory of the etiological significance of Pfeiffer's bacilli in influenza has therefore had a rather „academic" character; it has been a generally accepted theory of remarkably slight practical importance.

It must however be stated that there have always been sceptics who have not felt convinced of the truth of this theory. (ELMASSIAN, ROSENTHAL, SACQUÉPÉE, BESANÇON & DE JONG, RITCHIE, KRUSE (2)).

As is already evident from the title and introduction, the present work sets out to investigate the occurrence of the haemoglobinophilic bacteria as well as their classification.

But this is not two subjects which are independent of one another; on the contrary it is natural to look upon occurrence as subservient to classification, — as one of the factors responsible for the latter, since giving an account of an organism's natural habitat is tantamount to describing the circumstances under which it is capable of thriving in nature and this is just as much a characteristic of the microbe as for example its conditions of growth in vitro, its morphology etc.

For purely practical reasons it will be convenient, both in the review of the literature, which will be given first, and in the subsequent report of the author's own investigations, to deal first with the occurrence and afterwards with the classification. The conditions relating to the occurrence can then also be grouped under the latter.

The review of other authors' experiences may be divided up as follows:

First of all PFEIFFER's fundamental paper as well as other investigations during the pandemic 1889—92 are recounted (pp. 17—20).

Then a résumé is given of the views which suggest themselves concerning the occurrence of Pfeiffer's bacilli under various conditions on the basis of all the available literature (pp. 20—24).

As an illustration of this résumé an abstract of the lite-

rature is given in the form of a collection of short summaries of the individual authors' works (pp. 24—40).

Since the question of the occurrence of Pfeiffer's bacilli in the blood and various internal organs of man, and its power of multiplying in animal experiments and of passing into the blood-stream, has been the subject of rather varying interpretation, this is subjected to analysis (pp. 40—45).

After this question, which is important for the interpretation of the occurrence and classification of Pfeiffer's bacilli has been discussed, the way is open for dealing with the „internal classification" of this bacterium, that is to say the question of mutual differences among the strains.

Firstly the morphology is dealt with (pp. 46—48), then the biochemical reactions of the organism (pp. 48—51); finally the results of the serological investigations (pp. 51—56) and a few supplementary comments (pp. 56—58).

Since the investigations into the occurrence and classification of Pfeiffer's bacillus were made principally with particular reference of the question of its significance in influenza, and as this is the most important of the questions which arise in connection with Pfeiffer's bacillus, I have considered it appropriate to make this the subject of discussion both in connection with the account of PFEIFFER's investigations and the review of the occurrence and classification of the bacillus. In order that this discussion shall not be too incomplete the most important of the other facts to which significance has been attributed in this connection are also shortly referred to (pp. 58—64).

In the account given of the classification of Pfeiffer's bacillus several of its characteristics are mentioned. A complete presentation of the biology of this microbe has however not been attempted and various facts interesting in themselves are therefore entirely omitted as they have no particular importance with respect to the points at issue. Several of these facts will be referred to in discussing my own investigations. The same applies to the question of the distinction between Pfeiffer's bacillus and other nearly related species, especially other haemoglobinophilic bacteria.

Pfeiffer's Work.

The fact that PFEIFFER's discovery (1,2, (see also PFEIFFER & BECK (1)) immediately produced such a convincing impression depended upon the three following points:

(1) that the „influenza-bacillus" was found with great regularity and in extraordinary numbers, sometimes in „absolutely pure culture"", in all the typical influenza cases examined; (2) that it was a hitherto completely unknown organism which in addition (3) was distinguished by the characteristic, which also had never previously been known, that, as already stated, it could only be cultivated on media containing red blood cells or dissolved haemoglobin.

These three points formed a striking contrast to the observations other investigators had made in the course of the pandemic; they had in fact only found already familiar organisms: Streptococci, Pneumococci, Staphylococci, to which, on account of their general occurrence in other diseases, could hardly be ascribed any specific significance in influenza, the more so because some observers found principally one, while other observers found principally another of the organisms mentioned. As PFEIFFER's work also was carried out with great capacity and presented in a clear and elegant form, it is intelligible that it might evoke a feeling of relief that at last we had found the solution of the problem.

Looking at PFEIFFER's work however from the critical position we have reached after the later contradictory experiences, particularly in the last pandemic, the deficiencies of his proof are very evident.

PFEIFFER had shown that his bacillus was constantly found in cases of influenza he examined and occurred in such intimate relation with the principal pathological changes that it might well be the specific primary influenza microbe. But was it characteristic for influenza? Was it not also present in other diseases and in normal persons?

On this question PFEIFFER (1) certainly says that „in very numerous control experiments" it could not be demonstrated in „ordinary bronchial catarrh, pneumonia and phthisis," but more detailed information is wanting and subsequent investi-

gations at any rate have not supported him. He does not appear to have examined healthy subjects. Later he announced (2) that in addition to typical influenza cases it was also found in „ambulatory influenza" where the most important symptoms were a cold and fever, and in „chronic influenza" which usually developed in a phthisical case. (Half a year after the epidemic had ceased Pfeiffer's bacilli could no longer be demonstrated in phthisical cases). The reason he regarded these cases as influenza depended partly on the fact that they had more severe general symptoms than an ordinary cold or phthisis, and in the case of phthisis because the sputum had a different appearance to what we are accustomed to see in lung tuberculosis, and also partly on account of the finding of „influenza-bacilli". But in accordance with our present view we may doubt the justification of calling such cases influenza. We are immediately up against the great difficulty that the disease does not display such characteristic symptoms and anatomical changes that it is possible clinically or patho-anatomically to determine what is influenza and what is not. We are here easily involved in a petitio principii: In order to prove that Pfeiffer's bacillus is the microbe of influenza one seeks to show that it occurs regularly in influenza and only in this disease; but in order to judge whether a case of the disease or an epidemic is influenza one must avail oneself of the bacteriological finding!

By inoculation into animals the etiological significance of the bacillus could not be shown. Pfeiffer worked especially with rabbits, for which it proved strongly toxic without however multiplying in the animal. By inoculating into the upper air passages of monkeys malaise with fever and some cough could be produced which however could not be described as influenza.

Pfeiffer's bacillus was first demonstrated in the final stage of the influenza pandemic or perhaps more correctly in the first of the epidemics following the true pandemic which died away in 1890, while influenza again flared up in 1891—92 bearing another epidemiological character, that is to say it took the form of isolated epidemics without any mutual connection. We may therefore enquire: Was it after all present in the pandemic 1889—90? Pfeiffer tries to prove this by

pointing out that already in 1890 he had observed the same bacterium in preparations sent to him by M. KIRCHNER who has even pictured one of these preparations in a communication on his bacteriological investigations in influenza. Although Pfeiffer's bacillus cannot be detected with absolute certainty by the microscope alone this claim is in all probability correct; but it is by no means proved that the bacterium at that time had approximately the same wide distribution as when PFEIFFER carried out his later investigations. As it must be assumed that in the microscopic examination of sputum which has been made by different observers a considerable number of times, stains have frequently been employed which are suitable to make Pfeiffer's bacillus visible (carbol-fuchsin, alkaline methylene blue), it is difficult to imagine this organism could have been overlooked if it occurred with the same regularity and in the same abundance during the whole epidemic.

As an example it may be mentioned that WEICHSELBAUM (1) in the early part of the pandemic examined the expectoration of 21 influenza patients, for the most part uncomplicated cases. The examination was made immediately after admission to hospital, therefore usually in the early stage of the disease. The sputum was investigated shortly after it was coughed up, carbol-fuchsin being used as a stain followed by slight decolourisation in alcohol. Although all the conditions for the demonstration of Pfeiffer's bacillus seem to have been fulfilled, WEICHSELBAUM found almost exclusively, bacteria having the appearance of Pneumococci, usually in large numbers.

It must however be admitted that, as is well known, a phenomenon may be overlooked often in a surprising way before it has been discovered; and it is feasible that PFEIFFER (2) is right in maintaining that some of the earlier observers may have seen his bacillus but looked upon it as a diplococcus or streptococcus.

As already observed it should be further noted that Pfeiffer's bacillus does not belong to the bacteria which stain most easily. According to concordant statements by numerous authors (PFEIFFER (2), BÄUMLER, KRUSE (1), LINDENTHAL, JUNDELL, BEALL, WOHLWILL; FILDES, BAKER, & THOMPSON, PREIN and others) it stains very badly, particularly in sputum and

similar secretions, with aqueous methylene blue and several others of the ordinary dyes used. There are practically only two stains which are recommended, namely LÖFFLER's methylene blue or other alkaline methylene blue solutions and particularly carbol-fuchsin (diluted 10—20 times and staining for 5—10 minutes). Thus it can hardly be stated with absolute certainty that Pfeiffer's bacillus only appeared in the last stage of the pandemic 1889—92 with the great frequency and in the abundance that PFEIFFER demonstrated, but we must content ourselves with supposing it the more probable.

In the investigations which were undertaken in the course of the years 1892—94 in various places PFEIFFER's discovery was on the whole supported although the bacillus was not always found with such regularity (WEICHSELBAUM (2), HUBER, CHIARI, PRIBRAM (1), BORCHARDT, E. NEISSER, BÄUMLER, PIELICKE, RICHTER, VOGES).

Account of the Occurrence of Pfeiffer's Bacillus.

In order to appreciate the significance of Pfeiffer's bacillus correctly, it is necessary to have a detailed picture of its occurrence at different times, in different neighbourhoods, in various diseases and also in healthy persons, as well as (possibly) outside the human body. The literature on all these conditions is so extensive that it would lead much too far to give an analysis of all the papers extant. In place of this an impression of the occurrence of Pfeiffer's bacillus which one obtains from perusing the accessible literature will first be given, and afterwards the majority of the statements made will be substantiated or at all events illustrated by short summaries of a selection of the most enlightening investigations.

The conception I think one should form of the occurrence of Pfeiffer's bacillus is practically embraced by the following 13 points, of which nos. 1—7 especially, but not exclusively, refer to the interval between the two pandemics, while nos. 8—13 deal with the conditions during the last pandemic.

1. Pfeiffer's bacillus has been constantly present since its discovery, fairly widely disseminated in different regions of the world.

2. But the occurrence has not been uniform. In the period between the two pandemics there have been places where for several years it has either not existed at all or at any rate was present in such small numbers that it was impossible to demonstrate it even with adequate technique.

3. The diseases in which it has been found are: first of all whooping-cough, measles, and „influenza"; then in a number of other infections which attack the respiratory organs and the mucous membranes of the upper air-passages: lung tuberculosis, pneumonia, bronchitis, bronchiectasis, angina, scarlatina, diphtheria; affections of the accessory sinuses, otitis, conjunctivitis.

4. In some „influenza" epidemics it is met with almost constantly, in others only in a minority of the cases, and again in others it is absent. There are probably all possible stages from constant presence to entire absence. No regular relation can be shown to exist between the clinical and epidemiological characters of an epidemic, and the presence or absence of Pfeiffer's bacillus.

5. In a number of the above-mentioned diseases as well as in healthy persons it occurs particularly in connection with pandemic or epidemic „influenza", but also occurs very extensively without any direct relation to epidemics resembling influenza.

6. Pfeiffer's bacillus sometimes functions as a harmless saprophyte, at other times it reveals pathogenic qualities in a more or less marked degree. The latter has not only been the case in „influenza" but just as much in various other diseases.

7. In several preliminary epidemics to the pandemic of 1918—20 it occurred with much the same distribution as it reached later in the pandemic.

8. During the pandemic Pfeiffer's bacillus was found practically constantly in certain areas and at certain times with suitable choice of material, either in pure culture or in overwhelming numbers, therefore under conditions very closely corresponding to PFEIFFER's description in the earlier pande-

mic. In other cases it was met with in considerably more limited distribution, and lastly many observers, including some of the most noted bacteriologists, have either not found it at all or only exceptionally.

These negative results are undoubtedly due in many instances to defective technique in the examination; but this is certainly inadequate to account for all the negative findings. It must be assumed that there are places where Pfeiffer's bacillus has actually been absent or has played a quite subordinate rôle during a part of the pandemic, and that as regards its frequency of occurrence in patients there have existed all possible transitions from the most scanty to the most abundant occurrence.

9. On the whole Pfeiffer's bacillus had a considerably smaller distribution during the first outburst of the pandemic in the summer months of 1918 than later on. There are however places where immediately after the appearance of the pandemic it was found in considerable numbers in the majority of the patients. On the whole it must be assumed that the distribution of this microbe increased to a marked extent in direct relation to the first appearance of the pandemic in the different countries. While, generally speaking, the distribution of the bacillus extended during the course of the pandemic, there were also regions where at an early date it was widespread and during the further course of the pandemic it was encountered less frequently.

10. Pfeiffer's bacillus occurs in influenza in intimate relation with the most important pathological changes and must be assumed to play a decided pathogenic rôle.

11. It is found essentially in the early stages of the disease, afterwards disappearing or decreasing considerably in the course of quite a few days. How quickly Pfeiffer's bacillus disappears depends probably to a great extent upon the other bacterial flora which may be very different in different places.

It is found presumably (the same length of time from the beginning of the disease) more frequently in uncomplicated influenza than in influenza with pneumonia, but the difference is not great.

Only exceptionally Pfeiffer's bacillus is absent in the early stage of the disease and does not appear until later.

12. Several observers demonstrated Pfeiffer's bacillus with considerable frequency in h e a l t h y p e r s o n s during the pandemic, but others hardly ever found it in such people.

This may perhaps be explained by assuming that the bacillus at first only developes in influenza patients and it takes some time before it can spread from these to healthy individuals to any great extent.

13. In certain cases during the pandemic it has been possible to demonstrate a constant r e l a t i o n b e t w e e n t h e d i s t r i b u t i o n o f i n f l u e n z a a n d t h e o c c u r r e n c e o f Pfeiffer's bacillus not only in patients with influenza symptoms but also in n o r m a l p e o p l e and in patients with o t h e r d i s e a s e s (cf. 5).

It will be appropriate here to give an account of the views which have been formed in estimating the value of statements about the finding of Pfeiffer's bacillus although part of the basis of them can only be given later in connection with my own investigations.

Reports of n e g a t i v e findings must be accepted with great reservation when there is inadequate information about the technique employed or whether the author in question has had experience in the detection of Pfeiffer's bacillus.

Reports of p o s i t i v e findings can as a rule be accepted without further questioning when the bacillus is demonstrated in culture, but microscopic examination alone is insufficient.

It must however be remarked that BRUSCHETTINI (1—4) has introduced some confusion by calling some microbes Pfeiffer's bacillus which must be considered to be quite different (see PFEIFFER & BECK (2) and ONORATO), and several authors have built upon his work without caution: TIZZONI (1), who however subsequently (2) distinguishes between Pfeiffer's bacillus and BRUSCHETTINI's bacillus, BOMBICCI, and GHEDINI.

Apart from this not only must the organisms which the authors themselves take to be Pfeiffer's bacilli, be assumed to be such but also PFEIFFER's „Pseudo-influenza bacillus", ELMASSIAN's and LUZZATO's bacilli, L. MÜLLER's trachoma ba-

cillus, JUNDELL's „Bacillus catarrhalis", JOCHMANN's (1) „Bacillus pertussis Eppendorf" and COHEN's „bacille de la méningite cérébrospinale septicémique" must all be looked upon as Pfeiffer's bacillus.

A series of summaries will now be given, as far as possible in chronological order, of papers selected from the literature touching upon the occurrence of Pfeiffer's bacillus. (In the absence of any other information its occurrence in the respiratory tract is meant).

The choice of papers for discussion has not been made exclusively on the basis of their importance but the object has been especially to give a representation of the different kinds of experiences which shall be as general as possible. Therefore there may be several among the unmentioned works which are more important in themselves than some of least significant of the summarised ones.

KAMEN (1) (Czernowitz 1896), in the first few years following PFEIFFER's discovery, examined the sputum of about 100 cases of „influenza" and found Pfeiffer's bacillus constant at the „height" of the disease and when he took the densest bits of the sputum.

LINDENTHAL (Vienna), from Dec. 1896 to Feb. 1897, at a time when „influenza" did not appear in epidemic form cultivated Pfeiffer's bacillus from 8 autopsies of cases of „sporadic influenza". In the majority of these cases it was obtained from the accessory nasal sinuses as well as from the lungs.

JUNDELL (Stockholm) found Pfeiffer's bacillus in a number of cases of „influenza" in an epidemic in the winter of 1896—97.

v. JAKSCH (Prague 1899) was unable to demonstrate Pfeiffer's bacillus in numerous cases which closely resembled influenza clinically, while he found it in one case which did not run a clinical course like influenza.

PALTAUF (1) (Vienna 1899), in an „influenza" epidemic which departed from the ordinary endemic „influenza" in that the lungs were attacked to a larger extent partly with abscess formation and necrosis, found Pfeiffer's bacillus in 45 out of 86 samples of sputum examined and in 56 autopsies (it is not stated among how many). In 13 cases Pfeiffer's bacillus was found by making cultures from the pelvis of the kidney and in 3 cases from the spleen.

In the winter of 1899—1900 there seems to have been a rather decided outbreak of „influenza" in Europe.

WASSERMANN (Berlin) in a small epidemic in the months of Jan. to March 1900, found Pfeiffer's bacillus in the sputum only

when the examination was made in the first 24 hours after the disease started; afterwards it gave way to other bacteria. Simultaneously with the disappearance of Pfeiffer's bacillus marked toxic symptoms arose. He explained this phenomenon as the result of a certain amount of immunity remaining over from the pandemic of 1889—92, which was of a bactericidal nature, the toxic effect being due to the disintegration of Pfeiffer's bacillus.

CLEMENS (Freiburg) at the same time, found Pfeiffer's bacillus in 12 out of 95 „influenza" cases.

SACQUÉPÉE reports an epidemic of considerable size in Rennes in 1900. Of 4200 men in the garrison 2200 were attacked in the course of 2 months. From a bacteriological point of view the epidemic was divided into 3 phases. A hitherto unknown bacillus was found in the first period; then Pneumococcus and Streptococcus were predominant and not until the end of the epidemic was Pfeiffer's bacillus found, sometimes in great abundance, in spite of the fact that it was systematically looked for from the very beginning of the epidemic.

SÜSSWEIN (Vienna 1901) found Pfeiffer's bacillus in the nasal secretion of 10 out of 21 cases of measles (not in an „influenza" epidemic). Most of the cases with Pfeiffer's bacilli were particularly severe with well-marked lung symptoms, cyanosis, high fever etc. Some of the cases with Pfeiffer's bacillus however were indistinguishable from those without the bacillus.

JEHLE (1901) found it in 18 out of 23 cases of measles. It could very often also be cultivated from the blood.

LORD (1902) found Pfeiffer's bacillus in 60 out of 100 patients with acute and chronic lung diseases taken at random. In half the positive cases it occurred in pure culture.

AUERBACH (Cologne) from Jan. 1902 to July 1903 examined the tonsils and larynx of 700 patients and found Pfeiffer's bacillus in 38 (divided between angina, diphtheria, scarlatina, and measles).

In both years Pfeiffer's bacillus was found most frequently in January to March, concurrently with the greatest ordinary „influenza" morbidity.

KERSCHENSTEINER (Munich 1903) not infrequently found Pfeiffer's bacillus in tuberculosis and other chronic lung diseases. He asserts that it need not have any significance as regards the course of the disease.

WYNEKOOP (Chicago 1903) reports the frequent finding of Pfeiffer's bacillus in laryngitis, pharyngitis, angina, and conjunctivitis.

WOLLSTEIN (1) (New York 1906) often found Pfeiffer's bacillus in children with tuberculosis (in 14 out of 32 patients), bronchitis (12 out of 46), broncho-pneumonia (19 out of 66), empyema (3 out of 7), and measles (9 out of 27). She says that those

children who had Pfeiffer's bacilli displayed more severe symptoms than the rest.

A number of papers dealing with the presence of Pfeiffer's bacillus in whooping-cough will now be given.

ELMASSIAN (Paris 1899) cultivated a bacillus from 8 out of 32 cases of whooping-cough, which closely resembled Pfeiffer's bacillus and was only distinguished from it by its power of growing on media containing serum or ascitic fluid. In order to judge whether it was different from Pfeiffer's bacillus he compared it with some cultures from epidemic respiratory infections („grippe") and with an organism which MEUNIER had described as Pfeiffer's bacillus. He found that all the cultures behaved in a similar manner.

As no tests were made to ensure that the ascitic fluid and serum were free from haemoglobin there is no reason for regarding these bacilli as other than Pfeiffer's bacilli.

LUZZATO (Graz 1900) gives a parallel description of an organism which was found in great quantities in many cases of whooping-cough. He names it „Bacillus minutissimus sputi" and separates it from Pfeiffer's bacillus partly by its growth on serum-containing media and partly by a definite granular formation of the colonies. But the last characteristic would do well for Pfeiffer's bacillus with which it is probably identical.

JOCHMANN & KRAUSE (Hamburg 1901) examined the sputum of 31 cases of whooping-cough and found a haemoglobinophilic bacillus in 18 of them as well as in 3 autopsies of whooping-cough with broncho-pneumonia, to which he gave the name „Bacillus pertussis Eppendorf". JOCHMANN (1) (1903) subsequently reported the result of the further investigation of the sputum of 42 cases of whooping-cough, all in the convulsive stage. In all the cases the same bacillus was found. Lastly JOCHMANN & MOLTRECHT cultivated it from 23 out of 25 autopsies of bronchopneumonia in whooping-cough.

These investigations covered a period of 2—3 years and were carried out at all the seasons of the year. There was no question of a special hospital infection with the bacillus as the sputum was examined shortly after the child's admission. There was no „influenza" epidemic in the neighbourhood.

The bacillus completely resembled Pfeiffer's bacillus microscopically and as regards the appearance of its growth on blood-agar, its increased growth around the colonies of other kinds of bacteria, and absence of growth on blood-free media. (The findings of the two following authors are verified in the same way). The reason JOCHMANN hesitated to identify it with Pfeiffer's bacillus was only because it had no obvious connection with „influenza".

SMIT found Pfeiffer's bacillus in all of the 24 cases of whooping-cough examined in Utrecht in the spring and summer of 1903, and in 7 out of 8 cases of whooping-cough in Amsterdam in the spring of 1904.

DAVIS (1) (Chicago 1906) found it in 56 out of 61 cases of whooping-cough; also in measles (13 out of 22 cases), varicella (7 out of 11), bronchitis (5 out of 12), while it was only found in 3 out of 17 cases of „influenza". Lastly it was obtained from the throat of 2 out of 20 healthy persons.

WOLLSTEIN (2) (New York 1909) found the bacillus in 10 out of 20 cases of whooping-cough.

With regard to the question of the occurrence of Pfeiffer's bacillus in conjunctivitis L. MÜLLER (1900) and ZUR NEDDEN (1903) among others may be referred to.

In the winter of 1904—05 „influenza" was rather widespread over Europe and America.

BESANÇON & DE JONG (Paris) during this epidemic could only occasionally find Pfeiffer's bacillus. From a review they give of the results obtained in different countries it appears that it was found in most places but was very inconstant.

It was obtained relatively frequently by

JOCHMANN (2) (Breslau), namely in 13 out of 36 cases in broncho-pneumonia, usually in abundance. He states however he found it considerably more extensively during an epidemic in Kiel in 1899. Apropos of WASSERMANN's report of the epidemic in 1900 JOCHMANN remarks that in those cases where Pfeiffer's bacillus was present it could usually be demonstrated for a long time.

JOCHMANN states that when Pfeiffer's bacillus is found in diphtheria it has no effect on the course of the disease in some instances, while in others it may cause bronchitis and broncho-pneumonia, but these cases do not however resemble influenza nor do they differ clinically from the corresponding infections produced by Streptococcus or Pneumococcus.

KRETZ (2) (Vienna 1905) reports a number of positive findings of Pfeiffer's bacillus where there was a marked connection between the quantity of bacilli and well-developed symptoms.

STERNBERG (Vienna 1905) found Pfeiffer's bacillus in 13 out of 70 cases which clinically might be suspected to be „influenza", while in the preceding winter he could only demonstrate it in 4 out of 56 samples of sputum.

KLIENEBERGER (1) (Frankfurt a. M.) found Pfeiffer's bacillus in 8 out of 27 „influenza" cases. He claimed that the inconstant finding of the bacillus could not be attributed to defective technique, since in that laboratory they had worked for several years with haemoglobinophilic bacteria.

These contributions all concern the epidemic in the winter of 1904—05.

After the termination of the epidemic, KLIENEBERGER (2) (Königsberg) found Pfeiffer's bacillus in 8 out of 14 cases of tuberculosis, 9 out of 18 cases of chronic bronchitis, and in 4 out of 5 cases of bronchiectasis and gangrene of the lung.

TEDESKO (Vienna) in the years 1896—1906 could demonstrate Pfeiffer's bacillus in the majority of cases investigated by cultivation from several hundred autopsies, both in „influenza" and in various other diseases. In later years it was found comparatively seldom in pure culture however but commonly in association with two or more other bacteria.

In this country ELLERMANN (Copenhagen 1906) reported the cultivation of Pfeiffer's bacillus from „influenza" cases.

WOHLWILL (Hamburg 1908), in the summer months when „influenza" was at a low ebb, was able to cultivate Pfeiffer's bacillus from the small bronchial tubes in 29 out of 158 autopsies, that is to say in 16 out of 73 cases of phthisis, in 6 out of 6 cases of whooping-cough and 2 out of 2 cases of measles.

He was however never once able to cultivate it from the heart blood in several thousand autopsies.

POLLAK (Brünn 1908) only found Pfeiffer's bacillus in 8 out of 73 cases by cultivation from the purulent bronchial secretion post mortem, although cases resembling influenza were frequently present. In a small „influenza" epidemic in January 1908 it was only found in 1 out of 23 cases.

SCHELLER (1), during and after an „influenza" epidemic in Königsberg in the winter of 1906—07, carried out a considerable number of examinations of „influenza" patients (chiefly sputum, but in some cases pneumonic lung post mortem), of tuberculosis (sputum), and of healthy persons (cultivation from the throat). At the height of the epidemic Pfeiffer's bacillus was found in 62 out of 68 „influenza" patients, in 10 out of 29 tuberculosis patients and in 25 out of 109 healthy people. Simultaneously with the gradual remission of the epidemic in the course of the next few years Pfeiffer's bacillus decreased not only in „influenza" patients but also in tuberculous and healthy persons, until in the winter of 1908—09 it practically could not be obtained from any of the three groups.

Among the healthy subjects Pfeiffer's bacillus was found in the epidemic more often in persons who had had „influenza" than in those who had not been attacked.

Pfeiffer's bacillus therefore did not occur exclusively in „influenza" patients but was found more often in them than in other persons and there was a close connection between the extent of the epidemic and the occurrence of Pfeiffer's bacilli.

SCHELLER states that the pathological changes in the lungs were quite characteristic of influenza and that Pfeiffer's bacillus could be seen in sections of the affected lung itself.

These investigations are advanced by SCHELLER (1, 2, 3) and PFEIFFER (4) as a weighty argument in favour of the primary importance of Pfeiffer's bacillus in influenza.

In opposition to this SELIGMANN & WOLF very much doubt whether there really was a true influenza epidemic at the time SCHELLER made his investigations. They maintain that at the date in question there was no general outbreak of influenza in Prussia and that in the winter of 1906—07 when according to SCHELLER the epidemic was at its height, fewer deaths were reported in Königsberg from influenza than in the following years.

EYRE (London), in the period 1902—09 found Pfeiffer's bacillus in 20 out of 102 cases of primary broncho-pneumonia and in 13 out of 62 cases of broncho-pneumonia complicating other diseases, — in measles in 4 out of 20, in whooping-cough in 3 out of 14 and in diphtheria in 6 out of 18 cases.

TURNER & LEWIS (Edinburgh 1910) frequently obtained Pfeiffer's bacillus in disease of the accessory sinuses.

HASTINGS & NILES (New York) during the years 1903—10 found Pfeiffer's bacilli in 5 out of 17 influenza cases but only in a few cases of other diseases of the respiratory tract.

HOLT (New York) in 1909—10 looked for Pfeiffer's bacillus in 530 patients and in 35 autopsies, and found it very generally present (as the most abundant organism after Pneumococcus) in the winter and early spring, but it completely disappeared in the course of the summer. It was found in pneumonia (in 47 out of 124 patients), bronchitis etc. (63 out of 133), tuberculosis (6 out of 23), otitis (4 out of 57), various diseases outside the respiratory organs (49 out of 254). There is no mention of an epidemic outbreak of influenza. He claims to have shown a certain relation between the occurrence of the bacillus and the clinical symptoms. The latter however could hardly be termed classical influenza symptoms and he concludes by saying that „a much more extended study and many series of observations must be made before it will be possible to establish relationships between bronchial or nasopharyngeal cultures and clinical symptoms in respiratory infections".

SELTER (1) (Bonn) as late as the winter months of 1902 and 1903 frequently found Pfeiffer's bacillus in sputum examinations but never in great numbers and hardly ever by direct microscopic examination, but usually by cultural methods in association with other bacteria. In the succeeding years in spite of numerous attempts each winter he could no longer find it.

In the winter of 1908—09 an extensive „influenza" epidemic broke out in Bonn which was thought to have come from France. The clinicians asserted that it could in no way be distinguished from pandemic influenza. Neither SELTER nor WALB found Pfeiffer's bacillus in any of the cases, but Pneumococcus was constantly present (or a similar organism) which was also put down as the cause of this epidemic in a French publication.

CURSCHMANN and ROSE also give an account of „influenza" epidemics respectively in Leipzig in the winter of 1907—08, and in Strasburg in March and April 1909, where Pneumococcus was almost exclusively found and never Pfeiffer's bacillus.

HIRSCH (Frankfurt a. M. 1912) and many others report the frequent occurrence of Pfeiffer's bacillus in otitis, sometimes in pure culture.

KRUSE (2) (1914) maintains that Pfeiffer's bacillus is probably not the cause of pandemic influenza (which he assumes is an infection due to a filterable virus, because it „wie ich nach meinen seit 1889 datierenden Erfahrungen versichern darf, nur zeitweise bei grippeähnlichen Erkrankungen und später wieder in vielen Fällen, die mit Grippe nichts zu tun haben (Keuchhusten, Masern u. s. w.) gefunden werden."

LUETSCHER (Baltimore 1915) found Pfeiffer's bacillus in acute bronchitis (22 out of 47 cases), chronic bronchitis (6 out of 13), bronchopneumonia (22 out of 42), lobar pneumonia (5 out of 83), bronchiectasis (2 out of 4), asthma (1 out of 5), phthisis (3 out of 21), laryngitis (7 out of 38), coryza (6 out of 38), disease of the accessory sinuses (4 out of 29) and in 7 out of 60 excised tonsils. In most cases it was obtained in pure culture and considerable numbers. LUETSCHER comes to the conclusion that Pfeiffer's bacillus is not only present in influenza pandemics and epidemics but also is „the cause of approximately 30 % of the pulmonary lesions in interepidemic periods."

In the 5 years immediately preceding the pandemic (1913—18) Pfeiffer's bacillus was demonstrated at the Rockefeller Institute, according to STILLMAN, in 20 % of 672 cases of pneumonia even though it was not systematically sought for. It was merely encountered as a secondary finding in cultivations from mice in which sputum had been inoculated in the examination for Pneumococcus.

In Europe also the occurrence of Pfeiffer's bacillus is reported from many places in the last few years before the pandemic, especially in „influenza" epidemics.

THALMANN examined the sputum of 489 men from Nov. 1910 to Oct. 1912 during an epidemic in the garrison in Dresden and found Pfeiffer's bacillus in 359, often in large numbers. He further states that those cases in which it was not found were mostly not typical influenza.

HÜBSCHMANN (1) (Leipzig 1914) found Pfeiffer's bacillus in 2 cases of broncho-pneumonia as the predominating organism in the small bronchioles. During Jan.—March 1915 he (2) found it in 31 out of 110 autopsies in the purulent or muco-purulent secretion of the finer bronchioles which in his experience was the site of election of Pfeiffer's bacillus. (PFEIFFER, HERZOG, LEICHTEN-

TRITT, LÖWENFELD, OLSEN, MATERNA & PENECKE and many others had the same experience in the pandemic).

PALTAUF (2) (1915) cultivated Pfeiffer's bacillus in pure culture from many cases of bronchitis complicating typhus fever in a war-prisoners' camp.

According to ARNETH a larger number of „influenza" cases than previously, occurred among the troops in the spring months of 1915. In a number of cases Pfeiffer's bacillus could be demonstrated.

HAMMOND, ROLLAND, & SHORE discovered numerous cases of „purulent bronchitis" (influenza?) in the army in Northern France during the winter of 1916—17 and cultivated Pfeiffer's bacillus from 10 out of 20 of these patients. ABRAHAMS, HALLOWS, EYRE, & FRENCH (Aldershot 1917) found it in 7 out of 8 cases of the same disease.

LEVINTHAL (1) reports the regular finding of Pfeiffer's bacillus in „influenza" and explains that according to information from HILDEBRANDT in Freiburg he was able (spring and autumn 1916) to confirm the diagnosis bacteriologically in nearly all the cases of clinical influenza while other febrile diseases of the respiratory organs usually gave a negative result.

In an epidemic of a benign catarrhal infection which occurred in the middle of April 1918 among the American troops in the neighbourhood of Bordeaux, Pfeiffer's bacillus was found in the majority of cases when the examination was made at the beginning of the disease (Office international d'hygiène publique, procès-verbaux des séances. Oct. 1919, p. 116).

On the strength of these findings in the few years preceding the true pandemic it is not surprising that Pfeiffer's bacillus was found in various localities immediately after it started.

The first reports of influenza in such intensity that in can be regarded as belonging to the pandemic, came as is known from Spain where (according to PULIDO) it began in the first half of May 1918. (Nothing is said about the true origin and mode of spread of the pandemic).

In that country RUIZ Y FALCO & TAPIO found Pfeiffer's bacillus in the majority of cases examined, as early as May.

In June and July the pandemic extended over the rest of Europe and in many places bacteriological investigations were started with special reference to Pfeiffer's bacillus, immediately after the disease appeared in the locality.

Among the workers who found this bacillus without delay must first be named its discoverer.

R. PFEIFFER (3) (see also LEICHTENTRITT (1)), on the 27. VI. 1918 immediately after influenza appeared in a small hospital in Silesia, found the bacillus in 4 out of the first 5 cases examined sometimes in enormous quantities. In some other cases however it could not be demonstrated.

The investigations were continued by LEICHTENTRITT (1) (Breslau) who in the course of the summer and autumn of 1918 found Pfeiffer's bacillus in 155 out of 289 samples of sputum from influenza etc., in 24 out of 36 autopsies on influenzal pneumonia, and in 58 out of 228 samples of sputum from cases of phthisis. It was further found in influenza 5 times in the spleen and 3 times in the brain.

LEICHTENTRITT's work was continued by LOEWENHARDT (1,2), who from the early days of 1919 to March 1920 found Pfeiffer's bacillus in 122 out of 160 samples of influenzal sputum, in 65 out of 125 throat cultures from influenza patients, in 32 out of 56 autopsies on influenzal pneumonia, in 19 out of 179 samples of tuberculous sputum, and in 16 out of 218 samples of sputum from other diseases of the respiratory organs. In cultures from the throats of 62 healthy persons it was found in 2 only, and both fell sick with influenza 2 days later.

LOEWENHARDT compares in the form of curves the incidence of influenza in the summer epidemic 1918, in the autumn epidemic of the same year and in the epidemic in Jan.—March 1920 with the absolute number of findings of Pfeiffer's bacillus in patients at different dates and finds on the whole an excellent agreement, the positive findings being considerably more frequent during the epidemics than in the intervening periods. It is stated in the text that the relation between the number of positive findings and the total number of examinations in the various periods varies in the same direction as the absolute number of positive findings, but no further details are given.

The frequency of the presence of Pfeiffer's bacillus in patients with other diseases than influenza decreased steadily from June—December 1918 to January—March 1920.

That Pfeiffer's bacillus was not constantly present in LEICHTENTRITT's and LOEWENHARDT's material is explained by them as being partly due to the fact that they were not sure of the diagnosis of influenza in all the cases and partly that the samples could not always be dealt with as quickly as desirable. Thus LOEWENHARDT, in 45 throat cultures from influenza patients where the samples were dealt with immediately after they were taken, found Pfeiffer's bacillus in 41 cases, while it could only be discovered in about 30% of the cases if merely 3—4 hours passed from the time the samples were taken to the time they were deposited in the incubator.

In the Institute under the direction of PFEIFFER, PREUSS also carried out some investigations of non-influenzal patients. In the summer of 1920 which still „in die Ausläufer der letzten Pandemie hineinfiel", Pfeiffer's bacillus was found in 25 out of 224 of such patients, and in 11 out of 129 healthy persons, while in the winter of 1920—21, „als in Breslau kaum noch Influenzafälle vorkamen"", it was only found in 3 out of 102 patients and in 2 out of 76 healthy people.

Pfeiffer's bacillus was obtained more often from those persons who had had influenza than from those who had not.

Among 37 cases of measles it was only found in 3.

Having connectedly discussed the investigations of PFEIFFER and his collaborators, we will turn to the positive findings immediately after the commencement of the pandemic. Of other authors who have reported on such findings may be mentioned:

UHLENHUTH (Strasburg), v. HOESSLIN, MAHLO, and also CROFTON who cultivated Pfeiffer's bacillus from the first patients who were examined in the summer epidemic, while in the preceding years he had never found this bacillus in spite of numerous examinations of sputum by the same method, and

MC CLELLAND (Louisiana), who began his investigations immediately after the epidemic on the 24. IX. 1918 appeared in the locality. From the 25. IX to 3. X. he found Pfeiffer's bacillus by cultivation from the throat in 1749 (91%) out of 1919 influenza patients.

MICHAELIS (Berlin) could not demonstrate Pfeiffer's bacillus in the first week after the start of the summer epidemic, but by the second week he found it in many cases and likewise in the autumn months.

MESSERSCHMIDT, HUNDESHAGEN, & SCHEER (Strasburg), who worked under UHLENHUTH, found the bacillus in 47 out of 86 specimens of sputum from June to Aug. 1918, while they found it in 18 out of 20 samples during September. That the more inconstant results in the summer months were not due to a more defective technique of cultivation than was employed later is obvious from the fact that the microscopic examination of the sputum yielded similar results. In the summer months Pfeiffer's bacillus was found microscopically only quite exceptionally in large numbers. The typical picture of bacilli in compact masses as they were described by PFEIFFER in the previous pandemic, was rare in the summer months but became more common in October.

In the autumn Pfeiffer's bacillus was found in some cases in the kidneys and liver in influenza when the examination was made a few hours after death.

Pfeiffer's bacillus was not found in 11 patients with chronic bronchitis, in 15 tuberculous nor 100 healthy soldiers in a barracks where some cases of influenza subsequently appeared.

NEUFELD & PAPAMARKU (1—2) (Inst. f. Infekt.-Krankheiten, Berlin), who however only began their work towards the end of the summer epidemic, found Pfeiffer's bacillus in a number of cases both in influenza and in tuberculosis patients. In contrast to this they state that „während der vorhergehenden Jahre im Untersuchungsamte des Instituts, trotzdem seit 1912 eigens darauf geachtet wurde, niemals Influenzabazillen, insbesondere auch nicht bei Tuberkulösen, gesehen wurden".

WILSON reports similar experience; for many years he used blood agar in the examination of sputum but only found Pfeiffer's bacillus during the pandemic.

These 9 contributions give an idea not only of the occurrence of Pfeiffer's bacillus in the first stage of the pandemic, to some extent even on its first appearance in the locality, but also of its increasing frequency in the beginning of the pandemic and during its further course. As regards the latter point most reliance naturally must be placed on those cases where the same investigator found Pfeiffer's bacillus at different times in a markedly different degree of distribution.

Of other authors who found an increasing frequency of occurrence during the pandemic, may be cited:

SOBERNHEIM & NOVAKOVIC (Bern), who only found Pfeiffer's bacillus in a small number of cases in sputum of post mortem material etc. during the summer epidemic of 1918. In many cases however it was demonstrated microscopically but they did not succeed in cultivating it. In the renewed outburst of influenza in October „the bacteriological results were completely changed in favour of Pfeiffer's bacillus." Even in the first examinations of sputum and post mortem material it was regularly found, and as the investigation continued it was cultivated in 18 out of 23 cases. At the same time the other bacteria changed, that is to say Pneumococcus which was previously plentiful in the sputum, had now almost disappeared. It is stated that the methods employed were the same in both epidemics.

FRÄNKEL (Heidelberg) could not demonstrate Pfeiffer's bacillus in the summer epidemic but found it in the autumn epidemic in sputum in 18 out of 70 patients and in 8 out of 11 autopsies.

The relation between the course of the pandemic and the distribution of Pfeiffer's bacillus has been further investigated by the following:

ROSENOW (Rochester), whose investigations covered 4 „waves" of the pandemic. Only at the beginning of the first epidemic was Pfeiffer's bacillus cultivated in large quantities from influenza sputum; later it was hardly found at all. The medium was continually controlled by the inoculation of a pure culture. Altogether the microbe was found in 13 % of 571 samples of sputum, and in the lungs of 5% of 107 post mortem examinations.

DICK & MURRAY (Chicago) examined the relations of Pfeiffer's bacillus during the decline of the influenza from Oct. 1918 to Jan. 1919. In patients with influenza symptoms it was found in these 4 months respectively in 74, 54, 43 and 36% of the cases. Simultaneously Pneumococcus increased in extent.

All these various investigations during the pandemic give us the impression that there were marked differences in the distribution of Pfeiffer's bacillus at different times and in different localities.

A very large proportion of the investigations undertaken immediately after the beginning of the pandemic yielded either absolutely negative results, or the bacillus could only be found in a few cases. This state of affairs was particularly noticeable in Germany.

In many cases the negative result was undoubtedly due to defective technique. PARK cites several instances of experienced investigators being able to demonstrate Pfeiffer's bacillus in large numbers in the same hospitals where others had failed to find it.

This is however not sufficient to explain all the negative findings.

MANDELBAUM (Munich) examined a large number of samples of influenza sputum in June and July 1918 without being able to find Pfeiffer's bacillus either microscopically or by cultivation. One of the dyes used for staining the preparations was dilute fuchsin solution. Similar negative results, both microscopically and by cultivation, were obtained in his examinations of the „whole bronchial tract" in numerous autopsies. That the negative results were not due to lack of experience in the demonstration of Pfeiffer's bacillus is evident from the fact that MANDELBAUM had often cultivated it from bronchopneumonia in children who had died from measles, in the last few years before the pandemic.

SELTER (Königsberg) (2,3), who had also previously had experience in detecting Pfeiffer's bacillus, could only find it once in over 100 cases either microscopically or culturally, although the cultures were made 2—3 hours after the samples were taken from

the patient, and the autopsies were performed a few hours after death.

As a further proof of the inconstant occurrence of Pfeiffer's bacillus in contradistinction to PARK's contributions, examples can be cited of the same investigator being able simultaneously to demonstrate this microbe in one group of patients but not in another.

PRELL asserts that PRIBRAM could only demonstrate Pfeiffer's bacillus in the patients of one certain hospital among four, and that VAGEDES only found it in one army on the West Front and not in another.

That Pfeiffer's bacillus usually occurred very widely distributed in the later stages of the pandemic is evident from the innumerable papers, of which only a few will be referred to and mainly those that shed some light upon the various specific problems.

OLSEN (1,2) (Hamburg) found Pfeiffer's bacillus 166 times out of 220 autopsies on influenzal pneumonia cases. A number of the positive cases were in the summer months of 1918. (This is also reported by OLSEN's Chief, SIMMONDS). Pfeiffer's bacillus often occurred in considerable numbers and not infrequently almost in pure culture. It was found principally in fresh pneumonic infiltrations, but it could be obtained also in cases examined post mortem even 2—3 months after the beginning of the disease.

Pfeiffer's bacillus was found much less often by GRAETZ and SCHOTTMÜLLER who likewise carried out their investigations in Hamburg. SCHOTTMÜLLER examined the sputum of several hundred influenza patients. He made particular point of obtaining it as far as possible in the first few days of the disease and it was examined immediately after it was coughed up. He also controlled the suitability of his medium partly by showing that a pure culture of Pfeiffer's bacillus would always grow well on it, and also that when Pfeiffer's bacillus had once been grown from the sputum of a patient it could easily be demonstrated again by a fresh cultivation. Before the pandemic SCHOTTMÜLLER also found Pfeiffer's bacillus very frequently in necrotic foci in the lungs of children who had died of whooping-cough and measles.

It is difficult to assign a cause to these rather divergent results from the same town.

In addition to MC CLELLAND the following authors among others found Pfeiffer's bacillus in almost all cases examined:

TYTLER, JANES, & DOBBIN (also in about 90% of the cases).

WILLIAMS (New York 1918) examined over 300 patients and found Pfeiffer's bacillus in 80—100% of the cases in the different groups. The earlier in the disease the examination was made, the more often was Pfeiffer's bacillus found in large quantities in the naso-pharynx. Cultivations from this place taken through the mouth, yielded positive results more constantly than cultivations from other parts of the mucous membrane. The next best result was from the tonsils.

In a „Girl's Home" where there had been no influenza, Pfeiffer's bacillus was found in only 2 out of 34 girls and both of these were kept apart on account of a slight „cold". In contrast to this Pfeiffer's bacillus was present in 14 out of 39 persons in a „preventorium" where there had been influenza.

DUVAL & HARRIS (New Orleans) in 1918—19 found the bacillus in every one of 75 patients examined also in the lungs in 16 out of 17 autopsies.

OPIE, FREEMAN, BLAKE, SMALL, & RIVERS (Arkansas) examined 23 influenza patients for Pfeiffer's bacillus by several different methods and found it in all, at least in one of the examinations, but with no single method did they succeed in getting constant positive results. Injection of sputum into a mouse gave the most positive results viz. 18, then came direct cultivation from the sputum (14), from the throat (13), and from the nose (5). The examinations were fairly evenly distributed over the period from the 1st. to the 6th. day of the disease.

SMALL & STANGL (Chicago) found Pfeiffer's bacillus during an epidemic in Jan. and Feb. 1920, in 65 out of 67 influenza patients. In non-influenza patients it was found in 9 out of 26 before the epidemic, and in 21 out of 30 during the epidemic.

As an indication that Pfeiffer's bacillus was also widely distributed in influenza in other parts of the world than Europe and North America, the following may be cited:

KRAUS & KANTOR (Buenos Aires) found it in 36 out of 60 cases. In 29 autopsies it was found in the spleen in 11, in the liver in 5 and in the kidneys in 4 cases.

LISTER & TAYLOR found Pfeiffer's bacillus in 53 out of 56 post mortem examinations on miners who had died from influenza in the Transvaal and Johannesburg.

MALONE (1) (Calcutta) with suitable technique found Pfeiffer's bacillus in the majority of cases in the sputum and in empyema of the accessory sinuses.

OKAWARA, TANAKA, WATANABE, KOYAMA, & SATO, in the pandemic which spread over Japan from Sept. to Nov. 1918, found Pfeiffer's bacillus in 14 out of 46 samples of sputum and in 19 out of 28 samples of nasal secretion.

GREIG & MAITRA (Karachi, India) report on the frequent occurrence of Pfeiffer's bacillus in the accessory nasal sinuses; they constantly found it associated with Pneumococcus in this situation in 19 influenza autopsies. The same applies to E. F. MÜLLER (Hamburg) and CROWE & THACKER-NEVILLE (Baltimore). The last-named authors made cultivations from numerous cases of inflammation of the maxillary sinus for some years before the pandemic and also shortly after it had ceased. They found Pfeiffer's bacillus with practically the same frequency in both periods. In the years 1912—18 it was found in 15 out of 70 cases; from Feb. to April 1919 (the examinations were started 6 weeks after the epidemic had ceased) in 8 out of 30 cases.

As regards the occurrence of Pfeiffer's bacillus in healthy persons (in addition to the authors already referred to, PREUSS, WILLIAMS and others) the following have reported:

SILER found it in 595 out of 2197 healthy soldiers in the British army in France during the epidemic.

PRITCHETT & STILLMAN (New York 1919) cultivated Pfeiffer's bacillus from 74 out of 177 healthy persons who denied having had influenza. The positive findings were rather more frequent in the throat than in the saliva. The investigations were continued by WINCHELL & STILLMAN who found Pfeiffer's bacillus in 74 out of 190 children in a Children's Home where influenza was said to be absent. In 19 cases it was shown to persist for 4—5 months in the same individual. No alteration could be observed in the frequency of occurrence of the microbe in healthy individuals from Dec. 1918 to June 1919 although influenza died out in this interval.

In the investigations at the Rockefeller Institute during the winters of 1918—19, 1919—20 and 1920—21 Pfeiffer's bacillus was found altogether in 332 out of 1077 healthy persons. (STILLMAN).

LORD, SCOTT, & NYE found Pfeiffer's bacillus in 26 out of 34 soldiers who had been quite well for the last 3 months.

WOLLSTEIN & SPENCE (1920) found it in 10% of 266 healthy children under 2 years.

EDINGTON demonstrated Pfeiffer's bacillus by cultivation in 3—9 men out of each of 10 lots of 50 men. In comperative examinations of 200 men he found that cultivation from the naso-pharynx via the mouth yielded better results than from the nose or tonsils (Cf. WILLIAMS, and PRITCHETT & STILLMAN).

SKAJAA (Bergen) found Pfeiffer's bacillus in 19 out of 20 soldiers in Nov. 1919.

Of authors who deal particularly with the occurrence of Pfeiffer's bacillus in morbid conditions other than influenza, may be mentioned:

Sellards & Sturm (Massachussets, 1919) who found it in 25 out of 31 cases of measles. It usually disappeared quickly after the symptoms ceased.

Seligmann & Wolf (Berlin), in 1919, by using the „coughing method" demonstrated it in 30 out of 94 cases of influenza, in 22 out of 57 cases of measles, in 20 out of 44 cases of whooping-cough, in 12 out of 52 cases of tuberculosis, in 2 out of 21 cases of diphtheria, but in no case out of 31 scarlatina patients and 16 healthy persons.

Pilot & Pearlman (Chicago) from April to July 1920 made cultivations from the excised tonsils and adenoids of 155 persons and found Pfeiffer's bacillus in the tonsils 62 times and in the adenoids 47 times.

Stillman (New York) in the winter of 1920—21 when there were only a few cases of typical influenza in the town, cultivated Pfeiffer's bacillus from the throat and other places in 30 out of 35 patients with lobar pneumonia.

That on the whole Pfeiffer's bacillus is principally met with in the beginning of the disease is maintained by Neufeld & Papamarku, Olsen and many others. Some Italian authors have found this to a particularly marked extent.

Piras and Gosio (1) assert that in order to demonstrate Pfeiffer's bacillus it is important to make the examination in the first 12—15 hours of the illness.

That this provision is not always necessary, is evident at any rate from the paper cited of Opie & others (p. 37).

The occurrence of the bacillus in influenza in the blood and various internal organs apart from the respiratory tract is reported, in addition to the already mentioned authors (Paltauf (1), Leichtentritt; Messerschmidt, Hundeshagen, & Scheer, Kraus & Kantor), amongst others by the following:

Courmont, Durand, & Dufourt found Pfeiffer's bacillus by cultivation of the blood post mortem in 4 cases (twice in pure culture and twice associated with Streptococci); they did not find it however in kidney, spleen or liver, nor in 86 blood tests taken in vivo.

v. Hoesslin; Dick & Murray, and Spooner, Scott, & Heath each report finding Pfeiffer's bacillus twice in the blood; the two last-named groups of authors each examined the blood of 80 patients, in vivo and post mortem respectively.

TYTLER, JANES, & DOBBIN cultivated Pfeiffer's bacillus from the heart blood in 2 out of 50 autopsies.

DUJARRIC DE LA RIVIÈRE cultivated Pfeiffer's bacillus from a catheter specimen of urine in 10 influenza patients while it could not be obtained from the blood. This occurrence in the urine is in agreement with the finding of Pfeiffer's bacillus in the kidneys previously spoken of.

Whether ORTICONI & BARBIÉ's strikingly frequent finding of Pfeiffer's bacillus in the blood (in 38 out of 62 cases) is reliable, is open to doubt. They state firstly, to which no particular importance can be attributed, that the bacillus was a little larger than the ordinary Pfeiffer's bacillus, and secondly that it grows „difficilement" on ordinary media. But one of the essentials in the diagnosis of Pfeiffer's bacillus is that in pure culture it practically never grows on ordinary bloodfree bouillon or agar.

Concerning the occurrence of Pfeiffer's bacillus in animals I have only found one communication.

OLSEN (3) states that in pneumonia in dogs that had died of distemper, there were found „in grossen Massen hämoglobinophile Stäbchen, die sich mit den üblichen Differenzierungsmethoden (Kultur, Agglutination, Komplementbindung) bisher in keiner Weise vom Pfeifferschen Influenzabacillus unterscheiden liessen".* This account is however nò good enough to convince one that it really was Pfeiffer's bacillus and not the Bacillus haemoglobinophilus canis (FRIEDBERGER) which is distinguished from Pfeiffer's bacillus by some important characters, although ODAIRA found representatives of the two organisms to be identical from a serological point of view. Concerning „Bacillus septicaemiae canis" (PARANHOS) see p. .

There is no information about the occurrence of Pfeiffer's bacillus or other haemoglobinophilic bacteria „in Nature" apart from the human or animal organism.

The Occurrence of Pfeiffer's Bacillus in the Blood, Meninges Etc. Virulence.

Up to the present we have only discussed the occurrence of Pfeiffer's bacillus in the blood and in those organs to

* I have later had the opportunity of speaking with Dr. OLSEN, who stated that the bacilli exhibited the „symbiosis-phenomenon". They must, then, probably be considered as Pfeiffer's bacilli.

which it can only gain access by way of the blood-stream in so far as it was secondary to its presence in the respiratory tract. But we have left untouched the rather voluminous literature on haemoglobinophilic bacteria as the cause of meningitis and septicaemic or pyaemic infections. The reason for this is that several authors have considered that organisms found under such conditions, — or, generally, those that can be cultivated from the blood — should not be regarded as Pfeiffer's bacilli, because it ought to be one of the characteristic points of recognition of Pfeiffer's bacillus, that it is speedily destroyed when it enters the blood-stream.

In order to adjudicate upon this question we must first consider some experiences of the pathogenicity of Pfeiffer's bacillus for animals.

Even in the early days of his fundamental work PFEIFFER (2) reported that he could never cultivate his bacillus from the blood of influenza patients. It is true that post mortem it could in some cases be cultivated from spleen and kidneys but was only present in very small numbers. In over 100 inoculation experiments in rabbits carried out in various ways he observed a marked toxic action but no occurrence in the blood or multiplication in the organism; on the contrary the bacilli died rapidly.

DELIUS & KOLLE found that after intraperitoneal inoculation into rabbits, guinea-pigs, and mice, Pfeiffer's bacillus can easily increase in numbers at that site, while from the blood and the different organs isolated colonies at most, could be cultivated. On introduction into the blood or subcutaneous tissue the majority of the bacilli were quickly destroyed. SPOONER, SCOTT, & HEATH, GOSIO & MISSIROLI, WOLF and others had the same experience.

Several authors (KAMEN (2), JACOBSON, SAATHOFF), by inoculating Pfeiffer's bacillus together with certain other bacteria (alive or dead), could increase its virulence so that it could easily be cultivated from the heart blood.

But also by inoculation (especially into the peritoneal cavity of the mouse or guinea-pig) of pure cultures of Pfeiffer's bacillus cultivated from the respiratory tract in influenza, whooping-cough, tuberculosis etc., many authors have been able to produce a septicaemic infection or at any rate they could

demonstrate the bacilli in the blood in large quantities. (Cantani, Slatineano, Davis (1), Malone (1), Okawara & others).

As regards the cause of these divergent results we can naturally think of different possibilities, but it must be established that we cannot conclude directly, from the fact that a bacillus can be cultivated from the blood after inoculation of a mouse or guinea-pig, that it is different from Pfeiffer's bacillus.

Virulence, a term which principally evokes the conception of the power to multiply in the animal organism, is, as is well known, a character which is capable of undergoing great variations in the same bacterial strain. In consequence it is very difficult to employ it as a basis for a classification of bacteria when they do not possess a particularly well-marked and constant degree of virulence.

Tho what degree the virulence of individual strains of haemoglobinophilic bacteria can vary, and especially how greatly it may diminish after quite a few subcultures on artificial media, and what other difficulties there may be in the investigation of the virulence of these bacteria as well as individual differences in the susceptibility of the animals, is dealt with in the works of Cantani (2), Umeno & others, Tanaka, Yabe, Wolf, and Blake & Cecil.

The author who has been most emphatic that haemoglobinophilic bacteria which are the cause of meningitis and septicaemic infections, must be distinguished from Pfeiffer's bacillus, is Cohen, who found such bacteria in pure culture in meningitis in 3 children and also cultivated them from the blood. He found they were very virulent for rabbits and guinea-pigs, and laid particular stress on the fact that they produced a fatal septicaemia on intravenous injection into rabbits.

From several other authors' works it appears that haemoglobinophilic bacteria which are the cause of meningitis can usually be demonstrated in the blood of patients, and also in the blood of animals injected with them much more often than the „ordinary" Pfeiffer's bacilli.

It is not the intention here to proceed further with the complete literature on meningitis and septico-pyaemia produced

by haemoglobinophilic bacteria, but merely to utilise as much as is necessary for the discussion of the position of these bacteria in the classification of organisms.

When meningitis and septico-pyaemia are mentioned together it is because we have to deal with a whole group of diseases which turn upon the occurrence of the bacilli in question in the blood as the chief phenomenon. We have to deal partly with meningitis without other lesions, and partly complicated with purulent processes produced by the same microbe in other situations, particularly in the joints. Further, we have pyaemia and septicaemia (endocarditis) without meningitis. Frequently we find the organism at the same time in the ordinary sites of election of Pfeiffer's bacillus (lungs, throat, accessory nasal sinuses, and middle ear).

Wollstein (3) like Cohen, on injection of haemoglobinophilic bacteria from meningitis cases, into rabbits could usually cultivate them from the animal's blood in large quantities, but this was generally not the case with the haemoglobinophilic bacteria from the respiratory tract. She looks upon this difference in virulence only as one of degree which cannot form the basis of any division into two well defined groups. It appears from Henry's and Ritchie's works that the haemoglobinophilic bacteria from meningitis are on the whole more virulent for different animals than Pfeiffer's bacilli obtained from the respiratory organs but that this difference is not always so marked that it can be made the criterion of a definite boundary between the „meningeal" and the „respiratory" strains. This view is, on the whole, supported by the other literature bearing on the subject. (In this connection particular mention may be made of the lists of litterature by Cohen, Henry, Ritchie, Torrey, Anderson & Schultz, and Bender).

As regards morphological and „cultural" characters the haemoglobinophilic bacteria from the meninges and blood exactly resemble Pfeiffer's bacillus, according to practically all the work upon the subject, — even that of Cohen and Cohen & Fitzgerald. Besides differences in virulence between meningeal and respiratory strains, certain serological distinctions have been demonstrated (Cohen, Wollstein (3)). In view of the great serological differences which have been demon-

strated in the last few years in the group of bacteria which everybody agrees to call Pfeiffer's bacilli, no particular significance can be attached to this. Another argument in favour of not classing the meningeal strains as Pfeiffer's bacilli is that the corresponding meningitis commonly arises without any connection with influenza epidemics; and so long as Pfeiffer's bacillus is credited with being the bacillus of influenza, it would be difficult to class the other bacteria as influenza-bacilli also. But with the surrender of the theory of the specific importance of Pfeiffer's bacillus for influenza, this argument naturally falls to the ground.

Most authors have therefore decided to call haemoglobinophilic bacteria from meningitis and septicaemic infections Pfeiffer's bacilli, when their morphological and cultural characters are identical with this organism even though they possess a particular virulence.

To show how untenable it is to support COHEN's sharp distinction, a contribution of BENDER (from PFEIFFER's Institute in Breslau) which has recently appeared, will be made the object of a further discussion of the problem.

BENDER starts by assuming that COHEN's bacillus is different from PFEIFFER's and tries to show that the haemoglobinophilic bacillus he found in a case of meningitis is identical with the former and distinct from the latter. On injecting 4 cultures, which had been grown for from 6 weeks to 3 months, intravenously into 3 rabbits, 2 of them were unaffected while the third died 17 hours after the injection. Pfeiffer's bacillus was grown in pure culture from the heart blood, spleen, and liver but could not be demonstrated by direct microscopic examination. „Angesichts der grossen injizierten Bazillenmenge ist hier der Tod jedenfalls durch Vergiftung bedingt", for which reason he does not put much reliance on this result but believes, in view of the other 2 rabbit experiments and several other negative animal experiments, he has shown that his bacillus is identical with PFEIFFER's and distinct from COHEN's. But this conclusion is hardly justified. In the first place it is very possible that the bacillus was originally virulent but lost that property with the rather lengthy cultivation on artifical media As already mentioned such a loss of virulence can take place very rapidly. The bacilli with which COHEN could produce septicaemia in rabbits were certainly cultivated still longer, but it is highly probable that the rapidity with which the virulence diminishes may be very different in different cases depending amongst other things on the constitution of the nutritive medium. Pfeiffer's bacillus is in fact a rather fastidious organism and the conditions it is subjected to

by cultivation on media of variable constitution must undoubtedly be very diverse. Then again it was possible to cultivate the bacillus from the blood 17 hours after the injection in one of BENDER's rabbits which indicates that it possesses no trifling resistance against the bactericidal action of the blood and thus far is in favour of its identity with COHEN's. If we further compare this experiment with corresponding experiments of COHEN and PRASEK & ZATELLI (who BENDER considered worked with COHEN's bacillus), and also WOLLSTEIN, the only difference (apart from COHEN's inoculations in series into fresh rabbits, which was not tried by BENDER) consists in the fact that these authors not only cultivated the bacillus from the heart blood but also demonstrated it microscopically „in considerable numbers". How much weight may be attributed to this difference, it is difficult to know. While BENDER's rabbit died 17 hours after the injection, COHEN's died 5 and 12 hours after respectively, PRASEK & ZATELLI's 14 hours after, and WOLLSTEIN's 18—30 hours after. This does not speak well for the view that in the first case there was a purely toxic action while in the other cases there was multiplication without primary toxic action.

The reason for making this work the object of a detailed review is merely to afford an example of the want of clearness and certainty which besets all attempts to classify haemoglobinophilic bacilli on a basis of virulence. It is of course feasible that by means of very arduous and protracted experiments with the use of a large number of animals more definite boundaries could be established, but I consider it better to agree with HENRY that „the investigation of such a series of haemophilic 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."

A qualification of this statement however, must be made. As regards toxin formation or to use a more guarded expression, toxicity, it may certainly be claimed that this character is often very prominent, but it is undoubtedly not justifiable to propose it as a criterion of Pfeiffer's bacillus, and investigations on this point have up to the present scarcely made any appreciable contribution to the classification of this species of bacteria. The problem assumes a different aspect with regard to serum reactions as will later be explained.

Morphology.

After having seen that we cannot make use of virulence for grouping the haemoglobinophilic bacteria, we will now consider differences of another kind in the group which as before we will call Pfeiffer's bacillus.

This question has already been touched upon in PFEIFFER's (2) fundamental work. From 3 autopsies on cases of bronchopneumonia complicating diphtheria, bacilli were cultivated which were considerably larger than ordinary Pfeiffer's bacilli with a greater tendency to form threads (with what justification PFEIFFER and many others call these threads „Scheinfäden" is not evident). But in their remaining characters, especially their growth only on blood agar and the appearance of the colonies, they entirely resembled Pfeiffer's bacilli. He termed these divergent forms „pseudo-influenza bacilli" and regarded them as distinct from, but closely related to „true influenza bacilli".

Since then many investigators, in fact most of those who had worked more extensively with Pfeiffer's bacillus have observed similar forms. Even after only a few years had passed there was general agreement that the distinction between the „true influenza bacillus" and the „pseudo-influenza bacillus" on this purely morphological basis was untenable. PFEIFFER himself also subscribed to this view.

After going through the descriptions of the morphology of Pfeiffer's bacillus by the various authors who have given detailed information about it (PFEIFFER (2), PIELICKE, LINDENTHAL, GRASSBERGER (2), SLATINEANO, SÜSSWEIN, ALBRECHT & GHON, CANTANI (1), FICHTNER, KORENTSCHEWSKY, BEALL, WOLLSTEIN (1,3), TEDESKO, ELLERMANN, WOHLWILL, DAVIS (1,2), HÜBSCHMANN, LEVINTHAL, MC. INTOSH, HUNDESHAGEN, FROMME, LEICHTENTRITT, OLSEN (2), DICK & MURRAY, SPOONER, SCOTT, & HEATH, LEGROUX, SKAJAA, CARPANO (1), SELIGMANN & WOLF, PREUSS, WADE & MANALANG and others), one gets the following impression of the morphology of Pfeiffer's bacillus.

In sputum etc. (but not in spinal fluid) it is met with nearly always in the form of very small rods, usually quite short, so that they can be mistaken for diplococci. The length is however often rather variable but the thickness is fairly

uniform. In cultures on suitable media the same form is usually observed although there is on the whole rather more polymorphism, particularly as regards the length of the individual elements. It is far from rare however to find divergent forms which may have a very different appearance: first of all thread-forms often of considerable length (20—30 μ or more). They may be of the same thickness as the short forms or rather thicker. Very frequently a few threads or long rods are seen in an otherwise typical preparation. Next, forms like PFEIFFER's „pseudo-influenza bacillus" are met with, that is to say rods which are longer and thicker than the typical form, and often curved. Lastly a very variegated picture may be seen, — large, round, oval, pear, or spindle formations, rods furnished with large or small protuberances or irregular swellings, and sometimes branchings. The marked polymorphic forms are comparable with the involution forms of the plague bacillus. WADE & MANALANG have described cultures which consisted exclusively of long threads with rounded bodies of various sizes, sometimes situated at the end and sometimes at the side, in the latter case either on a small branch or directly on the flank of the thread.

There may be pronounced individual differences in the tendency of the various strains to produce such forms but an actual classification on these grounds cannot be made partly because there are all possible transitions, and partly because the same strain may often vary very considerably in such a way that at one time the typical picture may be found, at another a more or less atypical picture in different generations of the same strain, and also that the different forms may be seen side by side in the same preparation.

The irregular forms appear principally in old cultures and on poor media (ascitic agar, CANTANI's (2) bile agar, FICHTNER's sputum agar, blood agar containing citrate or other salts in greater concentration than normal, too acid or too alkaline media). PREUSS found them especially at the end of the pandemic.

But also under apparently unaltered conditions of growth the same strain may at one time grow typically and at another, in a very irregular manner.

Sometimes the first culture on an artificial medium is

abnormal and the later cultures typical, and sometimes the converse is the case.

According to WOLLSTEIN (3) and others Pfeiffer's bacilli from meningitis are usually bigger and longer than from the respiratory tract. This difference is however far from constant, and in all it has not been possible to demonstrate any connection between the habitat and the morphology (DAVIS (2)).

On the whole there is general agreement that the morphology of Pfeiffer's bacillus in every respect exhibits well-graded intermediate forms so that any grouping on that basis cannot be undertaken.

Biochemical Reactions.

We have now seen that the morphology is no more suitable than the pathogenicity for animals, as a basis for classification. As these factors, up to the beginning of the last pandemic, were the only ones which had been examined from this standpoint (apart from the first attempts at a serological analysis), it cannot be wondered at that we were still not in a position at that time to form an opinion as to how far distinct differences between the individual strains of Pfeiffer's bacillus existed, or whether they were all to be regarded as mutually identical.

But during and after the pandemic numerous papers have appeared dealing partly with the biochemical reactions of Pfeiffer's bacillus and partly with its serological reactions.

Of the biochemical reactions indol formation should first be mentioned. That Pfeiffer's bacillus can form indol was first proved by RHEIN (1919). Subsequently JORDAN (1), MALONE (2), RIVERS (1,2), RIVERS & KOHN, POVITZKY & DENNY, and YABE have all examined a number of strains of Pfeiffer's bacillus for indol formation and they have all obtained the surprising result that the strains fall into 2 fairly sharply defined groups: some form indol easily while in others this power is quite lacking. Intermediate forms, namely strains possessing weak or doubtful indol formation seem only exceptionally to occur. The overwhelming majority of strains from in-

fluenza and meningitis form indol, while those that do not produce it occur in a wider distribution, but usually in fewer numbers than the indolpositive strains, in healthy people.

Indol formation, as is well known, is a characteristic of certain groups of Gram-negative bacteria (Coli etc., cholera and various other vibrios, Proteus, and some of the dysentery organisms). It might seem possible therefore that indol-positive and indol-negative Pfeiffer's bacilli had hitherto been wrongly classed as the same bacterium, and that in the future they must be rigidly separated from one another and be looked upon as just as distinct as, for example, coli and typhoid bacilli. YABE writes thus: „The absolute distinction between strains of influenza bacilli is in the property of indol formation". It will later on be further discussed whether such a view is justifiable.

The first investigations of the action of Pfeiffer's bacillus on carbohydrates and alcohols were made by LEVINTHAL (1) (1918) who, on cultivating it on the medium he devised with the addition of litmus and 1% of various substances (glucose, laevulose, lactose, mannite, maltose), found a slight red colouration of the plates containing glucose only, all the other substances being unacted upon. MESSERSCHMIDT, HUNDESHAGEN, & SCHEER with the same technique obtained slight acid production on the glucose plates, lactose, saccharose, and mannite remaining untouched. Some strains fermented maltose and dextrin.

A number of authors (STILLMAN & BOURN, RIVERS (2), RIVERS & KOHN, POVITZKY & DENNY, THJÖTTA, MALONE (3)) have devised more elaborate experiments, mostly with the use of fluid media and they have found a more pronounced fermentation of the different kinds of sugars than the foregoing authors. But it must be pointed out that the power of Pfeiffer's bacillus to form acid cannot be compared with that of the powerful acid-producers. The greatest degree of acidity that can be produced in media containing glucose corresponds to P_H 6 to 6.4 (while Streptococcus for example can produce a P_H value of 5 and under). Furthermore the power of fermentation is largely dependent upon the constitution of the medium. Thus RIVERS, in his first experiment in which he

used bouillon containing fresh rabbit blood, obtained practically no fermentation either in glucose or in a whole series of other carbohydrates. Afterwards he got inconstant results by cultivating in peptone water heated together with blood and then filtered. He obtained the most marked reactions by solidifying the last-named medium with agar. But the same strain may give different results on the same medium on repeated trials.

The acid-producing power is therefore so weak that the alteration in reaction in many cases is limited by the very capacity to produce acid. In media which are poor in buffers and favourable for acid production so much acid however may be formed that the power to tolerate acid is the limiting factor. This follows from the authors' experience that the final P_H when media poor in buffers and favourable for acid formation are employed, is independent of the initial reaction even though it varies from P_H 6.8 to P_H 8. There was no difference in the final P_H between the different strains.

The following table shows the results of the various authors:

	Xylose	Glucose	Galactose	Laevulose	Maltose	Saccharose	Lactose	Raffinose	Dextrin	Inulin	Mannite	Salicin	Arbutin
Levinthal		(+)		0	0		0				0		
Messerschmidt a. o..		+			+ 0	0	0		+ 0		0		
Stillman & Bourn ..		+	+	(+)	+ 0	+ 0	0		(+) 0	0	0		
Rivers)*	+	+	+	+	+ 0								
Povitzky & Denny .		(+) 0											
Thjötta		+			0	0	0	0		0	0	0	
Malone		+ 0											
Gosio (2)													0

This indicates that the fermentation of the pentose, xylose, and the 3 hexoses, glucose, galactose and laevulose, as well

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* Rivers & Kohn however discovered a meningeal strain which did not ferment any of the 5 carbohydrates.

+ = marked acid production.
(+) = very weak acid production.
0 = no acid production.
+0 = reaction different in the case of different strains.

as the lack of fermentation of the disaccharide lactose, the trisaccharide raffinose, the polysaccharide inulin, the alcohol mannite and the glucosides salicin and arbutin, are common to all Pfeiffer's bacilli; while the action upon the disaccharides maltose and saccharose, and the polysaccharide dextrin, can be utilized to distinguish the individual strains of the Pfeiffer's bacillus group.

Serological Reactions.

Before the last pandemic there was very little information about the serological reactions of Pfeiffer's bacillus.

The first contributions date from 1903 when CANTANI (3) showed that Pfeiffer's bacillus could induce the production of agglutinins in man as well as in animals immunised with the microbe, and VAGEDES found the serum of 8 out of 27 influenza patients to be slightly agglutinating.

Later WOLLSTEIN (1,2) discovered that in whooping-cough and influenza, Pfeiffer's bacilli cultivated from the patients were agglutinated by the same patients' sera. This was especially the case in whooping-cough. Every serum agglutinated every strain which was cultivated from a whooping-cough patient, but the culture from the corresponding patient was always agglutinated to a greater extent than cultures from other patients. With a strain of Pfeiffer's bacillus from other sources the sera of all whooping-cough patients gave a negative result. With serum of rabbits inoculated with Pfeiffer's bacillus no difference could be demonstrated between Pfeiffer's bacilli from pneumonia, tuberculosis, influenza, conjunctivitis or whooping-cough.

ODAIRA prepared rabbit serum against Pfeiffer's bacillus, Bac. haemoglobinophilus canis, „Cohen's bacillus", and Bordet's bacillus and found by agglutination and complement fixation that the two first were practically the same, and the others different both from these and mutually among themselves. Only one or two strains of each kind of bacterium were used. COHEN's serum reactions with meningeal strains and other races of Pfeiffer's bacillus have already been mentioned. The investigations of OLMSTEAD & POVITZKY and others also showed that various strains of Pfeiffer's bacillus were different, but a clear perception of the facts was not obtained until the more extensive investigations during the pandemic were undertaken.

The serological reactions during the pandemic were carried out with two objects in view: (1) to search for specific anti-

bodies against Pfeiffer's bacillus in the serum of influenza patients and thus obtain information about its pathological significance; (2) to look for mutual differences between the strains of Pfeiffer's bacilli. It is almost entirely agglutination that has been used, as this reaction is the simplest to perform and gives the clearest results. The other antibody reactions have hardly given any appreciable information about the classification of Pfeiffer's bacillus beyond what has been obtained from agglutination, and they will therefore only be incidently touched upon in the following account, which will only be a summary of the most important investigations and their results.

While several workers have been unable to demonstrate any specific agglutinin against Pfeiffer's bacillus in the blood of influenza patients, many others, as LEVINTHAL (1), NEUFELD & PAPAMARKU, FROMME, SOBERNHEIM & NOVAKOVIC, have found a marked, specific production of agglutinin in a number of the patients examined. GAY & HARRIS observed agglutination and complement fixation not only in a number of influenza patients but also in some of the persons who were injected with influenza vaccine (containing Pfeiffer's bacillus). DUVAL & HARRIS found that the majority of influenza patients exhibited agglutination and later in the disease, complement fixation with Pfeiffer's bacillus.

It is however not only for Pfeiffer's bacillus that antibodies are produced but for various other organisms found in influenza.

KOLMER, TRIST, & YAGLE found agglutination and complement fixation for Pfeiffer's bacillus in a number of influenza patients, and to a lesser degree for Streptococcus and Micrococcus catarrhalis, while HOWELL & ANDERSON more often obtained complement fixation with Streptococcus viridans than with Pfeiffer's bacillus.

In testing the sera of their patients many authors have used several strains of Pfeiffer's bacillus. SPOONER, SCOTT, & HEATH found no difference between the reaction of the serum against the patient's own strain and foreign strains, and LORD, SCOTT, & NYE who found complement fixation with Pfeiffer's bacillus in healthy carriers, obtained the same result when using a monovalent or polyvalent antigen. The majority of authors however state that on carrying out the tests with several strains they found more or less difference between them. (FÜRST,

Fleming, Wollstein (4), Wilson, Hartley, Edington, Rapoport, Utheim, who used both agglutination and complement fixation).

Malone (1) tested 39 sera with the homologous strains (that is to say from the same patient) and got 32 positive and 7 negative agglutinations, while 52 sera tested with a mixture of 4 heterologous strains gave 22 positive and 30 negative reactions. Lastly 28 sera were tested simultaneously with homologous and heterologous strains; 23 of them gave a positive reaction with the homologous strain and only 10 with the heterologous. A negative reaction with the homologous strain coupled with a positive with the heterologous, was never found, and the titre was always lower for heterologous strains than for homologous.

These investigations on the whole create a decided impression that strains of Pfeiffer's bacillus, even though they are cultivated from influenza patients in the same locality, cannot usually be regarded as mutually identical from a serological standpoint. More precise information in this direction can however be obtained by means of animal experiments.

The most comprehensive investigations have been carried out in America at the instigation of Park's emphatic contention that the mutual identity or distinction of different strains of Pfeiffer's bacillus must be of great importance in the problem of their primary or secondary rôle in influenza.

Park, Williams, & Cooper prepared rabbit serum with 20 chosen from 160 strains, the latter being made up of 15 strains taken from autopsies and more than 60 from patients in the beginning of the disease. They tested all the strains against all the sera by simple agglutination and partly also by agglutinin absorption. A certain amount of cross agglutination of one strain with the serum corresponding to another strain was found, but by absorption tests only 4 „pairs" could be discovered, that is to say 8 strains with identical pairs.

Valentine & Cooper in the first place examined 9 strains from autopsies of influenza patients and found them all different by agglutination and absorption of agglutinin. Afterwards they examined numerous strains cultivated from influenza early in the disease, from influenza convalescents, and from the throats of healthy people, — in all, 171 strains, which were tested against 25 sera, — and only exceptionally found identical strains among them. Even strains which were cultivated early in the disease from persons who might be expected to be infected with influenza from the same source

(soldiers in the same camp; children in a children's home) were proved to be distinct from one another. This was the case even in a family, the culture being taken from different members who fell ill almost simultaneously. In the absorption experiments 3—4 times the bacterial concentration necessary to completely absorb the agglutinin of the homologous serum, was used, but practically none of the agglutinin in the serum corresponding to the other strains was absorbed. In some special experiments on the effect of bacterial concentration it could even be considerably further increased without absorbing any of the heterologous agglutinin.

POVITZKY & DENNY tested 100 strains obtained from influenza cases in 1918, and 90 strains from a later outbreak, against 30 sera prepared with strains obtained in 1918 and also before and during the last outbreak. These authors also found nearly all their strains different from one another.

The greatest number of strains in VALENTINE & COOPER's, and POVITZKY & DENNY's material collectively, which were identical with one another, was 5.

BELL tested 36 strains with 27 sera, — just as the three preceding groups of authors did by agglutination and absorption of agglutinin — and found the majority were different from one another. There was however a gradual change from one to another as regards the power to absorb agglutinin.

COOKE obtained similar results in complement fixation experiments with 16 strains against 48 sera.

RIVERS & KOHN examined 13 meningeal strains which were isolated in the course of 7 years and found 2 groups of 3 and 7 identical strains. 1 occupied an intermediate position between the 2 groups, and 2 stood quite apart. 4 strains cultivated from the blood of children with pneumonia were all different from one another.

7 of RIVERS & KOHN's 13 meningeal strains had previously been examined by POVITZKY & DENNY, who in fact, had found another grouping. (In one or two other cases also it is reported that 2 investigators obtained different results with the same strains. Whether this was due to confusing the strains or to other causes, it is hardly possible to determine).

Among other workers who have also found marked differences between strains of Pfeiffer's bacillus may be mentioned FLEMING & CLEMENGER, SMALL & STANGL, SELIGMANN & WOLF, BIELING & JOSEPH, BIELING, BIELING & WEICHBRODT, COCA & KELLEY, CHESNEY, MAITLAND & CAMERON, SKAJAA.

No distinct grouping on the strength of the serological reactions has been effected.

In opposition to the previous authors, HUNTOON & HANNUM found that some strains, from places far apart, both by direct

agglutination and by absorption, were very closely related. ROOS obtained similar results. These divergent results cannot however invalidate the experience of the numerous other authors which refers to a much larger material and to a great extent was obtained with very elaborate and accurately described technique. The divergent results of the two last-named authors are probably due to the technique employed, but it is not possible to throw any further light upon the question (non-specific serum action? Inhibition of agglutination after „absorption" as a result of altered milieu?) In this connection it may be stated that YOKOI found that Pfeiffer's bacillus and Meningococcus by complement fixation, and by agglutination combined with absorption, were identical! Such a result must certainly depend upon a defective technique and under such circumstances one can naturally not expect to find mutual differences between strains of Pfeiffer's bacillus.

It is of great importance to know whether the serological reaction of an individual strain keeps constant for a long while and in changing conditions, or whether it is easily and rapidly transformed.

Judging from our experience of other kinds of bacteria e. g. Pneumococcus and Meningococcus, the serology, and especially the agglutination of a strain, is one of its most constant characters which in the large majority of cases practically does not change either on cultivation for a long period or on passage through animals. We must therefore presume the same holds in the case of Pfeiffer's bacillus, if there are no important facts pointing in the opposite direction. For the technique with which differences in the agglutination of Pfeiffer's bacilli have been demonstrated is just the same as that employed to divide Meningococcus and Pneumococcus into well-defined types.

The direct observations that have been made as to the constancy or liability to change of the agglutination reactions of Pfeiffer's bacilli do not on the whole indicate that they would act very differently to other bacteria.

BIELING & JOSEPH found that a strain of Pfeiffer's bacillus retained its agglutinability unchanged even after 11 mouse passages. PARK, WILLIAMS, & COOPER, on investigating many strains, found they did not change their type either on cultivation for a long time or by animal passage. PARK & COOPER observed 3 chance laboratory infections in man and found in each case that the infecting culture and the bacillus cultivated from the patient were serologi-

cally identical. CECIL & STEFFEN found the same thing in several inoculation experiments in man. VALENTINE & COOPER and POVITZKY & DENNY made repeated investigations of the same persons, some patients and some healthy carriers, and they usually found the same type in all the examinations of the same person. In one case the type remained unaltered after a year had passed. Sometimes however the type would change. It is reasonable to suppose that this was due to its being superseded by another type. Thus one case was observed where 49 out of 50 colonies from the same inoculation, were of one type, and the remaining one, of another type. On further investigation a couple of weeks later only the last type was found. In a family it was observed that a certain type was present in overwhelming numbers in each member, but also in the case of several of them another type was present in smaller numbers which was identical with the dominating type in another member of the family.

An observation exists however which seems to suggest a marked lability from a serological point of view. ANDERSON & SCHULTZ cultivated Pfeiffer's bacilli in a meningitis case from the spinal fluid, blood, nose, throat, and naso-pharynx, and found them all different by agglutination. They look upon it as improbable that the patient was infected with 5 independent strains and draw the conclusion that Pfeiffer's bacillus can easily change its agglutination reactions. Unfortunately absorption experiments could not be carried out as the different sera had been used up. They state they obtained better results with agglutination when it was allowed 2 hours at 37° and subsequent standing in the ice-safe than when incubated at 53° or 56° for a longer time, which is difficult to understand. It is therefore feasible that ANDERSON & SCHULTZ's curious results are due to defective technique; but in any case they are so peculiar and of such fundamental importance that as soon as the opportunity occurs the experiments ought to be repeated.

In view of the wide distribution of Pfeiffer's bacillus there is nothing extravagant in the conception that a person may harbour more than one strain. Thus BELL found as many as 3 different strains in the same person, but 5 seems to be a rather large number. For the present we must be sceptical that the same strain of a bacterium may be different as regards agglutination according as it is isolated from one or other site of the mucous membrane of the upper air passages.

General Classification.

The biochemical reactions, just as the immune-reactions, show that Pfeiffer's bacillus cannot be regarded as a single well-defined bacterial type but rather that this name includes a

whole group of bacilli in which marked differences in various directions can be demonstrated.

The question now is whether the various reactions referred to (and a few others of more doubtful* value, e. g. nitrate reduction and gas production) afford any grounds for a rational division of the group. It must be insisted that some constant connection be demonstrated between the different characters. According to those authors who have investigated their strains in various different directions, this is generally speaking not the case. Between the cultures of Pfeiffer's bacilli from influenza patients and those from healthy people the difference can be demonstrated that amongst the latter more indol-negative strains are found and more strains which ferment di-and polysaccharides (maltose, saccharose, dextrine) than amongst influenza patients (STILLMAN & BOURN). But there is no constant distinction between Pfeiffer's bacilli from influenza cases and healthy persons; one cannot determine experimentally anything certain about the origin of a given culture. SELLARDS & STURM found no characteristic difference between strains derived from influenza and measles; the variations of resistance found against drying, freezing, and the action of bile and NaOH, can hardly be regarded as more than chances.

It is of special interest to know whether the distinction between indol-positive and indol-negative corresponds to any deeper biological difference between these two groups. If such is the case this difference must be apparent in other ways besides indol formation. In other words there must be a correlation between the conditions relating to indol and the other characters. From what is known up to the present, this is not the case. It may specially be mentioned that YABE found no difference (to which any importance can be attached) between indol-positive and indol-negative strains either as regards toxicity or complement fixation reactions. A strain A could just as well give a reaction with a serum corresponding to a strain B, when A and B were two strains differing as regards indol formation as when they did not. A particularly striking absence of correlation between indol formation and serological reactions was found by POVITZKY & DENNY, in that meningeal strains, 3 of which produced indol while the

* See for example, CONN & BREAD.

fourth did not, were all identical as regards agglutination and absorption of agglutinin.

Pfeiffer's Bacillus and Influenza.

An account has now been given of the most important observations concerning the occurrence and „internal classification" of Pfeiffer's bacilli, so that we are in a position to discuss the views arising out of this knowledge, about the relation of Pfeiffer's bacillus to influenza.

It is the current opinion that the most convincing proof of the etiological significance of a bacterium in a disease consists in the possibility of producing that disease by the inoculation of a pure culture of the said organism. In the case of Pfeiffer's bacillus there are facts which apparently fulfil this condition, viz. a laboratory infection which KRETZ was the victim of (see TEDESKO), and BLAKE & CECIL's experiments. By the inoculation of Pfeiffer's bacilli, the virulence of which had previously been enhanced by passage through 11 mice and 13 monkeys, in the nose and mouth of 12 monkeys the last-named authors produced in each case a disease bearing symptoms quite similar to those of influenza and which distinguished itself from infections due to Streptococcus and Pneumococcus, among other things, by its marked effect upon the general condition.

But as BLAKE & CECIL admit, it is not possible to decide with certainty whether such an infection is identical with pandemic influenza in man, since experience obtained from human pathology has clearly shown that it is impossible to define the limits of the term influenza alone by the symptomatology of the individual case .

Inoculation of pure cultures of Pfeiffer's bacillus in the nose, throat etc. of man has not in the majority of cases produced symptoms of disease (WAHL, WHITE, & LYALL, BLOOMFIELD, ROSENAU and others). In a number of cases however it has been possible to call forth a catarrhal infection with symptoms more or less like influenza (DAVIS; CECIL & STEFFEN).

It may also be mentioned that the inoculation in small animals particularly guinea-pigs, produces a marked hyperae-

mia and some haemorrhagic inflammation of the various organs, notably the lungs (Gosio & Missiroli and others).

All these facts in connection with the occurrence of Pfeiffer's bacilli in the fine bronchioles and to some extent in the lung tissue itself as well as the formation of specific antibodies in the patients, indicate that the symptoms and pathological changes of influenza to a large extent can be produced by Pfeiffer's bacilli, but they are inadequate to prove its primary significance in this disease.

Several authors insist that the bacillus is the true influenza microbe, that is to say the primary virus of influenza, which lies at the root of the whole pandemic.

Pfeiffer and his collaborators in defence of this attitude affirm in particular that the bacillus is closely associated with influenza and is only exceptionally met with apart from influenza epidemics. But when Leichtentritt (2) after having mentioned that Selter and Neufeld had not found Pfeiffer's bacillus in a number of years, says: „Gegen solche ausführlichen Forschungen verschwinden die vereinzelten Influenzabazillenbefunde bei akuten Infektionskrankheiten, wie sie von einzelnen Autoren, Jochmann, Wohlwill, Klieneberger u. a. m. beschrieben wurden . . . .", this must be looked upon as a rather one-sided view.

If one reviews, without any preconceived notions regarding the significance of Pfeiffer's bacillus, the whole of our knowledge relating to its occurrence, the following conception, in the author's opinion, best accords with the observed facts.

Pfeiffer's bacillus is a microbe which is mainly adapted to living in the mucous membranes of the respiratory tract of man. It is able to exist for a time in healthy persons after being transferred to them from other sources, but in the long run it can only maintain its foothold in the healthy mucous membrane with difficulty. It finds its ideal conditions for development in mucous membranes which are already in a catarrhal state such as is produced by another bacterial infection, but the nature of the infection is a matter of indifference. It may be influenza taken in its widest sense, whooping-cough, measles, or tuberculosis. A catarrhal condition which affects large tracts of the mucous membrane of the respiratory passages is common to these infections, especially the

first three. These diseases therefore afford particularly favourable conditions for the development of Pfeiffer's bacillus. Its growth is also favoured but to a less extent by infections which involve a more limited area of mucous membrane, — simple angina, diphtheria, scarlatina, and by catarrhal conditions or the disposition to such, induced by non-bacterial causes. The last case will explain its occurrence as the only bacterium in bronchitis and similar complaints. The reason Pfeiffer's bacillus occurs most constantly in whooping-cough and measles, is on account of the great distribution and endemic character of these diseases, so that the soil for its nourishment is always present. When once the bacillus has spread itself to its utmost extent in this favourable „soil" nothing will force it to relinquish its foothold. The conditions are otherwise in such a pronounced epidemic disease as influenza. Consider an epidemic in its very early stages. It spreads over a population the large majority of which do not harbour Pfeiffer's bacillus. At first it only exceptionally occurs in the patients, but in those patients in whom it is present it will multiply rapidly and spread from them, first of all in the same congenial soil, which may happen by transference together with the specific virus of influenza to healthy persons who thus become infected with influenza as well as Pfeiffer's bacillus, and also by transference to other influenza patients who then get an additional infection with Pfeiffer's bacillus. Furthermore it can be transferred to healthy persons without their contracting influenza. The frequency with which this takes place seems to vary greatly at different times and places even though its distribution in influenza is the same. This can be explained by assuming that before the appearance of the influenza it lacked the capacity of developing in healthy persons. This power was first developed in its new conditions of life in influenza patients and it is likely to take some time before it has acquired the characters which are essential to its flourishing in healthy persons. Both from originally healthy people and from those who retain their Pfeiffer's bacilli after the influenza has finally disappeared, Pfeiffer's bacillus may be transferred to other healthy persons and to influenza patients. In this manner the bacillus gradually spreads until sooner or later it is present in almost every influenza patient and in a

number of healthy individuals as well. Perhaps the microbe eventually acquires so great an increase in its vitality that it thrives not only as a saprophyte in healthy persons but also may be able by itself to produce infections resembling influenza. When the influenza dies out Pfeiffer's bacillus may exist in normal people for some months still, but as it is incapable of living for a prolonged period as a pure saprophyte it will, as before the influenza appeared, gradually be limited to occurring in the endemic diseases.

The reason that in some cases Pfeiffer's bacillus is found to have a great distribution among influenza patients immediately after the beginning of the epidemic in that particular locality, may partly be due to wide distribution among the patients from whom the infection came, and partly to its being widespread in the same place before influenza appeared there. The converse will be observed when a locality in which Pfeiffer's bacillus is rare becomes infected with influenza not complicated with this microbe.

If one of the smaller „influenza" epidemics develops in a place where Pfeiffer's bacillus is not found, or only rarely, it may happen that the epidemic runs its whole course without Pfeiffer's bacillus being demonstrated in the patients at all.

We can easily imagine that with regard to the distribution of Pfeiffer's bacillus in different „influenza"-epidemics there may exist all possible transitions from complete absence to constant occurrence, occasioned partly by the original distribution of the microbe in the place in question and partly by the interplay between this and other competing or amicable organisms.

As far as one can see, these views are in good agreement with the actual facts, that is to say that on the basis of what is already known about the occurrence of Pfeiffer's bacillus it is reasonable to conclude that it is not the primary influenza microbe. But this opinion forces itself upon us to a still greater extent from our knowledge of the manifold differences between individual strains of Pfeiffer's bacillus. In this connection it will suffice to reckon with serological differences. It has already been intimated that the serological reactions of a bacterium in as far as these can most simply and clearly be demonstrated by agglutination and agglutinin absorption,

must in general be regarded as a very constant characteristic and that the facts prove that Pfeiffer's bacillus in this respect is not very different from other kinds of bacteria.

Even if from a bacteriological point of view (SAHLI), or from epidemiological experiences (BUCHANAN) we relinquish the idea that a definite virus lies at the bottom of the whole pandemic, we must assume that when influenza spreads itself in a short time throughout a certain town or even in a given house (barracks, children's home, family residence) then the same virus must be the cause of all the influenza cases. If we find therefore that the strains of Pfeiffer's bacillus from the various patients are marked by extreme differences among themselves this must be due to the fact that the mode of distribution of this microbe is quite unlike that of influenza. The most likely explanation is that the types of Pfeiffer's bacillus met with were present in the given population before the arrival of the influenza. It is naturally not necessary that Pfeiffer's bacillus should be originally present in all the persons in whom it can be demonstrated after they have contracted influenza. To put the matter in a nutshell, we may imagine that among a population of 10000 there are 1%, that is 100 persons, who harbour 100 different types of Pfeiffer's bacillus, in such small numbers perhaps, that they cannot be found on examination. Nevertheless the conditions would be present for the demonstration of this bacillus in all the patients after the influenza had flourished for some time, and on investigating bacilli from say 50 patients chosen at random we should find the majority of the strains were different from one another.

In addition to the objection already advanced against this view, that Pfeiffer's bacillus might be be very labile in its serological characteristics, two more must be mentioned:

Firstly it has been presumed that the technique used in demonstrating serological difference between strains of Pfeiffer's bacillus is too refined so that unimportant variations have appeared on an unduly increased scale. It can only be said that in all the essentials the technique is the same as that employed in differentiating the types of Meningococcus and Pneumococcus, the soundness of which is generally admitted, and where there is no reason to believe one type is easily and frequently transformed into another. And it is the same tech-

nique as was used to demonstrate the constancy of the types of Pfeiffer's bacillus in animal passage and transference in man.

Secondly it might be urged: It is admitted that all the types of Pfeiffer's bacillus found cannot be the primary influenza microbe; but a particular „pandemic type" might well be discovered which really was the influenza bacillus. To this it must be replied that from the information available we must assume that only a very few strains of Pfeiffer's bacillus cultivated from influenza patients can belong to this type. But then the principal reason for regarding Pfeiffer's bacillus as the influenza microbe is lost, namely its widespread occurrence in influenza. If it had not been possible to find this microbe in more than a few per cent of influenza patients one would certainly never have entertained the thought that it could be the influenza bacillus. Just as little justification is there then for the contention that a distinct race of Pfeiffer's bacillus, which also at most could be demonstrated in a few per cent of the patients, could be the primary influenza microbe and that all the other Pfeiffer's bacilli with exactly the same general biological characters were secondary organisms.

Although therefore it must be said that our knowledge of the occurrence and other relations of Pfeiffer's bacillus strongly supports the idea that it is not the influenza bacillus in its true sense, it must be admitted that there are still obscure points which must be cleared up before we can confirm this with certainty. For instance we have not yet progressed far enough to see clearly in what way the four following points can be brought into harmony with one another.

1. Pfeiffer's bacilli cultivated from influenza cases even in an epidemic which is well-defined as regards time and extent, consist as a rule of a considerable number of different types.

2. The type must in general be regarded as a constant character.

3. In certain cases Pfeiffer's bacillus can be demonstrated almost constantly, immediately after the advent of influenza to the locality.

4. Apart from influenza epidemics Pfeiffer's bacillus can seldom be found in healthy individuals.

It must be pointed out that numbers 1, 2, and 4 can only be regarded as probable. Our experience up to the present is far from sufficient to prove their general validity. But let us assume all four clauses are proved. What will be thought about the relation of Pfeiffer's bacillus when through a single person influenza is

introduced into a hitherto influenza-free neighbourhood and in the course of a short time spreads throughout it, and then this microbe is found from the first in large quantities in all the influenza patients? On the assumption of the absolute validity of the four statements it cannot have been introduced with influenza because then it ought to be the same type in all the patients. We are almost compelled to assume it was present in all the individuals concerned before the influenza arrived but either in such small numbers that it could only rarely be demonstrated, or in such an attenuated condition that it could not be cultivated. If we give up the idea of the absolute validity of one or more of items 1, 2, and 4, there are of course, other explanations possible. For example we might suppose that in those cases where Pfeiffer's bacillus is found in all the patients immediately after the arrival of influenza in a locality, either we should have been able by a thorough examination to obtain it in all healthy persons before the epidemic, or by comparing the strains of Pfeiffer's bacilli from patients infected with influenza from the same source we should find they were serologically identical.

These considerations are sufficient to show that there are still many aspects that need further explanation before we can establish the negative with the greatest certainty, namely that Pfeiffer's bacillus is not the true influenza bacillus.

There is so much however to support this view that the great majority of bacteriologists have more or less decisively rejected the classical conception of the rôle of Pfeiffer's bacillus as the microbe of influenza. But even for him who completely abandons the conception of PFEIFFER, the bacillus, — in virtue of its wide distribution, its pathogenic qualities, and its characteristic nutritional requirements, — will be a worthy object for continued bacteriological research

THE AUTHOR'S INVESTIGATIONS

Investigations into the Occurrence of Pfeiffer's Bacillus.

The following account of my own investigations is divided into 4 sections.

First of all (pp. 72—97) an account is given of the occurrence of Pfeiffer's bacillus in influenza, in other diseases, in healthy persons, and in animals. It has not been my intention to investigate the factors which determine the distribution of the bacillus, but only to try to describe as accurately as possible the facts relating to its occurrence. With this goal in view the numerical results have been treated statistically as far as the material seemed to be adapted for this purpose.

In the next section (pp. 98—204) the whole of the material collected which deals with such bacteria as for the present are called Pfeiffer's bacilli, is investigated as regards a large number of characters that might be expected to be of importance in the classification of these organisms. Only for about 150 strains however is the investigation complete; in the case of the others (about 650 strains) only a small number of characters are dealt with, usually just those few which are regarded as especially characteristic of Pfeiffer's bacillus. The number of strains however which are examined more thoroughly, is so large that it is justifiable to conclude the results obtained in the case of the exhaustively examined strains must apply to the whole material.

The whole investigation has shown on the one hand that all the strains have so much in common that they naturally fall into the same group (species), and on the other hand it has given a complete picture of the differences which are to be observed in this group. In addition care has been taken to give an account of various technical questions.

After investigation of the relations of the most important representative of the haemoglobinophilic bacteria, three of the best known of the organisms which generally are classed in the same category are examined in a similar manner. One of the results attained by this procedure is the justification of our continuing to class all the organisms going under the name Pfeiffer's bacillus, as one species, as it is shown that the differences between this and other species are distinct and regular (pp. 205—224).

Finally, the last section is devoted partly to giving an account of the procedure adopted in this investigation and partly to discussing the different media which are adapted to the cultivation of Pfeiffer's bacillus, a term which is used as equivalent to haemoglobinophilic bacteria in the strictest sense (pp. 225—247).

The characters of Pfeiffer's bacillus which are employed in this investigation are the following:

1. The morphology and behaviour to staining by GRAM's method. Pfeiffer's bacillus is a Gram-negative rod. The first half of this term needs no further elucidation. But we must without delay attempt to define what is meant by the word „rod". It happens in fact, that by no means every single Pfeiffer's bacillus is distinctly rod-shaped. On the contrary it is very common to find that the majority of individual bacilli in a preparation show so little difference between their length and breadth, that they might well be called cocci. The reasons Pfeiffer's bacillus must however be classed with the rod-shaped organisms are explained in the following:

(a). In every culture of Pfeiffer's bacillus the majority of the individual bacilli will show a perceptible difference between length and breadth; and when two elements are arranged as a pair or constitute the two halves of a bacillus which is dividing, the longest dimension, at least for some of the organisms, is at right angles to a line separating the two elements.

The latter in particular always permits of a sure differentiation from Gram-negative cocci, which are either round or oval with the longest diameter parallel to the line of demar-

cation between the pair of elements. At any rate in the specimens I have had to deal with there has never been any difficulty in deciding whether a pure culture consisted of Gram-negative cocci or cocco-bacilli. (By a cocco-bacillus we do not understand an intermediate form between a coccus and a bacillus (rod), but a coccus-like bacillus). One gets a decided impression that there are no transitions between these two forms, but rather a distinct gap.

Yet Pfeiffer's bacillus, especially in sputum, but also in cultures, may greatly resemble Gram-positive cocci (Pneumococcus, „Diplostreptococcus") in shape. As Gram staining was always used there was again no difficulty in distinguishing it from these.

(b). There are quite continuous transitions between coccus-like forms and well-marked rod-forms. Furthermore, there are often in the same preparation both quite short and longer forms; and the same strain can in different „generations" now present itself markedly coccus-shaped, now markedly rod-shaped; finally different strains, of which one consists of quite short elements, while the other appears in well-marked rod-form, may be so quite similar in their other properties, that it would be unreasonable to designate the first as a coccus and the second as a rod.

2. The appearance of the growth on blood-agar. On this medium (i. e. peptone broth agar with a content of 20—25% fresh defibrinated unheated horse blood), Pfeiffer's bacillus grows in pure culture as translucent flattened colonies, only a fraction of a mm across. The culture often develops a darker colouration of the blood-agar in the immediate neighbourhood, but never haemolysis.

3. The appearance of the growth on haemoglobin-agar, that is to say agar with the addition of one of the soluble blood derivatives which have proved favourable for the growth of Pfeiffer's bacillus (haemolysed blood; pepsin-digested blood). The growth on these is far more luxurious than on blood-agar. With scanty inoculation, semi-translucent, usually rather flat colonies of 1—3 mm. diameter make their appearance. The centre of the colony is as a rule, — but not always, — darker than the periphery and forms a little eleva-

tion there. With a small magnification (x 50—100) characteristic granulations are usually observed in the central portion.

4. No growth on haemoglobin-free media. For the demonstration of this feature inoculation on ordinary agar and agar with the addition of (blood-free) ascitic fluid is employed.

5. The „symbiosis reaction". On agar with the addition of such a small quantity of dissolved haemoglobin (i. e. haemolysed blood) that Pfeiffer's bacillus in pure culture only grows scantily, the growth is increased to a considerable degree round the colonies of other kinds of bacteria which have been inoculated in the vicinity of the growth of Pfeiffer's bacillus.

6. The rate of growth. On media which are suitable for the cultivation of Pfeiffer's bacillus there will always be macroscopic colonies after 24 hours incubation at 37°, and after 48 hours there is not much more growth. (The reason for introducing this character, in which indeed Pfeiffer's bacillus does not differ from the majority of the ordinary bacteria which can be cultivated, is with view to distinguishing it from the whooping-cough bacillus which grows much more slowly).

In most cases all these 6 points are taken into account; first an inoculation is made in Petri plates containing haemoglobin-agar* (in a number of cases also on blood-agar); after 1—2 days in the incubator the colonies which have appeared are examined microscopically (staining by GRAM's method, counterstaining with carbol-fuchsin (Ziehl-Neelsen) diluted 1 + 9, for 5—10 minutes), and also by subculture on haemoglobin-agar, blood-agar, and blood-free agar (ordinary agar, ascitic agar or both); lastly the „symbiotic reaction" is carried out.

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* Until June 1920 haemolysed blood was used for „haemoglobin", and after that date pepsin-digested blood.

For the first month (July 1918) however, agar smeared with a drop of fresh human blood was used (as advised by PFEIFFER & BECK (1)).

Concerning further details about the constitution and preparation of the media the reader is referred to pp. 225—232.

The technique described above was in the main developed in the course of the first 2 months' work with Pfeiffer's bacillus. The choice of the method of investigation was naturally made on the basis of the statements in the literature about the characteristic qualities of this microbe.

With regard to comparison with the investigations of others it must be pointed out that most of the authors who have reported investigations on Pfeiffer's bacillus in a large number of persons have restricted themselves to observing the appearance of the colonies in the original culture, making a microscopic examination and demonstrating the absence of growth on haemoglobin-free media.

As one of the objects of my research has been to investigate how Pfeiffer's bacillus may be most rationally separated from other kinds of bacteria, it was naturally not a foregone conclusion that this technique and the boundaries fixed by it would bear the criticism of the subsequent more thorough investigations. This point will be further discussed later on. It need only be mentioned here that in the light of my later experiences hardly any errors have been committed in the investigation of influenza cases, but that in the inoculation from healthy persons decisions have probably been arrived at in isolated cases, which in view of later experience must be judged as inaccurate, without it having been possible afterwards to rectify the error. It may be that a few haemolytic, haemoglobinophilic bacteria have been called Pfeiffer's bacilli; and again one or two of the first series of investigations of healthy persons presumably might have given a rather larger number of positive findings of Pfeiffer's bacilli if they had been performed with the aid of the technical experience I have since gained.

On the whole it must be contended however that uncertainty in the correct limitation of Pfeiffer's bacillus has only played a very subordinate rôle.

The investigations into the occurrence of Pfeiffer's bacillus can naturally be divided up according to the conditions under which they were carried out, and fall therefore into the following groups:

1. Influenza: (a) Summer epidemic 1918.
   (b) Autumn and winter epidemic 1918—19.
   (c) Epidemic in the beginning of 1920.
   (d) „ „ „ „ „ 1922.
2. Whooping-cough.
3. Measles.
4. Tuberculosis.
5. Meningitis.
6. Healthy persons: (a) Recently enrolled soldiers.
   (b) Other healthy people.
7. Animals.

A minor part of the investigations of the first few months were undertaken by the then head of the department, Prof. Oluf Thomsen, and by my deceased colleague, Dr. Frederik Thorborg.

1. Influenza.

For the comprehension of what follows it is necessary to state that influenza here in Denmark occurred in 4 epidemics, July—Sept. 1918; Sept. 1918—May 1919; Jan.—April 1920; and Jan.—April 1922. The first epidemic was very mild, but there was a considerable mortality in the two following ones, while the last was again more benign in nature although more serious than the first. With respect to the number of cases of the disease reported per month the reader is referred to the graphic representation on p. 86.

(a). Summer epidemic 1918.

In July 1918 inoculations from the naso-pharynx were made from 135 soldiers with influenza, mostly uncomplicated.

The technique used in these cases and in the subsequent inoculations was as follows: the secretion was removed by means of cotton-wool on a rod bent at an angle, which was introduced through the mouth up behind the soft palate. Care was taken that it did not touch any mucous membrane except that of the naso-pharynx. The material was rubbed off on to a

water-agar plate and spread in a thin layer with the rod. The bent portion of a glass rod was now rubbed several times over the agar plate and then once on the surface of the nutritive medium.

Out of the inoculations of the first week Pfeiffer's bacillus was found in 5 persons, and later in a further 4. In the same month cultivations from 23 sputa were made, also principally from uncomplicated influenza, it was found in 4 of these.

In these and other examinations of influenza patients the sputa from the hospitals (Garrison-Hospital, Øresundshospital, Blegdamshospital) were collected directly in sterile Petri plates and then conveyed to the Serum Institute where a fragment of purulent or muco-purulent sputum was transferred to the nutritive medium and afterwards spread over the surface with a bent glass rod.

Microscopic examination has also been made of rather more than 100 sputa, partly from uncomplicated cases, fresh and old, and partly from cases with pneumonia. Only in a few instances and in small numbers were bacteria found which resembled Pfeiffer's bacillus.

(b). Autumn and winter epidemic 1918—19.

In October 1918 cultivations were made from 26 samples of sputum taken from influenza patients with pneumonia. Pfeiffer's bacillus was found in 6 of these.

In the same month cultivations were made from 37 cases of influenzal pneumonia by lung puncture post mortem. Pfeiffer's bacillus was found 9 times. 17 cases of pneumonia however, where the cultivations were made during the autopsy, gave only one positive result.

In the period from 12. XI. 1918—25. III. 1919 rather more than 200 sputa were examined. In the following account all of those are excluded however, that appeared to consist mainly of mucus from the throat or mouth, or that were more than 24 hours old. There remained 162 sputa from 98 patients. Most of them were muco-purulent, consisting of a mass of mucus with streaks of pus or more rarely of more homogeneous, markedly purulent pieces. Only a few sputa had a pronounced „pneumonic" appearence (Pfeiffer's bacillus was not found in these).

Microscopic preparations (Gram-staining + dilute carbolfuchsin) were made of all the sputa as well as the examinations by cultivation. The microscopic picture and the results of cultivation were far from always agreeing. In about half the cases where Pfeiffer's bacillus was cultivated it could not be observed with certainty in the microscopic preparations. In many cases on the other hand numerous bacilli were found microscopically with the appearance of Pfeiffer's bacillus but they could not be cultivated. There were however many sputa where the microscopic picture was quite „classical" (as in figure I in PFEIFFER's (2) article) with dense masses of Pfeiffer's bacilli enclosed in pieces of mucus, and where the bacilli could also be cultivated in large numbers, sometimes even in pure culture. The Pfeiffer's bacilli were nearly always extracellular.

The lack of correspondence between the microscopic picture and the cultivation experiment may perhaps be explained in some cases as the consequence of too deep staining of the preparation, so that Pfeiffer's bacillus did not stand out distinctly enough. This factor however could presumably only be of subordinate importance, because in those cases where Pfeiffer's bacillus was found microscopically it was usually very distinct.

On the other hand there is no doubt that Pfeiffer's bacillus can often be demonstrated by cultivation even though it occurs in too small numbers to assert itself in a direct microscopic examination.

A negative result on cultivation in spite of a positive direct microscopic finding is undoubtedly in some cases due to the fact that the bacilli were dead, because the sputum had been kept too long. In other cases it may be caused by confusion with other small Gram-negative bacteria. It must be emphatically asserted that it is impossible by direct microscopic examination of sputum etc. to accurately distinguish between Pfeiffer's bacillus and other small Gram-negative rods, e. g. the whooping-cough bacillus. According to the experience of many authors Pfeiffer's bacillus in sputum can also easily be confused with Micrococcus catarrhalis. I have no experience of this special point, but cannot deny it because the sharp distinction between Gram-negative cocci and Gram-negative rods, referred to above, may not always be so simple to demonstrate in sputa as in cultures.

For the certain demonstration of Pfeiffer's bacillus therefore, cultivation must be insisted upon, but direct microscopy may in many ways supplement it.

By cultivation, Pfeiffer's bacillus was found in 61 (38%) of the 162 sputa, that is to say in 34 (35%) of the 98 patients.

In the following table will be found the number of positive and negative findings which were made on the 1st., 2nd., 3rd., etc day of the disease.

Day of Disease	+	0	Day of Disease	+	0	Day of Disease	+	
1	1		16		4	35		1
2	2	4	17		4	36	1	
3	7	3	18		3	37	1	
4	1	5	19		1	38	1	2
5	3	6	20	1	1	39		1
6	6	9	22	2	2	45	1	
7	2	4	23		1	46		1
8	2	7	24	1	2	47		1
9	1	9	25	2	1	51		1
10	3	4	27	1		52	1	
11	1	4	30		2	54		1
12	3	5	31	1	2	59		1
14	2		32	1		73		1
15	3	1	34	1	1	80		1

The next table records the occurrence of Pfeiffer's bacillus in the various stages of the disease.

	+	0
1. Uncomplicated cases, in the febrile stage	11	12
2. „ „ after temperature had become normal	3	7
3. Cases where pneumonia developed later	2	1
4. Pneumonic cases before temperature had fallen to normal	30	55
5. „ „ after „ „ „ „ „	6	21

All the 162 examinations do not appear in the table, because, in the case of 5 patients, in whom Pfeiffer's bacillus was found on the first examination, I undertook a series of new tests. These repeated examinations are left out because the few patients would otherwise have too great weight in comparison with the other material.

We see that Pfeiffer's bacillus is encountered with great frequency in the early stages of the disease but it may quite likely be found at a much later date and even after the fever is over. It will also be observed that it is very common to

find the bacillus in pneumonia. Perhaps it is rather less frequent here than in uncomplicated cases. The figures are too small to permit of any definite conclusion however.

The sputa also, which displayed the „classical" microscopic picture with dense masses of Pfeiffer's bacilli from which they could be cultivated almost in pure culture, are met with in the different stages of the disease, both in complicated and in uncomplicated cases.

Furthermore, material from 41 autopsies on cases of influenzal pneumonia from 5. XII. 1918—25. III. 1919 was examined.

From each corpse cultivations were made as a rule from 2 or 3 different places in the affected lung, principally from the peripheral portion of the pneumonic area and from places where particularly fresh pneumonic lesions were present. In some cases additional cultivations were made from the upper air passages.

Altogether, Pfeiffer's bacillus was found in 21 (51%) of these 41 autopsies.

In 7 cases I was able to express drops of pus from the small bronchioles out on to the cut surface of the lung and I then made cultivations from these drops as well as from the pneumonic lung tissue. Pfeiffer's bacillus was cultivated from the drops of pus in 2 cases, and from the lung tissue in 3.

In 2 cases this microbe was found practically in pure culture (without its being seen however in the microscopic preparation). In cases where it was associated with other bacteria (Staphylococcus, Streptococcus, Pneumococcus, sometimes also with other kinds) it was always in the minority compared with the latter, at any rate in the microscopic preparation. In the cultures colonies of Pfeiffer's bacillus were present in some cases in about the same numbers as the colonies of other bacteria.

Out of 4 cases of catarrhal, membranous, or ulcerous inflammation of the larynx and trachea Pfeiffer's bacillus was cultivated twice and Staphylococcus twice.

Pfeiffer's bacillus was found once in cultivations from 51 pleural exudates, some made during life and some post mortem.

The culture plates (blood-agar, haemoglobin-agar) were inoculated on the spot and then conveyed, — at a rather cool tempe-

rature — to the Serum Institute, where they were placed in the incubator about an hour after the samples were taken.

In order to get an idea of the rapidity at which Pfeiffer's bacillus is destroyed in the lungs after the patient's death, I carried out the following survey.

20 autopsies, which yielded positive findings of Pfeiffer's bacillus, were made 6, 12, 18 18, 22, 22, 24, 24, 26, 30, 32, 34, 34, 35, 36, 36, 36, 38, 38, and 38 hours after death respectively. Instances of the growth of Pfeiffer's bacillus in large quantities occurred just as frequently among the late as among the early autopsies.

For the sake of comparison it may be stated that 18 autopsies, in which the microbe was not found, took place 14, 21, 26, 26, 27, 27, 28, 30, 30, 31, 33, 33, 34, 36, 38, 39, 40, and 54 hours after death respectively.

These results indicate that Pfeiffer's bacillus is not particularly prone to die out in the first day and a half after the patient's death, (for if such were the case the positive findings would be chiefly met with among the earliest autopsies), and we may perhaps venture to assume therefore, that practically the same result would have been obtained even if the examinations had been made immediately after death.

From the cultivation experiments it appears that Pfeiffer's bacillus occurs with rather greater frequency in the autumn and winter epidemic than in the summer epidemic. As the technique of cultivation was not so good at first as it became later on, this conclusion can scarcely be drawn from this evidence. A comparison between the microscopic pictures of the sputa from the two epidemics would be a more rational procedure, because this does not depend on any special technique which needs practice, and may therefore make a direct comparison between the preparations from the two periods.

It appears then that out of about 50 samples of sputa which were examined microscopically during the summer epidemic, only two contained bacteria that could be assumed to be Pfeiffer's bacilli (the preparations were kept, and on re-examination Pfeiffer's bacilli were not found in greater number). On dividing the sputa from the autumn and winter epidemic (with the exclusion of the „repeated examinations") into 3 groups of about 50, we get however, 10, 8, and 9 obviously

positive microscopic findings in the 3 groups respectively. As we cannot suppose that the method of collecting sputa in the summer epidemic will explain the scanty positive findings at that time, the figures point strongly to the fact that Pfeiffer's bacillus has really increased in distribution between the first and second epidemic.

(c). The epidemic in 1920.

In the period from 28. I.—9. III. 1920 Pfeiffer's bacillus was cultivated from 4 out of 15 sputa, and from the pneumonic lung tissue of 6 out of 59 autopsies. In 20 of these autopsies cultivations were also made from the pleural exudate without Pfeiffer's bacillus being found once.

These investigations were chiefly made in order to obtain some samples of Pfeiffer's bacillus from this stage of the epidemic, and I was therefore not present at the autopsies, but the pieces of lung tissue and pleural exudates were sent to me. As a considerably longer time elapsed than in the case of the previous investigations, before the cultures could be made one cannot expect to draw any conclusion from the relatively scanty positive findings as to whether Pfeiffer's bacillus occurred less frequently in this epidemic than in the previous one.

(d). The epidemic in 1922.

Immediately after the rather sudden outbreak of the epidemic in the garrison of Copenhagen, I examined (on the 7. I. and 8. I) 38 soldiers with uncomplicated influenza, for Pfeiffer's bacillus at the military hospital.

The examinations were made in 2 wards, which had been empty till the day of the examination, so that a hospital infection with Pfeiffer's bacillus must be regarded as excluded. On this occasion only cultures from the throat were examined, not sputa. After the specimen was taken the inoculation rod was put in a test tube and transferred to a portable incubator the temperature of which was kept at 32—35°.

This incubator was constructed of two glass cylinders (jam jars), one inserted inside the other. In the intervening space between the cylinders melted zinc nitrate ($Zn(NO_3)_2$, $6H_2O$) was poured, a salt the melting point of which is about 35°. The outer cylinder was surrounded by an insulating cover of newspaper etc. On solidifi-

cation of the salt so much heat is evolved that the temperature in the inner container keeps fairly constant for several hours. The same result could obviously be obtained with water instead of zinc nitrate but a much larger quantity would have to be used which would make the apparatus considerably heavier and larger.*

In this incubator the inoculated swab was conveyed to the Serum Institute where cultures were made about an hour after the sample was made. Water-agar plates were not used, but the glass rod was rubbed several times over the infected cotton wool swab and then rubbed on the surface of the nutritive medium.

In the table below, „0D", „1D", and „2D" signify that the sample was taken the same day, the first day and the second day respectively, after the commencement of the disease. The occurrence of Pfeiffer's bacillus is denoted by: „0" = bacillus not found; „I" = only a few colonies; „II" = rather numerous colonies, but only constituting a small proportion of the total number of colonies; „III" = colonies of Pfeiffer's bacillus at least as numerous as the other colonies; „IV" = practically a pure culture of Pfeiffer's bacillus.

	0	I	II	III	IV
0 D.			4		1
1 D.	10	1	6	6	
2 D.	4		2	3	1

Just as in 1918—19 Pfeiffer's bacillus was found most constantly at the beginning of the disease, but it was not the rule that it disappeared very quickly. Altogether it was found in 24 (63%) of the 38 soldiers.

In a large number of the examinations of influenza patients the microbe was verified by all the tests referred to on pp 68—70 (for the demonstration of haemoglobinophilia only ascitic agar, not ordinary agar, was used as a rule). As however it soon became evident that the colonies in the primary culture on haemoglobin agar were of such a characteristic appearance that they could be detected with great certainty by microscopic observation I have in a number of cases been satisfied with this in connection with the microscopic examination. There is no reason to believe that this abbreviated examination has lead to any error.**

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* I have, however, later realized that is very doubtful if the transport by elevated temperature is advantageous.

** Pfeiffer's bacilli from other than influenza patients were always verified by a complete examination.

As general procedure I have not washed the particle of sputum used for cultivation although several authors (JOCHMANN (1), LEVINTHAL (1,2)) have laid great stress on washing; others however (ROSENOW) have not considered it necessary. It is clear that if one wants especially to investigate the bacterial flora of the bronchial secretion one ought to remove the outer layer of the sputum as far as possible by suitable washing. But the object here was only to investigate whether Pfeiffer's bacillus was present at all in the patient, and it would rather be an advantage to obtain a mixed sample containing material from the bronchial tubes, the throat, and the mouth, with the proviso however that the other bacteria present in the last-named places were not capable of overgrowing Pfeiffer's bacillus to any considerable extent.

In cultivations from 6 samples of sputum with and without washing I have been unable to discover any sign whatever that the cleansing gave a better chance of demonstrating Pfeiffer's bacillus.

In order to estimate the importance of rapid examination cultures were made in some cases from the sputum immediately after they were received as well as on the following day after being kept in Petri plates at room temperature in the meanwhile. It was found with great regularity that in the course of a single day Pfeiffer's bacillus decreased considerably in numbers or even disappeared entirely.

We must therefore reckon with the fact that in looking for this microbe in sputum etc. a day's delay before cultivating will diminish the number of positive findings to a marked degree, so that the greatest possible expedition in the examination is important.

As regards the investigations reported here it cannot be said that this desideratum is so well fulfilled as could be wished, but better in the investigations of 1922 than in the earlier ones, and perhaps this fact, — in conjunction with the investigations earlier in the disease, — is sufficient to explain the higher percentage in the last case.

The results of the examinations for Pfeiffer's bacillus in influenza can shortly be summed up thus: the bacillus was found in all 4 epidemics and widely distributed in the last 3, but it was probably considerably more restricted in the first. It cannot be decided whether any change in its distribution has taken place during the last 3 epidemics. It was found most constantly in the beginning of the disease but as a rule it did not disappear as quickly as indicated in the investigations of WASSERMANN, PIRAS, and GOSIO. It was found nearly as often in pneumonia as in uncomplicated influenza.

2. Whooping-cough.

On the 19. III and 20. III. 1919 I collected the sputa of 12 children with typical whooping-cough (in Dr. AD. MEYER's clinic) and obtained Pfeiffer's bacillus from all of them, in 11 cases a luxuriant growth sometimes even in pure culture.

How many of the children had influenza before admission is not known, but they came from entirely different parts of the town and so they could hardly have been all attacked. Furthermore influenza did not prevail in the clinic during these children's stay there. We cannot thus assume any direct connection between the marked occurrence of Pfeiffer's bacillus and influenza.

On the 12. VII. 1919 the sputum of 5 children (in the same clinic) was examined. Pfeiffer's bacillus was present in large numbers in 2 of them.

On the 19. IX. 1919 an examination was made (at the Blegdams-Hospital) of 21 children. Cultivations were made from the sputum of 7 of these, with 5 findings of Pfeiffer's bacillus. In 13, cultivations were made only from the throat or mouth, with 6 positive results. In one patient the throat culture gave a positive result, but the sputum a negative one. Thus the microbe was found in 12 of the 21 patients.

In many of the sputa it was present in considerable quantities; in other cases it was more scanty.

With a few exceptions the children had coughed for 1—4 months. The large majority of cases were complicated with bronchitis or broncho-pneumonia.

In all cases an inoculation on BORDET's potato-glycerin-blood-agar was made in addition to haemoglobin-agar, for the isolation of the whooping-cough bacillus which was only found in one case. This small result was however to be expected in view of the late stages in the disease at which the examinations were made.

3. Measles.

On the 9. I. 1920 inoculations from the throats of 31 measles patients were made at the Blegdamshospital.

At this time there had been no influenza of epidemic dimensions in the country for 7 months. (The 3rd. epidemic did not gain a foothold till a fortnight later).

In most cases the cultivations were made from the region around the tonsils, — in a few cases from the naso-pharynx. In some patients with a cold, cultivations were made from the nose.

Pfeiffer's bacillus was found in 15 of the patients, in the throat and mouth of 14, in the nose of 1, while inoculations from the nose of 4 other patients gave negative results.

The following table shows the occurrence in uncomplicated cases, in cases with bronchitis, broncho-pneumonia or diphtheria, and in patients who were febrile or afebrile at the time the examinations were made. „+" and „0" signify the presence or absence of Pfeiffer's bacillus.

	Uncomplicated		Bronchitis		Broncho-pneumonia		Diphtheria		Total	
	+	0	+	0	+	0	+	0	+	0
Febrile	2	2	1	1	2	2	0	0	5	5
Afebrile.....	4	2	4	3	1	5	1	1	10	11
Total...	6	4	5	4	3	7	1	1	15	16

Thus no relation could be observed between the factors referred to and the presence or absence of Pfeiffer's bacillus.

4. Tuberculosis.

During the second epidemic cultivations were made at the autopsies of 7 cases of phthisis without any sign of influenza, partly from cavities and partly from areas of peribronchitis. Pfeiffer's bacillus was found in 5 cases, three times in large numbers and almost pure culture, and twice in smaller numbers.

During the third epidemic it was not found in 5 samples of the contents of tuberculous cavities sent for examination.

5. Meningitis.

In 5 cases of meningitis in children Pfeiffer's bacillus was found in pure culture in the spinal fluid both microscopically and by cultivation .

With regard to the first 4 patients' clinical histories the reader is referred to the contributions of MAX CHRISTIANSEN and the author, mentioned in the preface. It will suffice to say here that one of the patients was cured, and 3 others died.

The 5th. patient was treated in Department No. 5. of the Kommune-Hospital from the 13. IX.—16. XI. 1921.* The main points in the history are as follows:

C. L. E. N. 8 years old. Son of a smith. On the day of admission was run over by a motor-car. Fracture of ribs and clavicle, and various contusions were diagnosed. 22. IX. cough, 23. IX. temp. $\frac{40,7}{40,4}$. 25. IX. left-sided pneumonia was diagnosed. 17. IX. stiffness of the neck. On lumbar puncture being performed „diplococci" were found, which, from their shape were assumed to be Pneumococci, and so the patient was treated with Pneumococcus serum. In the spinal fluid taken on the 29. IX. Gram-negative „diplococci" and a few Gram-negative rod-shaped elements were found in a stained film. Cultivations on blood-agar and ascitic agar grew Pfeiffer's bacillus only.

On cultivating the blood (about 1.5c.c.), taken on the I. X., in bouillon and on agar containing digested blood, no growth appeared.

By the 17. X. the stiffness of the neck had disappeared but there were still increased reflexes. On the 16. XI. the patient was discharged with a tendency to strabismus but otherwise cured.

No connection could be established between the 5 cases.

It is worth noting that all these 5 cases of meningitis with Pfeiffer's bacillus arose at a time when there was no influenza epidemic.

It appears from the whole of the literature on the subject, that there is no clear connection between influenza and meningitis due to Pfeiffer's bacillus.

A rather larger number of cases of meningitis caused by

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* For permission to report this case I have to thank the Chief Surgeon, Dr. P. N. HANSEN.

this microbe were certainly reported during and after the last influenza pandemic, than in the preceding years, which is only to be expected in view of the increased distribution of Pfeiffer's bacillus and the greater attention that has been paid to it. But if this form of meningitis is to be looked upon as an influenza meningitis we should expect a much greater augmentation of its frequency in direct relation to influenza.

Meningitis due to Pfeiffer's bacillus is undoubtedly much more widely distributed than appears to be the case from the hundred or so published cases. When the attention is not specially directed to Pfeiffer's bacillus it can easily be confused in the spinal fluid, with Meningococcus or Pneumococcus, — particularly with the latter if GRAM's method of staining is not used. Cultivation also will often fail if the spinal fluid hos to be kept for some time before inoculation. By microscopic examination of the spinal fluid and exudate from the surface of the brain I have in 2 cases observed small Gram-negative rods which probably could be taken to be Pfeiffer's bacilli, but the cultures of which did not grow.

6. Healthy persons.

The occurrence of Pfeiffer's bacillus was investigated in 2240 healthy persons, exclusively by cultivations from the throat.

Unless otherwise mentioned, each person has visited the Serum Institute so that there is no question of conveying the material for inoculation from another place with the consequent danger of the death of Pfeiffer's bacillus before the cultures were made.

Nearly all the examinations of healthy people reported from other places were made on definite groups of individuals, the numbers of each group having been in contact with one another for a longer or shorter time (soldiers, personel of laboratories and hospitals etc.), so that the absence or different degrees of distribution of Pfeiffer's bacillus have only been demonstrated in the group examined and it is not possible to draw conclusions about the occurrence of the bacillus in the population as a whole.

A number of my investigations of healthy persons are of

a similar nature, but in addition, a long series of newly-enrolled soldiers has been examined, 1—2 days after their arrival, — in the last investigation however, 3 days after. They were persons who had journeyed directly from their usual places of abode in very different parts of the country and can therefore be regarded as „random samples" of the young healthy manhood of the whole country. Consequently, from the investigations of a not too small number of such persons carried out at various times, it will be possible to get a fairly reliable measure of the occurrence of Pfeiffer's bacillus among young healthy men in the whole country and subsequently the variations in its distribution among them. Such variations must in all probability, be an indication of similar variations in the population as a whole.

Although the analysis of the mutual differences between strains of Pfeiffer's bacillus will not be dealt with till later, it will be convenient, in connection with the purely numerical estimation of the findings in healthy people, to pay some attention to the distinction between „typical" and „atypical" Pfeiffer's bacilli. Regarding the significance of the expressions it need only be remarked here that practically only typical Pfeiffer's bacilli are met with in influenza, while the atypical are especially found in healthy persons. No sharp boundary however can be drawn between the two groups.

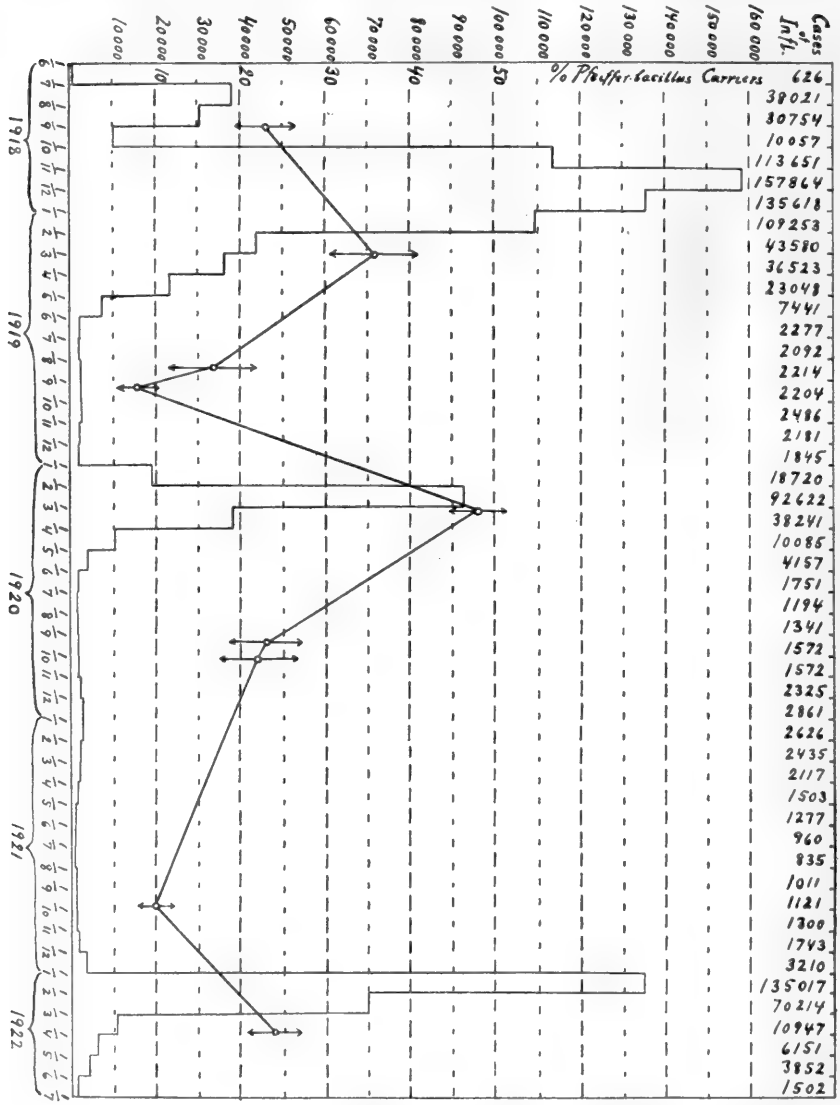
a. Newly-enrolled soldiers.

The results of these inoculations are given in tabular form as well as graphically.

3. IX. and 4. IX. 18	142	22 + 10 = 32	(23 ± 3,5 %)
5. III. 19	67	14 + 10 = 24	(36 ± 5,0 %)
13. VIII. 19	54	5 + 4 = 9	(17 ± 5,1 %)
10. IX. 19	173	6 + 7 = 13	(8 ± 2,0 %)
6. III. 20	200	70 + 26 = 96	(48 ± 3,5 %)
14. IX. 20	99	13 + 10 = 23	(23 ± 4,2 %)
5. X. 20	84	17 + 2 = 19	(22 ± 4,5 %)
24. IX. 21	195	12 + 7 = 19	(10 ± 2,1 %)
21. III. 22	194	26 + 20 = 46	(24 ± 3,1 %)
	1208		

The first column gives the date of examination, the 2nd. column the number of soldiers investigated. Then follows the

number of typical Pfeiffer's bacilli + the number of atypical Pfeiffer's bacilli = the total number of Pfeiffer's bacilli. Finally the percentage of positive findings is given in parenthesis together



with its mean error. With p% positive findings among n soldiers the mean error is $\sqrt{\frac{p\ (100 - p)}{n}}$ %. In this calculation there is the assumption that the choice of the persons examined is „fortuitous". This requirement must be fulfilled

in the present case because each man was questioned about his place of residence for the last 3 months, and these places have always been distributed indiscriminately over the whole country.

In the graphic representation the number of influenza cases reported each month for the whole country are plotted as a staircase curve and also noted along the upper margin of the figure. The other curve in the diagram gives the percentage of positive findings of Pfeiffer's bacillus. The magnitude of the mean error is also indicated by the double arrows.

What this mean error expresses is that uncertainty of the percentage values, which is a result of the limited extent of the material. It will be seen that the mean error is so small compared with the large variations in the occurrence of Pfeiffer's bacillus that we cannot assume a very different result would have been obtained even if the investigations had encompassed similar material of 10 or 100 times the dimensions.

The excellent agreement between the distribution of influenza and Pfeiffer's bacillus among the population, as depicted by the curve, hardly needs any further comment. Naturally we cannot decide whether this agreement is direct or indirect. Future investigations will show the manner of distribution of Pfeiffer's bacillus at different seasons of the year apart from influenza periods. That the potent factor is not the time of the year alone, seems however probable from a comparison between the 4 investigations in September 1918, 1919, 1920, and 1921.

We have now seen that the shape of the curve cannot be explained as due to a fortuitous error in consequence of the restricted scope of the material. This does not however exclude that it might be caused by variations in the constitution of the nutritive medium or other irregularities of the technique. This explanation must however also be rejected, because it would be a most improbable occurrence that a curve should be formed in this manner, the form of which corresponded so well to the influenza epidemics. It is instructive to compare the last of the above investigations (24. IX. 21) which gave 10% of Pfeiffer's bacilli, with the last of those reported below from the Jægersborg camp (17. IX. 21). In the

latter case 34% of Pfeiffer's bacilli was found. The colonies usually occurred in considerably greater numbers on the plates than was the case in the cultures of the 24. IX., and in spite of the fact that on 17. IX. the technique was not so perfect, partly because the inoculated swabs had to be transported before cultivation, and partly because only dark red (i. e. slightly heated) blood-agar was used in the cultures, while on 24. IX. the same medium was employed and in addition another containing dissolved haemoglobin which in comparison with the blood-agar, proved superior. The comparatively poor findings of Pfeiffer's bacilli on the 24. IX. cannot therefore be accounted for as the result of defective technique.

It must however be admitted that the technique employed in the examinations on 3 and 4. IX. 1918, and 5. III. 1919 was not so good as in the later inoculations. In the first case it was due to want of practice in detecting Pfeiffer's bacillus in the primary cultures from healthy persons (the colonies are not always so characteristic as in influenza); in the second case the plates remained so long without subculturing that a number of cultures which were presumably Pfeiffer's bacilli died out before they could be verified by a complete cultural examination. The percentages in these two series ought therefore to be increased by some unknown amount. But this correction would only further strengthen the connection between influenza and the distribution of Pfeiffer's bacillus in healthy persons.

b. Other healthy persons.

On 14. I. 1919 cultivations were made from 22 soldiers sleeping in the same room, and who had all been on duty at least 3 months. 17 of them stated they had had influenza, in most cases about 3 months previously. Pfeiffer's bacillus was found in all of them for the most part in considerable numbers. In 19, typical Pfeiffer's bacilli were present (in some atypical as well), and in the remaining 3, only atypical.

On 24. I. and 25. I. 1919, cultivations were made from 133 marines who, with a few exceptions had been on duty at least 3 months, — the majority of them for 5—6 months. In 63 (48%) Pfeiffer's bacilli were found, 49 typical and 14 atypical.

In consequence of some meningitis cases having arisen in a military camp (Jægersborg), inoculations were made in July

1920 (see THOMSEN & WULFF) in the course of 3 days, from the entire personel, in all, 225 men (recruits who had been there for about 1½ months). It was the original intention only to search for Meningococcus for which reason only ascitic agar was used for cultivation. This proved to contain a little blood*, as a plenty growth of Pfeiffer's bacilli appeared on a large number of the plates. I seized the opportunity of examining 165 of the plates more closely (the rest which referred to the first day, were rejected at that date). In 54 (33%) of the plates Pfeiffer's bacilli were found, typical in each case. (By using haemoglobin-agar instead of ascitic agar, a rather higher figure would probably have been obtained, by including some more of the specially fastidious atypical Pfeiffer's bacilli).

On 6. IX, 7. IX. and 10. IX. 21. inoculations from 452 recruits in the Værløse camp who had been there from 25. IV. were made. The inoculated swabs were conveyed in test tubes just as in the case of influenza in January 1922. They were however carried in the more primitive „hay-box", in which the temperature on reception at the Institute was 22—24°. The lapse of time between the taking of the samples and the inoculation of the plates was 1½—2½ hours.

In this and the following investigations agar with dissolved haemoglobin was not employed, but in place of it, blood-agar. (The mixture of melted agar and blood was heated at 50°—60° until it acquired a dark red colour. On such slightly heated blood-agar Pfeiffer's bacillus grows more vigorously than on ordinary blood-agar).

Pfeiffer's bacillus was found in 63 persons (14%); 46 were typical and 17 atypical.

On 17. IX. 1921 inoculations were made from 230 recruits in the Jægersborg camp (the same camp as that in which the investigations of July 1920 were carried out). They had been on duty from 25. V. The technique was the same as that used in the Værløse camp. Pfeiffer's bacillus was found in 78 men (34%); 65 were typical and 18 atypical.

It must be mentioned that in both the last-named investigations, Pneumococcus, Meningococcus and the Diphtheria bacillus were also looked for. No relation could be demonstrated

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* Which could also be demonstrated by the benzidin test.

between the occurrence of Pfeiffer's bacillus and the other kinds of bacteria.

In both the camps (in the Jægersborg, in both investigations) it was noted how the sleeping places of the carriers of Pfeiffer's bacillus were distributed among the different barracks and in each barrack. The distribution gave the impression of being fairly fortuitous; no typical grouping could be demonstrated.

During January and February 1922 repeated inoculations were made from healthy persons, namely the employers at the State Serum Institute and from 2 families in the dwellings connected with it. The occurrence of Pfeiffer's bacillus will be seen from the following table where „A" denotes the first inoculation (5. I.), „B" the 2nd., which was made between 19. I and 21. I., and „C", the 3rd. (16. II.).

The examined persons are indicated by numbers. The Roman figures give the number of colonies on the plate (cf. p. 79).

	A	B	C	
1	0			
2	0	0		
3	0	0	0	Same family.
4	0	0	0	
5	III	0	0	
6	0	0	0	
7	0	0	0	
8	III	0	II	Mother and Son. — Same family.
9	III	0	I	
10	0	0		
11	0	0	0	Each time, haemolytic haemoglobinophilic bacteria in dominating numbers.
12	0	0	0	
13	0	0	0	
14	0	0	0	
15	0	0	0	
16	0	1	colony I (several colonies).	
17	0	0		
18	0	0		
19	0	0	0	
20	0	0	0	Influenza 9—16. I.
21	0	0	0	— 30. I—4. II.
22	0	0	0	
23	0			21. I, 25. I, 9. 2.: III—IV. Influenza 16—20. I.

	A	B	C	
24	0	0	0	
25	0	0		
26	0	0	III	
27	0	0	III	
28	0	0	0	
29	0	0	0	
30	0			
31	0	0	0	Influenza between „A“ and „B“. „Cold“ for last week before „C“.
32	0			
33	0	0		
34	0	0	0	
35	0	0	0	
36		0	0	
37		0	0	Sister of 23.

All the Pfeiffer's bacilli found were typical except 27 C, which was atypical (yellow colour). After the previous experience we might have expected a marked increase in the distribution of Pfeiffer's bacillus among the personel and residents of the Serum Institute. That this has not proved to be the case may perhaps be explained by the fact that the majority of the persons examined live under good hygienic conditions as regards dwellings etc.

The constant and abundant occurrence of Pfeiffer's bacillus in the soldiers examined on 14. I. 1919 cannot be directly explained as the residue of influenza. The influenza infection that 17 of them stated they had passed through, took place about three months previously, and from my own experience of influenza patients as well as that of others, it appears that Pfeiffer's bacillus often vanishes after the course of a few days and probably in the vast majority of cases it will have disappeared at the end of 3 months, if unusual conditions do not come into play. What it is that causes Pfeiffer's bacillus — just as Meningococcus — particularly to spread among, and persist in, soldiers I am unable to explain. In view of the fact that it is usually inclined to disappear spontaneously in healthy persons, close association and the consequent easy transmission of the microbe from one person to another is not sufficient explanation.

The relatively great distribution among soldiers is also illustrated in a marked degree by the comparison already set

up between the inoculations in Sept. 1921, from the Jægersborg camp and from newly-enrolled soldiers.

Neither in the Værløse nor the Jægersborg camp had there been influenza during those months the men examined in September had been in their respective stations. Only a few soldiers had reported sick of other catarrhal infections.

The problem as to how far every healthy carrier of Pfeiffer's bacillus is to be looked upon as a carrier who has never got rid of the bacillus since an attack of influenza, can in all probability be answered in the negative from the experience gained in the military camps. That between May and September 1921 Pfeiffer's bacillus must have extended in distribution among the recruits in the Jægersborg camp without in every instance having been passed on from an influenza case, can hardly be doubted.

With the object of discovering the numerical connection between past influenza and the occurrence of Pfeiffer's bacillus, each man in some of the batches of soldiers examined was questioned about previous influenza („Spanish disease").

In the case of the soldiers examined on 3. and 4. IX. 18. the influenza could at the most, only date a month back; in those examined on 24. and 25. I. 19, 5 months had usually elapsed; in those examined on 5. III. 19. the influenza infection took place 1—7 months previously; and in the two next batches (13. VIII and 10. IX. 19.) it dated ¾—1 year back. Lastly, which of the soldiers examined on 6. III. 20., had had influenza in the epidemic of 1920, was noted.

In the following table the occurrence of Pfeiffer's bacillus is recorded separately in those soldiers who said they had had influenza and in the remainder.

	+ Influenza			0 Influenza			Total
	+ Pf. b.		0 Pf. b.	+ Pf. b.		0 Pf. b.	
	typical.	atypical.		typical.	atypical.		
3—4. IX. 18 ...	7		18	24		83	132
24—25. I. 19 ...	25	4	30	24	10	40	133
5. III. 19.....	7	4	13	7	6	30	67
13. VIII. 19	1	2	15	4	2	30	54
10. IX. 19	4	2	50	3	4	110	173
6. III. 20	2	1	3	71	25	40	142

In the elaboration of the results of the examinations of 3 and 4. IX. 18 at that time, no difference was made between typical and atypical Pfeiffer's bacilli. The reason only 132 of the 142 men examined are accounted for, is that information about influenza was only available later, and in 10 cases it could not be obtained.

These figures show that Pfeiffer's bacillus is encountered to a considerable extent also among persons who deny having had influenza. Even if this information about previous influenza cannot be reckoned very reliable, it is incredible that all the carriers of Pfeiffer's bacillus who said they had not suffered from influenza, should actually have had this disease. It is therefore justifiable to conclude from the figures that people often become carriers of this microbe without having had an attack of influenza. It is particularly interesting that in direct response to the influenza epidemic in the beginning of 1920, there has proved to be such a marked dissemination of Pfeiffer's bacilli among healthy persons the majority of whom stated they had not been attacked during the epidemic .

In order to investigate how far it would be possible from the figures stated in above table to draw any conclusion at all as to a possible connection between previous cases of influenza and the incidence of Pfeiffer's bacillus, the first three groups (the first three lines in the table) have been treated statistically.

In the left half on the table below, the frequency of occurrence of Pfeiffer's bacillus expressed as a percentage, is recorded for soldiers who had had influenza and for those who had not, separately. Then the difference between the two percentages is given. It will be seen that Pfeiffer's bacillus all the way through occurred more widely among past influenza patients than among others, whether we take all Pfeiffer's bacilli into account or only the typical ones.

In order to determine whether these divergencies are large enough to be accounted as more than „fortuitous", the mean error of each difference is calculated. If p_1 and p_2 are the two percentages, and the corresponding number of soldiers examined, n_1 and n_2, the mean error of $p_1 - p_2$ is given by the equation $\mu = \sqrt{\frac{p_1(100-p_1)}{n_1} + \frac{p_2(100-p_2)}{n_2}}$; μ is thus also

expressed as a percentage. Lastly, the value $\frac{p_1 - p_2}{\mu}$ is calculated.

As a test of the truth of the calculations, in the right half of the table they are set up in another way, viz, by estimating the frequency of occurrence of previous influenza among carriers and non-carriers of Pfeiffer's bacillus. It will be seen that the two methods of calculation give identical results.

	Occurrence of Pfeiffer's bacillus among:					Occurrence of influenza among:				
	"+ Infl." (p_1)	"0 Infl." (p_2)	p_1-p_2	μ	$\frac{p_1-p_2}{\mu}$	"+ Pf. b." (p_1')	"0 Pf. b." (p_2')	$p_1'-p_2'$	μ'	$\frac{p_1'-p_2'}{\mu'}$
3—4. IX. 18 (all kinds of Pf. b.)	28,0	22,4	5,6	9,9	0,6	22,6	17,8	4,8	8,4	0,6
24—25. I. 19 (all kinds of Pf. b.)	49,2	46,0	3,2	8,7	0,4	46,0	42,8	3,2	8,6	0,4
24—25. I. 19 (only typical Pf. b.).	42,4	32,4	10,0	8,3	1,2	51,0	40,5	10,5	8,9	1,2
5. III. 19 (all kinds of Pf. b.)	45,8	30,2	15,6	12,4	1,3	45,8	30,2	15,6	12,4	1,3
5. III. 19 (only typical Pf. b.)....	29,2	16,3	12,9	10,8	1,2	50,0	32,1	17,9	14,8	1,2

It appears that Pfeiffer's bacillus in all instances was of more frequent incidence in previous cases of influenza than in other subjects; the divergences are however so slight that they may very well be casual. The present material may perhaps be said to indicate a connection between previous cases of influenza and the occurrence of Pfeiffer's bacillus, it is however far from being sufficiently large to evidence such a connection.

In the inoculations of July, Sept. and Oct. 1920, no questions were asked about influenza because, from the experience

gained in previous cultivations, it was not thought likely it would give any information. But „cold in the head" and „sore throat" during the last 3 months before the examination, were inquired after. No relation could be detected as the following figures show:

	Total No.	No. of these with Catarrhal Infection	No. with Pf. b.	No. of these with Catarrhal Infection
July 1920	225	81 (36 %)	54	15 (28 %)
14. IX. 20	99	27 (27 %)	23	6 (27 %)
15. IX. 20	84	39 (45 %)	19	10 (53 %)

This naturally is no more convincing as a proof that catarrhal affections do not favour the spread of Pfeiffer's bacillus than the slight connection between past influenza and the finding of the same microbe proves that influenza is of trifling importance in its distribution.

The chief result of the investigations concerning persons not suffering from influenza is therefore, shortly expressed, that Pfeiffer's bacillus in Denmark was very widely distributed in such people, in the years in which the investigations took place; that the distribution at any rate in healthy persons was considerably greater in connection with each influenza epidemic and afterwards decreased greatly in a few months (whether an increase of distribution in patients with whooping-cough, measles etc. follows influenza is not clear from my work); that Pfeiffer's bacillus became widely disseminated among persons who had not had influenza, and that the degree to which this took place was largely influenced by the conditions under which the individuals lived.

My experiences of the occurrence of Pfeiffer's bacillus do not alter the views put forward in the introductory chapters (pp. 58—64).

7. Occurrence in animals.

On 27. IX. 20 inoculations were made from the mouths of 60 guinea-pigs, which had arrived from Germany a few days before. In 5, organisms were found which according to all the previously (pp. 68—70) described tests, must be classed as Pfeiffer's bacilli. They all belonged however to the „atypical" forms. In one of the animals two different varieties were found.

In 43 mice and 33 horses no haemoglobinophilic bacteria were discovered.

The table below records the whole of the strains of Pfeiffer's bacilli cultivated. To each investigation in which Pfeiffer's bacillus was found is assigned: (1) a capital letter which indicates where the material came from, and (2) a number.

1. Influenza.

Strains cultivated from influenzal pneumonia post mortem, or lung puncture:

I 1— 9 (Puncture, Oct. 1918).
„ 10 (Autopsy. „ „).
„ 11—31 (Autopsy. 5. XII. 18—25. III. 19)
„ 32—37 („ 28. I.—9. III. 20)

Strains cultivated from sputum or from the throat.

I 1— 13 (Throat cultivation or sputum, July 1918)
„ 14— 19 (Sputum. Oct. 1918)
„ 20—100 („ 12. XI. 18—25. III. 19)
„ 101—104 („ 28. I.—9. III. 20)
„ 105—128 (Throat. 7—8. I. 22.)

2. Whooping-cough.

P 1—12 (19—20. III. 19)
„ 13—14 (12. VII. 19)
„ 15—26 (19. IX. 19)

3. Measles.

Ms 1—15 (9. I. 20)

4. Tuberculosis.

T 1—5 (5. XII. 18—25. III. 19)

5. Meningitis.

Me 1—5 (1920—21)

6. Healthy persons.

H 1— 32 (3—4. IX. 18)
„ 33— 54 (14. I. 19)
„ 55—117 (24—25. I. 19)
„ 118—141 (5. III. 19)
„ 142—150 (13. VIII. 19)
„ 151—163 (10. IX. 19)
„ 164—259 (6. III. 20)
„ 260—313 (July 1920)
„ 314—336 (14. IX. 20)
„ 337—355 (5. X. 20)
„ 356—418 (6—10. IX. 21)
„ 419—496 (17. IX. 21)
„ 497—515 (24. IX. 21)
„ 516—527 (Jan.—Feb. 1922)
„ 528—573 (21. III. 22)

7. Animals (Guinea-pigs).

GP 1—5 (27. IX. 20)

Lastly there are:

8. Cultures received from abroad.

Pa 1—5 (Paris)
R 1—2 (Rome)

In the following portion of this work these signs will always be employed to designate the various cultures of Pfeiffer's bacilli investigated.

If several strains of Pfeiffer's bacilli were simultaneously isolated from the same person, each of them is denoted by a small letter (a, b — — —). Different cultures from the same colony are denoted by a and b in parenthesis (see p. 204).

Analysis of the Bacterial Group „Pfeiffer's Bacillus".

Introductory Remarks.

When we place a collection of organisms in a group (species, race etc.) on the basis of a series of characters, the classification will be rational provided the three following conditions are fulfilled:

1. Constancy. Each of the characteristics as far as it can be examined, must be invariably present. (By a character is partly understood that feature (as regards morphology, growth etc.) which is brought out by given conditions, and partly — in a wider sense — the way in which the feature is dependent upon the conditions. If we consider a manifestation of life as a mathematical function of the conditions, $y = f(x_1, x_2 \ldots)$, then we may use as a distinguishing character either the value of y when definite values are assigned to $x_1, x_2 \ldots$, or what is more rational but also much more difficult, — the nature of the function f ( ) itself).

2. Discontinuity. Each of the qualities employed to characterise a group, must appear in a discontinuous manner, that is to say either in the quantity which is looked upon as characteristic of the group, or in quite another quantity.

3. Correlation. There must be a connection between the characters employed, so that there is a group of characters which go together, thus affording the same dividing line whichever of them is used as a basis of classification.

These requirements which have been laid down, are the ideal ones, but in bacteriology it will seldom, perhaps never,

be possible to find a series of characters which will be adhered to in the strictest sense. It may even be frequently necessary as a schematic aid to orientation, to divide organisms into groups although it is very uncertain how stable the characters are, and even though Nature provides even gradations and free combinations so that none of the three requirements of a rational grouping are present; and in the more favourable cases one must usually be content with a good approximation to constancy, discontinuity, and correlation, the characters being proved to be stable only within certain limits, and the stepping-stones between the different gradations of the individual quality, not being absent, but only occurring rarely.

These considerations are the most important influence in all the following investigations into the classification of the haemoglobinophilic bacteria.

The reason Pfeiffer's bacillus has been exclusively dealt with so far is because it is the most important of the true haemoglobinophilic bacteria, and we will therefore begin with an analysis of this microbial group with the twofold object in view: to define the boundaries of the group and to investigate its inner „structure".

There is frequently mention of other nutritive media as well as those already discussed. As the whole question of nutritive media will be dealt with in a special chapter, the reader is referred to this for various details in connection with the preparation of media, and only the most essential information about the different media will be given, step by step, as they come under notice.

To begin with it will be advantageous to deduce a provisional separation * in the form of a definition, of the bacteria we are going to class as Pfeiffer's bacilli. In the course of the investigation it will appear whether any of the points in this definition should perhaps be modified, and what factors can further be included in a general description of this species of bacterium.

1. As the presence of Pfeiffer's bacillus is investigated by cultivation on various media containing blood or blood derivati-

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* The description on pp. 68—70 contains too many facts to be used as a definition.

ves and incubated at 37° for 1—2 days, the first criterion of the identity of this bacillus is that it shall be able to grow under these conditions and form well-developed colonies in the time stated. Moreover it should be possible to grow a pure culture in subcultures on the same medium.

2. It is necessary that the bacterium shall be a Gram-negative rod.

3. In pure culture it should not grow on agar or ascitic agar (by which is meant, here and in what is to follow, ordinary peptone broth agar, and the same medium with the addition of blood-free ascitic fluid).

4. On blood-agar plates (of suitable constitution) it should not produce haemolysis.

The significance of the conditions laid down under (1), namely (a) rapid growth, and (b) power of growing on agar containing dissolved haemoglobin, will be clear when the question of the boundary between Pfeiffer's bacillus and BORDET's bacillus is dealt with later on.

That (2) gives a sharp distinction has already been adequately explained on pp. 68—69.

With regard to (3) it may be remarked in anticipation that there has practically never been any difficulty in deciding whether an organism should be termed haemoglobinophilic or not. The bacteria examined fall naturally into two groups according as they are markedly dependent on haemoglobin or practically independent of it.

In a number of cases however a pure culture on ordinary agar has given a slight growth. But it is here exclusively a question of bacteria which under various conditions, particularly in giving haemolysis on blood-agar, naturally fall into a group which it is reasonable to distinguish from Pfeiffer's bacillus. If this group is eliminated, the distinction between haemoglobinophilic bacteria (giving growth only on media containing haemoglobin) and non-haemoglobinophilic bacteria (giving growth on media with and without haemoglobin) is very well marked.

Point 4 is also a good distinctive mark, in that by using a blood-containing medium of suitable constitution for those bacteria which possess other characters in common with Pfeiffer's bacillus, either there is no haemolysis even after several

days in the thermostat, or there is rapid and well-marked haemolysis.

That these points (with one small modification — see p. 196) together constitute the essential and complete characteristics of the group „Pfeiffer's bacillus" in the case of the bacterial flora coming from man (which of course does not exclude the fact that other characters may be valuable), will appear from the following investigations of these conditions and the others mentioned on pp. 68—70 as well as a number of other reactions.

The Haemoglobinophilic Character of Pfeiffer's bacillus.

By „haemoglobinophilia", — the character which has given the name to the whole group that interests us here, — it must be understood that it is not specially the substance haemoglobin which is necessary for the growth of the bacteria. On the contrary those authors (Thalhimer (1,2), Aguliion & Legroux, Olsen (3), Terada, Davis (6), Thjøtta & Avery (1,2)), who have examined the growth of Pfeiffer's bacillus on agar containing „pure" crystallised haemoglobin, have found that it was very little, if at all, active in this respect. (That it can be made active in various ways, will be later referred to). It has also been shown that the media which afford the richest growth of Pfeiffer's bacillus, are those which do not contain any actual haemoglobin at all, but only the different derivatives of it, the chemical nature of which it has hitherto been impossible to determine with any degree of certainty.

That Pfeiffer's bacillus is haemoglobinophilic therefore means that it can grow on certain media which in themselves are unable to cause growth, when either red blood corpuscles or suitable transformation products of these are added.

A thorough analysis of the statements in the literature on the growth of Pfeiffer's bacillus without „haemoglobin"* would be a rather prolix task and it is unnecessary for the investigation we are engaged upon. Only the following points will be mentioned:

* „Haemoglobin" in inverted commas is used in the following pages as a common term for red blood cells and all such products of them that make possible the growth of Pfeiffer's bacillus and other bacteria with similar nutritive requirements.

That Pfeiffer's bacillus is not absolutely haemoglobinophilic, but under certain conditions can grow in media not containing haemoglobin or its derivatives, has hitherto only been proved by THJØTTA & AVERY (2) who cultivated it in USCHINSKY's fluid + raw potato, and by RIVERS (4) who used an inorganic salt mixture with the addition of glycerin, ammonium lactate, asparagin, tryptophan, egg and yeast extract.

All the other authors who have reported growth on media with the addition of other substances than „haemoglobin" have employed peptone broth or peptone broth-agar as substratum. As broth and usually peptone also are prepared from meat which commonly contains some blood and also the special muscle haemoglobin (MØRNER), it cannot be excluded that peptone broth may contain haemoglobin derivatives which under certain conditions may be of importance for the growth of Pfeiffer's bacillus, in spite of the fact that there is a general consensus of opinion that this microbe (and other bacteria with similar nutritive requirements) in pure culture, cannot grow on ordinary peptone broth agar. It should be emphasized that the applicability of this characteristic will naturally not be interfered with, even if the future should prove that „haemoglobin" may be replaced by quite other substances to a still greater extent than hitherto.

The failure to grow on ordinary agar can only be taken as a constant character when the culture of Pfeiffer's bacillus is pure. In the presence of other bacteria, — or more generally, other organisms, e. g. yeast cells or pieces of tissue of the higher plants or animals which have never been contaminated, growth may take place on ordinary agar or other media, which otherwise would not support it. In many cases this can be explained on the ground that the media contained a sufficient amount of haemoglobin derivatives in such a form that they do not produce growth without the further addition of the „activating" substances mentioned. This point will be further discussed under the heading „Symbiosis Phenomenon".

That some authors (RICHTER, NASTIUKOW (1,2), CANTANI (2), and others) have obtained a growth of Pfeiffer's bacillus on agar containing bile or yolk of egg, may perhaps be explained by assuming these products include bodies which are closely allied to haemoglobin (the bile pigments, as is well known,

arise from the blood pigment, and haematogen is formed from the ovo-vitellin of egg yolk by digestion, which according to BUNGE is of importance in the formation of blood). That CANTANI (2) also found cholesterin, egg albumin, serum albumin, and semen active, can be explained partly by activation of the haemoglobin derivative (haematin) in the agar, and partly, — in the case of the last two substances, — that they themselves contained haemoglobin. But the growth-producing power of all these bodies is undoubtedly rather unreliable, and many authors have hardly been able to detect it.

Opinion is divided as to how far serum or ascitic fluid can replace „haemoglobin". The common view is that Pfeiffer's bacillus cannot grow on agar with the addition of these fluids if they are free from haemoglobin. In explanation of the various reports of the growth of Pfeiffer's bacillus on such media, there is a good possibility that the serous fluid did contain haemoglobin. In most cases this possibility is either disregarded, or it is stated that haemoglobin could not be demonstrated spectroscopically, which assurance is absolutely worthless, because a haemoglobin concentration that is much too small to be demonstrated spectroscopically, may be sufficient to allow the growth of Pfeiffer's bacillus, especially in the presence of other kinds of bacteria.

On the authority of the literature on the subject it must however be stated that although Pfeiffer's bacillus may be able to grow, in certain cases even in pure culture, on medium containing serum or serous fluids without „haemoglobin", it much prefers the pigments of the blood.

I will now pass on to report my own experiences of the various aspects of the haemoglobinophilia of Pfeiffer's bacillus.

This term has a positive side: growth on „haemoglobin"-containing media, and a negative side: no growth on „haemoglobin"-free media.

As regards the positive side I will only remark here that I have certainly not systematically investigated whether the kind of medium is a matter of indifference in demonstrating growth on „haemoglobin" containing media. But everything indicates that all the bacteria, which are termed haemo-

globinophilic,* are dependent upon the same „haemoglobin"-modifications, so that the kind of „haemoglobin" medium employed is without significance, in deciding what shall be admitted under the term „haemoglobinophilic bacteria". With regard to details concerning the different „haemoglobin" media, the reader is referred to the chapter on cultivation technique.

But the negative side of the term haemoglobinophilia will be discussed a little more closely.

In the investigation of this condition ordinary agar and ascitic agar were used for the examinations in the summer and autumn of 1918, 24 & 25. I. 1919, 5. III. 1919, July 1920, 14. IX. 1920, 5. X. 1920, and for the meningitis and guinea-pig cultivations; only ascitic agar was employed for the examinations in the winter of 1918—19, for whooping-cough later in 1919, for measles, and for the following inoculations from healthy persons: 14. I. 1919, 13. VIII. 1919, and 10. IX. 1919. Lastly only ordinary agar was used for the examinations of 6. III. 1920, Sept. 1921, and Jan. & Feb. 1922.

In using ordinary agar it was found, in the case of all the bacteria, which from their properties appeared to be Pfeiffer's bacilli, that by cultivating in pure culture no growth at all was obtained. (It is hardly necessary to point out that in subculturing from a medium rich in haemoglobin a slight growth might appear in the first subculture on account of some haemoglobin being carried over; but further cultivations could then not be obtained).

With ascitic agar the conditions were rather more complicated.

During the time my work has been in progress ascitic fluid has been widely used at the Institute for Meningococcus investigations, and the fluid from a considerable number of patients altogether, has been used. The value of the ascitic agar as a nutritive medium has therefore constantly been controlled by the capacity of Meningococcus, even in primary culture, to grow on it all times. Moreover it was not further investigated with particular reference to Pfeiffer's bacillus in the first year, but only those ascitic agar plates which were in use at the time, were employed in the investigations into haemoglobinophilia. Cultures, which from their other characters seemed to be Pfeiffer's bacilli, gave no growth on ascitic

* In the strictest sense.

agar, with great regularity. A slight growth was noted on this medium in a single case (I 71) only.

Later on my attention was directed to the advisability of controlling the ascitic agar more accurately, and by degrees I came to insist on the following requirements for an ascitic fluid suitable for the verification of Pfeiffer's bacillus: (1) The ascitic fluid should not in any case give a marked reaction with the benzidin test. This test is carried out as follows: To 1 vol. of a freshly prepared 5—10% solution of benzidin in glacial acetic acid, is added 1 vol. of oxydol (or water + perhydrol), and after mixture, 1 vol. of ascitic fluid. (2) The ascitic agar when finished should be neutral or weakly alkaline to azolitmin paper,* as it has been shown that this reaction is most favourable for the growth of Pfeiffer's bacillus. (Ordinary agar may conveniently react rather more alkaline to azolitmin paper). To adjust the reaction, the marked alkalinity of the ascitic fluid is usually reduced by adding an appropriate amount of hydrochloric acid. (3) Agar plates, with and without ascitic fluid, are inoculated with various cultures of the whooping-cough bacillus, Gonococcus, and Meningococcus, preferably such as have only been grown for a few generations in vitro. A much richer growth should appear on the ascitic agar than on that without ascitic fluid (in so far as any growth takes place at all, which will never be the case with the whooping-cough bacillus when it has only been subcultured a few times, but in the case of Gonococcus and especially of Meningococcus growth may occur on ordinary agar).

Lastly as a fourth control it can be insisted that Pfeiffer's bacillus is unable to grow on the medium. As a number of cultures are usually examined together they will to a certain extent serve as controls to one another.

The three first requirements were only regularly satisfied from July 1920, an ascitic fluid (denoted, for short, by „1,023") sp.gr. 1,023, from that date onwards being most frequently used, which only gave a very slight blood reaction. The reaction was adjusted as described, every time a batch of ascitic

* For the preparation of this, and for other matters relating to the reaction of media see pp. 225—232.

agar was prepared. The finished ascitic agar was frequently tested particularly by inoculation with the whooping-cough bacillus.

On this improved ascitic agar it was found that a slight growth would appear more often, but it was always much less than on haemoglobin agar. In the inoculations in July 1920 (see pp. 88—89) all the strains of Pfeiffer's bacillus as usual gave no growth on ordinary agar; nor did the strains H 260—281, which were tested on agar containing the ascitic fluid mentioned above, without the addition of acid, grow on this medium although the whooping-cough bacillus and Gonococcus grew well. For the other strains (H 282—313) ascitic agar with corrected reaction was employed and a number of them gave distinct though scanty growth on it, even in the second culture.

Similar observations were made in the inoculations of 14. IX. 1920. None of the cultures which were called Pfeiffer's bacilli grew in the 2nd. generation on ordinary agar. On freshly prepared ascitic agar made with „1,023", and on which Gonococcus and the whooping-cough bacillus grew well, some of the strains that in other respects must be called Pfeiffer's bacilli gave a weak growth still in the 2nd. generation.

In the inoculations on 6. III. 1920, only ordinary agar was used for the diagnosis of Pfeiffer's bacillus as mentioned above. Later on the same strains were tested by inoculating on agar with the addition of ascitic fluid with a sp.gr. of 1,017, which gave deep blue colour with the benzidin test but was not visibly red. The majority of the strains gave a weak growth which could be maintained for three generations. For comparison the 5 old strains I1, 5, 6, H 14 and 19 were inoculated and exhibited the same growth of slight intensity.

But there was no growth on further subculturing, for which plates were used which had been kept 5 days in the ice-safe, a period which according to my usual experience, only slightly impairs the value of the ascitic agar as a nutritive medium for the microbes for which it is specially suitable. Even in the presence of decided growth-promoting organisms (Staphylococcus, „white air-coccus") growth was absent.

In agreement with what is found in the literature on the subject, it is justifiable, from my experience therefore, to c o u n t

complete absence of growth on ordinary peptone broth agar as a characteristic of Pfeiffer's bacillus, but we must be more cautious in our interpretation of growth on ascitic agar. It would obviously lead to absurd results to exclude all bacteria that occasionally can be made to grow on one or other batch of ascitic agar, from the species „Pfeiffer's bacillus". The boundary between growth and absence of growth on ascitic agar is much too indistinct and what is more, it is far too susceptible to slight variations in the constitution of the medium. As will appear from what follows, quite different results may be obtained with the same strains when the medium is prepared in different ways even though the same agar and ascitic fluid are used each time. Still greater variations will be encountered by employing different ascitic fluids. It must be remembered that such fluids, quite apart from the blood content, can vary considerably in constitution and in their value as nutritive media for bacteria. Nor is it excluded that that amount of haemoglobin which is too small to give the benzidin reaction, may be useful to Pfeiffer's bacillus. Ascitic fluid seems to inhibit this blood reaction to some extent so that a greater concentration of haemoglobin is required for a positive result than in a watery solution or a solution in broth. (Nevertheless most of the ascitic fluids I investigated reacted strongly with benzidin, without being perceptibly redtinted).

Although ascitic agar does not lend itself to purely routine work in the same way as ordinary agar, it would be improper not to include it among the means which serve to distinguish Pfeiffer's bacillus, because the fact that an organism grows excellently on agar containing dissolved „haemoglobin", in conjunction with the fact that on carefully prepared ascitic agar it grows at most, only scantily, is very characteristic of Pfeiffer's bacillus and some closely allied species. When the test on ascitic agar is carried out with all the four requirements mentioned above, fulfilled, the interpretation of the result will certainly not involve any difficulty.

It was mentioned that some strains of Pfeiffer's bacillus which had been cultivated for over 1½ years, behaved exactly like recently isolated strains as regards growth on a slightly blood-containing ascitic agar. This indicates that the nutritive

requirements of Pfeiffer's bacillus are not greatly altered by prolonged cultivation. In order to investigate this more closely I repeatedly inoculated all the strains which were still living at the time, on haemoglobin-free media.

On 26—28. XII. 1919 the following strains were subcultured in pure culture on ordinary agar (weakly alkaline to azolitmin paper: I 1, 5, 6, 20, 21, 22, 23, 27, 29, 35, 38, 45, 51, 57, 59, 71, 72, 91, 100; I 5, 21; P 14, 15, 16, 22, 23, 24, 25, 26; H 14, 19, 34, 35a, 37a, 37c, 49b, 122, 127, 133, 151, 152, 154, 155, 156, 157, 158, 159, 160, 161; Pa 4, 5. In no case was there any growth after 4 days in the incubator. The same cultures were inoculated on ordinary agar together with various other bacteria (white air-coccus, Streptococcus, B. faecalis alcaligenes) in the same way as was done in the symbiosis reaction described later. Some of the strains were inoculated directly from haemoglobin agar, and showed a quantity of growth but only in the immediate neighbourhood of the alcaligenes culture. The other strains were inoculated from „symbiosis" cultures on plates poor in haemoglobin. None or only very slight growth took place. We thus see what a marked effect the minute quantity of haemoglobin which is transferred with the culture, can exert when it is combined with the action of the other bacteria.

On 2. III. 1920 the same strains were inoculated on agar and ascitic agar in company with a haemolytic Streptococcus which with suitable experimental technique, has a good growthpromoting action on Pfeiffer's bacillus. Both Pfeiffer's bacillus and the Streptococcus were inoculated in parallel streaks at about ½ cm. distance, alternately. The agar was alkaline to azolitmin paper, but did not react to α-naphthol-phthalein. For ascitic fluid the weakly haemoglobin-containing „1,017" (see p. 106) was used. The ascitic agar mixture was slightly alkaline to azolitmin paper. In order to avoid transferring a greater amount of haemoglobin than necessary, cultures of all the strains were first produced on agar with dissolved blood corpuscles in a concentration of $^1/_{10000}$, in „symbiosis" with the same Streptococcus. After 1½ days in the incubator there was a very rich growth on this medium. Subcultures were then directly made for the actual test. As a control that the agar and ascitic agar were efficient, some haemoglobin was added to a number of them, and they were inoculated with some of the cultures of Pfeiffer's bacillus, and they showed particularly good growth. On agar plates with the streaked „symbiosis" cultures a distinct though slight growth appeared after an interval of 4 days. From these, subcultures were made on to new plates, using the same technique. (The agar plates used in this second culture were made on the day before, while the ascitic agar plates had been kept 5 days in the icesafe). No growth of Pfeiffer's bacilli occurred this time.

On 9. IX. 1920 the following strains were inoculated on ordinary and on ascitic agar: I 1 (a), 1 (b), 5, 6, 20, 21, 22 (a), 22 (b), 23, 27, 29, 35, 38, 45, 54, 57 (a), 57 (b), 71, 72, 91 (a), 91 (b), 100, 103; I 5, 21, 32, 33, 34, 35, 36, 37; P 14, 15 (a), 15 (b), 22, 23, 24, 25, 26; Ms 1, 4, 9, 10, 13, 15; Me 1; H 14, 19 (a), 19 (b), 34, 35a, 37a, 37c, 49b, 122, 127, 133, 151, 152 (a), 152 (b), 154, 155, 156, 157, 158, 159, 160, 164, 168, 171, 172, 176, 178, 179, 181, 197, 205, 260, 261, 263, 264, 265, 266, 268—81, 283, 284, 286—89, 291, 293—296, 298—304, 306—313; Pa 4, 5.

The agar reacted strongly alkaline to azolitmin paper but did not react to α-naphthol-phthalein (see p. 226). To control the value of the agar as a nutritive medium, a very small amount of haemoglobin (dissolved blood corpuscles) was added to some of it. Plates of this were inoculated with various strains of Pfeiffer's bacilli together with the „white air coccus" which produced a very rich growth of the Pfeiffer's bacilli.

The ascitic agar was prepared from the same agar and the ascitic fluid „1,023". Hydrochloric acid was added till the reaction became weakly alkaline to azolitmin paper. As a control of the efficiency of the medium a plate was inoculated with 2 cultures of Gonococcus which gave a good growth and 3 cultures of the whooping-cough bacillus which gave a medium growth, while on the agar without ascitic fluid one Gonococcus cultivated grew moderately, the other and also the whooping-cough bacillus cultures, not at all.

The agar and ascitic agar were inoculated from cultivations of all the strains of Pfeiffer's bacilli mentioned on FILDES-agar (see p. 229), streak inoculations of these being alternated with streaks of the „white coccus".

After 4 days in the incubator there was no trace of growth on the agar plates.

The following strains also did not grow on the ascitic agar plates: I 6, 38, 45, 54, 57, 71; I 32, 33, 34; Ms 1; H 14, 19 (a), 35 (a), 122, 152 (a), 152 (b), 158, 160, 171, 176, 181, 205, 269, 272, 291, 295, 303, 304, 306, 307, 312. The remaining strains grew more or less luxuriantly on the ascitic agar plates but exclusively in the immediate neighbourhood of the coccus colonies. At a distance from these there was no growth in any of the cases. All the strains which grew were again inoculated on the same medium, always in association with the „air coccus". During the course of the experiments freshly prepared ascitic agar plates were required; they were controlled by the previously mentioned method. With each inoculation the plates were examined after 4 or 5 days incubation.

In the inoculations on ascitic agar in the second generation, two cultures were made from each strain. It should be noted that the results of these parallel tests diverge considerably from one another. From all the strains which grew cultures were again made, which represented the 3rd. generation, and the following strains showed growth but only around the coccus colonies: I 1 (a, b), 5,

35, 57, 59; P 15 (a, b), 23; Me 1 a, b, c, d; H 34, 157, 159, 280, 183, 284, 286, 289, 302, 308, 309, 310, 311, 313. But I 36 and H 155 grew particularly well and there was also distinct growth outside the sphere of influence of the „air coccus". In the next subculture these two strains still grew in the same manner.

Finally on 1. XI. 1921 the following strains were inoculated on ordinary and ascitic agar: I 1 (a, b), 5, 6, 20, 21, 22 (a, b), 23, 27, 29, 35, 38, 57 (a, b), 59, 71, 72, 91 (a, b), 100, 103; I 5, 21, 32, 33, 34, 36, 37; P 14, 22—25; Ms 9, 13, 15; Me 1a, b, 2, 3, 4, 5; H 19, 34, 49b, 122, 127, 133, 151, 152 (a, b), 154, 155, 156, 157, 159, 164, 168, 176, 179, 181, 197, 205, 260, 261, 263—266, 268, 269, 272, 274, 276, 280, 287, 288, 289, 291, 293—296, 298, 299, 300, 302, 305, 307—312, 314 a, b, c, d, 318, 319, 320, 322—325, 327—339, 341—350, 352; GP 1, 2, 3, 4 a, b, 5; Pa 4, 5; R 1, 2.

In the preparation of the ascitic agar the fluid „1,023" was used. The finished medium reacted slightly alkaline to azolitmin paper, and 4 strains of whooping-cough bacillus which did not grow on agar without ascitic fluid, grew well on it. The ascitic agar was used in a freshly prepared condition. A number of the strains were also inoculated on ascitic agar plates which had been incubated for 24 hours, but this made no difference.

The following strains grew slightly in the 1st. culture on ordinary agar: H 314 a, b, c, d, 318, 319, 322, 324, 330; GP 1, 2, 3, 4b; but in no case was there growth in the 2nd. culture. On ascitic agar most of the cultures gave no growth even in the 1st. culture. In the 2nd. culture the following showed slight growth: H 289, 314b, 318, 319; Pa 4.

Of the two strains which succeeded in growing in pure culture on ascitic agar in the experiment on 9. IX. 1920, I 36 did not grow in the 1st. culture, and H 155 only grew slightly.

It appears from this experiment that even those strains that had been cultivated for over three years, during which time they had been subcultured more than 200 times, — on medium rich in haemoglobin it is true, — had retained their dependence on haemoglobin unchanged.

We might imagine that it would perhaps be possible by gradually reducing the haemoglobin content of the medium, to train Pfeiffer's bacilli to be satisfied with a smaller amount and finally to do entirely without it. Apropos of this some experiments will be mentioned which partly deal with this question and partly give some information about growth in different concentrations of haemoglobin.

In August 1918 plates were prepared containing dissolved blood corpuscles in roughly the following concentrations (calculated as blood corpuscles, not haemoglobin): 1 : 10, 16, 30, 50, 80, 120, 200,

350, 600, 1000, 1600, 2500, 4000, 7000, 10000, and 15000. Al these plates were inoculated with 6 strains of Pfeiffer's bacillus. On the first 7 plates there was a rich growth even after 15 hours' incubation, perhaps a little more marked on the first one or two than the last. On Nos. 8—13 (1 : 350—4000) the amount of growth gradually decreased. On the last three plates the growth was slight after 40 hours incubation, but as it was the same on all three plates, it was probably due to the haemoglobin carried over when the inoculations were made.

On 23. II. 1920 agar plates were prepared (with a moderate alkaline reaction to azolitmin paper) with the following concentrations of dissolved blood corpuscles (1) 1 : 100, (2) 1 : 300, (3) 1 : 1000, (4) 1 : 3000, (5) 1 : 10000, (6) 0. All the strains mentioned on p. 108 were inoculated on these media from a medium identical with No. 1.

On (1) the usual good growth occurred in all cases. On (2) the growth was rather less; on (3) the growth was rather poor in each case, but a little better than on ordinary blood plates; on (4) the growth was about the same as on ordinary blood plates although considerably better in the neighbourhood of chance colonies of other bacteria; (5) was still able to support a slight growth of all the strains except H 161. The growth was not entirely produced by the haemoglobin carried over at the inoculation because a considerably greater number of strains showed practically no growth on the haemoglobin-free plates (6).

The lowest limit of (approximately) optimum growth is therefore at about 1% dissolved blood corpuscles, and the lowest limit of growth is round about $^1/_{100}$%. This agrees quite well with the experiment of 1918, in which $^1/_2$% and $^1/_{50}$% were found respectively. It would be quite wrong however to assume these values are generally applicable, since the amount of haemoglobin necessary depends largely on the constitution of the (peptone-broth) agar, on the nature of the haemoglobin, and on the presence of other kinds of bacteria or other growth-promoting substances having a similar effect. The importance of the first of these three points is seen from an experiment (1. II. 19) in which autoclaved agar was used while in all other cases the agar was sterilised at 100°. This alteration was the cause of Pfeiffer's bacillus, even on a 1—2% content of dissolved blood corpuscles, only growing poorly or entirely failing, but it grew luxuriantly when the concentration was increased to about 10%. The peculiar result that the haemoglobin agar which up to March 1920 had constantly been used with excellent results, then began to fail, will be further discussed in the chapter on nutritive media.

We will now revert to the experiment of 13. II. 1920. From media Nos. 3, 4, 5 & 6 subcultures were made on plates with the same haemoglobin concentrations. The results are tabulated below where „1 : 1000" etc. denotes the concentration of dissolved blood

cells. I, II, & III signify 1st., 2nd., & 3rd. culture on the medium in question.

	1 : 1000			1 : 3000			1 : 10000			0	
	I	II	III	I	II[3])	III[4]) [5])	I	II	III	I	II
I 1 (a)	+	+	+	+	+	(+)	+	+	+	0	
I 1 (b)	+	+	+	+	+	(+)	+	+	0	0	
I 5	+	+	+	+	+	+	+	+	0	0	
I 6	+	+	+	+	+	+	+	+	0	0	
I 20	+	+	+	+	+	(+)	+	+	+	+[2])	0
I 21	+	+	+	+	+	(+)	+	+	+	+[2])	0
I 22 (a)	+	+	+	+	+		+	0		0	
I 22 (b)	+	+	+	+	+	(+)	+	+	+	0	
I 23	+	+	+	+	+	+	+	+	0	0	
I 27	+	+	+	+	+	(+)	+	+	(+)	0	
I 29	+	+	+	+	+	(+)	+	+	+	0	
I 35	+	+	+	+	+	(+)	+	+	+	0	
I 38	+	+	+	+	+	+	+	+	+	0	
I 45	+	+	+	+	+	0	+	+	+	0	
I 54	+	+	+	+	+	+	+[2])	+	+	0	
I 57 (a)	+	+	+	+	+	0	+	+	(+)	0	
I 57 (b)	+	+	+	+	+	0	+	+	(+)	0	
I 59	+	+	+	+	+	0	+	+	(+)	0	
I 71	+	+	+	+	+	+	+	+	0	0	
I 72	+	+	+	+	+	+	+	+	(+)	0	
I 91 (a)	+	+	+	+	+	+	+	+	+	0	
I 91 (b)	+	+	+	+	+	+	+	+	+	0	
I 100	+	+	+	+	+	+	+	+	(+)	0	
I 5	+	+	+	+	+	+	+	+	(+)	0	
I 21	+	+	+	+	+	(+)	+	+	(+)	0	
P 14	+	+	+	+	0		+	0		+	0
P 15 (a)	+	+	+[2])	+	+	+	+	0		+	0
P 15 (b)	+	+	0[1])	+	+	+	+	0		+	0
P 16	+	+	0[1])	+	+	+	+	0		0	
P 22	+	+	+	+	+	+	+	+	+	0	
P 23	+	+	+	+	+	(+)	+[2])	+	+	+[2])	0
P 24	+	+	+	+	+	+	+[2])	0		0	
P 25	+	+	+	+	+	(+)	+[2])	0		0	
P 26	+	+	+	+	+	+	+	+	(+)	0	
H 14	+	+	+	+	+	(+)	+	+	+	0	
H 34	+	+	+	+	+	+	+	+	0	0	
H 35 a	+	+	+	+	+	(+)	+[2])	+	(+)	0	
H 37 a	+	+	+	+	+	+	+[2])	+	(+)	0	
H 37 c	+	+	+	+	+	+	+	+	0	0	
H 39 a	+	+	+	+	+	+	+	+	+	0	
H 39 b	+	+	+	+	+	+	+	+	(+)	0	
H 49 b	+	+	+	+	+	(+)	+	+	(+)	0	
H122	+	+	+	+	+	0	+)	0		0	

	1 : 1000			1 : 3000			1 : 10000			0	
	I	II	III	I	II[3]	III[4] [5]	I	II	III	I	II
H127	+	+	+	+	+	(+)	+	+	+	0	
H133	+	+	+	+	+	(+)	+	+	+	0	
H151	+	+	+	+	+	(+)	+	+	+	+	0
H152 (a)	+	+	+	+	+	+	+	+	(+)	+[2]	0
H152 (b)	+	+	+	+	+	+	+	+	+	+[2]	0
H154	+	+	+	+	+	(+)	+	0		0	
H155	+	+	+	+	+	+	+	+	(+)	0	
H156	+	+	+	+	+	(+)	+	+	+	0	
H157	+	+	+	+	+	(+)	+	+	+	[2]	0
H158	+	+	+	+	+	+	+	+	+	0	
H159	+	+	+	+	+	+	+	+	+	0	
H160	+	+	+	+	+	+	+	+	0	0	
H161	+	+	0[1]	+	0		0			0	
Pa 4	+	+	+	+	+	+	+[2]	0		+[2]	0
Pa 5	+	+	+	+	+	+	+[2]	0		0	

(1). Observed twice.
(2). Slight growth after 4 days.
(3). In many cases no visible growth after 2 days, but distinct growth after 4 days.
(4). (+) signifies that there was growth only in one of the two plates constituting each test.
(5). In the next subculture (4th. culture) there was no growth or only a trace, in each case.

The cultivation was continued on the plates containing $^1/_{1000}$ blood corpuscles to the 5th. or 6th. generation without any sign of adjustment to the new conditions. On the contrary the growth gradually diminished.

A number of strains (50 in all) were inoculated on ordinary broth in test tubes. In no case was there any visible growth.

The object of performing the experiments of inoculation on haemoglobin-free media in such large number and of reporting them so minutely, is to bring forward two points: (1) whether distinct individual differences between the various strains can be detected with regard to their dependence on haemoglobin, and (2) whether haemoglobinophilia can be regarded as a constant character.

As regards the first point there seem to be certain differences of such a kind that some strains can be accommodated with less haemoglobin than others, but nothing definite can be stated about this question, and large, well-marked differences at any rate, do not exist.

With regard to the second point it is a well-known fact that bacteria often change their nutritive requirements and conditions of growth very much on cultivation for a long period, so that after some time they can grow on media on which they could not thrive immediately after they were isolated. In the case of bacteria usually classed with the haemoglobinophilic, somewhat similar phenomena may take place also. BORDET's whooping-cough bacillus can, for instance, without any difficulty be trained to grow on ordinary agar which under ordinary conditions it is quite incapable of. If the same were the case for Pfeiffer's bacillus haemoglobinophilia would lose considerably in value as a specific character, or at all events would have to be used with greater caution.

PFEIFFER (2) had already drawn attention to the importance of this point, having convinced himself that „auch die ältesten Culturen haben keine weitergehende Anpassung an saprophytische Lebensbedingungen erfahren und gedeihen wie im Anfang ausschliesslich auf Blutagar". DAVIS, STILLMAN & BOURN, WADE & MANALANG and others, had the same experience.

TOCUNAGA observed that Pfeiffer's bacillus after being cultivated for a long time (10 months) could gradually do with less haemoglobin, but it could never grow on absolutely haemoglobin-free media. The statements of JUNDELL, WATAGUTI, and TOCUNAGA about training Pfeiffer's bacillus to grow on haemoglobin-free, s e r u m - c o n t a i n i n g media, must, for reasons already mentioned, be regarded as uncertain.

In the material I have worked with, there has been absolutely no evidence that by prolonged cultivation Pfeiffer's bacillus may lose its haemoglobinophilic quality, and it seems therefore to be legitimate, as an obligatory character of this microbe, to demand c o n s t a n t haemoglobinophilia in the meaning of the word elucidated above.

Several authors (WOLFF, MARX, FROSCH & BIERBAUM, RITCHIE, DAVIS (4)) have described bacteria which immediately after isolation were markedly haemoglobinophilic, but later lost this character. There need hardly be any doubt about excluding all such organisms from the designation Pfeiffer's bacillus, as in other points too they diverge from same, so that in demonstrating the latter it is not necessary to repeat the test of haemoglobinophilia after several months' cultivation, which would also be, as a rule, practically impossible to carry out. We will return later to these temporary haemoglobinophilic bacteria.

The complete picture obtained from the experiments reported in this chapter is as follows:

Among the non-haemolytic, Gram-negative rods, which have been cultivated on media containing dissolved haemoglobin, from the mucous membranes of the air passages, the lungs, pleura, and meninges of man, the negative test for growth on ordinary agar affords a good limitation of a group of bacteria which also possess this character in common, that ascitic agar supports only slight growth at most and much scantier than media containing dissolved haemoglobin. Both these qualities persist practically unchanged even after years of cultivation on artificial media. Characteristic individual differences between the different strains as regards dependence upon haemoglobin could not be discovered.

The Symbiosis Phenomenon.

Since GRASSBERGER (1) in 1897 observed that Pfeiffer's bacillus grows particularly well around colonies of Staphylococcus innumerable investigators who have worked with it have confirmed the phenomenon. It must be understood that it is not a question of symbiosis in the true sense. In the first place it is only the Staphylococcus that favours the growth of Pfeiffer's bacillus and not vice versa; and in the second place, immediate contact between the two is unnecessary. The effect can make itself felt over a distance of a couple of cm. It is produced therefore by a substance with a considerable power of diffusion. A Staphylococcus culture can futhermore preserve its activity after being killed by gentle heating. (Staphylococcus has only been named as an example of a growth-promoting organism; many other bacteria have exactly the same action).

For a long time „symbiosis" featured as a somewhat isolated phenomenon in the biology of Pfeiffer's bacillus. Latterly however we have come to look upon this phenomenon in its relationship to the other biological properties of this micro-organism. We may imagine that 3 things are necessary for the growth of Pfeiffer's bacillus.

(1) Some salts and other substances which do not play

a unique rôle in the metabolism of Pfeiffer's bacillus, but are just as important for the growth of the majority of other common bacteria. Ordinary peptone-broth (partly also peptone or broth alone) contains these substances.

(2) A body „X" (or perhaps better, a group of bodies, which resemble one another as far as they interest us here), which is principally found in haemoglobin and several of its derivatives, and is characterised by possessing the same „oxygen conducting power" as haemoglobin and by resisting heating to 120° for a short time (¾ hour is named).

(3) A body „V" which occurs very widely distributed in the animal and vegetable kingdom: bacteria, yeast cells, carrots, tomatoes, beans, muscle, spleen, liver, kidney, and so forth. Serum and serous fluids may be slightly active. In the case of ascitic fluid, according to Davis (6), this only happened when it had a high specific gravity and was fresh. Perhaps however the activity is due in the last case to the content of substances derived from blood corpuscles (Thjötta & Avery (1)). It is much less thermostabile than „X", as it is more or less quickly destroyed at 100° and instantaneously at 120°. (The signs „X" and „V" are those used by Thjötta & Avery).

We will assume that we always use the same medium (peptone-broth agar containing the substances referred to under the heading (1) in sufficient quantity) and add to it substances which will furnish it with the growth factors „X" and „V". It will then be seen that we can attain the same maximal limit of growth for Pfeiffer's bacillus by several different ways. We can imagine that for this maximal growth a definite concentration of ‚X" and also of „V" is required in all cases. If there is more of one or both factors, the surplus is useless. If we add to the medium a mixture of „X" and „V" which is inadequate to produce maximal growth, two possibilities will arise: (1) „X" is in excess. (2) „V" is in excess. (We set aside the special case when they are present in „equivalent" amounts). In the first case the addition of „V" only (that is to say without „X") will increase the growth, in the second case it will be without effect.

An attempt will now be made to give an account of the conditions of growth of Pfeiffer's bacillus under different circumstances, on the basis of the above scheme. It will then

appear that we possess a good means of obtaining a view of many things which at first sight seem to be rather complicated.

As the central factor in the group of substances which are of special importance for the growth of Pfeiffer's bacillus, stands crystallised „haemoglobin", i. e. oxyhaemoglobin. There will be no difference between reduced haemoglobin and oxyhaemoglobin as we are working entirely under conditions where the oxygen of the air has free access. Methaemoglobin, as far as it has been investigated, behaves like oxyhaemoglobin.

It has already been stated (p. 101) that crystallised haemoglobin in itself is inactive or nearly so. (The possibility that different products of crystallised haemoglobin even under otherwise perfectly similar experimental conditions, will not always prove so absolutely inactive is not excluded, since even after repeated crystallisation it can not always be regarded as a substance of constant constitution (Herzfeld & Klinger)).

But agar containing crystallised haemoglobin can be made active in two ways: (1) by the addition of „V", e. g. in the form of yeast extract. It therefore contains „X", even in considerable amount. Crystallised haemoglobin alone produced no growth even in so strong a concentration as 1 : 10, but in association with yeast extract it produced growth in a dilution of 1 : 2000000. (Thjötta & Avery (2)).

(2) By heating. Olsen (3) found agar containing crystallised oxyhaemoglobin or methaemoglobin inactive. But when he heated the haemoglobin agar to boiling point (and filtered off the precipitate produced, which however is unimportant in the present case), that is to say, when he prepared Levinthal agar from it, he obtained exactly the same luxuriant growth as on ordinary Levinthal agar prepared with blood. In the transformation of the haemoglobin caused by heating it in agar, „V" is therefore liberated in large quantities, „X" being present all the time.

Good Levinthal agar contains so much „V" that further addition of it is without effect. The symbiosis phenomenon can therefore not take place. But if the Levinthal agar is heated for long, most of the „V" substance will be destroyed. As the „X" substance is now present in excess, bacteria, yeasts etc. can exert their action .

We will go a step further and autoclave the blood agar. It will then be quite inactive itself, but can be reactivated by bacteria, yeasts and other substances containing „V".

That CANTANI and many others have been able to obtain a growth of Pfeiffer's bacillus on ordinary agar without the addition of blood, in the presence of growth-promoting bacteria, may be due to a small content of a substance with a pure „X" effect (hematin?), (see GHON & PREYSS, — cf. p. 102).

Amorphous haemoglobin (THALHIMER), haemoglobin solution prepared by haemolysing washed erythrocytes and then filtering off the stromata (PFEIFFER (2)), the same haemoglobin solution unfiltered, and haemoglobin solution made from blood corpuscles with their coating of serum intact, all have practically the same effect, — they contain „X" in abundance and a considerable deficiency of „V". By using a strong concentration of haemoglobin however there will often be sufficient „V" to give maximal growth (in some cases „V" will be present in such trifling amount that this is not possible). But on decreasing the concentration, the increase of growth on the addition of „V" in the form of bacteria etc., shows that „V" was present in less quantity that „X". THJÖTTA & AVERY (2) obtained growth with dissolved blood corpuscles in a haemoglobin concentration of 1 : 10000, but when yeast extract was added a haemoglobin concentration of only 1 : 1000000 was necessary. The content in „V" substance of impure haemoglobin preparations may perhaps come from leucocytes or blood platelets. This question does not seem to have been investigated. (Compare a remark in GRASSBERGER (1, p. 475) on a medium made with pus, and FICHTNER's sputum medium).

That the stroma does not play any important part is proved by the fact that haemoglobin solution retains its activity after this is removed by filtration.

The chemical nature of the „X" and „V" bodies is far from clear. According to OLSEN (3) haematin and haemin act like pure „X", while haematoporphyrin is quite inactive. TOCUNAGA obtained a rich growth on agar with iron-free globin, prepared from crystallised haemoglobin.

It seems therefore that both globin and the ironcontaining component contain „X", while „V" is only present in globin.

Like crystallised haemoglobin, the impure haemoglobin pre-

parations referred to, and blood, or blood corpuscles will split off some „V“ on heating (best seen in agar or broth), while „X“ continues to be present in large quantities. The same thing may take place in other ways: Treatment with strong acid (Fleming), digestion with trypsin (Matthews) or pepsin-hydrochloric acid (Fildes).

We can naturally know nothing of the relation of the two components in intact erythrocytes, because in media containing whole blood corpuscles only those substances which diffuse out into the medium itself, have any effect upon the growth of Pfeiffer's bacillus. If the blood corpuscles are not exposed to destructive actions in preparing the medium there will presumably be only a very small quantity of these substances outside the corpuscles, especially just after the medium is made, and the weak growth of Pfeiffer's bacillus on ordinary blood agar is then easy to understand. This microbe is unable to liberate the erythrocyte's store of haemoglobin with the help of haemolytic substances.

In blood agar, that is agar containing whole blood corpuscles, it seems in some cases to be the „V‘ substance that (outside the blood corpuscles) is in excess. Brown & Orcutt, and Skajaa for instance, found that on blood agar only haemolytic bacteria (which furnish dissolved haemoglobin from the blood corpuscles and consequently the „X“ substance) can produce increased growth of Pfeiffer's bacillus, while the non-haemolytic (which only act by providing the „V“ substance) are inactive. Skajaa found the same non-haemolytic strains to be active on a medium containing dissolved „haemoglobin“ which may be assumed to contain „X“ in excess.

In other cases „X“ is present in blood agar in excess, as non-haemolytic bacteria can also increase the growth of Pfeiffer's bacillus on this medium (Davis (5), Jordan (2)).

As far as they have been investigated, the blood derivatives that act like „X“ also give a reaction with guaiac and benzidin.

Thjötta & Avery (2) found (as mentioned on p. 102) that raw potato was effective when it was the sole addition to broth or Uschinsky's fluid. It thus contains both „X“ and „V“ as it was inactivated by autoclaving but was again made active by the addition of yeast extract. In harmony with this it gave the benzidin reaction.

It is however not universal that the „X" function and a positive oxygen-carrying reaction go together. THJÖTTA & AVERY found banana was active in the same way as potato, but it did not react with benzidin however. Perhaps RIVERS' (4) egg medium (see p. 102) also does not conform to the rule.

Various metallic salts which are quite inactive as growth factors for Pfeiffer's bacillus, can react with guaiac. Benzidin is more specific for haemoglobin derivatives (GREGERSEN) and will undoubtedly react more rarely with substances devoid of the „X" function.

It is greatly to be recommended on OLSEN's (3) advice, that the (guaiac or) benzidin test be carried out on every medium, before using it in the investigations of haemoglobinophilia.

In order to give some idea of the symbiosis phenomenon in the form which must be considered most rational at the present time, it has thus proved necessary to give a review, — although a very short one — of the whole question of nutritive substances for Pfeiffer's bacillus, a review which will also serve as an introduction to that section in which the technique of cultivation will be dealt with.

Besides the already named authors, may be mentioned CANTANI, GHON & PREYSS, KALKBRENNER, and PRINGSHEIM. My own experiences, which will now be related in detail, are also taken into account in this review.

One cannot work long with Pfeiffer's bacillus before the augmented growth around colonies of chance contaminating cocci and other kinds of bacteria, attract one's attention. Illustrations of such fortuitously arising „symbiosis" effects will be found in the work of THOMSEN, KRISTENSEN, & THORBORG (Figs. 7, 8, 9, & 13).

As similar phenomena are usually not observed in the case of other species of bacteria, I consider that this phenomenon should be regarded as one of the most characteristic features of Pfeiffer's bacillus and therefore ought to be included in the purely routine demonstration of this organism even in large investigations.

An account will now be given of my experiences concerning

(1) the technique of demonstrating the symbiosis phenomenon; (2) the question as to how far all strains of Pfeiffer's bacillus conform to a standard of „symbiosis"; (3) various conditions relating to the mode of action of the growth-promoting bacteria.

After at first having tried different methods of performing the symbiosis test, I finally evolved a definite method of procedure which, in its simplicity, proved itself to possess considerable advantages over the arrangements of the test commonly employed.

On a plate of ordinary agar containing about 1‰ haemolysed blood corpuscles, Pfeiffer's bacillus is inoculated in two parallel streaks at about 1 cm. distance. Between these streaks a suitable species of bacterium is inoculated at a single point on the agar surface. „Symbiosis" then shows itself by the presence of a very rich growth of both streaks of Pfeiffer's bacillus in the vicinity of the colony of foreign bacterium, — within the limit of 1 to 2 cm. from it, — while the growth outside this zone of activity is quite weak (see plate I).

This technique comprises many factors, each of which is important in itself.

Firstly it can be noted that the agar ought not be sterilised at a higher temperature than 100°, as it is not always possible to obtain a rich growth of Pfeiffer's bacillus on autoclaved agar. In a large number of my investigations I have however used agar to which ascitic fluid has been added, in which case the agar could be autoclaved without detriment.

Of greater importance is the nature and amount of the „haemoglobin" added. It is sufficiently evident from the above that we can only be certain of getting a growth round the colonies of foreign bacteria (by „foreign" is meant, different from Pfeiffer's bacillus), on agar containing „haemoglobin". But it is not even on every batch of „haemoglobin"-agar that these organisms will evince their growth-promoting action.

It is postulated above that when both „X" and „V" are present in large quantities, the further addition of one of the components (no matter in what form) will have no perceptible effect.

It is a matter of common experience (Grassberger (2), Hundeshagen, Brown & Orcutt, Pringsheim, Wolf, Skajaa),

which I can also fully confirm, that on these media which already produce a rich growth of Pfeiffer's bacillus without the assistance of foreign bacteria, the presence of other species of bacteria does not further increase the growth. We might at first imagine that in pure culture it would not be possible to obtain such a good growth as when the growth-promoting power of other bacteria was in action. That in actual fact this is certainly not the case indicates that the „V" substance in the haemoglobin derivatives and that formed by foreign bacteria, act upon Pfeiffer's bacillus in exactly the same manner, and thus far they are to be considered identical. If they had acted differently we should have expected that the presence of one of the substances by itself could not have produced the same effect as the combined action of both.

We must therefore employ „haemoglobin" in so low a concentration that a pure culture is far from attaining its maximal growth. But this is not all. It is further required that the „haemoglobin" shall contain „X" in considerable excess of „V". In my experience an ordinary (impure) solution of oxyhaemoglobin made by haemolysing horse red corpuscles with distilled or tap water, has always satisfied this requirement, so that the same amount of haemoglobin which in the absence of other bacteria can only support a weak growth of Pfeiffer's bacillus and therefore only contains quite a small amount of „V", has at the same time a sufficient quantity of „X" to produce a maximal growth (that is to say, just as good as in a pure culture on the best medium) when the deficit of „V" is supplied by the colonies of a suitable species of bacterium.

That every „haemoglobin" solution does not lend itself to the symbiosis test is shown by an experiment where the „symbiosis" test consisted of inoculations from a culture of Pfeiffer's bacillus, on to agar plates containing haemolysed blood corpuscles and others containing pepsin-digested blood. Both media were prepared with several different concentrations of „haemoglobin", so that in pure culture various degrees of a weak growth of Pfeiffer's bacillus down to complete absence of growth on the plates with the weakest concentrations, occurred. On all the plates containing haemolysed blood the symbiosis reaction occurred in quite a typical form, while the

growth of Pfeiffer's bacillus made on the plates made with pepsin-digested blood was only very slightly increased around the coccus colonies.

In another experiment the utility of the haemolysed blood of the sheep, goat, rabbit, and pigeon was compared. The haemoglobin of human blood has also been used in investigating some of the strains of Pfeiffer's bacillus. The blood of the first three species of animal and the human blood gave quite as good „symbiosis reactions" as horse blood, but pigeon blood, — in several different concentrations — proved to be quite unsuitable.

We may imagine that the pepsin-digested (horse or sheep) blood, and the haemolysed pigeon blood contain relatively much „V", and thus have not the considerable excess of „X" which is necessary for a marked symbiosis reaction. Without the presence of foreign bacteria pigeon blood gave a considerably richer growth than horse blood in the same concentration. It was necessary therefore to reduce the concentration of pigeon blood very greatly to get even a weak growth in pure culture and so it is possible that there is too little „X" to support a vigorous growth even with the addition of „V" in large quantities.

In the preparation of the haemoglobin solution, blood corpusles have usually been used which were only partially freed from serum: To find out whether the amount of serum, — at most, very slight, — which the medium contained, had any effect on the „symbiosis", I arranged a simple experiment with haemoglobin solution made from horse blood corpuscles which were separated by the centrifuge and then washed three times with salt solution. The symbiosis phenomenon on agar with the serum-free haemoglobin occurred in just as marked a degree as usual. (Davis (6) had the same experience).

The next important point is that the growth-promoting organism must be inoculated at a distance from the culture of Pfeiffer's bacillus, so that the two species of bacteria do not come into contact with one another. The question of the choice of the growth-promoting organism will be returned to later. For the present I need only remark that for the first few months I tried several different cocci and finally settled upon a „white air-coccus", that is to say a Gram-positive mi-

crococcus from the air, a chance contamination, which grows in pure white colonies. The same strain has been continually used in the agar inoculations. For the sake of brevity it may be referred to as „air coccus".

That the symbiosis phenomenon may be indefinite or even entirely absent, when the organism which is to evoke it, is brought into too intimate contact with Pfeiffer's bacillus, is proved by a big series of experiments that I carried out in 1918 to investigate the growth-promoting action of a large number of different species of bacteria on some strains of Pfeiffer's bacillus. The latter was inoculated in streaks and the other bacteria were inoculated at certain points in them. It was then found that the expected symbiosis effect was very weak or quite absent in many cases.

Fig. 8, plate I shows Pfeiffer's bacillus inoculated partly as a streak and partly as a four-sided area. The air coccus was also inoculated at 4 points outside the cultures and at 2 points in it. It will be seen how the colonies situated outside the cultures of Pfeiffer's bacillus produce greatly increased growth in the nearest portions of these, while the colonies inoculated actually in the cultures are quite inactive in this respect. It may be mentioned that a corresponding experiment where a Streptococcus took the place of the air coccus, gave precisely the same result, in spite of the fact that the Streptococcus colonies in the cultures of Pfeiffer's bacillus were larger than the ones outside.

In figs. 2—7, portions of 6 agar plates containing a small amount of dissolved haemoglobin will be seen. The plates were inoculated with Pfeiffer's bacillus, the two first so thickly that the individual colonies could not be identified with the naked eye, the following ones more or less scantily. The air coccus was also inoculated at a point on the plate. Where the symbiosis effect did not come into action, the growth of Pfeiffer's bacillus was always slight. The irregular growth of the air coccus in fig. 3 is due to the presence of condensation water on the plate. It will be seen that neither the limited growth of the air coccus in fig. 2, nor the extensive growth in fig. 3 were able to produce the slightest increase in the growth of Pfeiffer's bacillus in their vicinity. On the next plate (fig. 4) where the individual colonies could be distinguished with the naked eye a marked increase in the growth of Pfeiffer's bacillus took place, but only in quite a small zone around the air coccus. On the succeeding plates the extent of the effect increases obviously in proportion to the more and more scanty inoculation.

Lastly, it will be observed in figs. 9 & 10, how the innermost

circle of the culture of Pfeiffer's bacillus in fig. 9 apparently absorbs all the growth-promoting substance, so that none of it can escape to the outermost portion of the culture.

Whether this is really the explanation, or whether the non-increase of growth in the outer circle in fig. 9 depends upon the fact that the heavy growth of Pfeiffer's bacillus in the inner circle inhibits the growth of the outer portion by robbing it of other nutritive substances than the „V" substance, I have been unable to decide. In both cases it is intelligible that the presence of the culture of Pfeiffer's bacillus in the immediate vicinity of the air coccus, will hinder the increase of growth at a greater distance. It is more difficult to understand how the increase of growth can entirely fail quite close to the coccus colonies. The most probable explanation is that „V" is absorbed by the nearest portion of the Pfeiffer's bacillus culture without however being able to produce an increase of growth. It is quite feasible that the growth of Pfeiffer's bacillus may be inhibited in the immediate neighbourhood of the growth of the other bacterium. (Production of harmful substances which unlike the highly diffusible „V", can only act at short range. — Deprivation of nutritive substances). The combination, inhibited growth near the colony and augmented growth at a greater distance from it, can easily be brought about by performing the symbiosis test on agar to which glucose or some other fermentable body is added as well as the haemoglobin. If Pfeiffer's bacillus is now inoculated in the form of a streak and close to it an organism that can ferment the sugar etc. with marked formation of acid, inhibition of the growth nearest the colony of the acid-producing organism will be obtained, caused by the acid, and further away, increased growth, as the „V" substance has its greatest effect there. I have seen this phenomenon very plainly, around Pneumococcus on ascitic agar plates containing a little haemoglobin and with the addition of invert-sugar. Mild degrees of inhibition of growth nearest the colony are often seen in the arrangement of the experiment commonly employed, for instance in several of the cultures in plate I, fig. 1. Something similar might be the cause of the absent symbiosis effect when the air coccus is immediately surrounded by the growth of Pfeiffer's bacillus.

With the aid of these experiences and ideas, one will understand various authors' reports, that the symbiosis phenomenon is „capricious" (OLSEN (3)); that the increase of growth is of variable extension (DAVIS (6)); that there can be observed inhibition of growth near the colony of the foreign bacterium, with or without increase in the periphery (JOCHMANN & KRAUSE, RIVERS & POOLE, DAVIS (6) and others); and that the effect is not so good when Pfeiffer's bacillus and the other organism are intimately mixed (DAVIS (6)).

It might perhaps be contended that although I have found that the increase of growth which takes place when Pfeiffer's bacillus is inoculated at a distance from the other bacterium, may be

absent when there is intimate contact between the two species of bacteria, there might possibly be cases where the converse holds and that the symbiosis test should therefore be carried out not only with the separated cultures but also with mixed ones. M. NEISSER's cultivation of Pfeiffer's bacillus in mixed culture — even in mixed colonies — on ordinary agar might seem to favour this view. NEISSER regarded this as a symbiosis in the more restricted sense, as he could not produce any increased growth of Pfeiffer's bacillus with dead xerosis bacilli. It may be pointed out that according to my experience the increased growth is produced, at any rate when a medium which is suitable is used, by a very diffusible substance, and as far as we can see, the distance at which the effect can be detected depends only upon the amount of „V" formed. Vigorously acting microbes can induce increased growth of Pfeiffer's bacillus at a rather (though not much) greater distance than weakly acting ones, suggesting that the growth-promoting substance is always the same qualitatively but is manufactured in different amount. But if, in certain cases, „symbiosis" could only be brought about by immediate contact or at a very short distance between the two species of bacteria, it would then be a case of the action of substances with a very slight power of diffusion and would therefore be a phenomenon of quite a different kind from the usual one, and it would only create confusion to associate the two phenomena. It must also be remembered that when the two species of bacteria are brought so near to one another that competition comes into play their growth will be determined by the interplay between different factors which can only be controlled and estimated very inadequately. The characteristic of the „symbiosis" of Pfeiffer's bacillus is just the marked „distance effect".

Besides the necessity of inoculating Pfeiffer's bacillus and the air coccus at a suitable distance from one another, it is of importance that the former be inoculated in streaks and the latter at points between them. We thus ensure that all the different distances between the air coccus and Pfeiffer's bacillus which have any value, will be represented and in such a way that the proximate portion of the Pfeiffer's bacillus culture does not prevent the effect at a greater distance. Further, those parts of the Pfeiffer's bacillus streaks which are outside the zone of activity of the air coccus, will show the nature of the growth out of reach of the symbiosis effect.

With a view to accuracy in judging the phenomenon it has been found to be convenient to inoculate Pfeiffer's bacillus in two streaks, and not be content with only one.

In recording the occurrence of Pfeiffer's bacillus in healthy persons a distinction was made between „typical" and „atypical" strains but a detailed explanation of these terms was postponed till later. We have now come to the point at which a discussion of them is appropriate.

In fig. 1, plate I will be seen the symbiosis tests of the white air coccus and 7 different strains of Pfeiffer's bacillus. A striking difference will be immediately observed between the two upper strains (I 1 and I 5) and the others (H 318, H 332b, GP 1, GP 2, GP 5), the symbiotic growth of the first two being considerably more marked and of a purer white colour than that of the last 5. Further the two first strains' growth is of a soft, homogeneous consistence, (which naturally cannot be seen in the illustration), so that one can move an inoculation needle about in it, just as if it were a mass of cream. In contrast to this the growth of the other strains has a harder, more friable consistence, so that by prodding it with an inoculation needle one breaks it up into small pieces which can be pushed along the surface of the agar without further disruption. These strains on the whole are also prone to grow in separated colonies, even with liberal inoculation. The reason for this it that only comparatively few of the inoculated bacilli develop.

I have called those strains of Pfeiffer's bacillus with the first kind of growth, „typical" and the others „atypical".* The justification for these terms is firstly, that this distinction in the manner of growth is usually so pronounced that it forces itself on the attention, and indeed even raises the question whether we have not to do with two different species of bacteria. The macroscopic appearance and the consistence of the culture are largely made use of as a means of recognition of bacterial species. Secondly it has been found that the „atypical" strains are almost exclusively met with in non-influenza patients, and every factor which affords a distinction between Pfeiffer's bacilli in influenza and Pfeiffer's bacilli in other diseases and in healthy persons, must be utilised. In order to avoid misunderstanding it is again expressly stated that it was not

* Other authors have used these designations in another sense:
typical = non-haemolytic,
atypical = haemolytic haemoglobinophilic bacteria.

exclusively atypical Pfeiffer's bacilli that were found in healthy people, in fact in the majority of cases the greater number of bacilli were typical. Thirdly, there is on the whole a connection between the macroscopic appearance of the growth and the microscopic picture, the macroscopically typical strains most often showing on microscopic examination small regular elements while the macroscopically atypical more frequently consist of coarser elements of a more irregular shape.

It will be further seen from the illustration that among the typical as well as among the atypical strains there are individual differences. Thus it will be observed that the uppermost strain (I 1) in contrast to the other typical one (I 5) is inclined to spread itself on the surface by shooting out tongue-shaped protuberances. In the case of the atypical strains the meagre growth of the uppermost will be particularly noted.

The difference between typical and atypical growth has nothing to do with symbiosis as such, but usually it is displayed most conspicuously in the symbiosis experiment, which is quite intelligible as we have here in the same culture all possible grades of growth from the weakest to the strongest. We thus get a more complete picture of the growth of the individual strain than could be done with ease by any other method. But besides this, the difference between typical and atypical growth will naturally assert itself on cultivation in pure culture on various media. On haemoglobin agar (or FILDES agar) it is least noticeable as only specially atypical strains stand out clearly from the typical. On blood agar the relation is almost inverted, as we find here so good a nuance in the appearance of the different strains that we venture to distinguish between a large number of different types of growth.

The most important of the characteristics of the atypical growth, — both in the symbiosis experiment and on other media where it obtains, — is the relatively dry and friable constitution of the culture. The maximal intensity of the growth is most often less than in the typical, but this is not always so. Among the atypical forms are to be found all degrees from the most intense symbiosis reaction to the very weak, or even in a few cases, absolutely negative reaction.

On the whole the strains which give a weak symbiosis reaction, also grow comparatively poorly in pure culture on

good haemoglobin (or FILDES) agar, a fact which confirms afresh our assumption about the identical effect of the „V" substance from bacteria and from blood.

The culture of the atypical strains often has a distinct yellow colour. I have met with a few cultures of Pfeiffer's bacillus which had a rather deep yellow colour (but never so intense as Micrococcus citreus for example). Such strains were called atypical even though the growth was soft and homogeneous.

The typical strains always give a good symbiosis reaction, in contrast to the variable intensity of the symbiotic growth among the atypical strains.

The distribution of typical and atypical strains in healthy persons has already been given and it has also been stated that all the cultures from guinea-pigs were atypical. In influenza and whooping-cough atypical strains were only exceptionally found, in all 5 and 3, respectively, but in measles 8 out of 15 were atypical.

The boundary between typical and atypical growth is not sharp. In the large majority of cases the cultures clearly belonged to the one or other group, but transition forms were sometimes met with. In such doubtful cases the nature of the growth on blood agar and the microscopic picture, will decide.

On 27. X. 21. a series of symbiosis tests were carried out with the two-fold object of investigating (1) whether the manner of growth of the individual strains kept constant during prolonged cultivation, or whether a given strain could become alternately typical and atypical and (2) whether there was any difference when the inoculation of Pfeiffer's bacillus was abundant or scanty.

Thirty-three strains were employed, of which 13 (I 1 (a), 5, 6, H 19, 315, 316, 320, 323, 328, 329, 331, 333, 334) in the original examination (which in the case of the oldest strains was 3 years previously) had proved to be typical, and 20 (Ms 4; H 127, 314b, 318, 319, 322, 324, 332b, 335, 336, 419, 420, 423, 424; GP 1, 2, 3, 4a b, 5) were originally labelled atypical.

In the repeated examination each strain was inoculated in 4 parallel streaks, the needle only being filled once, so that the first streak had a liberal amount of the culture and the

following ones a decreasing quantity. The definite macroscopic appearance of the individual strains was clearly evident; the 4 streaks of the same strain had always exactly the same colour, intensity of growth and consistence. This was beautifully seen on the plates when two strains of different appearance happened to be side by side.

In the great majority of cases the strains which were originally identified as typical and atypical had the same characters in the new examination. The only exception was H 333 which on the first examination was found to be typical and on the second, atypical, but not to a marked extent. (The growth was white but of rather solid consistence and there was only moderately well developed symbiosis). It is thus possible that it could have been called atypical with just as much right, on the first occasion. GP 1, originally atypical, was called a transition form at the new examination. H 127, originally atypical, was almost typical on re-examination, but of rather more solid consistence than the other typical ones.

The strains H 419, 420, 423 and 424 which were also examined on 22. X. 21 showed the following, with regard to „symbiosis" intensity.

	First examination	22. X.	27. X.
H 419	None	weak	rather marked symbiosis
H 420	„	„	weak
H 423	weak	„	little more intense symbiosis
H 424	none	„	weak

Strains showing a weak symbiosis reaction or none at all, were found notably in healthy soldiers in Sept. 1921. Several others of these strains were tested 2 or 3 times and gave practically the same results, — sometimes absent reaction, sometimes weakly developed.

Among the 35 atypical Pfeiffer's bacilli from the soldiers' camp in Sept. 1921, there were 13 which gave no symbiosis reaction whatever on the first examination. 4 of these on a repetition of the examination also gave an absolutely negative result. 1 of these 4 (H 424) was further tested on 2 occasions and then gave a weak reaction, as mentioned above.

As I have done, Davis (6) in particular has laid great stress on „symbiosis" as a mark of recognition of Pfeiffer's bacillus: „it was one of the most reliable and uniform criteria we have for this group..... I have tested many hun-

dreds of strains and have yet to find an exception". My finding of Pfeiffer's bacilli which did not give the symbiosis reaction is not however in agreement with Davis' experience. These strains cannot be excluded from the Pfeiffer's bacillus group, firstly because all possible transitions from marked to absent symbiosis exist; secondly, because the same strain may give a different result on a repetition of the test; and thirdly, because the microscopic and macroscopic morphology and mode of growth on different media of various strains decidedly creates the impression that they belong to the same group. In the following investigations of the remaining characters of Pfeiffer's bacillus, — included in which there are strains giving marked, as well as weak, symbiosis, — it will also be shown that no dividing line can be drawn between the first and the last.

Nevertheless the symbiosis phenomenon is a very valuable recognition mark of Pfeiffer's bacillus, when it is present; and it was found to be very pronounced in at least 95% of the 800 or so, strains I investigated. The worth of the test as a means of distinction from other species of bacteria (whooping-cough bacillus, Bacillus haemoglobinophilus canis) will be discussed in connection with the other biological characters of these bacterial species.

It will now be interesting to investigate the growth-promoting power of different species of bacteria, and whether any connection can be established between it and the other biological characters of the different species.

A comparison of the symbiosis test was made (Sept. 1920) between the bacteria mentioned below (the majority were old laboratory strains), and the Pfeiffer's bacillus strain „I 6". Cultures were made at the same time on blood agar and on plates of the medium used in the test itself (ordinary agar with a little haemolysed blood) the latter containing a very sensitive indicator (phenol red — china blue, see p. 162). The blood agar plates were observed for 2½ days. If there was no clear area around the colonies the strain was considered to be non-haemolytic („0"). Haemolysis which could not be observed after 1½ days' incubation was called „weak", even if very clear areas subsequently appeared. In the next column the

effect of the growth on the reaction of the medium is given; 0 = no change, Al = change in the alkaline direction, Ac = change in the acid direction. In the last column the intensity of the symbiosis effect is given roughly in 7 different degrees, o signifying, effect entirely absent, 1, weak but undoubted reaction, and 6, the complete and typical symbiosis reaction. Each test was performed in duplicate.

	Hemolysis	Change of reaction	Symbiosis
Staphylococcus aureus I	0	0	6
— — II	weak	Al	6
— — III	0	Al	6
— — IV	weak	Al	6
— — V	0	Al	6
— — VI	0	Al	6
— — VII	0	Al	6
— citreus	0	0	6
— cereus albus	0	Al	6
Micrococcus tetragenus	0	0	6
— — citreus I	0	Al	6
— — — II	0	Al	6
— ureae albus I	0	Al	6
— — II	0	Al	6
„White air-coccus“	marked	Al	6
Grey —	weak	0	6
Diplococcus crassus (Jäger)	0	Al	6
Sarcina lutea	0	Al	4
Streptococcus hæmolyticus I	strong	Ac	4
— — II	weak	Ac	2
— — III	strong	Ac	5
— — IV	strong	strong Ac	5
— — V	strong	strong Ac	5
Bac. coli I	weak	Al	1
— II	weak	Al	1
— III	weak	Al	1
— IV	weak	Al	6
— V	weak	Al	1
Bac. ozoenae	0	Al	2
— dysenteriae (Shiga)	weak	Al	4
— paradysenteriæ I	weak	Al	1
— — II	weak	Al	2
— — III	weak	Al	2
Vib. cholerae I	weak	Al	2
— II	stronger	Al	3
— III	weak	Al	1
— IV	stronger	Al	3
— V	weak	Al	2
— VI	weak	Al	3

	Hemolysis	Change of reaction	Symbiossi
Vib. „Havn“	0	Al	6
Diarrhoea of calves	weak	Al	1
Ratinbacillus	weak	Al	2
Bac. typhi muris	weak	Al	2
— suipestifer	weak	Al	1
— fæcalis alcaligenes	weak	Al	5
— enteritidis Gärtner	weak	Al	2
— — „Král“	weak	Al	2
— prodigiosus		Al	2
— anthracis	0	Al	6
Nocardia farcinica	0	0	0
Saccharomyces apiculatus		0	0
— cerevisiæ			1

In special tests on haemoglobin-containing ascitic agar, Pneumococcus (a strain of each of the 4 serological types), Meningococcus (a spinal fluid and a throat strain, the latter not pathogenic), Gonococcus (2 strains), and Micrococcus catarrhalis, were all very active.

It will be observed that there is no relation between the growth-promoting action on the one hand, and haemolysis, acid or alkali formation, and pathogenicity for man or animals, on the other. Moreover such different bacteria as cocci, anthrax bacilli, and vibrios can display the same growth-promoting activity. Davis (6) too was unable to demonstrate any relation between the growth-promoting power of different species of bacteria and their other characters.

Let us further consider a few of these points. As will be clearly seen from the table there is no relation between the haemolytic power on blood agar and the symbiosis effect on agar containing dissolved haemoglobin. This is not surprising, for as soon as the haemoglobin is brought into solution there can be no longer any question of haemolysis, that is to say, liberation of haemoglobin from the red blood corpuscles. But it might be expected that other factors are at work on blood agar. As mentioned on p. 119 some authors have found that the symbiosis effect on this medium was exclusively associated with haemolysis, while others found that the „V“ substance, which is independent of haemolysis, was the deciding factor here. My experience agrees with the latter finding. The 6 bacteria named below were inoculated in a symbiosis test with a culture of Pfeiffer's bacillus on blood agar and on agar with a little dissolved haemoglobin.

	Haemolysis	Symbiosis	
Staphylococcus citreus	weak	+	
— aureus	0	+	The same on each medium
— ureae albus	0	+	
Diplococcus crassus	0	+	
Haemolytic Streptococcus	strong	0	
White air coccus	weak	+	

The haemolysis therefore had nothing to do with the symbiosis effect. The coincidence between strong haemolysis and absence of symbiosis effect is not general because in the previous experiment the opposite was observed in the case of Streptococcus.

That the symbiosis effect cannot be explained on the ground that the reaction (hydrogen ion concentration) of the medium is altered in a favourable direction, is sufficiently evident from the fact that growth-promoting organisms are encountered among alkali-producers as well as among acid-producers. Various other experiments prove the same thing. Thus the same haemoglobin agar on which typical Pfeiffer's bacilli constantly gave a vigorous symbiosis reaction, was prepared with a series of different reactions. Within a somewhat wide (optimal) range (p_H. about 7—7.7) Pfeiffer's bacillus grew in pure culture with about the same weak intensity which is usual on this medium, — many times weaker than what is called forth by the symbiosis effect. (Compare Ghon & Preyss (1) and others, who found that Pfeiffer's bacillus in symbiosis culture tolerated a greater degree of acidity and alkalinity than in pure culture).

The fact that the growth-promoting power is widely distributed among bacteria and has no connection with the specialised functions of individual species or strains, fits in well with the interpretation given above, — that the effect is due to quite an „unspecific" substance, which is exceedingly widely distributed throughout the animal and vegetable kingdoms.

When the „V" substances from two such widely different sources as bacteria and the animal organisms, seem to be identical, we should so much the more expect that the same would be the case with the growth-promoting substance from different species of bacteria. The question ought however to be examined a little more closely. If we wish to employ the symbiosis reaction in the characterisation and classification

of (haemoglobinophilic) bacteria it is important to know whether we can count on the grouping of the strains according to their behaviour in the symbiosis test, which we get for example by employing the air coccus as growth-promoting organism, being the same as when the anthrax bacillus, for example, is used. If it is always the same growth-promoting substance we have to deal with, this must necessarily be the case. But if the substances which different species of bacteria form are distinct, this will be shown by the fact that one will act particularly upon some strains of haemoglobinophilic bacteria, and another particularly upon other strains. Such a condition of affairs would indeed make the symbiosis test more difficult to apply.

To investigate this, I chose, with the help of the big symbiosis experiment described above, 9 of the bacterial cultures employed which differed considerably from one another both in their growth-promoting action on the same strain of Pfeiffer's bacillus, and in their general biological characters. The idea was this: if only one growth-promoting substance for Pfeiffer's bacilli is produced by bacteria, then the variable intensity of the increase of growth of cultures of one strain of Pfeiffer's bacillus must depend upon the fact that the substance is produced in unequal amounts, and if we test the same 9 strains against any other Pfeiffer's bacilli (by which is here understood merely a haemoglobinophilic bacterium which can give the symbiosis reaction), these strains must, as regards growth-promoting effect, form the same series among themselves as before. If the effect of a strain „A" on a strain of Pfeiffer's bacillus is greater than that of a strain „B", then the effect of „A" on any strain of Pfeiffer-bacillus whatever must be greater than that of „B". But if the different strains form different growth-promoting substances of which a particular one acts on some strains of Pfeiffer's bacillus, and another especially on others, then the activity series will be different according as they are tested against one or other strain of Pfeiffer's bacillus, provided strains are employed which are as different as possible among themselves.

As growth-promoting organisms 5 strains were used, which had shown this property to a marked degree in the previous experiment (air coccus, Staphylococcus aureus, haemolytic Streptococ-

cus „III", Coli bacillus „IV", and Bacillus faecalis alcaligenes), and 4 that had shown a weak effect („Diarrhoea of calves", „Ratin", Coli bacillus „II", haemolytic Streptococcus „II"). All these were tested in a symbiosis reaction with the following 9 strains of Pfeiffer's bacillus: I 5 („typical" growth, 0 indol), I 6 („typical" growth, + indol), Me 1a („typical" growth, + indol) H 151 („atypical" growth, only weak symbiosis, 0 indol), H 160 („typical" growth, + indol), H 164 („typical" growth, + indol), H 171 („atypical" growth, only weak symbiosis, 0 indol), H 179 („atypical" growth, only weak symbiosis, 0 indol), and H 205 („atypical", that is to say, yellow growth, 0 indol). This is a very heterogeneous selection as regards habitat, mode of growth and biochemical characters.

Each test was put up in duplicate at least. All the strains of Pfeiffer's bacilli with the exception of H 151 and H 171 each gave exactly the same grouping of the 9 bacterial strains with respect to vigorous and weak growth-promoting activity.

In the case of H 151 and H 171 the results vere rather obscure, which cannot be wondered at in view of the fact that these strains of Pfeiffer's bacilli, as stated, only gave a weak symbiosis reaction with bacteria known to be good growth-promoting ones. On repeating the test with H 151; the relative activity of the various bacterial species agreed fairly well with that given above, with the exception of the two coli strains which acted with about the same intensity. The same was the case for H 171 towards which the other strains behaved in the usual manner. H 151 and H 171 were now tested in 6 new experiments with both coli strains. The action of these was weak in every instance and therefore difficult to judge, but on the whole „IV" had a stronger action than „II".

From this experiment we must look upon it as highly probable that practically the same result would be obtained whichever growth-promoting bacterium was employed, if only the latter possessed pronounced activity.

Further it has been shown, as will later be referred to, in an experiment with Bacillus haemoglobinophilus canis and the whooping-cough bacillus in association with a large number of other species of bacteria, that in no case was there a symbiosis effect. With regard to the absence of effect on bacilli other than Pfeiffer's bacillus, the different species of bacteria therefore also agree with one another.

It is therefore justifiable to assume that it is a matter of indifference what bacterium we employ in the symbiosis test if it has a marked growth-promoting power upon any strain of Pfeiffer's bacillus chosen at random.

The main results of these investigations into the problem of symbiosis are: (1) the demonstration of different facts concerning the technique for the investigation of the phenomenon, and (2) the demonstration of marked individual differences between strains of Pfeiffer's bacilli both with regard to the intensity of the „symbiosis" and in other ways.

Out of all the strains examined from 1918 to 1921 the following were noted as atypical on the strength of the appearance of the culture: I 25, 33, 34, 55; I 28; P 1, 2, 15; Ms 1, 3, 4, 5, 6, 11, 12, 14; H 8, 10, 11, 16, 22, 32, 36, 45, 50, 67, 87, 88, 95, 96, 98, 104, 106, 108, 120, 124, 125, 126, 127, 128, 130, 137, 141, 148, 149, 151, 158, 161, 171, 173, 176, 178, 179, 181, 185, 193, 199, 200, 201, 205, 206, 207, 210, 223, 226, 233, 234, 314a, b, c, d, 318, 319, 322, 324, 330, 332a, b, 335, 336; GP 1, 2, 3, 4a, b, 5.

Growth on Blood Agar.

The next mark of recognition of Pfeiffer's bacillus we find most frequently in reports on the presence of this bacillus, after haemoglobinophilia and the morphology, is the naked-eye appearance of the growth on blood agar, i. e. agar with the addition of blood in with the corpuscles are not dissolved (or agar smeared with a drop of blood). Quite small, but visible, colourless, „dew-drop" colonies are described as characteristic of Pfeiffer's bacillus. I also, have extensively used blood agar (agar mixed with blood) in the investigation of Pfeiffer's bacillus and in the case of the majority of strains I have found the appearance of the growth on this medium very characteristic. It was soon evident however that the appearance of the cultures of different strains displayed several differences, and that the character of the individual strain usually continued almost unchanged even after numerous subcultures.

The strains which are labelled typical from their growth in the symbiosis test, usually grow in more or less flat, perfectly clear colonies of a slightly moist appearance, while the atypical most frequently produce small, rounded, colonies with a drier, lustrous appearance. A number of different grades in the appearance of the culture are found and gradual transitions between the typical and atypical forms of growth.

The blood agar is often dark-coloured or blackish in an area stretching some millimetres beyond the edge of the culture, and is very ill-defined. When a number of different strains are inoculated on the same plate, the variable intensity of the dark colouration developed often produces a rather variegated picture. The great majority of the typically-growing strains can produce a dark colouration but many of the atypical do not possess this power. In some cases however, I have seen atypical cultures give rise to a very dark colouration.

All transitions from deep colouration to none at all can be seen. The colour depends largely on the intensity of the growth, so that the best growing strains on a blood agar plate are most likely to blacken the medium. If the preparation of the blood agar is such that a relatively rich growth occurs, the colouration is also deeper. It may be seen around a part of the culture where there is increased growth from symbiosis with a foreign colony of bacteria, while elsewhere there is no change in the colour of the medium. Two strains which grow equally well however, may behave rather differently as regards dark colouration.

The blackening of blood agar is therefore not an essential character of Pfeiffer's bacillus. Its dependence upon small changes in the constitution of the blood agar and the gradual transitions have prevented me from utilising it in the description and classification of the strains.

Another phenomenon which may also be of rather variable development is the differing tendency of the various strains to spread on the surface of blood agar. It is not superfluous to review the great differences which may be observed in this respect, for it is usually proclaimed as characteristic of Pfeiffer's bacillus (as a distinction from BORDET's bacillus), that when inoculated in narrow streaks it spreads a bit on both sides. This is commonly the case, but it is practically confined to the „typical" strains and then to a variable extent. Further the constitution of the medium has some influence on the spreading of the growth. On fresh blood agar which has been prepared without undue heating and which on account of the intact condition of the red blood corpuscles only gives slight growth, the strains will not be able to spread much along the surface either. On slightly heated blood agar

where the growth is thicker the spreading will take place to a considerably greater extent and the same applies to media containing dissolved haemoglobin. This „surface" growth in its milder degrees is merely characterised by the edge of the culture not being clear-cut but shelvy. In the more intense surface growths flat tongues project from the inoculated area which together form an undulating border of as much as 3—4 mm. in breadth.

The various points enumerated were (Jan. 1920) carefully investigated in the case of the strains given on p. 108. It would be idle to attempt to describe the variegated, finely graded pictures which were seen. The most marked feature which arose out of the experiment was that a few strains were inclined to grow with fringes to quite a large extent, namely I 1, and P 23. Of strains acquired later I 33 had the same peculiarity. These 3 strains, but particularly the first, have shown this characteristic on different media and after cultivation for a prolonged period, to a greater degree than any of the other strains kept going for a long time.

Pfeiffer's bacillus, — according to definition — gives no haemolysis on blood agar. This negative property has proved to be constant, since the hundred or so stock strains in Sept. 1920, did not give haemolysis in a single instance when inoculated on blood agar. The cultures were observed during a week in the incubator.

Appearance of the Colonies.

In cultures produced by liberal inoculation, Pfeiffer's bacillus displays its most characteristic and individualised macroscopic appearance on blood agar and in the symbiosis test on weakly haemoglobin-containing agar, while the growth is less characteristic on agar containing dissolved haemoglobin (in natural or transformed condition) in large quantity. But when it is a question of examining isolated colonies, the latter is the medium which gives the most characteristic pictures of Pfeiffer's bacillus in general and presents the most variable individual characters of the various strains.

In order to give an impression of the different appearances colonies of various strains of Pfeiffer's bacillus may present, photographs are reproduced in plate 2, of colonies of 15 strains of Pfeiffer's bacillus (as well as a colony of Bacillus haemoglobinophilus canis). In each case the inoculation was made (Nov. 1921) on FILDES-agar, and the plates were then kept in the incubator for about 2 days. There were colonies on all the plates which were so isolated from the rest that they could not have been inhibited by their neighbours to any appreciable extent as regards the full development of their size and morphological characters. Square pieces of the agar with such colonies were cut out, placed together on a slide, and photographed by transmitted light and with a magnification, $\times$ 10.* The first thing that will be noticed is the very unequal size of the colonies. The thickness of the medium was certainly not uniform, varying from 2—3 mm. This is however far from sufficient to explain the difference in the size of the colonies. In 4 cases the layer of agar was 2 mm. in thickness and the colonies represented from these plates were 1.7, 1.8, 1.8, and 3.3 mm. in diameter. In 6 cases the layer of agar was 2½ mm. thick and the colonies were 1.2, 1.3, 1.7, 2.3, 2.4, and 3.2 mm. in diameter. In 5 cases the figures were 3 mm. ond 2, 2.1, 3.0, 3.3, and 5.5 mm. respectively.

Marked differences will also be observed in the degree of translucency and internal structure.

It is not a question of fortuitous characters of the individual colony, as each of the plates displayed a homogeneous and characteristic appearance both on macroscopic inspection and on examination of the different colonies with a weak magnification.

Most of the strains had been examined twice before in the same way.

(For the first of these two examinations („I") which took place in Jan. 1920, agar containing haemolysed blood was used, and for the second („II"), in Sept. 1920, FILDES-agar).

The appearance of the individual colonies depicted will now be described and also compared with the records of the appearance of the colonies of the same strains in the earlier investigation. Included in the description is a phenomenon which in consequence of the weak magnification is not so

* In the reproduction, the medium around the colonies is replaced by a uniform back-ground.

distinct in the photographs as could be wished, namely the „granulations". By these are meant sharp but irregularly defined formations which are seen in most of the Pfeiffer's bacillus colonies best with a magnification of 100, when they have reached a sufficiently developed state. The schematic figures „Schemes of colonies" in plate 3 give an idea of their size and arrangement.

„I 1" spreads, from a dark centre, well on all sides and attains a diameter of 5½ mm. The edge is quite flat, very undulating and jagged, and the surface is rough in the peripheral portions. In the centre there are but few granulations.

The investigations „I" and „II" showed precisely the same form of growth. In „I" it was noted that after 1¾ days there were just a few scattered granulations in the centre; after another day in the incubator numerous well-distributed granulations were present. This is the strain that shows most markedly and constantly the fringe formation referred to above even around a culture produced by a heavy inoculation (compare plate 1).

„I 5" is considerably smaller but must be counted as a large Pfeiffer's bacillus colony. Its shape is totally different from that of „I 1", as it declines at first slightly and then with an increasing gradient from a sharply defined central plateau. The edge is only slightly undulating. The colonies are rather dark. There are a few central granulations.

In the investigations „I" and „II" the colonies were also large and had a slight tendency to granulation formation. In „I" it was noted that „the colonies are considerably lighter than I 1. Numerous fine undulations in the edge were present".

The cracks occurring in the two colonies described were produced in the preparation.

„I 6" is rather smaller than „I 5". It has a similar shape but the margin is narrower and flatter. The edge is smooth and circular. Around the centre there is a little group of granulations.

In „I" and „II" the colonies were also of medium size with a round border and granulations in the centre.

„I 21" is a rather large colony, somewhat dark, but possessing a narrow light-coloured fringe with an undulating edge. The granulations are present in scattered array, thickest around the centre.

In „I" was noted, — rather dark, medium-sized colonies with slightly undulating edge; centrally grouped granulations. In „II" the colonies were comparatively clear, with very undulating edges.

„I 23" is a large dark colony with a margin divided into two sections. The border is regular and circular. There are a few centrally-placed granulations.

In „I" and „II" was noted, — medium-sized dark colonies with

centrally-grouped well-distributed granulations and finely scalloped edge.

„I 27" is a small light-coloured colony with a very convex surface, round edge, and very granular centre.

In „I" and „II" the colonies were also small and light-coloured. The edge („I") was slightly undulating. The granulations were rather different.

In „I 57" two associated colonies are taken. They are large, dark, and flat with rather steep margins and circular outlines. There are a few central granulations.

In „I" and „II" the colonies were also dark, but they were only of medium size. The border was undulating or scalloped. The granulations varied to some extent.

„I 5" is a small, very light colony with centrally disposed, but rather widely distributed granulations. The border is uniformly circular.

In „I" and „II" the colonies were also light, with granulations in the centre. They were of medium size. The edge of „I" was round, of „II" very undulating.

„H 122" is a small colony and of similar shape to „I 5", but with a small central elevation. A few centrally grouped granulations present.

In „I" and „II" the colonies were also small. The edges were round and the granulations grouped in the centre, but highly developed.

„H 151" is quite a small colony with a dark centre and light margin. Edge circular. Granulations central, rather widely distributed.

In „I" also the colonies were exceptionally small, and dark with centrally placed granulations. The edge however was slightly undulating. „II" was not reported on.

Of the 10 strains described, H 151 was „atypical", and all the others „typical".

Besides these 10 strains, about 50 others were examined several times in cultures consisting of isolated colonies. The descriptions furnished give a fairly correct picture of the conditions which generally obtained. The first point to emphasize is the great difference that may be present between the Pfeiffer's bacillus colonies.

It is naturally important to fully appreciate that it is not sufficient to fix the attention on colonies of a definite appearance, but that the boundaries of the types of colony on the original plates suspected of being Pfeiffer's bacillus are very far apart.

Moreover the type of colony of the individual strain retains,

on the whole, its character relatively unchanged through a long period of cultivation. Some strains always form large colonies, others always small ones; some form colonies regularly of a darker colour than others; in some strains the tendency to grow with a rough surface and undulating edge is more developed than in others.

With regard to the number and distribution of the granulations in the colony there is also a certain individuality, but great variations are seen. These granulations are obviously rather „labile" formations, in other words their development is influenced to a large extent by apparently unimportant „fortuitous" effects. Thus they develop principally round the scratches on the agar surface which are often made in the inoculation (cf. PRINGSHEIM). They are produced chiefly in isolated, well-developed colonies and increase in numbers with increasing age. Sometimes they do not begin to form before the plates have been in the incubator for 2 or 3 days.

Besides the 10 strains described, 5 others will be seen in the plate, namely the same as — together with I 1 and I 5— are illustrated in the symbiosis test in plate 1. A more detailed description of these 5 colonies, — all from „atypical" strains, — is unnecessary. It need only be mentioned that the unusually dark colony „318" was entirely without granulations, while the other 4 had them. The rather irregular shape of the surface of „GP 1" was produced in preparing it.

After this, one might be tempted to think that it would be practically impossible to distinguish Pfeiffer's bacillus from other bacteria by the appearance of the colonies. But it is not so; the range of shape of colonies of Pfeiffer's bacillus is indeed great but not unlimited. For instance, such dense colonies (dark by transmitted, and light by reflected light) as ordinary Staphylococcus colonies, are never met with, nor such large and „waxy" colonies as those of the colon bacillus. In my influenza material I even found that I could regularly diagnose Pfeiffer's bacillus with certainty from the macroscopic appearance of the colonies on the original plate, but this was far from always possible in the case of Pfeiffer's bacillus obtained from healthy persons.

The granulations are to a certain extent characteristic of Pfeiffer's bacillus, as in the material used in the investigations

of this bacillus I have only seen identical ones in haemolytic, haemoglobinophilic bacilli, which will later be discussed. But I have observed quite similar bodies, even highly developed, in Bacillus haemoglobinophilus canis, which will also be referred to in a special chapter, as well as in pure cultures of Gonococcus, and sometimes also in Meningococcus.

In smears from the granular and non-granular parts of the same Pfeiffer's bacillus colony I have been unable to detect any difference as regards the appearance and stratification of individual bacilli.

A certain heterogeneous appearance assumed by the colonies of some strains of Pfeiffer's bacillus, chiefly the „atypical", must not be confused with the granular formation described here. The former do not consist of special, well-defined elements, but of a coarse structure of the whole colony, the single elements of which are considerably smaller than the granulations. This appearance is undoubtedly associated with a coarse microscopic morphology, especially with the presence of numerous thread forms.

In the last 3 chapters we have seen that whether we examine the „macroscopic morphology" of a number of strains of Pfeiffer's bacillus (that is to say the appearance of the culture with the naked eye or low magnification) in a symbiosis experiment on blood agar or in the form of isolated colonies on haemoglobin agar, we shall observe a multiplicity of forms, among which all transitions between the different morphological types seem to be represented. I have sought in vain to discover a definite connection between the individual characters such as the appearance of the growth on blood agar, the size of the colonies, the degree of transparency, the occurrence and arrangement of granulations. On the whole there seems to be a rather free combination of the several characters. What we presumably can rely upon is the rough division into „typical" and „atypical" cultures. Schematically the 2 groups may be characterised thus:

	Typical.	Atypical.
The individual colony.	Rather large, semitransparent with well developed granulations, but otherwise homogeneous.	Small, more opaque. Granulations absent or weakly developed. Culture often slightly heterogeneous.
Growth on blood agar.	Quite small, flat clear colonies which form a confluent culture of a fairly homogeneous surface.	Colonies more arched and grow more isolated from one another.
Growth in symbiosis test.	Vigorous symbiosis; the heavy growth is pure white by reflected light; it is confluent, and of soft homogeneous consistence.	All degrees from absent to marked symbiosis are found. Growth more greyish or yellowish, dry and friable.
Occurrence.	Influenza, whooping-cough, measles, meningitis, healthy persons.	Frequent in healthy persons and in measles, rarer in influenza and whooping-cough.

The great differences in macroscopic morphology between different strains of Pfeiffer's bacillus is undoubtedly not yet generally appreciated. RIVERS (1) and SKAJAA's experience however, agrees well with that reported here.

Microscopic Morphology.

If, in investigations into the occurrence of Pfeiffer's bacillus, we accept the description of its morphology given in one of the ordinary bacteriological textbooks, we shall undoubtedly be liable either arbitrarily to adopt a too stringent limitation of the findings counted as positive, or fall into a dilemma with regard to the extent to which we shall admit forms that diverge from the picture described as typical. There is every reason to believe that numerous workers have obtained too few positive results from this cause, particularly in examinations of healthy people.

In my investigations it has come to light that among bacteria which I gradually realised must be included in the

Pfeiffer's bacillus group, „irregular" forms were considerably more in evidence than we should expect from the current descriptions. On a closer study of the literature however it appeared that practically all forms I have observed, had also been described by other authors, but had not been generally adopted.

All my (circ.) 800 strains of Pfeiffer's bacillus were examined microscopically in smear preparations (GRAM's method and dilute carbol-fuchsin) at least once; a large number, probably 200—300, were examined 2 or 3 times; about 50 strains, 4 times, and 20 strains, 7 times. I have therefore a considerable number of microscopic slides of Pfeiffer's bacillus. Several hundreds of the preparations were later on examined anew, in order to get a collective impression of them. In spite of continuously striving to establish definite criteria for the morphology of Pfeiffer's bacillus I have been no more successful than other investigators. My experience agrees entirely with that reported on pp. 46—48, and it would serve no useful purpose to give again a full presentation of the facts. A few points only will be discussed.

In order to give a general impression of the heterogeneous picture that meets the eye, a small selection of microscopic pictures is reproduced in plate 3. It is to be noticed particularly how greatly the microscopic picture of any one strain can vary at different times and under different conditions.

It need hardly be said that the unusual forms are more numerous in the plate than in reality.

As examples of pictures which are called typical both by myself and by other authors, may be cited: I 37, I 53, and T 3.

It will be noticed (e. g. in the various preparations of I 14 and I 43) that the length of the bacilli is an extremely labile character, the same strain varying considerably in this respect without obvious cause. The thickness is more characteristic (which however cannot be seen clearly in the plate). Some strains consistently grow as slender rods, others as rods of a considerably coarser appearance (e. g. GP 1, and GP 5), but there is no fixed boundary between the two types. The coarser forms are, on the whole, also more inclined to be

grossly irregular, — partly irregularly swollen, and partly unevenly coloured.

The well-defined round bodies which were also observed by WADE & MANALANG seem moreover to be not well known. So much the more occasion therefore is there to announce that I have found these or similar bodies constantly present in Pfeiffer's bacillus, but exhibiting a different development and sometimes in very small numbers. They are subject to great variability, as they display a large range of variation in 3 different directions, — size, position in relation to the bacilli, and staining. The size may vary from a fraction of 1 μ to 3 — 4 μ. The situation may be, in a rod (or thread) either in the middle or nearer either end; at the end of a rod or thread; placed right on the side, or on a small side branch*; lying free. The colour may range from an extremely pale one to a coal-black hue. The absolutely black elements are usually small, at most the size of large cocci. Conversely it is particularly the largest, „blown-out" elements that are pale-coloured.

I have very often seen pictures like the T-shaped elements in the collection, „various", in the plate. They can hardly be explained otherwise than by assuming that a sphere situated at the end of the bacillus grows into a rod transversely to the original one. What part these spherical bodies play I have been unable to determine. They cannot be regarded as spores. About 50 strains were examined with MÖLLER's stain for spores, but distinctly acid-fast elements could not be detected. Further the slight resistance against heat and drying, which will later be referred to, excludes the presence of spores.

The reason all the different spherical elements are discussed together is because all transitions between any two types may be observed. This naturally does not exclude that we have several kinds of elements of a distinctly different nature to deal with, only we have at present, no basis for such a classification.

We might imagine that the absolutely black spheres were different from the red, but there are no facts to support even this. There are also connecting links in this case in

* On the whole, undoubted branching is not infrequently observed.

the form of elements with a more and more intense dark red and blackish-red colour. Not only the red, but also the black staining can be produced by simple fuchsin staining.

As a general method of staining, as previously referred to, I have employed GRAM's method and it would be easy to look upon the black bodies as Gram-positive. After examining a number of cultures where such bodies were much in evidence, it was found however, that they could just as easily be stained black by simple fuchsin staining (ZIEHL-NEELSEN's carbol-fuchsin diluted 1 + 9; 5 minutes staining without heating).

It has been found that the black staining of these elements is also produced by carbol-toluidin blue and to a less extent by methylene blue and neutral red. (In all cases they were stained 5 minutes). Further, they could be observed in Gram-stained preparations without counterstaining. They are therefore elements with an excessive power of absorbing dyes.

If these experiments give no other information we can at least conclude from them that in an ordinary Gram-stained preparation with counterstaining we are not justified directly in regarding every black element as Gram-positive. (An element which is stained black by GRAM's method and by every other method of staining can neither be called Gram positive nor negative.* Some of the black stained spherical elements could resemble Staphylococcus. That it is not a question of contamination with Gram-positive cocci is proved partly by the fact that they are also stained by fuchsin alone and partly because these, or other elements of exactly similar appearance, adhere to Gram-negative rods.

These spherical elements are by no means specific for Pfeiffer's bacillus although they seem to occur in greater numbers in it than in other bacteria. In the whooping-cough

* Later addition: When preparations consisting partly of Gram-positive bacteria (Staphylococcus) and partly of Pfeiffer's bacillus with the black-staining granules, are treated with carbol-fuchsin + iodine in potassium iodide + alcohol + carbol-fuchsin, that is GRAM's method in which methyl violet is replaced by fuchsin (the staining then naturally ceases to be GRAM's method), the granules of Pfeiffer's bacillus were always stained black while the Staphylococcus only took the fuchsin colour.

bacillus I have observed, although rarely, exactly the same black spheres as in Pfeiffer's bacillus; and on examination of some cultures of Gram-negative rods, chosen at random, spherical bodies or at any rate distally placed rounded swellings were found on going through the preparations with this point specially in view (for example in typhoid, coli, cholera, and many others. Cf. ALMQUIST (1,2)).

The black spheres in Pfeiffer's bacillus are relatively acid-fast. In a preparation stained with fuchsin they resisted 5% sulphuric acid for 1 minute which completely decolourised all the other elements. On the other hand they only partly withstood 25% sulphuric acid for 1 minute. Whether this relative acid-fastness is only due to the large amount of deposited dye or to a firmer combination it would be difficult to decide.

The spherical bodies are not artefacts produced in making the dry preparations, as they could also be observed in moist unstained preparations (suspension in salt solution).

The spherical bodies are not artefacts produced in making currence of these bodies is no reason for believing they were not present in their material. Apart from the cases where they were especially well-developed the fact had evaded my own attention until the spring of 1921 when I undertook a systematic search of several hundred preserved preparations with the particular object of determining whether a classification of Pfeiffer's bacillus could be made on the basis of the presence or absence of granules. To my surprise I found that with perhaps one or two exceptions they could be demonstrated in any preparation whatever. The fact they are not present in the majority of the illustrations is because they are often very scanty.

As regards the further behaviour of Pfeiffer's bacillus towards stains, only the following need be noted: All the strains I have examined are decolourised very easily by GRAM's method. The most distinct pictures are obtained by staining by carbol-fuchsin (1 + 9) for 5—10 minutes. Methylene blue in alkaline solution also stains well, but an ordinary watery solution of methylene blue, carbol-toluidin blue, and neutral red give less clear results.

In January 1920 about 50 strains were inoculated on haemoglobin broth. There was a good growth in all the tubes in about 24 hours, and each culture was then examined microscopically in a hanging drop preparation at 37°. The microscopic morphology in broth corresponded in all its essentials to that found in stained smears. Similar differences between the individual cultures were observed especially as regards the length of the elements. In most of the cultures the majority of the bacilli were situated more or less in masses. In some cases they were all collected in masses while in others a large number were lying free.

Undoubted m o t i l i t y was observed in no case, but it was very difficult to determine as Brownian movement, on account of the lightness of the bacilli, was often exceedingly lively. In one of the cultures (I 71) a single element was observed which seemed to be distinctly motile.

In order to investigate this matter further, staining of the same strains by ZETTNOW's (1,2) method was carried out. The technique was first practised and tested on a number of different motile species of bacteria, and constantly gave satisfactory staining of the flagella, which was also the case with preparations of typhoid, cholera, and proteus which were stained at the same time as Pfeiffer's bacillus.

In several of the preparations bacilli were found with long, only slightly curved threads of similar appearance to those depicted by ELLIS (1,2). In a few preparations some bacilli were also seen with curved threads which more resembled flagella. There were 1, 2, or a whole tuft. Unfortunately I was unable to continue these investigations, as apparently several months' work would be required to clear up the question of the motility of Pfeiffer's bacillus.

Pfeiffer's bacillus is regarded by all the authors who have expressed an opinion on the subject, as non-motile and from my own investigations as well as those of others, there is no doubt that it does not generally possess any marked motility. But there is nothing at present to exclude the possibility that by a thorough investigation of the question it might be discovered that Pfeiffer's bacillus as a rule could, under suitable conditions, form flagella. The question is however scarcely of great importance from the point of view of classification. Even if only a minority of bacteriologists will be able to entirely agree with ORLA-JENSEN (1, 2) that the presence or absence of flagella is of no importance in the classification of bacteria, the general view is that it cannot be reckoned among the best marks of distinction.

On the whole there was correspondence between the ma-

croscopic and microscopic morphology, so that cultures with an atypical macroscopic appearance also usually had an atypical microscopic appearance, that is to say, consisted of coarse markedly polymorphic elements, while the macroscopically and microscopically typical cultures usually consisted of small elements without excessive polymorphism.

As an example, it may be mentioned that, in the inoculations of 6—10. IX. 1921, out of 46 macroscopically typical growths, 42 were also microscopically typical; 3 were rather polymorphic without being distinctly coarse; 1 was noted as „polymorphic" at the first examination and as „typical" at the second. Out of 17 macroscopically atypical only 4 were microscopically typical, the other 13 being „polymorphic" or „coarse".

Relation to Oxygen.

In the chapters on haemoglobinophilia and symbiosis a detailed account is given of the relation of the Pfeiffer's bacilli investigated to the conditions of growth which are particularly characteristic to this bacterium.

We will now discuss the relation of Pfeiffer's bacillus to 2 other chemical factors, the concentrations of oxygen, and hydrogen ions, and also a physical one, — the temperature.

Pfeiffer's bacillus is generally regarded as a decided aerobic organism. According to SCHELLER (5) it never thrives at the bottom of a stab culture. Experience is divided however, on this point. In agar with Hommel's haematogen HUBER obtained growth all along the course of the needle in a stab culture, but he also states it was the only medium in which Pfeiffer's bacillus could grow in stab culture. According to KAMEN (1), PARK & WILLIAMS and RIVERS & LEUSCHNER, it can grow absolutely anaerobically. See also SPOONER, SCOTT, & HEATH.

In the autumn of 1918, I myself only obtained growth on the surface of stab cultures of the strains I 1—5 in agar with haemolysed blood. Later on however I made stabs of numerous strains on FILDES agar in test tubes and I regularly got a good growth along practically the whole course of the needle.

That Pfeiffer's bacillus thrives best in an abundant supply

of oxygen is plainly seen on cultivation in haemoglobin broth. Rapid and prolific growth takes place principally when the tubes are laid obliquely, — almost horizontally, so that the medium has a large surface exposed to the air. In such a tube Pfeiffer's bacillus develops chiefly at the upper end where the layer of fluid is quite thin.

In March 1920 all the cultures named on p. 108 were inoculated on haemoglobin agar in Petri plates which as usual were placed upside down. In the cover a small flat dish was put in which the solutions of pyrogallol and alkali were poured in such a way that as far as possible they did not become mixed. The space between the bottom and the cover was sealed with paraffin and plasticine, after which by gently rocking the plate the liquids in the interior dish were made to mix. In this way a large absorbing surface in relation to the air space is obtained. Under these conditions none of the strains grew.

The experiment was repeated in December 1920 with all the strains which were alive at that time (see p. 110 (1. XI. 1921)), among which there were also strains from guinea-pigs. I 103, I 32, 33, 36, 37; Ms 1, 13, 15; Me 1a; H 49b, 127, 156, 157, 159, 261—288, 318—327, 344, 346 and 352 grew poorly (much worse than in the presence of air), while the other strains did not grow at all. Presumably the anaerobic conditions were not so complete as in the previous experiment. The spinal strains were tested separately and did not give any, or only very slight growth.

It must therefore be said that on the whole the strains examined behave similarly as regards the need of oxygen. It would be of interest to get a clear idea of the causes of the divergent experiences of different authors on the relation of Pfeiffer's bacillus to oxygen.

Relation to the Reaction of the Medium.

In July 1919 ordinary haemoglobin agar of 4 different reactions was prepared. By colorimetric determination (indicators: neutral red, rosolic acid, and phenolphthalein) the p_H value was found to be, 6.5, 6.8, 7.4, and 8.6 in the different samples. Each of the 4 media was inoculated with the strains I 6, I 74, I 21, and H 34. The growth on the first 3 plates was good and about the same in each case; on the last it was scanty. Although the determinations are not very accurate it can be stated that Pfeiffer's bacillus is not particularly sensitive to variations in the reaction of the medium.

In Jan. 1920 I made plates of agar with only 0.2% dissolved blood cells having 11 different reactions; to 50c.c of the original agar were added (1) 0.05, (2) 0.04, (3) 0.03, (4) 0.02, (5) 0.01c.c. concentrated hydrochloric acid, (6) none, and to (7) 0.1, (8) 0.2, (9) 0.3, (10) 0.4, and (11) 0.5c.c. n. NaOH. No. 4 reacted neutral to azolitmin paper, and the colorimetric determination of No. 11 partly with neutral red and partly with α-naphthol-phthalein gave p_H = 7.6 (circ.). Attempts to an exact colorimetric determination of the reaction of the different media gave no trustworthy results. On the media Nos. 1, 2, 4, 6, 8, 9, 10, and 11 all the strains on p. 108 were inoculated with the result that after 1½ days in the incubator none of them grew on No. 1; on No. 2 all the strains with the exception of I 5 grew, but very poorly; on No. 4 all the strains gave a very fair growth, but weaker than on Nos. 6, 8, 9; 10 and 11. On these last 5 plates the growth was about equal (considerably weaker than on agar with an abundant supply of haemoglobin. Perhaps it was a trifle better on No. 10 than Nos. 6, 8, 9, and 11. On examining them again after a further 24 hours in the incubator no marked change was observed.

This experiment teaches that all the strains behaved in a similar manner with regard to dependence on hydrogen ion concentration; any marked individual differences could not be demonstrated.

Minimum Temperature for Growth.

The temperature limit for growth must undoubtedly be reckoned as one of the most constant characteristics of the different bacteria, and I have therefore investigated it for a large number of strains of Pfeiffer's bacillus, but only as regards the lowest temperature limit, where it might be assumed that any differences would be most marked, and where the determination from a technical point of view, was easiest to carry out. The results are tabulated below.

	March 1920.				November 1920.		
	20°	25° I	25° II	26°	19°	23°	28°
I 1 (a)	0	2	3	3	0	2	3
(b)	0	2	3	3	0	2	3
5	0	2	3	3	0	3	3
6	0	2	3	3	0	1	3
20	0	0	3	3	0	0	3
21	0	0	1	3	0	0	3
22 (a)	0	1	3	3	0	0	3
(b)	0	2	3	3	0	0	3
23	0	1	3	3	0	1	3

		March 1920.				November 1920.		
		20°	25° I	25° II	26°	19°	23°	28°
27		0	2	3	3	0	1	3
29		0	2	3	3	0	2	3
35		0	2	3	3	0	2	3
38		0	2	3	3	0	1	3
45		0	0	3	3			
54		0	2	3	3			
57	(a)	0	2	3	3	0	1	3
	(b)	0	2	3	3	0	0	3
59		0	2	3	3	0	3	3
71		0	2	3	3	0	1	3
72		0	(1)	3	3	0	0	3
91	(a)	0	2	3	3	0	3	3
	(b)	0	2	3	3	0	3	3
100		0	2	3	3	0	3	3
103						0	2	3
I 5		0	2	3	3	0	1	3
21		0	(1)	1	3	0	0	0
32						0	0	3
33						0	3	3
34						0	1	3
36						0	1	3
37						0	1	3
P 14		0	2	3	3	0	3	3
15		0	2	3	3			
16		0	2	3	3			
22		0	2	3	3	0	1	3
23		0	2	3	3	0	2	3
24		0	2	3	3	0	3	3
25		0	2	3	3	0	2	3
26		0	0	3	3			
Ms 1						0	1	3
4						0	3	3
9						0	1	3
13						0	1	3
15						0	3	3
Me I	a					0	3	3
	b					0	3	3
GP 3						0	1	3
4	a					0	1	3
	b					0	0	3
5						0	0	3
Pa 4		0	2	3	3	0	2	3
5		0	2	3	3	0	3	3
R 1						0	3	3
2						0	3	3
H 14		0	2	3	3			

	March 1920.				November 1920.		
	20°	25° I	25° II	26°	19°	23°	28°
19 (a)	0	2	3	3			
34	0	0	3	3	0	0	3
35 a	0	1	3	3			
37 a	0	2	3	3			
37 c	0	2	3	3			
49 b	0	2	3	3	0	3	3
122	0	2	3	3	0	1	3
127	0	(1)	3	3	0	1	3
133	0	2	3	3	0	1	3
151	0	1	3	3	0	1	3
152 (a)	0	2	3	3	0	1	3
(b)	0	2	3	3	0	1	3
154	(1)	2	3	3	0	1	3
155	0	0	3	3	0	0	3
156	0	2	3	3	0	3	3
157	0	2	3	3	0	3	3
158	0	2	3	3			
159	0	1	3	3			
160	0	2	3	3			
161	0	2	3	3			

	November 1920.		
	19°	23°	28°
H 164	0	1	3
168	0	0	3
176	0	0	3
179	0	1	3
181	0	0	3
197	0	3	3
205	0	0	3
260	0	1	3
261	0	1	3
263	0	0	3
264	0	0	3
265	0	1	3
266	0	0	3
268	0	1	3
269	0	1	3
272	0	0	3
274	0	3	3
276	0	0	3
280	0	1	3
287	0	1	3
288	0	1	3
289	0	1	3
291	0	1	3

		November 1920.		
		19⁰	23⁰	28⁰
293		0	0	3
294		0	1	3
295		0	1	3
296		0	1	3
298		0	0	3
299		0	0	3
300		0	1	3
302		0	1	3
306		0	0	3
307		0	1	3
308		0	0	3
309		0	1	3
310		0	1	3
311		0	1	3
312		0	0	3
314	a	0	0	3
	b	0	0	3
	c	0	0	3
	d	0	2	3
315		0	2	3
316		0	3	3
318		0	0	3
319		0	2	3
320		0	2	3
321		0	3	3
322		0	1	3
323		0	3	3
324		0	2	3
325		0	0	3
326		0	1	3
327		0	0	3
328		0	3	3
329		0	3	3
330		0	0	3
331		0	0	3
332	a	0	0	3
	b	0	0	3
333		0	0	3
334		0	0	3
335		0	1	3
336		0	1	3
337		0	0	3
338		0	1	3
339		0	1	3
340		0	1	3
341		0	0	3

November 1920.

	19°	23°	28°
342	0	1	3
343	0	0	3
344	0	1	3
345	0	0	3
346	0	2	3
347	0	0	3
348	0	2	3
349	0	3	3
350	0	0	3
351	0	0	3
352	0	1	3

These experiments were made by inoculating plates (made in March with haemolysed blood; in November, with pepsindigested blood) with fresh cultures of the different strains and then putting them in an air thermostat. A thermometer controlling the temperature, was placed by the side of the plates.

In experiment „I" the temperature was only about 23° for a large part of the time on account of faulty regulation, while the opposite happened in experiment „II" and the experiment at 26° in March, where it was about 1° higher than the temperature given, for a few hours.

The two last experiments in March were inadvertently only continued for about 1½ days, while the plates in the other experiments remained in the incubator for 5 days.

In spite of these technical deficiencies all the experiments are given as I do not consider the results obtained in March 1920 to the absolutely worthless.

The growth was estimated in different degrees; (1) = trace, 1 = weak, 2 = fairly good, 3 = luxuriant growth.

In cases where weak or absent growth occurred it cannot be due to the inoculation of too few living organisms because the weak growth in the great majority of cases consisted of a confluent mass of growth, not of isolated colonies.

It will be seen that the minimal growth temperature for the different cultures examined is not the same, but varies between 20° and 25°. The individual differences between the strains are particularly prominent in the 23°-experiment.

That these variations do not depend upon the exposure of the strains actually to different temperatures is evident from the fact that strains inoculated on the same plate (about 10 cm. in diameter) were just as different from one another, as those on different plates in the same experiment. That we are dealing with fairly constant individual characters of the strains is rendered probable

from a comparison between the experiments in March and November in the case of those strains examined at both these times. The 4 strains which gave no growth in the experiment „25° I" and were also examined in November at 23°, still did not grow. This and several similar concurrences are probably not fortuitous.

With regard to the minimum temperature for growth, the present collection of strains of Pfeiffer's bacillus therefore form a group in which it can vary over a range of about 5°, namely from 20° to 25°, and, — at any rate under the present conditions of cultivation — it must be accepted as a fairly constant factor for each individual strain.

As regards the position of the minimum temperature my experiments are in accord with those of AUERBACH (cited from CZAPLEWSKI) and LUERSSEN who obtained growth at 22°. PFEIFFER's (2), JUNDELL's, and DAVIS' statements that 26°—28° is the minimum temperature for growth, will therefore at best only apply to a few strains of Pfeiffer's bacillus. Presumably these divergencies depend on differences in the media used, the method of inoculation etc. I could not however, by inoculating different strains on FILDES agar and on blood agar, and keeping them at a temperature at which only a few of the strains grew, discover any indication that the minimum temperature for growth on the two media was different.

Resistance to Heating, Drying, and Keeping.

A fact that is very often put forward as characteristic of Pfeiffer's bacillus is its slight power of withstanding various deleterious agencies. Thus ONORATO found it was killed by heating to 45° for half an hour and drying for ¼—2½ hours, according to the temperature and the degree of humidity of the air. Many authors testify to the tendency of cultures of Pfeiffer's bacillus to die out quickly, in that they have to be subcultured at intervals of 3—6 days, if one wants to be certain of keeping them alive, but this does not exclude the possibility, of course, that in some cases they might continue to live for 2—3 weeks. But it must be remembered that this very limited resistance only refers to surface cultures and even from these DAVIS (2) obtained growth after 3 months, although only after the tubes were kept sealed. This must be regarded as an exception. Deep down in suitable media

and also in ordinary stab cultures, they have been preserved for a longer time than in surface cultures. TWORT & TWORT were able to keep Pfeiffer's bacillus alive in mixed culture with other bacteria for 5—6 months on DORSET's egg medium.

I investigated the resistance to heating, in March 1920 in the following manner.

On a haemoglobin agar plate fresh cultures of the strains mentioned on p. 108 were inoculated and the space between the top and bottom closed with cotton wool and plasticine. The plates were then sunk in a water bath for half an hour, which was kept at the temperature to be investigated. The plate was taken out, dried, opened and a liberal amount of each culture inoculated on a new plate which was kept under observation in the incubator for 2½ days. This method was very simple to carry out, and in other ways seems to have advantages: suspension of the cultures in another medium which may possibly damage them is avoided; a good quantity of the culture can be inoculated which will give a good chance of growth even if only a very few bacilli remain alive. By a suitable filling of the inoculation needle one can ensure the inoculation of any fairly constant amount of the different cultures. It is also easy to be certain of a uniform heating to the desired temperature.

The experiment gave the following result: At 54.5° and 50.5° all the cultures were killed. After heating to 48.5°, all grew except I 1, I 20, I 21, P 15(b), P 16 and H 19(b). At 46° all the strains preserved their viability; I 21 however only produced 1 colony.

In November 1921 another experiment was carried out with the same technique, with all the cultures mentioned on p. 110 but FILDES agar was used. The culture was killed after having grown for a day and a half. The following temperatures were employed: 54°, 50.5°, and 47.5°. After heating to 54° only H 314 d grew (which however, did not give any growth in the experiment at 50.5°). 50.5° killed all the strains except H 314 b, H 318, H 319, and H 324*). After heating to 47.5° the following strains did not grow: I 57 (a, b), 103; I 32, 36, 37; P 14; Ms 1, 4; Me 1a, b; H 34, 122, 164, 176, 179, 205, 261, 263, 264, 265, 269, 272, 274, 276, 280, 293, 295, 298, 306, 308—312, 320, 325, 327, 328, 333, 338, 341, 342, 343, 345—348, 350, 351, 352, GP 1; Pa 4, 5; all the others grew. It should be noted that the plates stood about 6 hours after the heating before inoculations were made from them.

Resistance against low temperatures I have found

* All these strains were distinctly atypical.

to be considerably greater than ONORATO did who found that Pfeiffer's bacillus is killed in a couple of hours at —15° to —20°.

Different strains were suspended in broth in test tubes which were placed for 24 hours in the freezing mixture. The temperature for the first 12 hours was —18° to —20°, and then rose during the remainder of the experiment to —12°. Inoculations made from them all gave growth. I thought it unprofitable to continue these experiments as the process of freezing introduced the combined effect of a low temperature and drying. In a greatly cooled, frozen suspension in salt solution or broth the bacteria will in fact, be surrounded by a very concentrated solution of salts and other substances. In addition, freezing does not set in at the same moment in a series of tubes which are exposed to exactly the same cooling effect, and indeed, as is well known, it may not take place at all for a long time, which I have also frequently observed.

I have made the following experiment with reference to the resistance of different strains of Pfeiffer's bacillus against drying.

In August 1918 strains I 1—17 were tested by rubbing a loopful of each culture on a small area of the bottom of a Petri plate, which was then put in the incubator at 37°. After 2 hours had passed each of the dried cultures was rubbed up with a little broth and inoculated on haemoglobin agar plates. There was growth in each case. The experiment was repeated, allowing 3, 4, 5½, and 8 hours in the incubator. Marked individual differences were found in the resistance of the cultures against drying. 2 grew in all 5 tests, while 4 did not grow in any of them. In the other cases the results were quite irregular as growth was just as likely to occur after prolonged drying and no growth after a short drying, as the reverse. The attempt to use this test as a means of distinguishing the individual strains was therefore abandoned.

On testing the strains H 13—32 (Sept. 1918), the majority gave growth after 8 hours' drying.

In March 1920 the strains mentioned on p. 108 were tested. After 5 hours' drying (dew-point of the air, 11°), almost all the strains grew but only a few (I 1 (a), I 29, I 54, H 122, H 127 and Pa 5) grew after 14 hours' drying.

In November 1920 all the strains on p. 110 were examined. The Petri plates containing the dried cultures were kept 24 hours in the incubator this time, the plates being placed upside down on a perforated iron shelf, so that the air had free access to them. The dew-point of the air was 13°, the percentage humidity therefore being about 25. None of the strains gave growth on cultivation

If we would make use of sensitiveness to dessication as a character of Pfeiffer's bacillus it would thus seem to be appropriate to employ a period of 24 hours for the drying process (with the proviso that similar temperature and humidity conditions prevail to those mentioned above).

In examining the duration of life of the various strains of Pfeiffer's bacillus I have, in a large number of cases, used cultures which after having been incubated 1—3 days, were preserved in the cold room (at about 5°). Almost all the strains mentioned on p. 110 were examined, some of them twice. The greatest length of life was observed in I 71 and I 91 which still gave a good growth after having been cultivated 24 hours in the incubator and then remaining a month in the cold room. But 6 days later these cultures did not give growth.

Distinct individual differences were noted between the various strains but as they seemed to be rather inconstant the experiments will not be given in detail.

The very limited resistance against heat, drying, and keeping, excludes the presence of spores in the cultures, unless in this instance they require very different conditions for their development than the ordinary vegetative forms. One might imagine however that spores might be formed under special conditions of cultivation. I have therefore made the following tests.

All the strains mentioned on p. 110 were inoculated on plates of FILDES agar, and kept in the incubator for 8 days. The plates were then immersed in a water bath at 54° for 40 minutes, in the manner previously detailed. In no case was there growth in the cultures made from them.

Twenty-four hour cultures of the same strains were also inoculated on to plates prepared with salt solution (½% salt) and agar. These plates remained in the incubator for 24 hours and were afterwards heated to 55° for half an hour. There was no growth from any of these plates on subcultivation.

Fermentation Tests.

In October 1918 the strains I 1—17 were inoculated on 6 sets of agar plates containing litmus, and glucose, laevulose, lactose, saccharose, maltose and mannite respectively. Small drops of horse

blood were put on the surface of the agar, in which the various cultures were rubbed up. Although there was a fairly good growth of Pfeiffer's bacillus no acid production was noted in any of the media in 1½ days.

In April 1920, the strains on p. 108 and a number of other cultures from influenzal pneumonia and healthy persons, as well as all the 15 cultures from measles cases, were inoculated on LEVINTHAL agar with the addition of litmus and about 0.4% of the following carbohydrates: glucose, laevulose, saccharose, and maltose The medium was of such a reaction that the litmus assumed a bluish-violet tint. Although Pfeiffer's bacillus grew particularly well there was not the slightest change of colour during the 4 days' observation.

Later, a number of experiments were performed in which FILDES agar with the addition of a mixture of phenol red and decolorised china blue was used, which in a rather stronger concentration than that given by MORISHIMA, is extremely sensitive. Unfortunately all these experiments failed. Most of the strains certainly gave a marked acid reaction, but it was very inconstant in the case of surface growths, though more constant in stab cultures. It appeared however that the same indicator change (acid-formation?) could be obtained, not only on agar without sugar added, but even when the agar was prepared from broth which had previously been fermented with Bacillus coli. Whether the change in colour of indicator in the experiments with the „sugarfree" agar was due to glucose, — partly unfermented by the coli bacilli and partly split off from the agar — I cannot decide. We know that absolutely sugar-free agar medium cannot be made. Curiously enough Pfeiffer's bacillus in stab culture in the agar prepared from coli-fermented broth produced a considerably greater degree of acidity than a number of other bacteria (various micrococci, dysentery, paratyphoid, Bacillus enteritidis Gärtner, Ratin bacillus), which were inoculated in the same way for comparison. Only the two last-named organisms gave a weak change in the acid direction, while Pfeiffer's bacillus caused a marked colour change.

Although I have not been able to throw much light upon the subject, I do not consider it superfluous to point out, on the basis of these experiments, that in fermentation tests with Pfeiffer's bacillus on agar, it is possible to get even a very pronounced acid reaction where it is not legitimate to assume there has been any fermentation of definite carbohydrates.

As the fermentation tests on agar were apparently worthless I proceeded to try them in liquid media.

Broth, previously fermented with Bacillus coli, was inoculated with the „air coccus". In a couple of days' time, 1% dissolved

horse blood corpuscles, 1% glucose, $^1/_{100}$ % decolorised China blue, and $^1/_{500}$ % phenol red were added. The mixture was filtered through a Berkefeld filter and run into test tubes which were inoculated with the strains on p. 110. The tubes were kept under observation in the incubator for a week. (For the first three days they were kept approximately horizontal, and afterwards, vertical). The average growth was rather weak. The only strains that decidedly changed the colour of the medium were H 274 and H 323, which produced weak acid reactions. The same result was obtained when the experiment was repeated. Since the acid reaction appeared however, when the medium was prepared with broth fermented with Bacillus coli without the addition of glucose (in which medium 2 strains of Bacillus haemoglobinophilus canis with strong fermentative properties, did not give any change of colour), there can hardly be any question of glucose fermentation.

The strains Me 2 and 3, which were later on tested separately for glucose fermentation in a liquid medium, gave a weak acid reaction (while a strain of Bacillus haemoglobinophilus canis which was inoculated for comparison, gave a very strong reaction). Whether it was a true fermentation reaction in this case, was not investigated.

On the whole therefore no certain and even moderately marked power of fermentation could be demonstrated either on solid or in liquid media.

As mentioned on pp. 49—51 other authors have found a distinct though rather „capricious" fermentative power by using suitable substances, without being able to determine precisely what properties a medium must possess in order that Pfeiffer's bacillus may exert its power of fermentation. There is absolutely no reason for concluding from the negative result of my fermentation experiments, that my strains of Pfeiffer's bacillus were of a different nature from those of the authors cited.

According to Gosio (2) lack of the power to attack arbutin is characteristic of Pfeiffer's bacillus as opposed to bacteria which it might be confused with. I therefore (March 1921) prepared Fildes agar containing 1% arbutin (in autoclaved watery solution) and inoculated these plates with all the stock strains of Pfeiffer's bacillus, — practically the same collection as that given on p. 110, together with Me 2 and 3. In no case did Pfeiffer's bacillus produce the slightest change of colour. For the sake of comparison many other species of bacteria were inoculated on the same medium. Most of these gave a positive reaction, which showed itself as a marked

dark colouration of the medium caused by a rather easily diffusing substance (oxidation product of the hydroquinone, splitt off).

Investigation of the Proteolytic Power.

In Dec. 1920 all the strains on p. 110 (except I 1, and I 38) were inoculated on peptone broth gelatine (containing a little over 1% gelatine), to which was added about 2‰ dissolved blood corpuscles. The tubes were incubated for a week. In most cases a good growth appeared. At the end of the week the tubes were cooled. In each case the gelatine set just as firmly as before inoculation.

The same strains were also tested as to their power of liquefying coagulated serum. The following medium was used. One part salt-free and sugar-free peptone broth in double concentration was mixed with 3 parts of ox serum, to which was added pepsin-digested blood in the usual concentration. This concoction was poured into Petri plates and coagulated by a short heating to 70°. Pfeiffer's bacillus grew splendidly on this medium. In no case was the slightest sign of liquefaction of the serum observed after a week in the incubator.

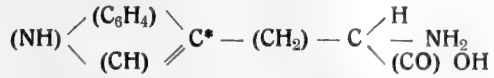
Indol Formation.

The absence of proteolytic power on the part of Pfeiffer's bacillus in the ordinary meaning of the word, does not mean that it is unable to decompose dissociation products of the proteins.

A dissociation which has particularly attracted the attention of bacteriologists is the liberation of indol from the tryptophane molecule.

According to Frieber all bacteria can split off the group $CH.NH_2$ from tryptophane, which is seen on the extreme right in the formula below, while comparatively few organisms can break the bond marked with an asterisk, which liberates indol and which can be detected by Ehrlich-Böhme's reaction. (The

earlier, much employed reaction of KITASATO-SALKOWSKI should, according to statements in the literature, be given up).



In certain cases bacteria can elaborate indol from open chains (LOGIE), but usually the only essential for indol formation is the presence in the medium of tryptophane or complexes from which it can easily be split off.

We must, on the whole, undoubtedly agree with M. NEISSER that the presence or absence of the power to form indol is one of the most constant biological characters of bacteria. He says: „wir müssen deshalb die bakteriologische Identität ohne sachgemässe Anstellung der Indolreaktion für unvollständig erklären".

As it soon appeared that there were both indolpositive (that is indol-producers) and indol-negative strains in the Pfeiffer's bacillus group, it was necessary to investigate whether, on the basis of this property, Pfeiffer's bacillus might be separated into two sharply-defined groups. To decide this we must know: (1) whether any sharp boundary can be drawn between the positive and negative indol reactions; (2) whether an individual strain is constantly indol-positive or indol-negative; (3) whether any connection can be demonstrated between the grouping of the strains according to the indol test and their other biological characters.

The results of my indol tests will first be reported in tabular form, and then the necessary explanations and comments, together with some supplementary investigations will be given.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I 1	0	0	0	0	0	0	0	0				
5	0	0	0	0	0	0	0	0				
6	+	+		+			6	+				
20	+	+	+			+	6	+				
21	+	+	+	+		+	6	+				
22	+	+	+	+	+	0!	6	+		7	1	2
23	+	+	+	+		0!	6	+		7	1	2
27	+	+	+	+		+	6	+				
29	+	+	+	+	+	+	6	+				
35	+	+	+	+		+	6	+				
38	+	+	+	+	+	+	6	+				

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
45	0	0	0	0		+!	6!			0	0	0
54	+	+	+	+	+	+	6					
57	0	0			0	0	0	0				
59	+	+	+	+	+	+	6	+				
71	0	0		0		0	0					
72	+	+		+	+	0!		+		7	1	1
91	0	0		0	0	0	0	0				
100	+	+		+	+	+	6	+				
101	+											
102	+											
103	0							0	0	0	0	0
104	+											
I 5	+	+	+	+	+	+	6	+				
21	+	+		+		0!	2	+	(All the positive reactions for I 21, weak).			
32	+							+	4	7	0,5	
33	+							+	4	7	2	
34	+							+	4	7	1	2
35	+								4	7	3	2
36	+							+	3	7		1
37	+							+	4	7	1	1
P 14	+	+		+		+	5	+				
15	+	+		+		+	6					
16	+	+				+						
22	0	0		0	0	0	0	0				
23	0	0		0	0	0	0	0				
24	+	+		+		+	6	+				
25	+	+		+		+	6	+				
Ms 1	+							+	1	3	0!	0!
4	0							0	0	0		0
8	+											
9	0!							+	4	7	1	1
10	+								4		2	2
13	+							+	1		3	2
15	+							+	4		0!	2
Me 1 a								+	4	7	2	2
b								+			0,5	3
c											0,5	3
d											3	2
H 14	+	+	+	+	+	+	6					
19	+	+	+	+	+	0!	6	+		7	2	1
34	+	+		+		+	6	+				
35 a	0	0		0		0	0					
37 a	0	0		0		0	0					
c	+	+		+		+	6					
49 b	+	+		+		+	6	+				
122	+	+		+	+	+	6	+				

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
127	0	0		0		0	0	0				
133	+	+		+	+	+	6	+				
151	0	0		0	+!	0	0	0			0	0
152	+	+		+		+	6	+				
154	+	+		+	+	+	5	+				
155	+	+		+		+	6	+				
156	+	+		+		+	6	+				
157	0	0		0	0	0	0	0				
158	0					0	0					
159	+	+		+		+	6	+				
160	+	+		+		+	6					
161	0	0		0		0						
Pa 4	+	+		+		+	6	+				
5	+					+	6	+				

	I	VII	VIII	IX	X	XI	XII
H 164	+		+	4	7	4	2
168	+		+	3	7	3	2
171	0			0	0	0	
172	+			4	7	4	1
176	0		0	0	0	0	0
178	0		0	0	0	0	0
179	0		0	0	0	0	0
181	0		0	0	0	0	0
197	+		+	4	7	0!	1
205	0		+!		0	0	0
260			+	4	7	3	2
261		2	+(weak)	1	4	0!	0!
263			+	4	7	1	2
264			+	4	7	1	2
265			+	4	7	0!	2
266			+	4	7	0!	2
268			+	4	7	2	3
269			+		7	3	3
271		6					
272		2	+	(weak)			
273		6					
274		6	+				
275		6					
276		6	+				
277		6					
278		6					
279		6					

	VII	VIII	
280	6	+	
281	6		
283	6		
284	6		
286	5	+	
287	6		
288	6	+	
289	6	+	
291	2	+	(weak)
293	6	+	
294	6	+	
295	2	+	(weak)
296		+	
298	0	0	
299	6	+	
300	6	+	
301	6		
302	6	+	
303	6		
304	6		
306	2	+	(weak)
307	2	+	(weak)
308	6	+	
309	6	+	
310	6	+	
311	6	+	
312	2	+	(weak)
313	6		

	VII a	VIII
H314 a	0	
b	+	+
c	0	
d	+	+
316	+	+
317	+	+
318	0	0
319	0	0
320	+	+
321	0	0
322	0	0
324	+	+
325	0	0
326	0	0
327	+	+
328	+	+
329	+	+
330	0	0

	VII a	VIII
331	0	0
332 a	0	0
b	0	0
333	+	+
334	+	+
335	0	0
336	0	0
337	+	+
338	+	+
339	+	+
340	+	+
341	0	0
342	+	+
343	+	+
344	+	+
345	0	0
346	+	+
347	+	+
348	+	+
350	+	+
351	0	0
352	+	+
GP 1	0	0
2	0	0
3	0	0
4 a	0	0
b	0	0
5	0	0
R 1		+
2		+

On testing later, Me 2 gave a weak reaction, Me 3 and Me 4, marked reactions.

In the column on the left the strains tested are given; columns I, II etc. give the results of each series of tests. When not otherwise stated, all the experiments in the same column were carried out simultaneously.

The test was performed with the usual technique of BÖHME. To 1 volume of broth culture is added ½ volume of the following mixture, — paradimethyl-amido-benzaldehyde, 4 gm.; 96% alcohol, 380;* concentrated hydrochloric acid, 80.* After slight stirring ½ volume of a saturated solution of potassium persulphate is added

* BÖHME does not state whether grams or c.c. I have assumed grams were meant, but I have since become aware that most authors probably have taken the values to be c.c.

(My experience has been that less than ½ voulme of the first reagent ought not to be taken, but about ⅓ volume of the second may be used without harm. By entirely leaving out the last, which several authors do, the reactions do not always seem to be so strong as when both reagents are used). When indol is present a purple-red colour develops. The result is not read until 10—15 minutes have elapsed, as the colour often appears rather slowly.

The series „I" was made in Jan. 1920, with the exception of I 101—104, I 32—37, all the Ms's, and H 164—167, which were examined a couple of months later. They were inoculated in ordinary broth containing 1% WITTE peptone and with the addition of enough haemolysed horse blood corpuscles to produce a weak pink colour (about 1‰ blood corpuscles), henceforward referred to os „haemoglobin broth". Before inoculating, the tubes were kept in the incubator for 24 hours to be sure they were sterile. This changed the weak red colour to a still weaker brownish tint which in no way hampered the judging of the indol reaction.

After inoculation the tubes were placed very obliquely in the incubator for 5 days. (The same time was allowed for growth in the following series when the contrary is not indicated. Both in this and the following series cultivations were made, before the indol test was carried out, from all the tubes on to haemoglobin or FILDES agar. Only those tubes from which a growth of other bacteria than Pfeiffer's bacillus occurred are omitted from the table.

For series II (March 1920), a sterilised watery solution of 1% LIEBIG's meat extract, 1% peptone „CHAPOTAUT", and ½ % salt was the medium used. For series III, ordinary broth with 1% peptone, „Chapotaut", instead of the commonly used WITTE's peptone, was employed. For series IV a watery solution of 2% peptone „Chapotaut" and ½ % salt, was used. Haemoglobin was added to all three media, as described above. All the three series were investigated at the same time.

The medium in series V was the same as in series I, but the cultures with which it was inoculated had previously been subcultured 25 times on ascitic agar with a little haemoglobin, in „symbiosis" with a large number of different species of bacteria. The object was to discover whether by cultivating Pfeiffer's bacillus under variable nutritive conditions, its biological characters could be altered (indol formation; for agglutination, see later).

In series VI (May 1920) haemoglobin broth was used which was inoculated with cultures that had been subcultured daily for 96 days on haemoglobin agar. (It is not known whether WITTE peptone, or „Peptone Berna" which is said to be prepared „par le procédé WITTE", was used in this case).

In series VII (Sept. 1920) and VIIa (Oct. 1920) trypsin-digested casein prepared according to COLE & ONSLOW's instruction, was used. One part of „stock broth" + 2 parts of water containing

0.3% NaCl, 0.2% secondary potassium phosphate and 0.1% magnesium sulphate with its water of crystallisation, were taken. (The last two salts can be dispensed with without danger; COLE & ONSLOW do not mention them). To favour the growth of Pfeiffer's bacillus the air coccus was previously grown on the medium for 2 days; 1—1½‰ dissolved sheep blood corpuscles were added after which the mixture was filtered through a Berkefeld filter.

In series VIII (Oct. 1920) cultures in haemoglobin broth with a little gelatine were used. (Before inoculation the medium had been heated to about 70°, which coagulated the haemoglobin). After the cultures had been in the incubator a week for the gelatine liquefaction test, which as previously stated, was always negative, the indol test was done on them. In a number of cases this was carried out by pouring a mixture of 2 parts of the paradimethylamido-benzaldehyde reagent and 1 part of the potassium persulphate solution, on the solidified gelatine medium. A positive reaction consisted in the development of a red ring at the boundary between the medium and the reagent. The remainder of the cultures were melted at 37° and then mixed with the two liquids in the usual manner. The reaction was very distinct with both methods of carrying out the test.

Experiments IX—XII which were performed in Aug. 1920, but for typographical reasons are placed last, constitute a connected investigation designed with the object of finding out the effect on the formation of indol, of varying the substances from which it is formed.

In series IX Bacillus coli-fermented broth with the addition of 1% crepton („vollständig abgebautes Fleischeiweiss nach Prof. Dr. Abderhalden" from Meister, Lucius, and Brüning) was used instead of peptone. In X the same casein medium as in VII was employed. In XI a watery solution of 1% peptone CHAPOTAUT + the same salts as in the casein medium, was used. In XII Bacillus coli-fermented broth with WITTE peptone was used. All the media were sterilised in the steam steriliser. They were inoculated with the air coccus. After 2 days' incubation at 37°, 2‰ dissolved sheep blood corpuscles were added, and then the media were filtered through a Berkefeld filter.

In series VII and IX—XII 4 days was allowed for incubation. To obtain a numerical expression for the strength of the reaction, a fuchsin solution was added to a series of tubes, 1, 2, 3, etc. containing boiled bacterial culture in such quantities that tube No. n contained fuchsin in a concentration $2^{n-1} \times 10^{-6}$. Tube No. 1 assumed a light rose colour; No. 7 became dark red. The strength of the indol reaction was then given by the number of the fuchsin tube the colour of which most closely matched the colour of the reaction tube, in intensity.

In series VIIa the positive reactions, which are only designa-

ted in the table by a „+", were all of the titre 5—6 except H 327 which was a little weaker (about 4).

With the exception of series VI the difference between the positive and negative reactions was always very sharp; either a marked red colour appeared or the liquid remained a pure yellow.

As a control that the reacting substance really was indol, the medium, in series I in the Jan. investigations and in series VI, was shaken up with amyl alcohol. In all the cases where a red colour was present the colouring matter was taken up by the alcohol, while the latter was never tinted red when the reaction had been read as negative.

The tubes in series IX—XII where the reaction was negative or weakly positive, were shaken with chloroform. Both the positive and negative reactions were confirmed by this test except that in some of the negative tubes a suspicion of red appeared. But as this was only observed in one of the tubes for each strain, and only in series IX, XI, and XII and not in X where it would be particularly expected that a weak reaction would first of all be obtained, this cannot be regarded as an indol reaction.

In using amyl alcohol for shaking out, a weak red colour could sometimes be seen in the negative tests, but only after several hours' standing, and never when the readings were taken immediately after the liquids had separated. As this separation takes place quicker with amyl alcohol which also extracts the colour more completely than chloroform, amyl alcohol seems to be preferable to chloroform for the purpose of verifying indol tests by the shaking out method. From my experience with Pfeiffer's bacillus this need only be resorted to in the case of weak positive reactions, as marked positive or negative reactions can be read with the same accuracy without it.

The reactions given for I 1, I 57 and the other strains where two colonies of different appearance were generated, were partly carried out with the original growth and partly with the two forms of colonies. In all cases however, the different cultures gave identical results.

The first impression we get from the table is that the reaction of each strain is usually either constantly positive or negative, irrespective of the time it is done and whether the growth took place on media of rather different constitution

Some of the reactions however, are divergent. These are printed in thick type. We will now consider them more closely. No explanation is forthcoming of the causes of the absent reaction for Ms 9 in series I and the positive reactions for H 151 and H 205 in series V. The change from negative to positive reaction in I 45 occurred simultaneously with a marked alteration in

its agglutination reactions. Further, in Jan. 1920 it formed colonies with a finely granular surface, while in September they were smooth. A comprehensive mutation must therefore have arisen, or else a contamination of the culture or its substitution by another must have taken place.

These four cases are the only ones in the material included in the table for which it is necessary to have recourse to the three possible explanations just given. This must be considered very satisfactory because it must not be forgotten that the sole guarantee of purity of the majority of the cultures consists partly in the fact that each strain was cultivated from an isolated colony on the original plate, and partly that there is good reason to suppose that contaminations with other bacteria than Pfeiffer's bacillus would practically always be detected and got rid of by plating out.

An absolutely pure cultivation e. g. by BURRI's or ØRSKOV's (1,2) method, would undoubtedly be fraught with considerable difficulties on account of the smallness and slight resistance of the bacillus, particularly if it had to be undertaken on an extensive scale; and I did not think that the relative certainty which could be attained by the usual plating out of each strain a number of times would repay the labour entailed and the considerable expenditure of material. It is impossible to deny that during the extremely numerous inoculations a mistake may have been made in one or two cases. In such an extensive statistical investigation as the present a few irregularities are only to be expected.

In series VI the positive reactions were on the whole rather weak. Several of them could only just be recognised as positive. I was unable, at the time, to subject these cultures (which as will be remembered, were subcultured 96 times in rapid succession) to a fresh examination on other media. It is however highly probable that the weakly developed indol formation or its complete absence in some cases, must be accounted for by the constitution of the peptone.

The significance of the kind of substance we give Pfeiffer's bacillus to manufacture indol from, is well seen in the series IX—XII all carried out at the same time. A particular „peptone" was used in each of these series and care was taken to choose peptones as different from one another as possible.

WITTE peptone, as far as is known, is a peptic product and as such it principally contains albumoses. Peptone CHAPOTAUT is considerably further broken down and contains about 20% amino-acids (see STICKEL & MEYER). Trypsin-digested casein (here referred to as „casein-tryptone") is also extensively split up and contains in particular, an unusually large proportion of free tryptophane (see COLE & ONSLOW). Lastly, erepton is said to be completely broken down into animo-acids.

We might at first think that the formation of indol would be largely dependent on whether one or other of these four very different products from which it could be formed was forthcoming. A very marked uniformity however was observed on the whole, as the cultures either formed indol in all the four media or in none of them. Occasionally the formation of indol, present in the other media, failed. But this was chiefly the case in the CHAPOTAUT peptone, which (as it was used as a pure peptone solution without broth) proved to be a very bad nutritive medium for Pfeiffer's bacillus, and this also explains why the positive reactions in this medium were weak and considerably more irregular than in the other media. In a few instances the reaction was also absent in the broth containing Witte peptone, which is quite intelligible as the positive reactions were usually weak on this medium also, and in those cases where the reaction failed it was comparatively weak on the other media. In WITTE peptone tryptophane is not easily accessible because it is not sufficiently liberated from the albumose complex.

The formation of indol in erepton and casein-tryptone media (IX and X) however, agree excellently with one another. Most of the strains seem to break down all the tryptophane (4 strains of Bacillus coli, inoculated in the casein-tryptone medium, gave an indol reaction of exactly the same intensity as the vigorously indol-producing Pfeiffer's bacilli), and thus the reactions „4" and „7" in the erepton and casein-tryptone media respectively make their appearance. Two or three strains proved to be weak indol-formers: intensity in erepton, 1; in casein-tryptone, 3—4.

In the case of all the strains H 260—313, which came from the same series of inoculations, and were all uniformly good growers and can therefore be compared with one another, those strains are noted where the reactions on the gelatine medium (tested by mixing the melted gelatine culture with the reagents) were weak. It will be seen that weak reactions on the 2 different media regulary coincide.

In a number of the cultures examined the growth was only weak or uncertain and it might be thought that the negative result of the indol test could be due in some cases to lack of growth. In order to test this I inoculated, in November 1920, all the strains which had reacted negative in the pre-

vious experiment, on a broth-agar medium containing caseintryptone (1 part COLE & ONSLOW's „stock broth" + 2 parts broth free from salt, sugar, and peptone, + 2% agar), to which after sterilisation pepsin-digested blood was added. This medium was put into large test tubes and sloped. All the strains gave a fairly good growth. After 4 days' incubation the contents of the tubes were melted, and a little distilled over by boiling, the indol reaction being carried out on the distillate.

In this way I achieved 2 things: (1) the assurance that there actually was a growth of Pfeiffer's bacillus in the cultures; (2) the production of a more sensitive reaction. In an orientation experiment it had been shown that the positive reactions were strongest when they were done with the distilled liquid. The indol passes over with the first fraction of the distillate and it will therefore be found in greater concentration in this than in the original culture.

With this mode of procedure the reactions were also negative with the following exceptions: Ms 4, H 127, H 205, H 314c and GP 5.

Plate cultivations of these strains were then prepared and inoculations were made in tubes of haemoglobin broth and the solid medium described, from various colonies that appeared. After 4 days' incubation the reaction was tested on the broth cultures and on the distillate from the agar cultures.

The results were as follows:

			Broth culture	Agar culture
Ms 4 from colony		a	0	Trace
	—	b	0	0
	—	c		0
	—	d		Weak
H 127	—	a	+	+
	—	b	0	0
	—	c		+
	—	d		+
H 205	—	a	+	+
	—	b	+	
	—	c		+
	—	d		+
H 314 c	—	a	0	Trace
	—	b	0	0
	—	c		0
	—	d		Trace
GP 5	—	a	0	Trace
	—	b	0	Weak
	—	c		0
	—	d		0

The positive reactions in the distillates from the agar cultures were not therefore due to this method giving different results to those obtained with the direct reaction on the broth cultures, but owed their origin to the fact that the strain was impure or a mutation had taken place.

These investigations into the formation of indol show that in almost all cases Pfeiffer's bacillus is either distinctly indol-positive or negative and that this property is not as a rule influenced by cultivation for a long time or by fairly wide variations in the experimental conditions. They also show that media containing trypsin-digested casein is specially suitable for this reaction. The question of the connection between indol formation and the other characters will be discussed later.

Agglutination.

For the preparation of agglutinating sera rabbits were injected intravenously with living cultures grown on haemoglobin or FILDES agar for about 24 hours and then suspended in salt solution. As a rule 5—7 injections were given in increasing doses at intervals of 5 or 6 days. The rabbits were killed and the blood collected 7 or 8 days after the last injection. The sera were kept in a frozen condition.

Sera were prepared with the following strains, and were used extensively in the agglutination experiments:

Strain isolated in pure culture		Serum prepared	Titre (estimated several times)
I 6	July 1918		1 : 800—1600
I 21	Jan. 1919	Febr.—April 1919	1 : 400
H 34	Jan. 1919		1 : 200
Pa 5	?	April—May 1919	1 : 400—800
I 35	Dec. 1918		1 : 200
I 57	Jan. 1919		1 : 200
I 91	March 1919		1 : 200—400
P 22	Sept. 1919		1 : 800
I 33	Feb. 1920	July—Aug. 1920	1 : 200
I 34			1 : 400
I 35			1 : 400
I 36			1 : 400
I 37			1 : 400—800
Me Ia	June 1920		1 : 200

After at first having tried a rather different technique partly in orientating experiments, and partly in the first part of the main series of experiments, I finally fixed upon the following method.

For the simple agglutination experiment and also for the absorption of agglutinins fresh suspensions of 36—42 hours' cultures on haemoglobin or FILDES agar in a solution of 0.3% NaCl and 0.3% phenol in distilled water, were always used. The bacterial suspension was prepared in a tube of about 15 mm. internal diameter and was made of such a density for the agglutination test that one could just read through the tube, with certainty, rather heavy letters, 3—4 mm. in height* on white paper held immediately behind the tube. The light — preferably clear daylight from a window — must fall perpendicular to the surface of the paper; the investigator therefore stands with his back to the window. Dilutions of the serum are made with the carbolised salt solution in a series of small tubes, so as to get the concentrations 1:10, 1:20, 1:40, 1:80 etc. in a volume of 0.1 c.c. To each tube is added 0.3 c.c. of culture, making the final dilutions of the serum 1:25, 1:50, 1:100, 1:200 etc. When it is found necessary, a tube containing only carbolised salt solution and the bacterial suspension, is put up as a control of spontaneous agglutination. The tubes are then placed in a water-bath at 50° for 4 hours and then read macroscopically. Typical complete agglutination consists of large clumps like those seen in typhoid agglutination. This is denoted by $\dot{+}$ while fine clumps are denoted by +, and partial agglutination by (+). Very slight agglutination is disregarded.

The following details concerning the technique may be given. The relatively long cultivation period was chosen to diminish the tendency to spontaneous sedimentation. In comparative tests it was found that young cultures had this tendency in a greater degree than old. For the same reason the salt concentration is made as low as 0.3%. In this way spontaneous sedimentation of most of the strains was completely avoided, or else it was reduced to such an extent that it did not interfere with the estimation of the agglutination. There were some strains however in which spontaneous sedimentation or agglutination was so marked that the investigation of the specific agglutination could not be undertaken. But these strains were usually so divergent in their morphology, — macroscopic and microscopic, — that they would scarcely have reacted with the sera, even if it had been possible to obtain the necessary experimental conditions. The addition of phenol was required when the test was done at 37°, as otherwise a growth of foreign bacteria took place, and it seemed advantageous to make use of it also when the test was done at 50°. Formalin ($1^0/_{00}$) was also tried.

* It naturally does not matter if the letters are a bit larger or smaller.

There seems to be no important difference between the two antiseptics

At first the agglutination test was carried out at 37° and read 6—8 hours later. From orientating experiments at different temperatures it appeared however to progress distinctly more rapidly and also to proceed further, round about 50° and therefore this temperature was employed in the major portion of the experiments. The temperature could vary a couple of degrees, exceptionally as much as 5° degrees on either side. At first the tubes were given 3 hours at 50°, but as it was found that in this interval the reaction did not always reach its final point the time was afterwards lengthened by an hour. There is nothing to indicate that these discrepancies can have had any important influence on the principal facts of the investigation and therefore they will not be further discussed in the description of the individual experiments.

The method used for standardising the bacterial suspensions must be discussed a little further.

The principle of using the density through which an object can just be distinguished, as a basis of the determination of the density of a bacterial suspension, has been used by several other authors but usually in a more complicated form. In my experience however, a degree of accuracy can be attained by this very simple method which is sufficient for the large majority of serological tests, and moreover it is difficult to do better with other methods.

The following experiments illustrate the accuracy of the method. A dense bacterial suspension was prepared. Variable quantities were measured into test tubes of 14—16 mm. internal diameter. This diameter was correct to within ½ mm. for each tube. With a graduated pipette salt solution was now added until the letters could just be read through the suspension. (During the process the investigator was ignorant of the amount of bacterial suspension which had been measured into the tube). If the volume of the concentrated bacterial suspension is V_1, the added salt solution, V_2, and the diameter of the tube d, the concentration* is given by the equation,

$$x = \frac{V_1 + V_2}{V_1} \cdot \frac{15}{d}$$

The last factor is based on the assumption that the concentration through which the letters can just be read is inversely proportional to the diameter of the tube, which is only approximately correct.

The experiments were made partly by myself and partly by a colleague who was used to estimating the concentration of bacterial suspensions by comparison with standard suspensions, but who had not used the technique described here before and only had it

* in a provisional, arbitrary unit.

explained to him verbally by me just before the experiment. We both worked in the same room but we had absolutely no influence on one another's results. My colleague got the following values for x in 11 determinations: 7.2, 6.9, 7.1, 6.8, 7.05, 6.7, 6.7, 6.65, 6.7, 6.85, 6.65; average, 6.845, mean error, 0.185 = 2.7%. I obtained the following for x in 12 determinations: 7.35, 6.65, 7.1, 7.0, 6.9, 6.9, 6.9, 6.75, 6.85, 6.65, 6.95, 6.9; average, 6.91, mean error, 0.19 = 2.7%. This degree of accuracy must be considered particularly satisfactory. Errors in pipetting, measuring the diameter of the tubes etc. are even included in the mean error; the mean error of the optical determination alone can hardly be put at more than 2%.

A third investigator who was not used to estimating the concentration of suspensions, obtained divergent results, namely in the first series: 8.65, 8.65, 8.55, 8.45, 7.95, 8.4, 8.6, 7.6, 7.2, 7.5, 7.4, 6.4; and in the following series (after having been assured that these figures were on an average „too high"): 6.4, 6.6, 6.5, 6.2, 6.8, 6.6, 6.3, 6.3, 6.2, (6.43 + 0.21 (3.3%)). These divergent results, — psychologically easily intelligible, — of an unpractised worker, can however hardly depreciate the value of the method.

The concentration determined in a test tube of 15 mm's diameter by the method described, is used in the direct agglutinations, but is not defined as the unit of concentration. As concentration 1 is defined that concentration through which letters can just be distinguished when the tube (which should not have too thick walls) is 1 cm. in internal diameter. In the agglutination experiments therefore suspensions of a concentration about $^2/_3$ were used and consequently the concentration in the mixture of culture and serum dilution was about 0.4.

In a couple of experiments with a suspension of a weighed amount of a surface culture (of semi-fluid consistence) it was found that the concentration 1 corresponds to 5—6 mg. per c.c.

If it is desired to prepare a suspension of the concentration n ($n > 1$), a suspension is first made which is obviously more concentrated. It is determined how many times (m) a sample of it must be diluted to get a concentration 1. The remainder therefore is to be diluted $\frac{m}{n}$ times to obtain a suspension of the concentration n.

If the concentration desired is $n < 1$, a suspension of the concentration 1 may first be prepared, which is then diluted to $\frac{1}{n}$ times the original volume.

Concentration determinations can be made not only with daylight but also with lamplight. In the latter case it is advisable however, always to use the same source of light, to place the tube always in the same relative position to it, and compare the determinations made by lamplight with those made by daylight.

The unit which is determined by clear diffused daylight a couple of metres from an ordinary window, should serve as the standard.

The advantage of this method over that of comparison with standard suspensions is that it takes advantage of a unit which is always available, because it only requires persons with normal vision, while standard suspensions may alter in the course of time, and may be lost.

The method however can only be applied to suspensions which are of such a constitution that the letters to be read through them preserve their sharp contour right up to the moment they completely disappear. If on the contrary the contours gradually become more and more indistinct the method cannot be used. This happens when the individual particles in the suspension are distinctly larger than an isolated bacterium, for example, in spontaneously agglutinating suspensions. Doubtless the indistinct contours arise from the comparatively large particles diffracting a considerable amount of light rays at small angles, while the light is deflected by sufficiently small particles more uniformly in all directions, which envelops the letters in a diffuse white haze.

The concentration 0.4 used in the agglutination experiments, is comparatively large. It has been found that this produces more rapid and obvious agglutination than is the case with thinner suspensions.

To obtain further insight into the agglutination conditions the simple test must be supplemented by a b s o r p t i o n tests.

As an orientation experiment for the most suitable technique for the absorption test, I first arranged some tests with homologous absorption at different temperatures and for different periods. A suspension of a concentration about 2 of strain I 6 was prepared. To 20 parts of this was added 1 part of the corresponding immune serum and the mixture was then divided into 6 portions, which were allowed to absorb at 4°, 17° and 37°, half of them for2 hours and the other half for 6 hours. The tubes were centrifuged and an agglutination test carried out with the supernatant fluid on a suspension of I 6. At 17° and at 37° both for 2 hours and 6 hours the absorption brought down the titre to 1:25, while in the cold it was 1:50 after 2 hours, and 1:25 after 6 hours but with partial agglutination in 1:50.

In another experiment in which two different sera were absorbed with their homologous strains in so small a concentration that a little of the agglutinin was left, it was shown that most of the absorption had taken place after 15 minutes but that it still went on slightly for from $2^1/_4$—7 hours.

An experiment with homologous absorption where the serum concentration as well as the bacterial concentration was varied, showed that a decrease of the former or an increase of the latter, increased the absorbed fraction of agglutinin. It was even more marked than EISENBERG & VOLK found. If it is desired to give the absorption specially favourable conditions for its action we must therefore increase the bacterial concentration and diminish the serum concentration. It was also noted in the same experiment that ab-

sorption progressed at least as far when after mixing the bacterial suspension and serum the tubes were allowed to lie in an almost horizontal position without touching them, as when they were stood op and frequently shaken.

In experiments with homologous agglutination by various sera no difference in the absorption power of cultures could be distinguished, which had been cultivated for 12, 24 or 39 hours.

As a standard method I have adopted a serum dilution of 1:20 and a bacterial concentration of 5. After mixing the serum and suspension I have allowed the tube to lie down without shaking for 2 hours at room temperature. The bacteria were then centrifuged down. A series of tubes containing 0.4, 0.2, 0.1 c.c. etc. of the supernatant serum was arranged and the volumes made up to 0.4 c.c. To each tube 0.1c.c. suspension of concentration 2 of the culture was added, for which the agglutinating power of the serum after absorption was to be estimated. In the actual agglutination test therefore there was serum in the dilutions 1:25, 1:50, 1:100 etc. and culture in the concentration 0.4.

The concentrations of serum and culture employed were (with a single exception — Pa 5) always sufficient to allow the homologous strain to remove the agglutinin „completely" by absorption from a serum, by which is to be understood that the serum after absorption gave no reaction even in a dilution of 1:25.

In many cases the serum and culture were employed in other concentrations. The details are given under each individual case.

As a preliminary orientation of the relation of the different strains to one another as regards agglutination, a simple agglutination experiment was carried out with each of the strains I 6, I 6, H 34, and Pa 5, and each of the sera prepared with these strains. The result was as follows:

Sera

Strains	I 6											I 21										
	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400			1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400		
I 6	+	+	+	+	+	+	+	0	0			0	0	0								
I 21	0	0	0									+	+	+	+	+	0	0	0	0		
H 34	0	0	0									0	0	0								
Pa 5	0	0	0									0	0	0								

Strains	H 34											Pa 5										
	1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400			1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400		
I 6	0	0	0									(+)	0	0								
I 21	0	0	0									0	0	0								
H 34	+	+	+	(+)	0	0	0	0	0			0	0	0								
Pa 5	0	0	0									+	+	+	+	+	0	0	0	0		

It will be seen that it is practically only in the homologous reactions that agglutination occurs.

This experiment was made with 61 hour cultures. At the same time 45 hour, 36 hour and 17 hour cultures were tested with exactly the same results, apart from the fact that the reactions in which the younger cultures took part were difficult to read on account of the greater tendency to spontaneous agglutination.

The following absorption experiments were then carried out with serum I 6:

			Agglutination							
			1/25	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200
Serum dilution 1 : 20,	Absorbed with strain I 6	in concentration 1	+	(+)	0	0	0			
— —	— —	— 4	0	0	0	0	0			
— —	— —	— 15	0	0	0	0	0			
— 1 : 80	— —	— 1			0	0	0			
— —	— —	— 4			0	0	0			
— —	— —	— 15			0	0	0			
— 1 : 320	— —	— 1					0			
— —	— —	— 4					0			
— —	— —	— 15					0			
— 1 : 80	— — I 21	— 15			+	+	(+)	0	0	0
— 1 : 320	— — —	— —					0	0	0	0
— 1 : 20	— — H 34	— —	++	++	++	++	+	(+)	0	0
— 1 : 80	— — —	— —			++	+	(+)	(+)	0	0
— 1 : 320	— — —	— —					+	(+)	0	0
— 1 : 20	— — Pa 5	— —	++	++	++	++	+	(+)	0	0
— 1 : 80	— — —	— —			++	++	++	0	0	0
— 1 : 320	— — —	— —					+	0	0	0
Serum dilution 1 : 20 without absorption			++	++	++	+	+	0	0	0
— 1 : 80 —					++	++	+	0	0	0
— 1 : 320 —							(+)	0	0	0

They were absorbed for 6 hours at room temperature with frequent shaking. The dilutions of the unabsorbed serum were made at the same time as those in the absorption experiment, and were kept at the same temperature.

It will be noticed that while the homologous strain even in a concentration of 4, absorbs all the agglutinin in a serum concentration as strong as 1:20, none of the heterologous strains absorb enough of the agglutinin to make it possible to demonstrate the deficiency with certainty although the experiment is arranged under conditions likely to be extremely favourable for showing the presence of absorption power.

Nor could any absorption whatever be demonstrated in a repetition of the experiment with serum I 6 and strains I 21, H 34 and Pa 5; serum I 21 and strains I 6, H 34, and Pa 5; serum H 34 and strains I 6 and I 21; and with serum Pa 5 and strains I 6, I 21 and H 34, with varying technique (weak serum concentrations — large bacterial concentrations).

An absorption test was made with the serum I 6 in a concentration 1:160 and the strain H 34 in a concentration 10 in such a way that after centrifuging fresh culture in the same concentration as before was ground up with the separated serum and this was repeated once more. In each of the three absorptions the mixture of serum and culture was allowed to combine for one hour. In spite of the repeated treatment of a highly diluted serum with a liberal amount of culture, the serum agglutinated its homologous strain in just as high a dilution as the untreated serum. The same was the case in similar experiments with three-fold absorption of serum H 34 in a concentration 1:40 with strain I 6 in a concentration 10.

The agglutinative properties of the four strains examined — which were chosen quite at random — proved therefore to be absolutely specific for each strain. No common relationship could be demonstrated.

In addition to the experiments described, numerous agglutination tests were performed in the orientation of the technique, which will not be reported further. One of the results of these tests was that inhibition of agglutination sometimes occurred in the two first tubes (1:25 and 1:50), but never in the following ones. It was also found that the milder degrees of group agglutination may be rather variable, and that strains with absorptive power for heterologous agglutinin were only present among those showing particularly marked agglutination with the homologous agglutinin. Group agglutination only in a dilution of 1:25, must for the last two reasons, be considered to have special significance.

In the systematic testing of agglutination all that is required therefore, is to first put up the test in 1:50 and 1:100 dilutions and then titrate those samples further which give a reaction in 1:100.

In this way agglutination tests were made from November 1919—February 1920 with all the 14 sera against a considerable number of strains of Pfeiffer's bacillus, about 15 strains at a time being tested against all the sera. As far as possible enough suspension of each culture was prepared for the further titration of the positive reactions.

The collection of reactions reported in the table below is therefore in reality composed of a large number of experiments each consisting of about 200 single reactions of 2 tubes each, and also of the further titration of the positive reactions. The principal result of the whole investigation, the great differences in agglutination between the individual strains, cannot be due to variations in the experimental conditions from day to day, because they are also fully developed in tests performed simultaneously. It was thought unnecessary therefore to indicate which experiments were carried out at the same time. The strains are arranged in the table according to their origin (influenza, whooping-cough, etc.), but the experiments were made in a rather different order.

From considerations of space the strength of the reactions is given by the number of dilutions giving complete agglutination. For example, „2" signifies complete agglutination, + or ++, 1:50 and 1:100, „2+" complete agglutination in these two tubes and incomplete agglutination, (+), in 1:200. In some cases partial agglutination in the first two or three tubes is recorded; this is denoted by 1 and 1+ respectively, which is not quite correct but was rendered necessary on account of the statistical treatment. An error of real significance is hardly introduced in this way.

In the case of each strain it is stated whether it was „typical" (t) or „atypical" (at), and whether it formed indol (+) or not (0).

The homologous reactions are given in heavy type.

Cultures				Sera													
				I 6	I 35	I 57	I 91	I 21	I 33	I 34	I 35	I 36	I 37	P 22	Mel a	H 34	Pa 5
I 1	(a)	t	0	0	0	0	3	0	0	0	0	0	0+	0	0	0+	0
	(b)	t	0	0	0	0	3	0	0	0	0	0	0+	0	0	0+	0
I 5		t	0	0	0	0	0	0	0	0	0	1+	3+	0	0	0	0
I 6		t	+	**5**	0	0	0	0	0	0	0	0	0	0	0	0	1
I 20		t	+	0	0	1	2	0+	0	0	0	1	1	2+	2	2	1
I 21		t	+	0	2	2	2	2+	0	1	1	2+	2	3	2	2+	3
I 22	(a)	t	+	0	2	0	0	0	0	0	0	3+	2	2	0	0	0
	(b)	t	+	0	2	0	0	0	0	0	0	3+	1	1	0	0	0

Cultures			Sera 16	I 35	I 57	I 91	I 21	I 33	I 34	I 35	I 36	I 37	P 22	Me 1 a	H 34	Pa 5
I 23		t +	0	0	0	2	0+	0	0	0	0+	0+	0	0	0	0
I 27		t +	0	1	0	1	2	0	1	0+	1	2	2	0	2	1
I 29		t +	0	3	0	0	0	0	0	0	0+	4	0	0	0	2
I 35		t +	0	3!	0	0	0	0	0	0	3	2	1	0	0	2+
I 38		t +	0	3	0	0	0	0	0	0	3	2	0	0	0	2
I 57	(a)	t 0	0	0	3!	1	0	0+	0	0	0+	0	2+	1+	0	0
	(b)	t 0	0	0	3!	1	0	0+	0	0	0+	0	2+	1+	0	0
I 59		t +	0	0	0	2+	2	0	0	0	0	0	0	0	0	2
I 71		t 0	0	0	0	0	0+	0	1	0	0	1+	2+	0	0+	4
I 72		t +	0	0	0	0	0	0+	1+	0	1+	3+	3+	0+	0	2+
I 91	(a)	t 0	0	0	0	3!	0	0	0	0	0	0+	0	0	0+	0
	(b)	t 0	0	0	0	3!	0	0	0	0	0	0+	0	0	0+	0
I 100		t +	0	2	0	0	0	0	0	0	3+	3	2	0	0	0
I 103		t 0	0	1	0	,1	0	0	0	0	0	1	0	0	0	2
I 5		t +	0+	0+	0	0+	3	0	0+	0+	0+	3+	3+	0+	3	0+
I 21		t +	0	0	0	0+	3!+	0	0	0	0+	0+	0+	0	0	0
I 32		t +	0	0	0	1	1	2	3+	0	1+	2	0	0	0	0
I 33		t +	0	0	0	2	0	2!	0+	0	1+	1	0	0	0	0
I 34		t +	0	0	0	1+	0	0	3!+	0	1	2	0	0	0	0
I 36		t +	0+	1	0	0	0	0	0+	0+	3!+	3+	0+	0	0	0
I 37		t +	0	0+	0	0	0	0	0	0	1	5!	0+	0	0+	1
P 14		t +	0	0	0	0	0	0	1	0	2	2	0+	0	0	0+
P 22		t 0	0	1	0	0	1	0	0	0	0	2	4!+	0	0	0
P 23		t 0	0	0	0	0	0	0	0	0	0+	2	0	0	0	0
P 24		t +	0	0	0	0	1+	0	0	0	0	1+	0	0	0	0
P 25		t +	0	0	1	0	2	0	0+	0	0+	1+	1	0	2	0
Ms 1		at +	0	1+	0	0	0	0	0	0	0	0	2+	0+	0	0
Ms 4		at 0 +	0	0	0	0	0	0	0	0	0	1	2	0	0	0
Ms 9		t +	0	2	0	0+	0	0	0	0	2	1+	1	0	0	2
Ms 13		t +	0	2	0	1	0	0	0	0	2	1	0	0	0	0
Ms 15		t +	0	0	0	0	2	0	0	0	0	2	1	0	2	0
Me 1	a	t +	1	0	0	0	0+	0	1+	0	1	2	1+	2!	0	2+
	b	t +	0+	0+	0	0	0+	0	0+	0	1	1	1+	2	0	1+
H 19	(a)	t +	0	2	0	1+	1	0	0+	0+	2	0	1+	0+	0	0+
	(b)	t +	0	0	0	1+	0+	0	3+	0	1+	0	0+	0	0	0
H 34		t +	0+	3	2	2	1	0	1+	0	1	2	2+	0	2!+	0
H 49	b	t +	0+	1	0	0+	1+	0	1	0	1+	0	0	0	0+	2+
H 122		t +	0	2+	0	0	0	0	0	0	3+	2	1	0	0	0
H 127		at 0	0	1+	0	2	0+	0	0	0	2	2	1	0+	0+	0
H 133		t +	0	2+	0	0	0	0	0	0	3+	4	2	0	0	0
H 151		at 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H 152	(a)	t +	0	0	0	2	0+	0	2	0	0+	2	0	0	0	0
	(b)	t +	1	0	0	0	0	0	0+	0	0+	0+	1	0	0	2
H 154		t +	0+	2	0	0	0	0	1	0	2	0+	0+	0	0	1+
H 155		t +	0	0	0	0	1	0	1	0	0	3+	1	0	1	1

Cultures			Sera 16	I35	I57	I91	I21	I33	I34	I35	I36	I37	P22	Mela	H34	Pa5
H 156		t +	0+	2+	0	0+	1+	0	2	0	0	2+	2	0	1+	1
H 157		t +	0	0	0	0	0	0	0	0	1+	2+	0	0	0	0
H 159		t 0	0+	1	0	0	1	0	1	0+	1	2	1	0	1	2
H 164		t +	0	0	0	0	0	0	0	0	3	2	1	0	0	0
H 168		t +	0	0	0	1+	0	0	0	0	0	0+	0	0	0	0
H 176		at 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H 179		at 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H 197		t +	0	0	0	0+	0	0	0	0	0+	4	0	0	0	0
H 205		at 0 +	0	0	0+	1	1	0	1+	0	0	0	0	0	0	0
H 260		t +	0	1	0	0	0	0	0	0	3	2	0+	0	0	0
H 261		t +	0	0	2	0	2	0	1+	1	1	2	2	2	2	2
H 263		t +	0	0	0+	0	0	0	0+	0	2	1	1	0	0	0
H 264		t +	0	0	0	0	0	0	1	0	2	2*)	0+	0	0	0
H 265		t +	0	0	0	0	0	0	1	0+	2	2	0+	0	0	0
H 266		t +	0	0+	0	0	0	0	0+	0	2	2	0	0	0	0
H 268		t +	0	0	0	0	0	0	0	0	0	0+	2	2+	0	0
H 269		t +	0	0+	0+	0	0	0	0+	0	3	2	0+	0	0	0
H 272		t +	0	0	2	0	1+	0	0+	0	0	1	2	0	1+	1
H 274		t +	1+	0	0	0	0	0	0	0	2	2*)	0	0	0	2+
H 276		t +	0	0	0	0	0	0	0	0	0	2*)	1	0	0	0
H 280		t +	0	0	0	0	0	0	0	0	0	0	3+	0	0	0
H 287		t +	0	2	0+	0	0	0	0	0	0	2*)	1+	0	0	0
H 288		t +	0	0+	0	0	0	0	2+	0	3	2*)	0+	0	0	0
H 289		t +	0	0+	0	0	0	0	0+	0	3	2*)	0	0	0	0
H 291		t +	0	0	2	0	2	0	1	1	0+	0+	2	1	1	1+
H 294		t +	0	3	0	0	0	0	0	0	3+	2*)	0	0	0	0+
H 295		t +	0	0	1	0	1	0	1	0	0	1	1+	1	1	0+
H 296		t +	0	0+	0	0	0	0	0	0	3+	2*)	1	0	0	0
H 298		t 0	0	0	0	0	0	0	0	0	0	2*)	0	0	0	0+
H 299		t +	0	0	1+	1+	0+	0	0	0	0	2	0+	0	3	0
H 300		t +	0	2	0	0	0	0	0	0	2	1+	0	0	0	2
H 302		t +	0	1	0	0	0	0	0	0	2*)	2*)	0	0	0	0
H 306		t +	0	0	1	0	2	0	1+	0	1	1	0+	1	0+	2
H 307		t +	0	0	0	0	1+	0	0+	0+	0+	0+	1+	1+	1+	1+
H 308		t +	0	2	1	0	0	0	0	0	3	2	0+	0	0	0
H 309		t +	0	0+	0	0	0	0	0	0	3	2	0+	0	0	0
H 310		t +	0	0	0	0	0	0	0	0	3	2	0	0	0	0
H 311		t +	0	0	0	0	0	0	0	0	3	2+	0	0	0	0+
H 312		t +	0	0	2	0	2	0	2	0	0	2	2	0	2	1
H 314	a	at 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	c	at 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H 315		t +	0	1	0	1	0	0	0	0	0	0	0+	0	0	0+
H 316		t +	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H 320		t +	0+	0	0	0	2	0	0	0	0	1+	1	0	0	2
H 321		t 0	0+	0	0	1	3	0	1+	0+	0	2	2	1	0+	2

Cultures			Sera 16	I 35	I 57	I 91	I 21	I 33	I 34	I 35	I 36	I 37	P 22	Me 1 a	H 34	Pa 5
H 322		at	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H 323		t	+	0	2	0	2	1+	0	1+	0+	1+	2	2+	2	0	2
H 325		t	0	0	1+	0	0+	1	0	0	0	2	1	4	2	0	0
H 326		t	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H 328		t	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H 332	a	at	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	b	at	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0+
H 333		t	+	0	2+	0	0	0	0	0	0	0	0	1	1+	0	2
H 337		t	+	1+	0	0	0	0+	0	0	0	0	0	1+	0	0	2+
H 338		t	+	0	0	0	2	0+	0	1	0	1+	2	0	0	0	0
H 339		t	+	0	1	0	2+	2	0	2	0	0	2	0+	0	0	0
H 340		t	+	0	2+	0	0	0	0	2	0	2	3+	2+	0	0	2+
H 341		t	0	0	0	0	0	0	0	0	0	0+	1	0	0	0	1
H 342		t	+	0	0	0	2	1+	0	0	0	0	0	0+	0	0	0
H 343		t	+	0	0	0	0	1+	0	2	0	0	0	0+	0	0	0
H 344		t	+	0	0	0	1	0+	0	3	0	0	1+	0	0	0	0
H 345		t	+	0	2+	1	3	2+	0+	3	1+	2	2	3+	2+	1	2
H 346		t	+	0+	0	0	0	1	0	2	0	3	2	0+	1	0	2+
H 347		t	+	0	0	0	0	0	0	2	0	2	4	2	0	0	2+
H 348		t	+	0	1	0	0	0+	0	2+	0	2+	3+	2+	3+	0	3+
H 349		t	+	0+	1+	0	0	1	0	2+	1	2	2	2	2	0	2
H 350		t	+	0	0+	0	0+	0	0	0	0	1	0+	0	0	0	0
H 351		t	+	1	0	0	0	0+	0	0	0	1	1	0	0	0	2
H 353		t	0	0	2	0	0	2	0	2	1+	2	1	2	2	0	1+
H 354		t	0	0	0	0	0	0+	0	0	0	1	1	0	0	0	0
Pa 4		t	+	1	1	0	0	0	0	2	0	2	2*)	2+	2	0	2+
Pa 5		t	+	0	0	0	0	0	0	0	0	0+	3+	0	0	0	4
R 1		t	+	0	0	0	0	0	0	0	0	0	2	1	0	0	2
R 2		t	+	0	0	0	0	0	0	0	0	0	0	2	0	0+	2

From a perusal of this table we get a clear impression of the numerous differences in agglutination among the various strains of Pfeiffer's bacillus. The absence of agglutination in the majority of experiments does not depend on simple inagglutinability. This is clear in the first place because the strains with which sera were prepared both in the present experiment and throughout the whole investigation, always gave typical agglutinations with their homologous serum. As regards the strains with which serum was not prepared the majority were agglutinated to a greater or lesser extent by one or more of the 14 sera. Great differences in the sensitiveness

* Only examined in 1:50 and 1:100.

of the strains to agglutinin do not seem to be present. This follows from the fact that the strength of the heterologous agglutination of each serum converges towards the homologous agglutination as a maximum. Only exceptionally are the heterologous reactions observed to be stronger than the homologous (H 345 and H 348 with serum Me 1a; I 5 and H 299 with serum H 34). If there were great differences in agglutinability between the various strains we should expect more irregularity. Since the serum corresponding to a strain A does not always agglutinate a strain B to the same extent as the serum corresponding to B, agglutinates A, we cannot regard the strength of the heterologous agglutination in any particular case as an accurate measure of „agglutinative" relationship of the strains. But if we take a large number of reactions into account whereby chance irregularities will to a great extent be eliminated, there seems to be a good deal of justification for such a view (see also p. 193).

In the following description of a number of agglutinin absorption experiments certain symbols are used for the sake of brevity: (1) $S_X + Y$, signifies, a simple agglutination test with serum corresponding to strain X against strain Y; (2) S_X, $Z + Y$ signifies, absorption test with strain Z and S_X followed by agglutination with the „absorbed" S_X against strain Y. The concentrations of serum and culture can be added in parenthesis.

It was of particular interest to determine whether the strains which were derived from influenzal pneumonia in the same epidemic, were the same or different serologically. If Pfeiffer's bacillus is the primary influenza organism we should certainly expect such strains to be identical with one another.

At the time when the technique of the agglutination experiments was worked out only two of such strains from the epidemic 1918—19 were available. But the epidemic in the beginning of 1920 afforded utilisable though limited material, Pfeiffer's bacillus being cultivated from 6 autopsies on cases of influenzal pneumonia. As appears from the table, 5 of these were used for the preparation of serum (I 33, 34, 35, 36, 37). All these sera were tested against all the 6 strains in a special agglutination experiment with the following result.

	Sera (1 : 25, 1 : 50 etc.)				
Cultures	I 33	I 34	I 35	I 36	I 37
I 32	0000000	++++(+)00	0000000	++(+)0000	+++0000
I 33	++++000	(+)(+)00000	0000000	(+)(+)(+)(+)000	++00000
I 34	0000000	+++0000	0000000	++00000	+++0000
I 35	0000000	++00000	+++++00	+++0000	++++000
I 36	0000000	(+)(+)00000	(+)(+)00000	+++++00	++++(+)00
I 37	0000000	(+)000000	0000000	+++0000	++++++0

(No distinction is made here or in later experiments between different grades of complete agglutination).

The following absorption experiment (with serum 1:20, culture in a concentration 5, and subsequent agglutination in dilutions of 1:25, 50, etc.) was then made, using simple agglutination as a control.

S_{33} *) + 33 : +++(+)0
$S_{33,32}$ + 33 : +++(+)0
$S_{33,33}$ + 33 : 00000
$S_{33,34}$ + 33 : +++(+)0
$S_{33,36}$ + 33 : +++(+)0
$S_{33,37}$ + 33 : +++(+)0

S_{34} + 32 : ++++0
S_{34} + 34 : +++(+)0
$S_{34,32}$ + 34 : 00000
$S_{34,33}$ + 34 : +++00
$S_{34,34}$ + 34 : 00000
$S_{34,36}$ + 34 : +++00
$S_{34,37}$ + 34 : +++(+)0

S_{36} + 32 : ++(+)00
S_{36} + 34 : +++00
S_{36} + 36 : ++++0
S_{36} + 37 : ++(+)00
$S_{36,32}$ + 32 : 00000
$S_{36,32}$ + 36 : ++++0
$S_{36,33}$ + 36 : ++++0
$S_{36,34}$ + 34 : 00000

*) „I“ is omitted.

$S_{36,\ 34} + 36$: ++++(+)
$S_{36,\ 36} + 36$: 00000
$S_{36,\ 37} + 36$: ++++0
$S_{36,\ 37} + 37$: 00000

$S_{37} + 32$: ++0000
$S_{37} + 33$: +(+)0000
$S_{37} + 34$: ++0000
$S_{37} + 36$: ++(+)000
$S_{37} + 37$: ++++00
$S_{37,\ 32} + 32$: 00000
$S_{37,\ 32} + 37$: ++++0
$S_{37,\ 33} + 33$: 000000
$S_{37,\ 33} + 37$: ++++00
$S_{37,\ 34} + 34$: 000000
$S_{37,\ 34} + 37$: ++++(+)0
$S_{37,\ 36} + 36$: 000000
$S_{37,\ 36} + 37$: ++++00
$S_{37,\ 37} + 37$: 000000

The investigation is not altogether consistently carried out but proves with sufficient clearness that I 32, and I 34 are to be looked upon as identical, while the other 4 strains are different from them and from one another. As a control of the suitability of the absorption technique employed it will be noticed that the agglutinations according to the scheme $S_{x,}$ Y + Y are negative in all cases without regard to whether X and Y are the same or different. This control is not regularly included in these and the remaining absorption experiments, but so many tests have been made that it can be taken for granted that a strain in all probability will absorb its „homologous" agglutinin with the technique employed.

One exception however was observed when Pa 5 (28. VIII. 20) only absorbed its homologous agglutinin very slowly. At an earlier date however (I. XII. 19) its absorptive power was normal, as even in a concentration of 1 it practically completely absorbed the agglutinin from serum in a concentration of 1:20.

Absorption experiments were also made with the strains showing marked group agglutination in rather more than 100 combinations of serum and culture. In cases of doubtful absorption the experiment was repeated often several times, partly with renewed absorption, as mentioned on p. 183.

Further, about 40 of the oldest strains were tested as regards absorption against $S_{I\,6}$, $S_{I\,21}$, $S_{H\,34}$, and $S_{Pa\,5}$ in such a manner that 2—3 cultures at a time were mixed together and tested against each serum. If absorption took place the experiment was repeated with each culture.*

With regard to their power of absorption the strains can be divided into 3 groups: (1) The larger number did not absorb so much of the agglutinin that its disappearance could be demonstrated with certainty; (2) Some of them absorbed slowly, so that with one absorption about half the agglutinin was removed, and with repetitions of the process gradually the bulk of it disappeared; (3) Some absorbed the agglutinin about as easily as the homologous strain so that the major part of it was removed by one treatment.

As an instance of a slowly absorbing strain, 154 (experiment in December 1919) may be cited.

	$^1/_{200}$	$^1/_{400}$	$^1/_{800}$	$^1/_{1600}$	$^1/_{3200}$
$S_{I\,6}$ + I 6	+	+	+	0	0
$S_{I\,6}$ (1 : 160), I 54 (circ. 10) + I 6: after 1st. absorption	+	+	0	0	0
— — 2nd. —	+	0	0	0	0
— — 3nd. —	0	0	0	0	0

It would lead too far to enter further into details of the absorption experiments. The combinations of serum and culture only need be mentioned with which certain absorption could be observed. (Strains which were thus proved to be associated, will be referred to later as belonging to the same type).

Weak absorption was observed in the following experiments:
Serum I 6 with strain I 54,
Serum H 34 with strains I 20 and I 21,
Serum I 36 with strain H 264, H 265, H 266, H 288 and H 289.
Strong absorption was observed in:

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* The plate cultivations which resulted in the splitting of strains I 1, I 22 etc. into (a) and (b) had not yet been made at this time.

Serum I 6 with strain H 303,
— I 35 — I 29 and I 38 (I 35 and I 38 were cultivated from the same person at different times).
— I 91 — I 1
— H 34 — H 299
— I 34 — H 344
— I 36 — Ms 13, H 260, H 263, H 269, H 294, H 296, H 302, H 308, H 309, H 310, and H 311.

The property of absorption was only observed in the strains showing strong agglutination with the serum which was absorbed.

The spinal strains Me 1a, Me 2, Me 3 and Me 4 were thoroughly investigated by agglutination and absorption tests (using sera corresponding to the first three strains), which gave the following results.

In the ordinary agglutination test Me 1a, Me 3 and Me 4 were about the same, but Me 2 was rather different from the others

In the absorption test Me 4 absorbed the agglutinin for Me 1a and for Me 3, while Me 1a absorbed the agglutinin for Me 3, but Me 3 could not absorb the agglutinin for Me 1a. No relationship was found between Me 2 and the other strains by the absorption tests.

Me 5 was partially agglutinated in a later experiment by the three sera but the reactions were not clearly defined.

The meningeal strains therefore seem to be closely related without however being identical. The numerous group agglutinations between Me 1a and the strains of different origin prove that the meningeal strains are not sharply separated from other Pfeiffer's bacilli, but occupy a natural position in the great Pfeiffer's bacillus group.

The agglutinatory relationship between the meningeal and the other strains was further confirmed by agglutination tests with the 14 sera used in the large series of experiments, except I 36 against the strains Me 1a, Me 2, Me 3. All three strains showed group agglutination in variable degrees with the majority of these sera.

Concerning the constancy of the agglutination reactions the following may be said. The homologous agglutination reactions, as far as they have been investigated, have kept very constant apart from small irregular variations, in spite of

numerous subcultures. The same seems to apply on the whole, to absorption, but the material in this case is small. The variability is greater in heterologous agglutination. This applies to strains which have constantly been cultivated on haemoglobin (or FILDES) agar, as well as to those grown in symbiosis with different bacteria. The strongest group agglutinations are usually more stable than the weakest.

The sera do not seem to have become either more or less specific on keeping.

The most important result of the agglutination experiments reported here is the demonstration of the marked differences between the strains. The finding of strains with identical agglutination reactions is the exception which proves the rule. If identity could not be shown to exist at all we should be on very unsafe ground in talking of heterogeneity.

My results agree in all essentials with those reported from other quarters. One fact has been brought to light which does not seem to have been observed by other investigators, — namely the demonstration of the wide occurrence of a distinct type in a well-defined group of persons. In fact all the strains which were able to absorb agglutinin from serum I 36, except Ms 13, were found in the inoculations, in July 1920, from soldiers in the Jægersborg camp.

An investigation was made as to how the Pfeiffer's bacillus carriers of identical type were distributed in the camp. Two of them slept in beds side by side, and two others above them, but the remaining carriers of this type were irregularly distributed over this and three other barracks. The type we have to deal with here does not seem to be very sharply defined. As far as can be seen there exist gradual transitions from the rapidly absorbing strains through the slowly absorbing ones to those without this property.

It will be seen that strains belonging to the same type have, on the whole, the same agglutination „spectrum", by which is meant the complete picture of the agglutination reactions of the 14 sera. Perhaps, under certain conditions, it would be just as rational to describe different serological types by their „spectra" as by absorption reactions.

Complement Fixation.

BIELING & WEICHBRODT's observation that the Pfeiffer's bacillus strains they had investigated in complement fixation tests with horse serum, agreed with one another, naturally raises the question whether it might be possible with the help of complement fixation to collect Pfeiffer's bacilli serologically under one heading.

From the extremely well-marked agglutination differences this must be considered improbable. I have nevertheless carried out a number of complement fixation tests, not with horse serum it as true, but with the same rabbit sera as were used in the agglutination tests. Many of the tests failed on account of inhibition on the part of the sera or suspensions. A couple of series of experiments however gave reliable reactions and will be reported, as they are very instructive.

Fresh cultures suspended in salt solution were used in a concentration which a preliminary experiment had shown did not cause appreciable inhibition in the amount used in the experiment. In each reaction, serum (inactivated for half an hour at 56°), culture, and complement in a total volume of 0.3c.c. were mixed in a series of tubes. The serum was employed in falling quantities: 0.02, 0.01, 0.005 c.c. etc. The culture and complement were constant in amount, in the case of the later, 1½ times the minimum haemolytic dose. After remaining ¾ hour at room temperature and ¾ hour at 37° 0.2 c.c. of a 2½ % suspension of sheep corpuscles sensitised with 2½ amboceptor units, was added. The results were read after the tubes had been kept for 1—2 hours at 37°. The figures recorded represent the degree of haemolysis.

On 7. IV. 21 strain „I 6" was tested against the following sera:

I 6	0.	0.	0.	0.	0.	0.	0.	0.
I 57	0.	0.	0.	20.	40.	60.	80.	100.
I 91	0.	0.	0.	0.	0.	20.	60.	80.
I 21	0.	0.	0.	0.	0.	0.	60.	100.
I 33	0.	0.	0.	40.	100.	100.	100.	100.
I 34	0.	0.	0.	0.	0.	10.	60.	100.
I 35	0.	0.	0.	0.	20.	60.	100.	100.
I 37	0.	0.	0.	0.	10.	30.	100.	100.
Me 1a	0.	0.	0.	10.	40.	60.	80.	100.
Pa 5	0.	0.	0.	0.	0.	0.	0.	20.
Two normal rabbit sera	in each case 100.							

On 13. IV. 21 strain „I 37" was tested against the same sera:

I 6	40.	40.	100.	100.	100.	100.	100.	100.	100.
I 57	0.	0.	20.	60.	100.	100.	100.	100.	100.
I 91	0.	0.	0.	20.	40.	100.	100.	100.	100.
I 21	0.	10.	20.	40.	60.	100.	100.	100.	100.
I 33	10.	20.	60.	100.	100.	100.	100.	100.	100.
I 34	0.	0.	0.	0.	20.	40.	100.	100.	100.
I 35	10.	20.	40.	60.	80.	100.	100.	100.	100.
I 37	0.	0.	0.	0.	0.	0.	0.	0.	10.
Me 1a	0.	0.	0.	40.	100.	100.	100.	100.	100.
Pa 5	0.	0.	0.	0.	10.	20.	60.	80.	100.
Two normal rabbit sera	in each case 100.								

In both the experiments all the sera were tested in the first three quantities without the addition of the culture. There was no inhibition in any of the cases.

It will be seen that complement fixation, at any rate with the technique employed, brings out a more extensive relationship between the various strains of Pfeiffer's bacillus than agglutination did; but the lack of homogeneity of the material makes itself felt to a very marked degree here also.

For instance, serum I 6 reacts very strongly with its homologous strain but practically not at all with strain I 37, and strain I 6 reacts considerably more vigorously with sera I 21 and Pa 5 than with serum I 37, while the converse holds in the case of strain I 37.

We can therefore conclude, with the knowledge of the experiments brought forward, with tolerable certainty that the present material cannot be collected under one heading by complement fixation tests with monovalent rabbit serum. Furthermore, not only these but also the other complement fixation tests decisively indicate that there are even gradations from strong heterologous reactions to entirely absent reactions, so that no natural classification within the Pfeiffer's bacillus group can be undertanken.

Summary of the Characters of Pfeiffer's Bacillus.

We have investigated a collection of bacterial strains which were defined by the rules given on pp. 99—100, all the strains

being cultivated from man and a few from guinea-pigs. We will now enquire whether there are any grounds for believing that all these strains together constitute a natural group and whether this can be further divided into sub-groups. By a natural group is merely understood one which is formed by a rational classification, as explained on p. 98.

The questions which the previous part of this investigation should have made it possible to answer are the following: (1) Have all the strains examined so much in common that it as justifiable to place them in one group? In connection with this it must also be enquired to what extent the characters examined permit of a sufficiently sharp limitation of the group. (2) Are there gradual transitions in the group, or does it naturally fall into sub-groups? In order that the last question may be answered absolutely in the affirmative it is requisite that a decided jump between the different grades of several of the characters, be demonstrated, and that a constant connection between the various characters be present.

With these different questions in mind we will briefly review the characters that have been investigated.

That all the bacteria grow rapidly, and are markedly haemoglobinophilic, non-haemolytic, Gram negative rods, follows from the definition on pp. 99—100, where the results of the following investigation are also foreshadowed in so far as it was remarked that haemoglobinophilia, absence of haemolytic power, decoloration by GRAM's method and the rod-shape are each of them characters which allow of a sharp distinction from bacteria which behave in a different manner. As regards haemoglobinophilia the original definition must however be modified as follows: Complete absence of the power of growing on ordinary agar; the same is usually also the case on, as far as possible, blood-free ascitic agar, on which medium however a slight growth is not excluded. But it is required that in all cases the growth on such „haemoglobin" media, which on the whole have proved particularly suitable to Pfeiffer's bacillus, shall be far richer than on ascitic agar.

The symbiosis phenomenon has proved not to be an obligatory character of Pfeiffer's bacillus; this is excluded by the gradual transitions from strains which constantly give this reaction to a marked degree, through strains in which

it is less marked, to those in which, at all events in a single test, it may be entirely absent. Furthermore the same strain when repeatedly examined may at one time exhibit no „symbiosis“ and at another exhibit it to a more or less marked degree. In the majority of cases where the symbiosis reaction is present it must however be reckoned as a special characteristic of Pfeiffer's bacillus, as will be clear from a comparison with BORDET's bacillus and Bacillus haemoglobinophilus canis.

All the morphological and „cultural“ characters, as they are described in the chapters on symbiosis, growth on blood agar, form of colony, and microscopic morphology, together constitute a rather wide, but not unlimited, „series“ within which all possible transitions are to be found. Nevertheless a rough but useful division into „typical“ and „atypical“ strains can be made (see the scheme on p. 145).

Absence of spore formation is a constant character; this applies, for all practical purposes, to the absence of motility. All the strains examined were distinctly aerobic, by the method of investigation employed.

As regards the reaction of the medium, they behave practically the same as most other bacteria which are cultivated from man. Differences within the group could not be demonstrated.

The investigation of the minimum temperature for growth shows three things: (1) the group as a whole, has rather restricted boundaries as regards this factor; (2) each strain, at any rate to some extent, has its own temperature minimum; (3) there appear to be gradual transitions with regard to the situation of the temperature minimum.

The resistance against heating was always slight; but is should be pointed out that while the death-point, under the experimental conditions, for most of the strains can be taken to lie between 46° and 50°, a few strains were more resistant, and the fact that all were distinctly atypical is greatly in favour of the fact it is more than a chance experimental irregularity.

Slight resistance to drying and keeping of surface cultures which are subcultured must be regarded as a characteristic of the whole group. Doubtless there are individual differences between the strains in this respect also,

but it is difficult to make the experimental conditions so uniform that they can be more accurately defined.

Other common characters are as follows: poorly developed power of fermentation of all the carbohydrates and alcohols investigated; complete absence of dark coloration of medium containing arbutin; absence of the ability to liquefy gelatine, and serum coagulated by heat.

Agglutination and complement fixation exhibit great complexity, but, as far as can be seen, there are innumerable continuous gradations, so that there is no basis for a division into serological sub-groups.

In agreement with other authors I have found that Pfeiffer's bacillus may be fairly toxic (for rabbits), but my experience of the pathogenicity of this organism is too small and scattered for me to discuss it further.

While in all the tests, the results of which have just been shortly recapitulated, there is a series of smooth gradations in the group, the indol test forms a marked exception, as the result of this test divides the strains into two sharply defined groups: indol-producers and non-indolproducers.

The question now arises whether this is the expression of a fundamental difference between strains of Pfeiffer's bacillus, and one which therefore must make itself felt in the other characters of the strains, or is it to be regarded as an isolated phenomenon without obvious connection with other facts?

The reactions most used in the classification of bacteria in the smaller groups are the serological ones, particularly agglutination. We will therefore first investigate whether any relation can be shown to exist between the indol reaction and agglutination.

Even a casual glance at the large agglutination table will show that indol-positive and indol-negative strains are not sharply distinguished from one another by agglutination, strains belonging to the one group often being agglutinated by sera corresponding to the other. To further investigate the problem the number of group agglutinations of different grades among indol-positive and indol-negative strains for each serum are given in the table below „0+", „1+", etc. being denoted

by „½“, „1½“, etc. The percentage of group-agglutinating strains and the average strength of the group agglutination among all the strains is also calculated.

For instance the table shows that serum I 6 does not agglutinate 56 out of 71 indol-positive strains, agglutinates 9 in a strength of ½, 4 in a strength of 1, and 2 in a strength of 1½; that the 15 which were agglutinated constitute 21% of the total 71 and that the average strength of the group agglutination for all the 71 was 0.16.

In the calculation the following strains were omitted: I 1(a), I 1(b), I 20, I 21, I 22(b), I 29, I 38, I 57(b), I 91(b), Ms4, Ms13, Me1b, H205, H260, H263, H264, H265, H266, H269, H288, H289, H294, H296, H299, H302, H308, H309, H310, H311, H314c and H344, on the ground that only one strain belonging to each agglutination type should be included, otherwise the types in which several strains were found would be too strongly represented. Strains with inconstant indol reaction are also left out. Lastly the homologous reactions are not included.

	Sera	Strains																																	
		Indol-positive																		Indol-negative																
		0		½		1		1½		2		2½		3		3½		4	%	Average	0		½		1		1½		2		2½		3		3½		4	%	Average
Indol-positive	I 6	56		9		4		2											21	0.16	19		2													10	0.05
	I 35	44		3		7		2		8		6		1					38	0.64	16				3		1		1							24	0.31
	I 21	37		8		6		8		10		1		1					48	0.67	15		2		2				1		1					29	0.38
	I 33	69		2															3	0.01	20		1													5	0.02
	I 34	36		8		8		7		8		2		1		1			49	0.70	17				2		1		1							19	0.26
	I 35	61		6		3		1											14	0.11	18		2				1									14	0.12
	I 36	27		10		9		7		10		1		3		4			62	0.99	13		3		2		1		2							38	0.43
	I 37	15		9		5		6		23		2		1		7		3	79	1.51	8		1		5		1		5				1			62	0.98
	Me 1a	54		4		4		2		4		2				1			24	0.36	18				1		1		1							14	0.21
	H 34	54		4		4		3		5				1					24	0.33	17		3		1											19	0.12
	Pa 5	32		5		7		3		14		9				1			55	0.96	14		2		1		1		2						1	33	0.55
Indol-neg.	I 57	60		2		3		1		5				1					15	0.26	21															0	0 00
	I 91	48		7		2		4		7		2		1					32	0.47	17		1		3											19	0.17
	P 22	18		12		12		6		13		6				4			75	1.15	15				1				2		2				1	29	0.67

By an „indol-positive“ serum is meant one corresponding to an indol-positive strain.

As far as can be observed from these results there is no connection between the indol reaction and agglutination. Some sera agglutinate heterologous strains only to a very limited

extent and this applies in the same manner to indol-positive and indol-negative strains; other sera agglutinate numbers of strains irrespective of whether they are indol-positive or indol-negative.

Of the 11 „indol-positive" sera 9 gave on an average better group agglutination with indol-positive strains than with indol-negative, but with two sera the converse was the case. All the 3 „indol-negative" sera agglutinated indol-positive strains to a greater extent than indol-negative.* If there is anything to be learned from the table it is that on the whole, indol-positive strains are agglutinated more readily than indol-negative both by „indol-positive" and „indol-negative" sera, but any correlation between agglutination and indol formation cannot be presumed from it.

We might think that both the agglutination and the indol reaction would form an adequate basis for a rational classification. But as no relation between them can be demonstrated, at least one of them must be rejected for such a purpose. It is probable that neither of them is sufficient for a natural grouping (that is to say, one that is founded on all the characters).

A connection between indol formation and the slight differences in the degree of haemoglobinophilia could not be found; nor between the former and the temperature minimum for growth or resistance against heating.

But a certain relation could be shown to exist between the indol reaction and typical or atypical growth, in that almost all the atypical strains which were tested for indol formation gave a negative reaction.

This was the case with H127, 151, 158, 161, 171, 176, 178, 179, 181, 205, 314a, c, 318, 319, 322, 330, 332a, b, 335, 336, GP1, 2, 3, 4a, b, 5; only H314b and d and 324 gave a positive reaction.

In contrast to this nearly 90% of the typical strains formed indol. The connection between kind of growth and the indol reaction is therefore very marked. Since the distinction between

* It would not cause any change here if the error introduced by the incomplete titration of some of the samples was corrected (see pp. 186—187).

typical and atypical growth in some cases is difficult to estimate, it might be thought that the indol-positive among the atypical strains should perhaps have been called typical, and the indol-negative among the typical, called atypical. This however is excluded. H314b and d were very markedly atypical, the growth being relatively poor, and very friable, while symbiosis was weak and inconstant. On the other hand most of the indol-negative among the „typical" strains were very decidedly typical both macroscopically and microscopically.

Atypical strains usually grow worse than „typical". It might therefore be thought that the absence of indol formation in the former was simply due to insufficient growth. RHEIN, for instance, states that Pfeiffer's bacillus only forms indol when there is a good growth.

This explanation however, is not probable. Obviously a certain amount of bacteria must be present in order to form a detectable quantity of indol, but in my experience Pfeiffer's bacillus (in liquid media) can produce a very marked indol reaction even when the growth is hardly visible. Of the 4 strains H314a, b, c, and d, which, on solid media, all grew poorly and about the same, indol could be demonstrated in a and c but not in b and d. In carrying out the indol test in the same way as RHEIN, I obtained considerably weaker reactions than by the method I usually employed.

Not only do the typical and atypical growths show a relation to the indol reaction but also to various other phenomena. As previously mentioned the few strains which were particularly resistant to heat, were all atypical. No connection could be shown to exist however, with the minimum temperature for growth.

Regarding the agglutination of the atypical strains it should be noted the reason they are so scantily represented in the table of agglutination tests is because a large number of the atypical cultures which it was desired to test, either could not be made into homogeneous suspension or were spontaneously agglutinated. Of the remainder, several did not react at all with any of the 14 sera, while nearly all the typical strains reacted with one or more of them. Some of the atypical strains however reacted without any definite „spectrum" being predominant. A closer investigation of the agglutination relations between different atypical strains would necessitate their being used in the preparation of the sera besides to 14 typical ones, which was not done.

The atypical strains on the whole possess a slighter „vitality“ than the typical, as they die out more easily during repeated subculturing. This is in agreement with the fact mentioned on p. 127 that in cultures of typical and atypical bacilli of the same age, the latter usually contain far fewer bacilli capable of developing than the former. In accordance with this it is natural to regard the abnormal and pale-stained elements of which the main portion of atypical cultures often consist, as degenerate forms.

We see therefore that the distinction between „typical“ and „atypical“ appears to stand for a fundamental difference, since a relation between this classification and several of the various principal characters of Pfeiffer's bacillus can be demonstrated.

The next correlation which interests us is the relation between the occurrence of Pfeiffer's bacillus and its other characters.

It has been repeatedly stated that in influenza and, perhaps hardly to the same extent, in whooping-cough, the majority of Pfeiffer's bacilli met with are typical, while the atypical are chiefly obtained from normal persons, (and from guinea-pigs). It may be added that the great variations in the occurrence in healthy persons chiefly — perhaps exclusively — refer to typical Pfeiffer's bacilli. It could not be expected to be otherwise in view of the connection between influenza epidemics and the distribution among healthy persons. This connection has a natural explanation in the fact that the same bacilli which first spread among influenza patients, afterwards extended to healthy individuals, and thus we cannot expect to find characteristic differences between the bacilli from the two different sources. Other explanations are however feasible and it will therefore not be amiss to compare the characters of Pfeiffer's bacillus from influenza, from other diseases, from healthy persons, and from guinea-pigs. Particular attention must be bestowed upon seeking for a difference between Pfeiffer's bacillus from influenza and whooping-cough. If no such difference can be detected in the material before us there will be still less cause than hitherto to distinguish between Bacillus pertussis Eppendorf and Pfeiffer's bacillus.

As the difference in the occurrence of the typical and

atypical strains has already been sufficiently established, we will confine ourselves to comparing the typical strains from different sources. It will be easily perceived that no connection among the typical strains can be demonstrated between the habitat on the one hand, and the dependence upon haemoglobin, the macroscopic and microscopic morphology of the culture, the minimum temperature for growth, the resistance against heating, and indol production etc. on the other hand. A similar statement could be prepared with regard to agglutination, as has been done in the case of the indol reaction. This however would hardly be worth while. It will be observed that agglutination between the different groups (influenza, whooping-cough, meningitis, and healthy persons) is practically just as common as between strains and sera belonging the the same group. The only difference is that positive reactions among the strains from healthy persons do not occur quite as frequently as among strains from influenza etc. This is easily explained on the assumption that besides the atypical forms others may be met with in healthy persons which do not usually occur in influenza.

(I did not succeed in making suspensions of the guinea-pig strains which could be used for agglutination. Otherwise no important difference could be found between these and the atypical strains from man).

It would naturally be interesting to find out whether the „mutations" observed in several strains and incidently already referred to, are genuine, or are due to contamination; but as previously said this would involve a very difficult piece of work from a technical point of view which the main portion of the investigation did not leave time for.

The most important phenomena will however be briefly mentioned.

Among the strains, numbering about 60, of which plate cultures were made with a view to investigating the form of colony, it was usually observed that all the colonies of a single strain which were so remote that they were not appreciably influenced by their neighbours, were practically of the same appearance. In cases however, two distinctly different types of colony were observed on the same plate, (a lighter and a darker, with or without

a „fringe", and of different sizes etc.). Subcultures were made from one colony of each kind. These subcultures were denoted by the addition of (a) and (b) to the original number of the strain in question (I 1, I 22, I 57, I 91, P 15, H 19, H 152). In new subcultures some months later from (a) and (b) cultures there was no longer any difference however, in the appearance of the colonies of 4 of the strains, nor as regards agglutination. The (a) and (b) subcultures of the remaining strains (I 91, H 19, and H 152) were still different as regards form of colony or agglutination, or both. There was no difference in the indol test between the two cultures.

It was further remarked that in several strains the culture consisted of light and dark portions, arranged in sectors, and separated from one another by sharp boundary lines. The granulation structure extended quite unaffected through the light and dark sectors. From one such culture, plate cultures were made; a mixture of light and dark colonies appeared on the plates. No difference could be made out in agglutination tests combined with absorption between cultures from the light and dark colonies.

We have now arrived at the end of the analysis of the bacterial group which — hitherto as a working hypothesis — we have included under the general heading „Pfeiffer's bacillus". We are confirmed in the opinion that all these strains ought to be placed in the same group by the existence of a series of common characters which bind them together, but which does not exclude the group being somewhat heterogeneous in several directions. But we have only observed a distinct discontinuity in one property, namely the indol test. This discontinuity cannot however disrupt the group because the necessary correlation with other characters is wanting. The group can be split up in a more rational manner, into the two sub-groups, typical and atypical Pfeiffer's bacilli, which however are associated with transitions forms.

It is principally the „inner structure" of the Pfeiffer's bacillus group that we have examined so far. We will now regard the group „from without", by comparing it with three different related bacterial types, the haemolytic haemoglobinophilic bacteria, BORDET's whooping-cough bacillus, and Bacillus haemoglobinophilus canis.

A Comparison between Pfeiffer's Bacillus and other „Haemoglobinophilic Bacteria".

1. Haemolytic, haemoglobinophilic bacilli.

Haemolytic, haemoglobinophilic bacilli were first described by PRITCHETT & STILLMAN and afterwards by STILLMAN & BOURN, RIVERS & LEUSCHNER and BLOOMFIELD (2). These authors describe, in a fairly unanimous manner, these organisms as Gram negative rods which as a rule, though not always, are coarser than Pfeiffer's bacillus and more inclined to form threads. On blood agar they produce clear haemolysis in the same way as typical haemolytic Streptococci. Like Pfeiffer's bacillus they are haemoglobinophilic and their growth is promoted by other organisms. They only have a slight power of fermenting the various carbohydrates. Only a few of them produce indol. In cultures which are not sub-cultured, they die out still more rapidly than Pfeiffer's bacillus. They are principally met with in healthy persons and do not appear to be pathogenic for man or animals.

Through using media on which haemolysis cannot show itself e. g. „chocolate agar", they have undoubtedly often been confused with Pfeiffer's bacillus. RIVERS & LEUSCHNER consider that in future they ought to be regarded as belonging to the group of Pfeiffer's bacilli. There is general agreement that in many respects they resemble them very much.

I, also, have found these bacteria widely distributed and exclusively in healthy persons. They were probably present in the earlier inoculations, but on account of the haemolysis produced they were not investigated further. In the inoculations of 6. III. 20. haemolytic, well marked haemoglobinophilic bacteria were discovered in 16 out of 200 persons examined.

In the inoculations on 14. IX. and 15. X. 20. they were also found but they were not investigated further. In the inoculations on 6.—10. IX. 21. about $^2/_3$ of the 452 plates were examined for these bacteria; they were found in 16 cases. On 17. IX. 21 they were found in 30 out of 230 persons. Further investigation of the strains from the last two series of inoculations showed that on the whole they were decidedly less haemoglobinophilic than Pfeiffer's bacillus. (The reason this was not noticed in the investigation of the 1920 strains, is presumably due to the fact that only the most haemoglobinophilic strains were taken into account and that a direct comparison between these and Pfeiffer's bacillus was not made). In the later investigations Pfeiffer's bacillus and the haemolytic ones were inoculated simultaneously on agar plates. It was noted (without knowledge of which strains were haemolytic) which cultures gave perceptible growth in the first cultivation on this medium and it was found that they were practically identical with the haemolytic. As Pfeiffer's bacillus and the haemolytic bacilli were thus examined under precisely the same conditions there can be no doubt that the latter really behave rather differently from Pfeiffer's bacillus as regards dependence upon haemoglobin. It was only possible to cultivate some of the haemolytic organisms further on agar; in the third culture on this medium only a few strains grew. In a number of cases only one colony appeared which however was sometimes rather large. The addition of ascitic fluid to the agar did not favour the growth of these bacilli in any of the cases, on the contrary it inhibited it so much that there was usually no growth on ascitic agar in the first culture.

The keeping properties of the haemolytic strains, in my experience, are also distinctly less than those of Pfeiffer's bacillus, a fact which makes it difficult to define more accurately the undoubted differences in the dependence upon haemoglobin between Pfeiffer's bacillus and the haemolytic bacilli as well as between different strains of the latter. In this group there seem to be gradual transitions from strains which are very haemoglobinophilic to strains which can grow fairly well without haemoglobin.

Haemolysis is a character which sharply distinguishes these

bacteria from Pfeiffer's bacillus, but only when a suitable medium is employed. On blood agar with 20—25% blood it may sometimes happen that the growth is too feeble and the amount of blood corpuscles to be dissolved, too large to give distinct transparency. By employing ordinary FILDES agar with the addition of 5 or 10% blood the haemolysis was always extremely marked. Only one strain failed to give marked haemolysis before the lapse of a couple of days and the clear margin remained narrow. In all the other cases there was complete haemolysis after 24 hours. In agreement with the American authors I found this haemolysis closely resembles that around colonies of the true haemolytic Streptococci. The blood agar beneath the culture and in a narrow, outwardly fairly sharply defined border around it, is completely transparent and partially decolorised.

Haemolysis is moreover a constant character. It has already been mentioned on p. 139 that a large number of haemoglobinophilic non-haemolytic bacilli (that is to say Pfeiffer's bacilli) had in no case acquired the power of haemolysing after many subcultivations, which could be demonstrated on ordinary blood agar. In October 1921 inoculations were made on the recently mentioned improved blood medium, of about 70 strains of Pfeiffer's bacillus, the oldest of which had been cultivated for more than 3 years, together with 7 strains of haemoglobinophilic bacteria which had been isolated about a year earlier and were then haemolytic. In the new investigation none of the Pfeiffer's bacilli showed haemolysis, while the originally haemolytic strains had all preserved this property. All the haemolytic strains from the inoculations on 17. IX. were kept until 1. XI., when they were again examined for haemolysis. The result was exactly the same as in the first investigation. Both times one of the strains (the one referred to above) gave slow and comparatively weak haemolysis, while all the others even after 24 hours' growth showed very marked haemolysis.

With regard to „symbiosis" my haemolytic strains behaved very differently. In some this character was entirely lacking, but in the majority it was present to a marked degree.

The macroscopic appearence of the cultures was as a rule „atypical"; in some cases the consistence was friable, more

often tenacious. Not uncommonly the appearance and consistence of the cultures however could best be described as typical. On blood agar the growth was always considerably heavier than in the case of Pfeiffer's bacillus and of a more whitish appearance by reflected light.

The appearance of isolated colonies differed a good deal. In some cases they could not be distinguished from Pfeiffer's bacillus, but they were more often darker, flatter and drier. Granular formation occurred chiefly in those colonies which most resembled Pfeiffer's bacillus colonies.

The microscopic morphology of all the strains, a number of which were examined several times, must be described as distinctly „atypical", that is to say that coarse forms and threads were predominant. In most cases the elements were coarser than we occasionally observe in Pfeiffer's bacillus (see plate 3). In other cases the microscopic picture however was indistinguishable from that of microscopically atypical Pfeiffer's bacilli. The spherical bodies were often well developed but scarcely differed from the corresponding bodies in Pfeiffer's bacillus.

A few strains were examined for anaerobic growth, gelatine and serum liquefaction, glucose fermentation, minimum temperature for growth, thermal death-point, and resistance against drying, but they did not differ from Pfeiffer's bacillus in any of these respects.

18 strains were examined for indol formation, with 4 negative results. Agglutination tests (1:50 and 1:100) with 3 of the haemolytic strains and 14 Pfeiffer's bacillus sera were all completely negative. Some other strains agglutinated spontaneously.

We must therefore regard the haemolytic haemoglobinophilic bacteria as a group which is closely allied to Pfeiffer's bacilli, but can be easily distinguished from them, first of all by the haemolysis itself, which is a very appropriate character common to the group, because (1) it is constant; (2) it is either absent, or present to a marked degree; (3) practically all the strains examined haemolyse in exactly the same manner. Further the group as a whole, though not always the individual strain, is characterised by a series of other common qualities which distinguish it from Pfeiffer's bacillus (occurrence, rela-

tion to haemoglobin, microscopic morphology, tendency to die out easily).

Although these bacteria are called „haemolytic, haemoglobinophilic bacteria" the latter adjective is not quite correct, especially because we ought certainly to include in this group strains which can grow quite well without haemoglobin. The reason for keeping the term nevertheless, is partly because the majority of the strains must be termed haemoglobinophilic, and partly because the group is identical with that described by other authors in a similar manner.

2. Bordet's Whooping-Cough Bacillus.

Although the bacillus described by BORDET & GENGOU in 1906 as the organism of whooping-cough, ought to be regarded, on the basis of their discovery and the work of later investigators (which will not be reviewed here), as a well defined bacterial species distinct from Pfeiffer's bacillus, it appears from comments made by BELFANTI, ANDREWES, and RIVERS (1) that this conception is still far from being generally held. I have therefore thought it not superfluous to subject this question to a fresh investigation on a large scale.

The extensive material relating to the whooping-cough bacillus which the diagnostic investigations of the State Serum Institute started at the instigation of MEYER & CHIEVITZ's (1—5) work, has constantly placed at my disposal, made it easy to examine a large number of strains of this bacillus from various points of view and thus obtain a solid basis for a comparison between it and Pfeiffer's bacillus. It should first of all be remarked that the investigation of these whooping-cough bacillus strains has not produced anything really new with regard to this organism, but on account of the large number of strains examined it has further established its characteristic qualities and the by no means few points where it is markedly different from the whole group of Pfeiffer's bacilli.

The differences between the two species of bacteria upon which most stress has hitherto been laid, are the serological ones. From the investigations of ODAIRA, INABA, SHIGA, EMAI, & EGUCHI, WINHOLT, OLMSTEAD & POVITZKY and others it

appears firstly that there is a marked difference between BORDET's bacillus and Pfeiffer's bacillus both as regards agglutination and complement fixation, and secondly that the different strains of BORDET's bacillus usually resembled one another fairly well.

As in my investigations strains of Pfeiffer's bacillus were found to be distinctly different from one another serologically, it was taken for granted to begin with that at any rate most of the strains in this group must also be serologically distinct from whooping-cough bacilli, and this aspect of the case was therefore only superficially investigated.

A rabbit serum prepared by the injection of whooping-cough bacilli agglutinated 4 different strains of the whooping-cough bacillus at 1:10000, while it agglutinated 4 strains of Pfeiffer's bacillus (from healthy persons) at most at 1:25 and incompletely at 1:50. At the same time the 4 same strains of each species of bacterium were tested as regards complement fixation with sera from 3 whooping-cough patients, and 3 control sera from other persons. Each of the 3 whooping-cough sera gave complement fixation with the 4 whooping-cough cultures, and all the strains reacted practically equally with the same serum. But none of the strains of Pfeiffer's bacillus gave a stronger reaction with the 3 whooping-cough sera than with the 3 control sera.

It was of greater interest to compare a number of whooping-cough strains one with another, particularly by agglutination, for if decidedly more uniform relations were found than among Pfeiffer's bacillus, it would indicate that the great differences observed among the latter could not be accounted for by special peculiarities of the technique employed.

Agglutination experiments were undertaken with the rabbit serum referred to, against 75 strains of the whooping-cough bacillus in dilutions of 1:2500, 3500, 5000, 7000, 10000, 14000, 20000 and 28000. Cultures grown on a mixture of equal parts of BORDET's potato glycerin agar (see BORDET & GENGOU), ordinary peptone broth agar and horse blood were employed. For the serum dilutions and the suspensions of the cultures 0.3% salt solution with the addition of $1^0/_{00}$ formalin was used. Each strain was further tested with serum corresponding to Pfeiffer's bacillus I 6, and with pure formalin salt solution. In these two control tubes there was weak agglutination in 3 cases only and then merely in one of the two. The experiment was arranged in 3 parts. Some sera which on first

examination gave comparatively weak reactions, were again tested in the last part of the experiment.

Of the 75 strains, 10 were agglutinated at 1:10000, 20 at 1:14000, 37 at 1:20000 and 8 at 1:28000. A small number of these are mean values of two experiments with the same strain. In a few cases the agglutination titers of the two tests varied as much as 1:7000 and 1:20000. Four of the experiments recorded are repetitions of experiments which gave only slight agglutination even in the stronger serum concentrations; no special divergencies were found in the case of these strains in the repetition of the test.

Al the strains were also investigated in an earlier experiment which on account of technical deficiency, will not be further discussed; the strains which in the experiment recorded only gave comparatively weak reactions, did not depart in this experiment from the others with regard to the strength of the agglutination.

Later, a further 26 strains of the whooping-cough bacillus were agglutinated by the same serum with the same results.

In the agglutination experiments with the whooping-cough bacillus no certain difference could therefore be observed between any of the strains.

The 75 strains, with the exception of one which had died out, were then examined in the following way.

On inoculation on blood agar the strains that had only been grown a comparatively short time, gave a little weaker growth than the others, but otherwise no difference whatever in the macroscopic appearance of the cultures, which will not be described in detail here, could be seen. It need only be remarked that none of the strains had a tendency to spread over the surface as is so often seen in Pfeiffer's bacillus, and that I have never met with any pure culture either of Pfeiffer's bacillus or other bacteria which by comparison with a growth of the whooping-cough bacillus on blood agar could be mistaken for the latter. All the strains grew distinctly more slowly than Pfeiffer's bacillus. In all cases there appeared, after a couple of days growth, a relatively extensive ill-defined haemolysis of the blood agar, very different in appearance from that of the „haemolytic, haemoglobinophilic bacilli".

On microscopic examination all the strains were decidedly Gram negative. The one that had been cultivated longest in vitro, namely for about a year and a half, and with which the serum mentioned was made, was distinctly rod-shaped.

The others were on the whole ovoid, sometimes oblong and sometimes almost spherical. In a number of the cultures scanty thread-shaped elements were observed, of moderate length and usually rather thick, and also transitions between these and chains of the ordinary ovoid bacilli. Sometimes ovoid elements of unusual size were seen. Marked polar staining was only exceptionally observed. On the whole however these strains as well as several hundreds of other strains of the whooping-cough bacillus have shown that the morphology of this organism is far more uniform than that of Pfeiffer's bacillus. The ovoid form is very characteristic of the whooping-cough bacillus, but given an individual preparation one cannot be certain from the microscopic appearance alone whether one has a whooping-cough bacillus or a Pfeiffer's bacillus to deal with.

On inoculation on ordinary agar none of the strains gave the slightest growth.

On haemoglobin agar only the 3 oldest strains grew, but the growth gradually became rather good. Some strains were tested for growth on FILDES agar with negative results. The oldest strains grew on LEVINTHAL agar (November 1921) but the younger one did not, even after repeated attempts. On „chocolate" agar both the young and old strains grew though not so well as on blood agar. Pfeiffer's bacillus which was inoculated at the same time as the whooping-cough bacillus on the four last-named media, grew excellently.

That it was the oldest strains that grew on haemoglobin agar was no mere chance. It is a known fact that the whooping-cough bacillus can be trained to grow on ordinary agar (BORDET & SLEESWYK and others), and that on the whole it becomes less „fastidious" after a long period of cultivation. Thus I have observed that by subculturing daily for a long time the cultures acquire a considerably increased energy of growth. The reason these facts are not very prominent in the present material, is undoubtedly due to the fact that the strains have only been subcultured with a 3—4 week's interval and after incubation they were kept in the cold room. The power of adaptation is a property that always distinguishes the whooping-cough bacillus from Pfeiffer's bacillus which preserves its growth energy and its dependence on haemoglobin

so constant even on cultivation for some years that I have never detected any changes in these factors. (According to Tocunaga, Gosio (3), Wyard, and Cohen & Fitzgerald, a certain, very limited, adaptation can however take place). This power of adaptation is naturally no obstacle to identifying the whooping-cough bacillus by the kind of growth it produces after a few subcultures subsequent to its isolation in pure culture from man.

On a s c i t i c a g a r all the whooping-cough bacillus strains grew about as well as on blood agar. The whooping-cough bacillus cannot therefore be regarded as haemoglobinophilic in the true sense.

S y m b i o s i s t e s t s were made with all the strains on ordinary ascitic agar and on ascitic agar with the addition of haemoglobin, both with the air coccus and a haemolytic Streptococcus. In no case was the growth of the whooping-cough bacillus influenced in the least by the other bacteria. It might be thought that the growth on ascitic agar in pure culture was too good to allow the symbiosis effect to show itself, and also that other species of bacteria might produce it. A medium of ordinary agar containing a few per cent of blood was therefore prepared; the mixture was kept for half an hour at 55°—60° (which made it rather dark coloured). On inoculation of 79 whooping-cough bacillus strains in streaks on this medium a fairly good growth appeared which however could certainly not be called the maximum. As growth-promoting organisms 24 different species of cocci, bacilli and vibrios were used, each of which were tested with 3 or 4 whooping-cough bacillus strains. At a few places a slight inhibition of the growth of the whooping-cough bacillus was observed close to the foreign colonies. In two only of the whooping-cough strains did there seem to be a mild growth-promoting effect, but on repeating the experiment with these two strains with the same „growth-promoting" organism not the slightest effect was observed.

Among other ways in which the whooping-cough bacillus is constantly different from Pfeiffer's bacillus may be mentioned the greater r e s i s t a n c e of the culture against keeping. I have regularly obtained a rich growth by subculturing from

the whooping-cough cultures on BORDET's potato glycerine blood agar after a month's keeping in the cold room.

All the facts mentioned prove that BORDET's bacillus is distinct from Pfeiffer's bacillus in a series of perfectly constant properties and also in that the various strains of the former species are more uniform than in the case of Pfeiffer's bacillus.

It must also be asserted that the „cultural" characters are at least as useful in the detection of the whooping-cough bacillus as the serological tests.

3. Bacillus Haemoglobinophilus Canis.

As a further test whether the methods used in the investigation of Pfeiffer's bacillus permit a clear distinction between this group and closely allied species, I procured some strains of the organisms which, according to the works of FRIEDBERGER and KRAGE, occur very commonly in the widely distributed blenorrhoea of the prepuce in dogs.

They are described as Gram negative rods which grow on blood agar in small colonies of similar appearance to those of Pfeiffer's bacillus and which do not grow on ordinary or ascitic agar, even after a year's cultivation on artificial media. According to FRIEDBERGER they are able, in contrast to Pfeiffer's bacillus, to grow on agar containing ferratin instead of haemoglobin, and they are satisfied with a particularly small concentration of haemoglobin. KRAGE obtained weak growth in broth containing sugar, which accords well with his statement that it turns haemoglobin broth acid. It is apparently able to ferment and make use of the sugar. It is also stated that the growth is not increased by other bacteria. FRIEDBERGER found that the culture decolorised haemoglobin but according to KRAGE this was not the case. On keeping they died out just as quickly as Pfeiffer's bacillus.

ODAIRA compared one strain of Bacillus haemoglobinophilus canis with two strains of Pfeiffer's bacillus and found them to be serologically identical.

Thanks to Prof. CARL HANSEN's kindness I was able (5. VII. 20) to undertake inoculations from the inner surface of the

prepuce of 15 dogs. From each dog a blood agar plate and a FILDES plate were inoculated. From 9 of the 15 dogs colonies appeared usually in large numbers which grew rather more slowly than Pfeiffer's bacillus and in contrast to these, better on blood agar than on FILDES agar. After 3 days' incubation the colonies on blood agar were about 2 mm. in diameter, of a homogeneous grey colour, and almost soft though little tenacious in consistence. They departed considerably in appearance from Pfeiffer's bacillus. On FILDES plates the colonies had the appearance of small Pfeiffer's bacillus colonies. In several cases well developed granulations were seen. On microscopic examination Gram negative rods were observed, usually rather polymorphic, varying from coccus-shaped forms to rather long curved rods.

They failed to grow on ordinary and ascitic agar but they could easily be cultivated on the same media as Pfeiffer's bacillus. Decolorisation of the blood pigments was never observed either on solid or liquid media.

They were also compared with Pfeiffer's bacillus in a number of different ways.

They d i d n o t d i f f e r from Pfeiffer's bacillus in the following points.

1. In the dependence upon haemoglobin. Like Pfeiffer's bacillus they grow well on blood agar, haemoglobin agar (with a liberal amount of haemoglobin), FILDES agar, LEVINTHAL agar, and „chocolate agar", but not at all on ordinary and ascitic agar.

On 1. XI. 21. — after they had been grown for 16 months — all the 9 strains were inoculated on agar containing 1% ferratin (prepared from pig's liver according to SCHMIEDEBERG's directions). Neither these strains nor three of Pfeiffer's bacillus grew.

On inoculation of the same 12 strains on agar with gradually diminishing content of pepsin-digested blood practically no difference could be observed in the „haemoglobin" requirements of the two bacterial groups. None of them grew on „haemoglobin-free" agar.

2. They do not produce haemolysis on blood agar. This was tested repeatedly, the last time in Nov. 1921, both on ordinary blood agar and on FILDES agar with the addition of 10% blood.

3. The appearance of the colonies on a transparent medium (FILDES agar) is represented in the various forms of Pfeiffer's bacillus.

4. They are Gram negative bacilli resembling morphologically atypical Pfeiffer's bacilli (see plate 3). They do not form spores.

5. They are decidedly aerobic.

6. They have the same minimum temperature for growth as the majority of Pfeiffer's bacilli.

7. They are killed at the same temperature as Pfeiffer's bacillus.

8. They die out in a couple of weeks in the cold room.

9. They do not liquefy gelatine or serum.

10. They do not produce a dark coloration of media containing arbutin.

11. Eight of the strains were vigorous indol-producers. One formed indol in a very slight degree.

In the following points they were d i s t i n c t from Pfeiffer's bacillus:

1. The good growth on blood agar. This was constant on repeating the test several times.

2. They showed no symbiosis reaction at all. All the 9 strains were tested a short time after they were isolated, on FILDES plates and on agar containing haemolysed blood. In no case did the white air coccus increase the growth. Nor in experiments with 13 other species of cocci and bacilli (each of the 9 strains was tested with 2 of these), nor in a fresh trial in October 1921 with several of the strains and the white air coccus, could any growth-promoting effect whatever be observed.

3. B. haemoglobinophilus canis however has a marked growth-promoting effect on Pfeiffer's bacillus (see below).

4. In the previously described experiments where a large number of cultures of Pfeiffer's bacillus were dried for 24 hours, the 9 strains of B. haemoglobinophilus canis were also included. While all the Pfeiffer's bacillus cultures were killed this was the case with only one of the strains of the last-named group.

5. All the 9 strains were capable of marked acid production in the same haemoglobin glucose broth the reaction

of which none of the strains of Pfeiffer's bacillus were able to change to any appreciable extent.

Agglutination experiments were not done as the cultures (on FILDES agar) could not be made into a homogeneous suspension.

From this summary it is clear that the bacilli from the prepuce of dogs are closely related to Pfeiffer's bacillus but however differ from it in important points.

DAVIS (6) has called attention to the fact that while almost all species of bacteria promote the growth of Pfeiffer's bacillus, it forms an exception itself. A growth of Pfeiffer's bacillus is helped neither by the same strain nor by any other strain of Pfeiffer's bacillus.

I was able to fully confirm this. Agar was used with the addition of so much dissolved haemoglobin that the bacillus grew in pure culture rather better than on blood agar but that the growth fell sufficiently short of the maximum for it to be considerably increased by the air coccus. A typical indol-positive Pfeiffer's bacillus was inoculated in the usual manner in parallel straks, and between was inoculated another Pfeiffer's bacillus, not however at points but covering a rather larger area. The test was carried out with the homologous strain, with typical indol-negative strains, and with various atypical strains among which were all the 6 from guinea-pigs. In no case was the slightest growth-promoting effect observed.

19 strains of haemolytic, haemoglobinophilic bacteria were also entirely inactive in this respect, as regards Pfeiffer's bacillus. But in the case of whooping-cough bacilli an undoubted though rather weak growth-promoting power was observed.

All the 9 strains of B. haemoglobinophilus canis were examined for growth-promoting effect which proved to be present in all the cases to a very marked degree.

The results of these tests indicate that the haemolytic, haemoglobinophilic bacteria may be looked upon as more closely related to Pfeiffer's bacillus than the two last species. This is in conformity with the other properties of these species of bacteria.

The Circumscribing and Grouping of Haemoglobinophilic Bacteria.

We have now studied four of the bacterial groups that are generally included under haemoglobinophilic bacteria: Pfeiffer's bacillus, B. haemoglobinophilus canis, haemolytic haemoglobinophilic bacteria, and BORDET's bacillus, and we have seen that the dependence on „haemoglobin' is a character which is constantly present in every strain of the first two groups and is very easy to demonstrate. It is therefore right to use this property as a distinguishing epithet, that is to say as a limiting quality for a number of otherwise more or less distinct bacteria. Neither on the basis of my own results nor on that of the other existing contributions could we mention a property which equally well links Pfeiffer's bacillus with other organisms in a large group. Particular attention has also been paid to the morphology, pathogenicity, and occurrence, but it follows perfectly well from the above that none of these characters would afford so clear and natural a classification. In my experience and that of others the haemolytic, haemoglobinophilic bacteria are characterised by their requiring the same haemoglobin derivates as Pfeiffer's bacillus for their growth although this necessity is not so absolute, and also by the haemolysis. This last property must be made the starting point for defining the group, as the haemoglobinophilia which is less developed than in Pfeiffer's bacillus, is neither strong nor constant enough to be used as a primary basis for this purpose.

BORDET's bacillus diverges so decidedly from these three species as regards nutritive requirements that it ought not to be included under the haemoglobinophilic bacteria, at all events in the true and more restricted meaning of the word.

Besides these four species „haemoglobinophilic bacteria" have been described under numerous other names, and an attempt will now be made to classify them in the most natural manner.

„The Committee on Classification of the Society of American Bacteriologists" has proposed (see DAVIS (6)) the generic designation „Hemophilus" and has provisionally included in this, — PFEIFFER's bacillus, BORDET' bacillus, MORAX-AXENFELD's

bacillus, DUCREY-KREFTING's bacillus, and KOCH-WEEKS' bacillus. This genus therefore comprises what we might call the haemoglobinophilic bacteria in its wider sense. It can scarcely be called an obvious natural classification. Apart from the fact that they are Gram negative bacilli, the only features common to the five species consist in that they require various protein products of animal origin in a not too denaturated form. But each species has its own particular nutritive requirements. Media which produce a very good growth of Pfeiffer's bacillus are quite unsuitable for BORDET's bacillus and vice versa. About the same marked differences in food requirements could no doubt be demonstrated between any two of the five species.

Thanks to Dr. OLAF BLEGVAD's kindness I have had the opportunity of cultivating MORAX-AXENFELD's bacillus from a case of conjunctivitis. The object was to find out how easily this organism could be distinguished from Pfeiffer's bacillus. It was found that good growth took place on LOEFFLER's serum (3 parts horse serum + 1 part broth containing 2% glucose; the mixture is coagulated in a sloped position in test tubes), and on ascitic agar, but not on ordinary agar, FILDES agar, or blood agar. The organism cannot therefore be called haemoglobinophilic. Furthermore it differed very markedly from Pfeiffer's bacillus in its characteristic morphology, and especially in its very intense, clearly defined liquefaction of coagulated serum; a growth on this medium took the form of a group of deep pits with steep sides. In consequence of the extremely slight resistance of this organism in the cultures, it died before it could be investigated more thoroughly, but the above facts are sufficient to show that it is very different from Pfeiffer's bacillus.

The same applies to DUCREY's bacillus, about whose nutritive wants there have certainly been very different views, but they cannot at any rate be assumed to be identical with those of Pfeiffer's bacillus. It also differs from the latter in its morphology, pathogenic qualities, etc.

Whether the KOCH-WEEKS' bacillus ought to be distinguished from Pfeiffer's bacillus has been the subject of much controversy which still cannot be regarded as finally settled. Of the authors who have latterly dealt with the question, PESCH

and HAMMERSCHMIDT believe that the two organisms are very different, while DAVIS (6) looks upon them as practically the same, only, however, on the basis of investigations of a single character (symbiosis).

Unfortunately it has not been possible for me to investigate this organism because the epidemic conjunctivitis in which it is found does not occur in this country, and I have tried in vain to obtain cultures elsewhere. Moreover for a proper comparison with Pfeiffer's bacillus one must have the opportunity of collecting a large amount of material of both bacteria directly from their natural habitats.

From my experience of the nutritive demands of Pfeiffer's bacillus however, the bare fact that several authors emphatically state that it grows better on ascitic and hydrocele agar than on haemoglobin-containing media (e. g. LEVINTHAL agar and pigeon blood agar), is in my opinion sufficient proof that the KOCH-WEEKS' bacillus and Pfeiffer's bacillus cannot be regarded as identical. It is curious nevertheless that the symbiosis phenomenon is present in the case of both organisms (in the same form?).

In connection with the KOCH-WEEKS' bacillus, MC KEE's bacillus must be mentioned which has the following points of similarity with it: it was found to cause conjunctivitis; it is not very haemoglobinophilic (it can be cultivated on haemoglobin agar or on haemoglobin-free media); and its growth is increased by other bacteria. It grows more slowly and feebly than Pfeiffer's bacillus on haemoglobin agar.

It is clear that when the term „haemoglobinophilic bacteria" is used so loosely that all the organisms which have been referred to here are included, it is a matter of indifference where the line is drawn. We will therefore take the expression „haemoglobinophilia" literally and only include organisms with food requirements similar to Pfeiffer's bacillus and B. haemoglobinophilus canis.

Of the organisms which have been termed by different authors „influenza bacillus-like", the following immediately fall out of line:

BECK's, KRAUS', VOLK's and KURITA's bacilli from rabbits;
FRANK's bacillus from swine;
Bacillus bronchicanis (bronchisepticus) (FERRY);

Bacillus septicaemiae anserum exsudativae (RIEMER—FROSCH & BIERBAUM—LÖFFLER). Although FROSCH & BIERBAUM could only grow their organism on haemoglobin-containing media for the first two months, yet they look upon it as identical with RIEMER's; similar LÖFFLER regards the bacilli he found as identical with RIEMER's and FROSCH & BIERBAUM's.

The phenomenon that an organism which at first is haemoglobinophilic later on loses this character was also observed in the bacilli which WOLFF and MARX cultivated from a rat and a tiger respectively. WOLFF's bacillus continued to be haemoglobinophilic for the first 3 months, but 2 months later it could be cultivated on ordinary agar. MARX's bacillus was only haemoglobinophilic for the first 3 generations. Both bacteria had a rather slimy and tough or sticky consistence, and WOLFF's bacillus formed colonies on blood agar up to „fast Erbsengrösse". According to WOLFF's account it differed from Pfeiffer's bacillus immediately after isolation in pure culture by its being able to grow on broth in a generation.

As already mentioned the authors who investigated on a large scale whether Pfeiffer's bacillus preserves its dependence on haemoglobin after cultivation for a long time (several months), found that this was the case. Bacteria which in the beginning are haemoglobinophilic but after the lapse of a few months are able to grow on ordinary media should therefore not be regarded as Pfeiffer's bacilli. Fortunately it appears from the above that even during the time that they are haemoglobinophilic it will be possible in the case of such organisms to demonstrate how they deviate from Pfeiffer's bacillus, so that it will be unnecessary in diagnosing the latter to insist on the proof that it is still haemoglobinophilic after, say, a year's cultivation!

RITCHIE's contribution only, may perhaps create a little more serious apprehension on this point. He found that the haemoglobinophilic bacteria that he isolated from meningitis cases grew feebly on haemoglobin-free agar after a long period of cultivation. Whether it was a question of bacilli which were less haemoglobinophilic than the ordinary Pfeiffer's bacillus or whether it was a peculiarity in his haemoglobin-free medium, cannot be decided. It is not a general rule that the haemoglobinophilic bacteria from meningitis cases can be weaned from haemoglobin. I inoculated

(31. III. 21) 5 strains of the previously mentioned Pfeiffer's bacilli from meningitis cases (which had been cultivated for from 1—1$^3/_4$ year), together with 5 strains from influenzal pneumonia on haemoglobin-free ascitic agar neutral to litmus and ordinary agar containing quite a small amount of haemoglobin, without observing the slightest sign that the meningitis strains were more easily satisfied as regards haemoglobin than the pneumonia strains.

PARANHOS has described a bacillus, „Bacillus septicaemiae canis", as a small haemoglobinophilic rod of slight resistance in cultures. This information is however quite inadequate to disclose the systematic position of the bacillus. (I have however only been able to avail myself of a short summary by the author of a larger contribution).

The same author cultivated a haemoglobinophilic organism from spinal fluid which however was Gram positive. I have been unable to find any other report of Gram positive haemoglobinophilic bacteria apart from that of a couple of investigators who assert that the ordinary Pfeiffer's bacillus may be Gram positive in preparations of sputum etc. and become Gram negative on cultivation. We have presumably here to do with some peculiarity in the staining technique of these workers. At all events it is too early to establish a special group of Gram positive haemoglobinophilic bacteria.

From 3 cases of infection of the urinary tract DAVIS (3) cultivated a Gram negative bacillus which grew better anaerobically than aerobically, and considerably more slowly than Pfeiffer's bacillus; it produced haemolysis on blood agar; the appearance and consistence of the growth was different from that of Pfeiffer's bacillus; it gave no symbiosis reaction and required a larger amount of blood than Pfeiffer's bacillus. It must therefore be called a haemolytic haemoglobinophilic bacterium but differs in several ways from the organisms previously described under this name.

In judging the literature on the finding of haemoglobinophilic bacteria (in man) we are usually up against the difficulty that they have not been thoroughly enough investigated to allow us to decide with certainty, whether they belong to the Pfeiffer's bacillus group or not. We have to limit ourselves to finding out whether there is anything in the description which negatives such a classification. If this is not the case it is presumably legitimate to regard them as Pfeiffer's bacilli (in so far as they were cultivated on media containing whole blood corpuscles; otherwise they might be haemolytic haemoglobinophilic bacilli).

Judging from the material we are now dealing with it would be wrong to exclude bacteria from the class Pfeiffer's bacillus on the ground of such factors as occurrence or virulence. That bacteria to which this designation must be given by no means exclusively occur in influenza, and that a clear distinction between virulent and avirulent forms cannot be drawn, is admitted. Thus the justification vanished for excluding MÜLLER's trachoma bacillus because it was cultivated from conjunctiva attacked by trachoma; JOCHMANN & KRAUSE's „Bacillus pertussis Eppendorf", because it was cultivated from whooping-cough; MEUNIER's bacillus; COHEN's bacillus and many others because they could produce septicaemia in man and animals. Nor can we exclude ELMASSIAN's bacillus, LUZZATO's „Bacillus minutissimus sputi", or JUNDELL's „Bacillus catarrhalis" on account of statements that they can grow on ascitic agar. If a number of strains were simultaneously cultivated on ascitic agar and it was repeatedly shown that some grew and others did not, then we could assert that the latter possessed particular nutritive requirements. But nothing can be concluded from the demonstration of growth on ascitic agar which has not been controlled with regard to its freedom from haemoglobin either by the benzidin test or by the inoculation of undoubted Pfeiffer's bacilli.

In accordance with these views, as far as I can see all the reports on the finding of haemoglobinophilic bacteria in man to which exception has not been taken in the above, may be looked upon as referring to Pfeiffer's bacillus. This also applies to the rarer habitats such as the biliary passages (HEYROVSKY, KLIENEBERGER (1), LAUBENHEIMER, KNINA), urinary tract (KRETZ, COHN, KLIENEBERGER (3)), uterus (THALHIMER (1)), Fallopian tubes (KISSKALT).

Our investigations into the classification of the haemoglobinophilic bacteria have lead to the following conceptions:

(1) Haemoglobinophilic bacteria (as a group designation) in the strictest sense of the word are identical with Pfeiffer's bacillus. On the other hand this species must be more extended than we have often been inclined to make

it. There are two species closely related to Pfeiffer's bacillus which in my experience and that of other authors, are not so intensely haemoglobinophilic as the latter, and which differ from it in a number of other ways: (2) the haemolytic haemoglobinophilic bacteria and (3). B. haemoglobinophilus canis.

Other groups of haemoglobinophilic bacteria are not known. But in a few cases strains of bacteria have been described (always bacilli) that with more or less propriety are termed haemoglobinophilic, though they have not been sufficiently examined to make it possible to determine their relationship to the groups mentioned and other bacterial species. It has not been proved that an organism has been discovered which is just as intensely and permanently haemoglobinophilic as Pfeiffer's bacillus.

Technique.

The various rules that apply to the utilisation of haemoglobin and its derivates in the cultivation of Pfeiffer's bacillus, have already been discussed. In this chapter therefore merely the purely practical side of the technique of cultivation will be dealt with. My own experiences will be related and then I will pass on to a review of the literature on the different media for Pfeiffer's bacillus.

For the primary isolation of the haemoglobinophilic bacteria I have only used agar media in Petri plates. The agar has practically always been the ordinary peptone broth agar: 25 kg. beef is minced; 25 litres water are added; the mixture is allowed to stand in the cold room till the next day, when it is boiled for a short time, and then the broth is pressed out of the meat. About 16 litres of water is added to this, it is again heated to boiling and the solid remnant of the meat is compressed once more. The two portions of broth, — about 50 litres in all, — are mixed. 1% peptone is added (WITTE's or a similar preparation; at first 1½ % was taken but the difference seemed to be unimportant), and ½ % salt. It is heated to boiling. 10 c.c. of the broth are titrated till the red colour of phenol-phthalein begins to appear. The calculated amount of n. NaOH is added, the broth is boiled for 5—10 minutes and then the reaction tested again. After filtration and cooling the broth should not give a colour with phenol-phthalein even when the indicator is present in excess, but it should become tinted on the addition of 0.2 to 0.3 c.c. $\frac{n}{10}$ NaOH to 10 c.c. The p_H value will then be about 7.8 to 7.9.

Then 2% agar is added. Next day the mixture is liquefied, filtered through cotton wool, distributed in 600c.c. flasks and sterilised in the steamer (100°) on 3 consecutive days. This brings down the p_H value to roughly 7.2 (determined colorimetrically in broth without agar, sterilised in the same way). The agar ought not to be autoclaved as it has been shown that this renders it a distinctly worse basic medium for the haemoglobinophilic bacteria.

It may sometimes happen that the finished agar has not acquired just the reaction desired; an appropriate amount of sodium hydroxide or hydrochloric acid can then be added after sterilisation.

It is stated fairly unanimously by a number of authors that the optimum reaction for Pfeiffer's bacillus lies between p_H 7.2—7.5 (nearest the first value).

For controlling the reaction of the finished medium I found it convenient to use azolitmin paper which I prepared myself from the instructions of HENRIQUES & SÖRENSEN (I used double the amount of azolitmin however), and also a 1‰ solution of α-naphtholphtalein (SÖRENSEN & PALITSCH) in dilute alcohol. In phosphate mixtures (SÖRENSEN) of p_H 6.8 azolitmin paper reacts neutral, that is to say, retains its violet colour, while α-naphthol-phthalein (in not too small quantity) shows a sharp change of colour (a gray colour as a transition from weak yellowish-brown to deep bluish-green) at p_H 7.4. With the aid of these indicators in conjunction with phenolphthalein (which begins to change colour at p_H 8.0) we can, within a range of from about 6.8—8.0, judge the reaction of the medium with a precision which will certainly be quite sufficient in most cases for ordinary bacteriological purposes. They can be employed in many cases where a more exact colorimetric measurement would be impossible or very difficult (very turbid and coloured media etc.).

It has been found that broth or agar media without any ascitic fluid have a reaction suitable for Pfeiffer's bacillus and many other bacteria when they react strongly alkaline to azolitmin paper without changing the yellow colour of α-naphtholphthalein. Ascitic agar however should react neutral or slightly alkaline to azolitmin paper. These facts apply

without regard to whether the reaction is determined before or after the addition of the „haemoglobin".

As „haemoglobin" human blood was used in the first investigations in July 1918, a drop of which was spread over the surface of an agar plate (of autoclaved agar). This is the original method of PFEIFFER. It was natural to assume that human blood might be better than that of other animal species when it was a question of growing haemoglobinophilic bacteria obtained from man; but in my experience and in that of practically all other authors this has proved not to be the case. Pfeiffer's bacillus could be grown with greater certainty on agar mixed with a liberal amount (20—25%) of defibrinated horse blood, but the growth was slight.

For the inoculations from healthy persons in September 1918 I used agar mixed with h a e m o l y s e d blood for the first time on a large scale. Although the agar was autoclaved this medium was very satisfactory, the growth being considerably richer than on blood agar. I was more certain of getting a good growth however when the agar was sterilised at 100° as mentioned above, and such agar was therefore employed as the basis of the „haemoglobin" medium in all the subsequent experiments.

Haemolysed horse blood was used as „haemoglobin" for a long time with excellent results, prepared in the following way: The flask containing the defibrinated blood was allowed to stand for a couple of hours so that the blood corpuscles could sink. The supernatant serum was then poured off as completely as possible and replaced by distilled water, which speedily dissolved the blood corpuscles. The haemoglobin solution was distributed in flasks with 20c.c. (corresponding to about 6c.c. blood corpuscles) in each, which can be preserved in the ice-box for several months before use. The freezing completes the haemolysis. On the addition of the liquefied contents of a flask of 600c.c. agar (liquefied and cooled to 50—40°) and then pouring the mixture into plates a moderately red, transparent medium was obtained which for the first 1¼ years consistently produced a rich growth of Pfeiffer's bacillus (isolated colonies 2—4 mm. in diameter) and which on the whole, was ideal. I was rather surprised to find that during the winter and spring 1919—20 it gradually became less satis-

factory, till finally it was impossible to obtain a good growth on it. This was not a transient phenomenon; on repeating the test in 1921 and 1922 with the blood of a number of different horses only a weak growth appeared. This happened whether the inoculation was made from cultures which had been grown for a long time (on FILDES agar) or whether it was made directly from the throat of a person (March 1922) who had been a Pfeiffer's bacillus carrier for about two months in consequence of an attack of influenza. The blood was taken from the „serum horses" of the Institute (principally diphtheria). The failure of the blood as a nutritive medium for Pfeiffer's bacillus might be attributed to the experiments on the increase of antitoxin by the injection of manganese salts which had been gradually started on a large scale during the first half of 1920. But in the last test in March 1922 blood was taken from 3 horses which had not received such injections (they had for some time been immunised against Staphylococcus, Streptococcus, and Pneumococcus respectively), without it proving more active.

I have thus not succeeded in probing the mystery of this peculiar phenomenon that apparently exactly the same medium which for a long time proved to be ideal, subsequently persistently failed, but it is none the less important to report the experience because it is of great significance in judging the technique for the cultivation of Pfeiffer's bacillus. Several authors maintain for instance that in criticising the work on this bacterium, — particularly when there are negative findings — it is of great importance that the investigator gives accurate information about how the media were prepared. This is naturally always desirable, but the important point is not whether one or other of the large number of media detailed below, each useful in itself, was employed. The actual proof that Pfeiffer's bacillus can always grow on the medium used is by far the most important point.

It was chiefly the „V"-function of the blood that failed because the latter had consistently been quite suitable for the symbiosis reaction (p. 120). It seemed however, as if the same effect could be obtained at first with $^1/_4$—$^1/_2$ $^0/_{00}$ dissolved blood corpuscles, as later on with 1—2 $^0/_{00}$.

All the horse and sheep blood I have tested was completely

satisfactory for the preparation of FILDES agar. Since June 1920 I have made this in the following manner which only differs from FILDES' directions in unessential details.

To 150c.c. of 0.9% salt solution are added 6c.c. pure, concentrated hydrochloric acid and then 50c.c. defibrinated horse or sheep blood and 2gm. pepsinum concentratum Langebek (FILDES uses 1gm. „pepsin B. P."). The mixture which need not be prepared from sterile materials or in sterile measuring cylinders, is now poured into a sterile flask and is kept at 56° for 5—6 hours (either in an air thermostat or in an oil bath). NaOH is now added until a sample diluted with distilled water to a light brown colour, turns red on the addition of phenol red (phenolsulphonephthalein) without giving the alkaline colour change with α-naphtholphthalein (see p. 226). Next, 1/4 % chloroform is added, the flask is closed with a sterile rubber cork and kept in the cold room. I have not observed any decrease in activity during a couple of months' preservation. (According to FILDES it lasts even for over a year at room temperature). 30c.c. of this liquid is added to 600c.c. agar. (It seems not to matter whether it is added while the agar is hot (about 90°) or after it has been cooled to 50°—40°). After pouring it into flasks a perfectly transparent medium is obtained but of a rather darker yellowish-brown colour than ordinary agar. With less than 5% of the blood solution the growth of Pfeiffer's bacillus was perceptibly weaker.

The potent factor in the transformation of the haemoglobin to a favourable nutritive medium for Pfeiffer's bacillus seems to be the hydrochloric acid. The pepsin is however important as a clear solution is only obtained in the presence of this substance.

The pepsin-digested blood only gave good results in my hands in the case of solid media but here it proved to be active with extraordinary constancy, although during the period it was in use many different batches of blood solution and agar have been prepared.

That FILDES agar gives good growth is evident from the fact that the colonies figured in plate 2 appeared on this medium. In my experience however it did not give the most copious growth of Pfeiffer's bacillus it is possible to observe. In cases where it is necessary to produce the greatest possible

mass of culture, agar with heat coagulated blood is probably to be preferred.

Besides the haemoglobin agar and FILDES agar other media were made use of in some cases in the large series of investigations as mentioned on pp. 88—89. As regards the „dark red" blood agar referred to, it has always happened that when the blood agar was prepared in the „normal manner" by mixing agar cooled to about 40° with blood previously heated to the same temperature, Pfeiffer's bacillus grew in minute colonies only, just visible to the naked eye. By heating the liquid mixture of agar and blood to such an extent that it assumed a dark red colour a medium was obtained on which Pfeiffer's bacillus grew considerably better. By further heating the blood agar till the colour became chocolate-brown the growth was still richer. This „chocolate agar" however I have only exceptionally used, as the transparent media with dissolved haemoglobin and pepsin-digested blood gave a sufficiently good growth for my purpose.

In some of the mass inoculations from healthy persons various other media besides those ordinarily used, were employed. Thus all the inoculations on 5. III. 19. were made on the usual medium containing haemolysed horse blood and also on agar containing the haemolysed blood in some cases of pigeon and in others of different samples of human blood. The pigeon and horse blood gave almost equally good results, while all the three samples of human blood were distinctly less effective.

The medium referred to on p. 88 which was used for the inoculations on 24. IX. 21. was prepared from agar to which was added 25% ascitic fluid, dissolved haemoglobin until the colour became moderately red, and 2—3% pepsindigested blood solution. (The last constituent is possibly superfluous). On this medium Pfeiffer's bacillus grew in pure culture but not much better than on ordinary („cold" prepared) blood agar. In the primary inoculations however the colonies were very well developed, being about 2 mm. in diameter. This was undoubtedly due to a symbiosis effect from colonies of other species of bacteria. In cases where the latter were only present in small numbers it could be observed that the colonies of Pfeiffer's bacillus were largest in their vicinity. In the

inoculations on 21. III. 22. this was the only medium used apart from a few cases where plate cultures were made on FILDES medium. Subsequent cultivation took place on FILDES plates.

While it is a general rule for the more fastidious bacteria (Meningococcus, Gonococcus, etc.) that the primary culture is more exacting in its demands upon the nutritive medium than the subsequent subcultures, the converse is the case for Pfeiffer's bacillus. This depends upon the simultaneous inoculation of growth-promoting bacteria which (in sputum, mucus from the throat, etc.) are practically always present in sufficient quantity to exert an effect. On the other hand the demands of Pfeiffer's bacillus for a medium of a definite constitution decrease after a long period of cultivation, as previously mentioned, to a less extent than is the case with the majority of other bacteria.

In the investigations on 21. III. 22. besides the medium mentioned, others were used for some of the inoculations, prepared in the same way except that the agar was made partly with HOTTINGER's broth (8 litres of broth from 1 kilogram of meat), and partly with COLE & ONSLOW's broth (40 litres of broth from 1 kilogram of casein. The digestion of the casein however, was carried out with Pancreatin „Rhenania", not with extract of pig's pancreas).

The first-named medium gave a rather slighter growth of Pfeiffer's bacillus than the ordinary peptone broth agar, while the last proved to be absolutely useless.

For subcultivation of Pfeiffer's bacillus I also (earlier) tried HOTTINGER agar (as FILDES plates), chiefly in strong concentrations. With the most favourable concentration (4 litres of broth from 1 kilogram of meat) the growth of Pfeiffer's bacillus was at times just as good as on ordinary FILDES plates and at times, not so good.

HUNTOON's agar is stated to possess specially good „vitamine" qualities (1) because the agar is boiled with the meat; (2) because it is not filtered through wool, paper etc. The hope of thus obtaining a particularly good medium for the most atypical Pfeiffer's bacilli which usually only grew slightly and were easily lost, was not realised however. I did not succeed in making a better „basic medium" than the classical peptone broth agar.

The liquid medium principally used was broth containing about $1^0/_{00}$ dissolved horse blood corpuscles. If broth

is first inoculated with the air coccus and after a couple of days in the incubator and the addition of the haemoglobin solution, is filtered through a Berkefeld filter, a better growth can be obtained than in ordinary haemoglobin broth. A few other observations will be referred to in connection with the following summary of the literature on the different media.

A considerable number of modifications of media for Pfeiffer's bacillus have been introduced in the course of time and particularly during the last pandemic. Most of the media which were tried in the first few years (HUBER's agar with HOMMEL's haemologen, NASTIUKOW's egg-yolk medium, CANTANI's (1) semen medium, GHON & PREYSS's (1) use of haematin agar in conjunction with growth-promoting bacteria, FICHTNER's sputum agar), are now obsolete.

It must also be remarked that various media which were used by TIJÖTTA, RIVERS and others in the study of the nutritive requirements of Pfeiffer's bacillus, have no practical importance apart from those cases where these conditions are being investigated.

The generally useful media may be divided into the following groups:

1. Agar smeared with blood.
2. „ mixed with undissolved blood without heating.
3. „ „ „ haemolysed blood.
4. Media where the effect of blood and growth-promoting bacteria are combined.
5. Agar with heated blood.
6. „ „ blood altered by the action of acid or alkali.
7. „ „ pepsin-digested blood.
8. „ „ trypsin-digested blood.
9. Selective media.
10. Media which are particularly suitable for preserving the cultures alive.

The reason agar receives so much attention in this summary is because solid media have been used to the largest extent

in the cultivation of Pfeiffer's bacillus but most of the methods applicable to solid media can also be used for fluid media.

By „agar" is understood, when the contrary is not stated, either the classical peptone broth agar or another medium of similar constitution („tryp-agar", „vitamine agar" etc.).

1. The classical medium for cultivating Pfeiffer's bacillus is agar smeared with a thin layer of blood (Pfeiffer (2)). Human and rabbit blood etc. were originally used but Pfeiffer soon found that pigeon blood was specially suitable. Pfeiffer's pigeon blood agar has given useful results in the hands of most investigators. During the last pandemic Pfeiffer himself adhered to this medium (Leichtentritt (1), Loewenhardt (2)). Agar smeared with other animals' blood and particularly with human blood must on the contrary be deemed a bad medium.

According to the experience of many authors (Czaplewski, Ellermann, Crofton, Wyard and a number of others) which I quite agree with, agar mixed with blood gives a growth of Pfeiffer's bacillus that perhaps is not exactly richer but at any rate is more easily perceptible, and this applies also to pigeon blood.

2. Agar mixed with unaltered blood, for the sake of brevity termed „blood agar", was undoubtedly the medium most used until after the beginning of the last pandemic. The first to give this method of preparation was Borchardt.

The majority of authors even those that praised the medium, have only got Pfeiffer's bacillus to grow however, both on agar smeared with blood and on agar mixed with blood, in very small colonies the size of a pin's head or there-abouts. These tiny „dew-drop" colonies have indeed even been commonly described as the characteristic form of growth of Pfeiffer's bacillus and the larger colonies which were also observed in the early days around foreign bacterical colonies, have been called „giant colonies". As is clear from the following, Pfeiffer's bacillus grows on really good media always in the form of these „giant colonies" which are naturally to be looked upon as the normal form of growth. The growth described as characteristic until the last few years can therefore only be called a very stunted growth.

A few authors (Williams, Crofton and others) have ob-

tained large colonies on agar with undissolved blood corpuscles the reason being uncertain (infection with foreign organisms?), but this is the exception.

In spite of the weak growth on blood agar it has, particularly for primary cultivation where growth-promoting organisms exert their favourable influence, proved to be a very useful medium when it is employed with requisite discretion. Duval & Harris (see p. 37), who found Pfeiffer's bacillus practically constantly, used blood agar on which this organism only grew extremely poorly (although the blood agar was „complement-free"). The colonies were often not visible for 48 hours.

Several authors have used blood agar for the primary culture, and for subcultivation one of the media containing heated blood, mentioned below. Blood agar is just the medium on which the various bacteria generally present in sputum etc. appear most characteristic. It is true this applies especially to Pneumococcus and Streptococcus, while the colonies of Pfeiffer's bacillus are at least just as recognisable on one of the good transparent „haemoglobin" media. On blood agar however a marked increase of growth of Pfeiffer's bacillus will most often be observed around colonies of Staphylococcus, Streptococcus etc. and this picture is extremely characteristic for Pfeiffer's bacillus.

Fildes, Baker, & Thompson found blood agar almost useless. Dick & Murray and others found that even after there was good growth on the original plate Pfeiffer's bacillus did not always grow on further cultivation.

Schottmüller states that blood agar plates must be used in the first 24 hours after they are made, while Levinthal (2) just as decisively maintains that they must be kept at least 24 hours before they can be used. It is clear that different factors are operative here. In my experience it does not matter whether the plates are used immediately after preparation or after being kept for some days. If blood agar plates are to be used however, attention should be directed to this point and it should be determined whether the length of time between the preparation of the plates and their inoculation is of importance under the given conditions.

3. The weak growth on media containing whole blood corpuscles is simply explained by the slight amount of haemoglobin

etc. which it may be assumed, diffuses out from them into the medium. It seems therefore that to obtain better growth the blood corpuscles must be destroyed in one way or another. The simplest way of accomplishing this is to produce haemolysis (by distilled water, freezing, or the addition of saponin) without altering the haemoglobin. As described above this method of procedure gave excellent results in my hands for a long time, but at a later date liberation of the haemoglobin by haemolysis only slightly increased its effect. These diverging experiences from the same investigator account for differences between those of other authors. Of the authors who have used haemolysed blood with good effect may be named: DELIUS & KOLLE, KORENTSCHEWSKY, ELLERMANN, NEUFELD & PAPAMARKU (1), RUIZ Y FALCO & TAPIO, MC INTOSH, BROWN & ORCUTT, GOSIO & MISSIROLI, SELLARDS & STURM. Some of these authors expressly state that the nutritive quality of the blood is enhanced by haemolysis. DAVIS (2), who found that haemoglobin even in a dilution of 1:180000 could support a growth of Pfeiffer's bacillus, made the observation with ordinary haemolysed human blood. (He says however that pigeon blood was better than human blood). In opposition to this HUNDESHAGEN was unable to notice any favourable effect from haemolysis as such.

We get the impression that haemolysis usually increases the activity of the blood corpuscles to an appreciable extent, but that the effect is not constant, and by this method we seldom get a medium which produces the maximum growth for Pfeiffer's bacillus.

Among media containing haemolysed blood THALHIMER'S (2) agar with amorphous haemoglobin must naturally be included. Carefully purified, crystallised haemoglobin, as already mentioned, give practically no growth unless the aid of growth-promoting bacteria is made use of, or unless it is subjected to radical chemical transformations.

4. Several of the older authors (about 1900) regarded it as an established fact that a good growth of Pfeiffer's bacillus could only be obtained in the presence of growth-promoting bacteria. Quite recently also growth-promoting bacteria, — Streptococcus, Staphylococcus, Subtilis — have been inoculated on the culture plates partly to increase the

growth of Pfeiffer's bacillus and partly to make it easier to recognise by means of the characteristic symbiosis phenomenon. (Brown & Orcutt, Roos). Harvey, Brown, & Cunningham cultivated Pfeiffer's bacillus on blood agar for the preparation of vaccine, in association with Pneumococcus and Streptococcus, which increased the growth of Pfeiffer's bacillus considerably while the growth-promoting organisms always kept in the background.

Savini & Savini-Castano employed growth-promoting bacteria even in the p r e p a r a t i o n of the medium. A mixture of glycerin and Staphylococcus culture was kept at 58°—60° overnight, after which blood was added and the mixture allowed to stand an hour at 56°—58°. The blood corpuscles are haemolysed by the glycerin; the solution thus produced which is said to keep for months, is added to agar or broth. The colonies that grew on it were „riesengross". It is doubtful whether this medium can compare with the more modern ones. The reason for dwelling upon it is that several different factors, each active in itself are made use of in its preparation: growth-promoting bacteria, haemolysis, and heating.

Whether the glycerin, which is frequently added to the media containing heated blood mentioned below, plays any important part I have been unable to find out. According to Wolf it was of no importance in the symbiotic growth on ordinary agar.

5. Although agar containing h e a t e d b l o o d was not used on a large scale prior to the last few years the first time such a medium was employed dates as far back as 1894 when Voges prepared his haemoglobin medium by putting some drops of human blood in a Petri plate and pouring melted agar at 100° over them whereupon the contents were well mixed. A transparent, light red medium was thus obtained. That the blood kept its red colour is intelligible as the agar was naturally instantaneously cooled to a considerable extent by contact with the glass plate. Voges pronounced this medium better than any hitherto known. Grassberger (2), who on the basis of Voges' contribution in 1898 employs a similar method of procedure points out the importance of the agar being hot. An hour's heating at 50°—60° was however adequate to bring forth the desired improvement of its value as a nutritive medium.

He found that blood thus heated was superior to blood haemolysed in various other ways.

According to AUERBACH (1904) the agar should be 60°—70° when it is added to the blood (pigeon). The colonies of Pfeiffer's bacillus then attained a diameter of 1 mm. which was then reckoned a good growth. Curiously enough he states that the medium is impaired when the agar is made so hot that the blood is tinted brown. None the less it was precisely by means of this further heating that the best and most commonly used media for Pfeiffer's bacillus were subsequently obtained. This step forward was made by COHEN & FITZGERALD in 1910. As it seems to be so little known (compare COCA & KELLEY) that this was the first contribution on the preparation of the typical „chocolate agar" the method will be given word for word: „on fait fondre en le portant a l'ébullition un tube de gélose peptonée; on le refroidit à 60° environ, puis on introduit dans le tube 1c.c. de sang de lapin défibriné; on mélange de façon intime le sang à la gélose en roulant le tube entre les mains, puis on le plonge dans un bain-marie chauffé à 80°; on l'y laisse durant trois minutes; le sang est cuit et le milieu prend une consistance plus épaisse de teinte brun chocolat". Pfeiffer's bacillus however must have been trained to this medium before „on constate que la culture présente l'aspect non plus de fines gouttelettes translucides, mais bien d'une couche épaisse, uniforme, de coloration grise, de consistance crémeuse: l'abondance de cette culture rappelle celle du staphylocoque sur agar peptoné".

In 1912 POVITZKY (cited from COCA & KELLEY) made the same discovery of the value of „chocolate blood" as a nutritive medium. In 1915 HUNDESHAGEN learnt this method and introduced it into Germany. LLOYD (1916) added to agar (according to DOUGLAS or COLE & ONSLOW) 5% ox blood, heated in steam at 100° for ¾ hour, the clot removed by centrifuging or filtering through glass wool, and finally sterilised at 100°. She recommends this medium for the cultivation of Pfeiffer's bacillus, Meningococcus, Pneumococcus etc. without a further description of the conditions of growth.

Nevertheless LEVINTHAL (1918) independently of the previous authors again discovered by a roundabout method the value of strongly heated blood agar as a nutritive medium.

Like LLOYD he removed the blood clot, but contrary to the procedure of this author he laid stress on the fact that after the addition of the blood the medium must only be heated at 100° for a few minutes which is a point of considerable importance.

During the latter part of the pandemic and afterwards these media with heated blood (in the various modifications) have been more used than any of the other groups of media mentioned. In America „chocolate agar" has principally been used, in Germany, LEVINTHAL's agar.

HUNDESHAGEN found that Pfeiffer's bacillus lived longer on heated blood agar when the clot was not removed than on LEVINTHAL agar, and that a better growth was frequently obtained on the first-named medium. These experiences, particularly the latter, may be regarded as general. We hardly ever read that „chocolate agar" has given inconstant results, whereas several authors report that LEVINTHAL agar at times could entirely fail or that it constantly gave a worse growth than the discoverer claimed (PRIBRAM (2), GIBSON & BOWMAN, WYARD, LOEWENHARDT (2) and several others). Even LEVINTHAL himself had a few mishaps with his medium. He believes that all the unsatisfactory results are due to too long heating after the addition of the blood. On the other hand (FILDES, BAKER, & THOMPSON) it has been emphasized that only glass wool ought to be used in filtering LEVINTHAL agar, not cotton-wool, gauze, or paper which may absorb the nutritive substances. Moreover filtration is not always necessary, the coagulated blood often sinks to the bottom so rapidly that the supernatant agar can be easily decanted off.

It is probable that most of the untoward results with LEVINTHAL agar could be avoided by paying sufficient attention to the two dangers mentioned, — too much heating, and contact with finely divided cellulose. Whether this will be enough in all cases can scarcely be decided at present. Even so it cannot be denied that the removal of the clot and the consequent diminished content in haemoglobin derivatives in LEVINTHAL agar is a defect in this medium, as it explains its sensitiveness to an uncautious technique of preparation, its limited keeping qualities, and the comparatively poor resistance against keeping of the cultures grown on it. In chocolate agar on

the contrary nutritive bodies will be able to diffuse out from the clot into the medium for a long time. It must be admitted nevertheless that the growth which can be obtained on successful LEVINTHAL agar can hardly be exceeded on any other medium. I have myself once prepared LEVINTHAL agar of excellent quality, but a later preparation did not function nearly so well. I also got just as good a growth on LEVINTHAL agar as on agar containing blood clot (heated to 100°), but the cultures on the latter medium lived the longer.

The temperature to which different workers heated the blood agar mixture varied from about 70° (the temperature at which the chocolate-brown colour appears) to 100°. COCA & KELLEY and others affirm that it is insufficient to heat it until the brown colour appears but that the full effect is only reached by heating to 96°—100° for 10 minutes or perhaps rather less. That this is so in every case does not seem to be definitely proved. SOPARKER (cited from LISTON) was satisfied with heating to 66° for half an hour (haemolysed human blood) with the results that the solution kept its red colour and transparency (except for a fine, brown precipitate which was filtered off).

„Chocolate agar" and LEVINTHAL agar are usually prepared by heating the blood after it is mixed with the agar, and several authors lay great stress on this. OLSEN and others found that blood heated to 100° together with the agar regularly gave better results than when it was heated separately and afterwards added to the agar. This is also in harmony with the older contributions of PFEIFFER, GHON & PREYSS (1) and others that blood heated apart from the agar was not improved as a nutritive substance for Pfeiffer's bacillus but on the contrary became worse.

Against this FLEMING found in comparative, quantitatively performed experiments that it was a matter of indifference whether the blood was heated with the agar or separately. Several American workers (WOLLSTEIN (4) and others) adopted the latter method in preparing their media.

I myself found that blood diluted with distilled water (I used sometimes 1 part blood corpuscles + 4 parts water, and sometimes 1 part blood + 9 parts water) decreased in activity on heating, while blood (1 part) heated to 100° with

salt solution or broth (9 parts) ond then added to agar gave the same good growth as blood heated with the agar; and the uppermost clear portion of the liquid proved to be just as active as the lowest portion containing the clot. That the milieu in which the blood corpuscles (or haemoglobin) are heated, is of considerable importance for its transformation in a favourable direction or the reverse can hardly be doubted.

If the blood is heated in the agar (or broth) we are on the safe side. There seems to be no particular advantage in heating it separately.

6. Although Ghon & Preyss found that blood was more deficient as a nutritive substance after simple heating at 100°, he obtained a better result by boiling the blood corpuscles with a solution of sodium carbonate. Later Hundeshagen prepared a medium with blood treated with alkali but renounced it when he became acquainted with heated blood agar.

Tocunaga has recently devised a medium in the preparation of which blood treated with alkali was also used. He says that colonies of Pfeiffer's bacillus attain a size of 0.8—1.2 mm. in 24 hours. This must be considered a rather poor growth compared with what can be obtained on the best media

Media containing alkali-treated blood can therefore be used but are not among the best.

Considerably better growth occurs when the blood is treated with strong mineral acids. Fleming (1919) introduced this method; he mixed blood with the same volume of normal HCl or H_2SO_4. A brownish liquid with a brown precipitate was obtained. After standing some minutes it was neutralised with NaOH. The deposit was removed by decantation or centrifugation and the clear liquid used to mix with agar. This method had the advantage that a sterile medium was obtained even though the original materials were grossly infected. Wyard and Skajaa found that this medium could be used but from their descriptions it appears that it did not always produce a good growth, and that the blood solution itself as well as the final agar would only keep for a few days.

Neither alkali treatment nor acid treatment seems therefore to give media that can be specially recommended.

7.. In 1901 Cantani (1) reported experiments dealing with

the preparation of a medium by digestion of blood with pepsin and HCl followed by filtration, neutralisation, steaming, and renewed filtration. By adding the liquid thus prepared to agar or broth he obtained media which gave an „excellent" growth of Pfeiffer's bacillus. He found the method practically useless however since the blood solution completely lost its activity after a few days' keeping.

Fildes however prepared (as stated on p. 229) pepsin-digested blood in 1919 with considerably better results which was superior to Cantani's medium by being extremely stable. Cantani's bad results in this respect presumably depend upon his using pepsin in too small amount (the repeated filtrations indicate this), by the absorption of the active substance in the act of filtration, and by the boiling.

By comparing the statements in the literature about Fildes' and Fleming's media (in connection with my own experiences (see p. 229) one forms the opinion the acid treatment of the blood is sufficient in itself to produce a good growth but that the pepsin causes all the „haemoglobin" to go into solution, and this is the reason a clear solution is obtained without filtration etc. and that on account of the greater content of active substance the solution keeps better.

Fildes' medium has the same advantage as Fleming's that it becomes sterile even though infected material is used.

8. Both these media were introduced to supersede Matthews' and Mc Intosh's media with trypsin-digested blood, not because they gave unsatisfactory results but because it was desirable to have a medium which was simpler to prepare than these. Fildes' blood solution satisfies this requirement in a marked degree, as it is only necessary to prepare a large supply about once a year and ordinary blood from the slaughter-house can be used.

Media containing trypsin-digested blood, — Gore has also described such a one (cited by Liston) — seem to give an excellent growth of Pfeiffer's bacillus. Both the first-named authors state that trypsin-digested blood also inhibits certain other bacteria, namely Pneumococcus, Streptococcus and various other Gram positive cocci, but not Staphylococcus, coliform bacteria etc. According to Mc Intosh this selective action is not developed until after digestion has proceeded for a

considerable time (10—14 days). The same author states that the medium suffers from the disadvantage that Pfeiffer's bacillus quickly dies out on it. For its keeping qualities he therefore preferred agar containing haemolysed and slightly alkaline pigeon blood. Whether the inhibition of growth, which unfortunately does not include the bacteria that we should most expect to overgrow Pfeiffer's bacillus, is really of use, is indeed rather doubtful. It must be looked at in the light of Mc Intosh's previous observation that Pfeiffer's bacillus from post mortem material is nearly always overgrown by other bacteria on ordinary blood agar.

The media applied by me did not possess this growth-inhibiting property; according to my experience however this was no disadvantage. On haemoglobin agar where the growth of other organisms is favoured somewhat, and on Fildes agar where they did not grow very differently than on ordinary agar, Pfeiffer's bacillus has always had an easy task in asserting itself above the other bacteria. In many cases the latter have increased the growth of Pfeiffer's bacillus by their symbiotic affect on less successful haemoglobin-agar, and on media containing ascitic fluid which was employed in the last ino-inoculations from healthy persons.

9. There does not seem therefore to be any reason for preferring the media in which an attempt has been made to inhibit the other bacteria by adding special substances (Avery: sodium oleate; Fleming: brilliant green; Bernstein & Loewe: gentian violet). Of these, Avery's medium has been largely employed. That this and the other selective media can be used is undeniable, but they undoubtedly require a particularly careful preparation since the inhibiting bodies can hardly be entirely without action on Pfeiffer's bacillus. On Avery's medium Pritchett & Stillman did not usually obtain a growth of Pfeiffer's bacillus at pH. > 7.5. This slight tolerance of variations in the reaction is undoubtedly to be regarded as a sign that the conditions of growth are far from optimal. The same authors did not always obtain large colonies on this medium; they varied from „pinpoint colonies to large round colonies of 1—3 mm. in diameter". Furthermore the various strains of Pfeiffer's bacillus, according to my investigations, are by no means all in possession of the same

energy of growth. Some of them, — the specially „atypical" it is true, which perhaps have no particular clinical significance but which without further reason cannot be disregarded, — were even difficult to keep alive on such haemoglobin agar which produced an extremely rich growth of the majority of strains of Pfeiffer's bacillus. It is thus very probable that the selective media will inhibit the delicately growing Pfeiffer's bacilli even if they do not inhibit the more hardy strains from developing.

10. Lastly it may be mentioned that Gibson & Bowman, and Legroux & Mesnard have described media on which Pfeiffer's bacillus only needs subculturing with several weeks interval (broth from minced meat, — minced liver). Lal could keep Pfeiffer's bacillus alive for 132 days on blood gelatine by keeping at 37° in tubes sealed with paraffin. According to Wollstein (4) and Tanaka Pfeiffer's bacillus can also be kept alive in blood broth for many weeks. Concerning Twort's mixed cultures see p. 159.

I have not however found it preferable to use any of these media but have kept my strains going in surface cultures on plates of haemoglobin agar and later on Fildes agar, usually by subculturing every 5th. day, incubating for 1—2 days, and then keeping them in the cold room. The certainty in recognising the growth of Pfeiffer's bacillus and any contaminations and the convenient subculturing from plate to plate have outweighed any advantage the cultures might have of greater resistance to keeping in test tubes.

The blood of many different species of animals has been used in the preparation of media for Pfeiffer's bacillus. Practically speaking it is only the blood of mammals and birds that is of importance. (Man, horse, ox, sheep, goat, dog, cat, rabbit, guinea-pig, pigeon, fowl, and duck). It is therefore important to know the significance of using the blood of one or other species.

Briefly it may be said that when using unaltered or simply haemolysed blood, pigeon blood gives a very good growth, human blood a very bad growth, while the blood of the majority of the other species of animals employed seems to give fairly uniform results. But if the blood is heated or subjected

to other drastic procedures (acid treatment, pepsin digestion, etc.) these differences pass away and good results can usually be got with the blood of any of the animals mentioned. For instance LEVINTHAL found that the blood of all the species of animals investigated was uqually suitable for his medium. The reason that MESSERSCHMIDT, HUNDESHAGEN, & SCHEER found sheep blood unsuitable for this purpose is given by LEVINTHAL as due to the fact that „Wassermann animals" were used which from frequent bleeding had become anaemic, and he points out that some workers principally used sheep blood for making LEVINTHAL agar.

RIVERS (1) found, on comparing a large number of samples of human blood with rabbit and guinea-pig blood, that human blood contained a bactericidal, growth-inhibiting substance in considerably greater concentration than the other two. This substance was destroyed by half an hour's heating 54°—56°.

GOSIO and GOSIO & MISSIROLI, in spite of numerous trials with the blood of the most different species of birds and mammals, could only obtain a good growth of Pfeiffer's bacillus on pigeon, dove, and plover blood. They even advanced the view that the Pfeiffer's bacilli which other authors declared they had cultivated on other media were „degenerated".

Prof. GOSIO has been kind enough to send me two strains of his typical Pfeiffer's bacilli (already referred to as „R 1" and „R 2") that had however been grown for 2 years in vitro, which, according to GOSIO, entails a certain amount of training to the blood of other animal species. When cultivated on FILDES agar and various other media these two strains grew exactly like other typical Pfeiffer's bacilli. As the power of adaptation of Pfeiffer's bacillus to a nutritive medium in vitro seems to be very limited it is improbable that these strains were originally quite different from all those I isolated. The enormous material of Pfeiffer's bacillus which, all over the world, has been cultivated on other media than pigeon blood agar, discountenances however, without going any further, GOSIO's view that true Pfeiffer's bacilli should be limited to those that only grow on media containing pigeon blood. There is no reason to believe that GOSIO's Pfeiffer's bacilli were different as regards their nutritive requirements from those that other investigators have worked with. He

must have prepared his media in such a way that only pigeon blood agar supported growth.

Among other conditions concerning media it may be mentioned that Pfeiffer's bacillus according to OLSEN (2) can tolerate variations in the concentration of (added) salt from 0—2%, while it is very sensitive to high concentrations of agar. If the latter exceeded 3% the growth was greatly checked.

According to JORDAN and ROSENOW it is important that the air surrounding the culture plates be kept moist. Most authors give no special information on this point. I myself have not taken precautions to keep the air moist although cultivation, as mentioned on p. 160, took place in an incubator where the air was rather dry. All that was noticed was that the growth seemed to be not so good once or twice during frosty and windy weather and consequent increased dryness and motion of the air in the incubator (but without altering of its temperature). Even on partially dried up plates I have also obtained good growth.

Practically all the methods applicable to solid media can also be used for liquid media for which reason the latter need not be dealt with further. FILDES' method however was unsuitable for broth in my experience (see also pp. 170—171).

A special method of isolating Pfeiffer's bacillus is the inoculation into animals. CARPANO (2) got constant positive results by injecting into the pleural cavity of a rabbit (after preliminary cultivation in blood broth). The animal died in a couple of days, when cultivations were made from the pleural exudate. OPIE & others as previously mentioned (p. 37), obtained more positive results in influenza by injecting sputum into a mouse than by various other methods.

That inoculation into animals would be suitable as a general method for the primary isolation of Pfeiffer's bacillus does not however seem probable, since a number of different indeterminable factors would probably render the result uncertain. In addition to this the quantitative relations of the different bacteria in the original material could not be determined by this procedure.

We have only spoken of the treatment of the material. The question of the c h o i c e of material naturally entirely depends

on the special object of the investigation and will not be dicussed in this connection. Some of the most important points were dealt with on pp. 30—31 and 37. It need only be stated here that NEGRETTE, OLSEN (1) SELIGMANN & WOLF and others had good results in the cultivation of Pfeiffer's bacillus by letting the influenza patients cough on the plates (with MEYER & CHIEVITZ's (4) „coughing-inoculation method" as a guide), but that this method failed in the hands of LOEWENHARDT (2), which he explained as due to the fact that the sputum was too tenacious to be distributed in fine droplets. In my experience also the coughing method was greatly inferior to the spreading of the sputum as a means of detecting Pfeiffer's bacillus. The utility of this method ought therefore to be carefully controlled under the given conditions before using it as the chief one.

The most important rules for the cultivation of Pfeiffer's bacillus may be stated as follows. As far as the examination concerns the occurrence of the bacillus in influenza it should be conducted as early in the disease as possible. As material, sputum and mucus from the naso-pharynx are used. It is of special importance to inoculate the material as soon as possible. Plates should be used which have been previously warmed in the incubator.

When a larger series of investigations into the occurrence of Pfeiffer's bacillus is to carried out it will be advisable to start with several different media and afterwards adhere to that giving the best results. The special conditions will determine which medium will be best. FILDES agar (for example, prepared with horse blood) and agar mixed with haemolysed pigeon blood ought specially to be recommended as media for the isolation of Pfeiffer's bacillus. If we further include ordinary blood agar (heated to a dark red colour) and LEVINTHAL agar the chief types of „haemoglobin" media will be represented. A good medium is characterized by two things: (1) that when the original culture of Pfeiffer's bacillus grows on it colonies are always formed of the size that ought to be attained on that medium; (2) that a pure culture of Pfeiffer's bacillus inoculated on it should always give a good growth. The most suitable incubation time to obtain a good result is presumably

24—36 hours. The plates ought however to be examined again after remaining another 24 hours in the incubator.

For the preparation of large quantities of culture (for serological work or vaccine production) „chocolate“ agar seems particularly suitable.

The reason that the extensive investigations into the occurrence of Pfeiffer's bacillus during the last pandemic did not bring forth the hoped-for explanation of the nature of the undoubted connection between this bacillus and influenza, depended to a large extent upon the fact that we were still rather groping in the dark as regards the purely elementary technique of cultivation. We must certainly recognise the necessity of again undertaking extensive investigations during another pandemic which will undoubtedly take place sooner or later, but according to a carefully prepared plan and at the same time making use of the experience of the last few years. Besides the special problem of the relation of Pfeiffer's bacillus to influenza, many other questions relating to this organism, particularly its manner of spreading among healthy and diseased persons and the biochemical factors which are important in its cultivation deserve further investigation which will probably also throw light upon questions of more general bacteriological interest.

In this review of the experiences of myself and others which is now concluded, it has not so much been my object to establish definite conceptions as to display an extensive collection of facts. It is my hope that even for those readers who are unable to share my particular views, these experiences may form a portion of the material from which further studies can spring.

Résumé.

1. The occurrence of Pfeiffer's bacillus in influenza patients was investigated during 4 epidemics in the years 1918—1922. It was found only rarely in the first epidemic, while in the next which was only separated from the first by an extremely short interval, it was found decidedly more frequently (in the sputum in 35% of the patients and in 51% of the autopsies on influenzal pneumonia). A more efficient technique would certainly have yielded a somewhat higher percentage in the case of the sputum.

Pfeiffer's bacillus must certainly have increased in distribution in influenza patients between the 1st. and 2nd. epidemics. Whether any real change in its occurrence in influenza took place later cannot be decided.

2. Pfeiffer's bacillus was found in influenza most often in the first couple of days after the beginning of the disease.

3. In whooping-cough this organism could be demonstrated still more frequently than in influenza, in the first series in all the 12 patients examined.

4. In measles it had a similar distribution to that of influenza.

5. It was found in pure culture in the spinal fluid of 5 cases of meningitis.

6. Pfeiffer's bacillus was met with, widely distributed among healthy persons. The occurrence however was very variable in the different groups investigated ranging from 8% to 100%.

7. As a direct consequence of each of the 4 epidemics a marked increase in the distribution of Pfeiffer's bacillus among the population as a whole was observed, followed in the succeeding months by a sharp decrease.

8. In military camps and barracks Pfeiffer's bacillus was encountered in distinctly wider distribution than was usual in the remainder of the population.

9. Pfeiffer's bacillus was found a little more commonly in persons who had had influenza than in others, but it may also occur in wide distribution among the latter.

From numbers 1, 7, and 9 it is concluded that its distribution in influenza was secondary to the influenza and that its distribution in healthy subjects was probably secondary to its occurrence in influenza.

10. In inoculations from the mouths of 60 guinea-pigs Pfeiffer's bacillus was found in 5. It could not however be demonstrated in horses or mice.

11. From the different sources named about 800 strains were cultivated. In all of these (with unimportant exceptions) the characters detailed below under a, d, g, h, and either e, or f, were demonstrated. The other characters were identified in a smaller number, in most cases in about 150 strains. As these may practically be looked upon as random samples of the entire material it is justifiable to assume that the characters which were demonstrated only in these strains were common to the whole material and to the complete flora of Pfeiffer's bacilli present in Denmark.

(a). Microscopically they were all rods which could however assume very different forms. The collection of forms for the whole group is very large (see plate 3), but not unlimited. The most important common feature is the slenderness of the bacilli, at least in a number of the individuals in a culture.

The collection of forms of an individual strain often constitutes a considerable proportion of the forms of the whole group.

(b). They are non-motile.

(c). Spores can never be demonstrated.

(d). They are distinctly Gram negative.

(e). In pure culture they cannot grow on ordinary peptone broth agar.

(f). In pure culture they are usually unable to grow on blood-free ascitic agar. Very occasionally however a slight growth is observed.

(g). For a rich growth red blood cells or certain of their

derivates are required in the medium. All the strains appear to react in the same manner towards the form of „haemoglobin" supplied to them. Thus they grow poorly on blood agar with intact blood corpuscles and abundantly on agar with heated or pepsin-digested blood. Different preparations of haemolysed blood can give growth of very dissimilar intensity. But all the strains go together, so that when for example any one strain gave a particularly good growth (for that strain) on a certain medium, it was the same with all the other strains inoculated simultaneously.

The lowest haemoglobin concentration which would support growth was about the same for all the strains examined.

It is therefore not only the dependence on „haemoglobin", but also the special nature of that dependence which is common to the group.

This character persists practically unaltered even after a long period of cultivation.

(h). On suitable nutritive media growth proceeds rapidly so that isolated colonies are always visible after 24 hours' incubation at 37°.

(i). All the strains (at any rate with the technique used) proved to be decidedly aerobic.

(j). No difference could be shown to exist with regard to the optimum hydrogen ion concentration of the medium for growth.

(k). The minimum temperature at which growth took place lay between 20° and 25°.

(l). The cultures were killed by drying for 24 hours (under further detailed experimental conditions).

(m). Surface cultures were killed by remaining 5 weeks in the cold room without being subcultured.

(n). On blood agar (even with the addition of pepsin-digested blood which increased the growth considerably) no haemolysis took place during a week's incubation.

(o). No appreciable fermentation power was observed in the liquid medium containing glucose employed.

(p). Arbutin was not broken down.

(q). No proteolytic action (for gelatine or coagulated serum) was found.

All these characters were common to all the strains examined.

The following characters were common to the large majority of strains.

(r). Increase of growth around colonies of other species of bacteria. In a few strains this character was absent or very poorly developed.

(s). Death after half an hour's heating at about 50°. A few strains however resisted a slightly higher temperature.

Since all the strains cultivated, so far as was investigated, possess such a large number of common characters, they ought, as has hitherto been the case, to be classed as the same species. This receives additional support from the following marked differences between these bacilli and closely related species.

12. The haemolytic haemoglobinophilic bacilli differ from Pfeiffer's bacilli (a) in the presence of haemolysis, (b) in only occurring as saprophytes, (c) in a less strict dependence on haemoglobin and usually also, (d) in a coarser morphology and (e) in a slighter resistance to keeping.

13. Bac. haemoglobinophilus canis differs from Pfeiffer's bacillus (a) in its richer growth on blood agar, (b) in its growth not being increased by other bacteria, (c) in promoting the growth of Pfeiffer's bacillus, (d) in its greater resistance to drying, (e) in having well developed fermentative powers.

Otherwise these two species resemble Pfeiffer's bacillus in so many points that they are naturally regarded as closely related forms.

14. Bordet's whooping-cough bacillus is considered to be more divergent. The aim here has been to show how constant and easily demonstrable the series characters is which distinguishes it from Pfeiffer's bacillus. These characters are:

(a). The appearance of the culture on blood agar.

(b). Haemolysis of a definite kind on this medium.

(c). The slow growth.

(d). Growth on blood-free ascitic agar.

(e). No growth or slight growth on the media which are particularly suitable for Pfeiffer's bacillus.

(f). The growth is not promoted by other bacteria.

(g). It has itself a growth-promoting action on Pfeiffer's bacillus.

(h). The greater resistance against keeping.

(i). Specific agglutination and complement fixation.

Most of these points were investigated with 75 strains.

15. Apart from a certain change in the intensity of growth on long-continued cultivation all the strains of this organism investigated entirely agreed with one another for all practical purposes on each of the points enumerated, and also in the absence of growth on ordinary agar in which they resemble Pfeiffer's bacillus.

16. In contrast to this great homogeneity in the species, BORDET's bacillus (Bacillus tussis convulsivae), there exist in the species Pfeiffer's bacillus (Bacillus influenzae), — in spite of a large number of common characters, — a multiplicity of individual differences between the strains.

The most important of these are:

(a). The division into indol-producers and non-indol-producers.

(b). The macroscopic appearance and consistence of the culture. This applies to mass cultures on different media and for single colonies.

(c). Agglutination and complement fixation.

(d). Minimum temperature for growth.

16. The division into indol-producers and non-indol-producers is very sharp. It has, with perhaps a few exceptions, proved to be independent of long-continued cultivation and wide differences in the experimental conditions.

Strong and weak indol-producers can be distinguished. The latter constitute only a small minority.

On suitable media the strong indol-producers form about as much indol as Bacillus coli.

17. On the basis of the macroscopic appearance and consistence of the culture a classification of Pfeiffer's bacilli into two not very sharply defined main groups, — „typical" and „atypical" is made (table p. 145). This difference is particularly well brought out by the same experimental arrangement as is employed to demonstrate the „symbiosis" phenomenon.

18. A definite method for showing the symbiosis phenomenon has been evolved, based on the investigation of a series of different factors of which the following may be specially alluded to (19—21):

19. The medium must contain a small amount of „haemoglobin"; details are given of the form in which it must be present.

20. The cultures of the growth-promoting organism and Pfeiffer's bacillus must not be brought into immediate contact.

21. It can be assumed that the different species of bacteria form the same growth-promoting substance, but in different amounts.

22. There are strains which must be classed as Pfeiffer's bacilli although the symbiosis phenomenon is poorly developed or even completely absent.

23. From investigations into the agglutination reactions it may be asserted that any two strains of Pfeiffer's bacillus chosen at random will prove in the majority of cases to be fundamentally distinct when compared by simple agglutination and absorption of agglutinins.

Out of 6 strains of Pfeiffer's bacillus cultivated from autopsies on influenzal pneumonia cases in the same epidemic, only two were identical as regards agglutination. The others were distinctly different from them and from one another.

24. No connection could be shown to exist between the agglutination reaction and the classification into indol-positive and indol-negative strains.

25. The larger number of typical strains however are indol-producers, and the majority of atypical strains, non-indol-producers.

26. As regards microscopic morphology there are in all probability true individual differences between strains of Pfeiffer's bacillus, but on account of the great variability of the individual strains and the gradual transitions between the different appearances observed it has been impossible to determine their exact nature.

The occurrence of round bodies has been shown to be a feature common to all Pfeiffer's bacilli, which exhibit wide variations with regard to size and staining reactions.

Some of these bodies are stained black by Gram's method and also with fuchsin or any simple staining method.

27. A comprehensive survey is given of the way in which the haemoglobinophilic bacteria probably ought to be classified. Only three species are considered as belonging to this group,

namely, Pfeiffer's bacillus, the haemolytic haemoglobinophilic bacteria, and Bacillus haemoglobinophilus canis.

Of these the first species only is to be regarded as haemoglobinophilic in the strictest sense.

Besides these three well defined species a few strains have been described which may possibly belong to same group.

28. An account and criticism of the nutritive media which have been used in the cultivation of Pfeiffer's bacillus has been given.

29. From my own experience and that of others it is maintained that in all probability Pfeiffer's bacillus is not to be looked upon as the primary specific virus of influenza. A number of considerations of the conditions relating to its occurrence in the healthy and the sick, are also given.

Our knowledge of the biology of the haemoglobinophilic bacteria still requires to be widened in many directions.

Résumé.

1. Forekomsten af Pfeiffers Bacil hos Influenzapatienter er undersøgt under 4 Epidemier i Aarene 1918—1922. Under den første Epidemi fandtes den kun sjældent, medens den ved den næste Epidemi, som kun ved et yderst kort Interval var adskilt fra den første, fandtes med betydelig større Hyppighed (i Ekspektorat hos 35% af Patienterne og ved 51% af Sektionerne af Influenzapneumoni). For Ekspektoraterne vilde en mere fuldkommen Teknik sikkert have givet et noget højere Procenttal.

Pfeiffers Bacil maa antages virkelig at være tiltaget i Udbredning hos Influenzapatienterne fra 1ste til 2den Epidemi; om der senere er sket nogen reel Forandring i dens Forekomst ved Influenza, kan ikke afgøres.

2. Pfeiffers Bacil fandtes ved Influenza med størst Hyppighed i det første Par Dage efter Sygdommens Begyndelse.

3. Ved Kighoste kunde denne Mikrob paavises med endnu større Hyppighed end ved Influenza, i den først undersøgte Serie endog hos samtlige 12 Patienter.

4. Ved Mæslinger fandtes den med lignende Udbredning som ved Influenza.

5. Ved 5 Tilfælde af Meningitis fandtes den i Renkultur i Spinalvædsken.

6. Pfeiffers Bacil er fundet med betydelig Udbredning hos sunde Personer. Forekomsten var dog meget forskellig i de forskellige undersøgte Grupper, idet den varierede fra 8 til 100%.

7. I direkte Tilslutning til hver af de 4 Epidemier er paavist en væsentlig Forøgelse af Pfeiffers Bacil's Udbredning i Befolkningen som Helhed og derefter i de følgende Maaneder en stærk Aftagen.

8. I Soldaterlejre og -Kaserner træffes Pfeiffers Bacil med

betydelig større Udbredning end gennemsnitlig i den øvrige Del af Befolkningen.

9. Pfeiffers Bacil er fundet noget hyppigere hos Personer, der havde haft Influenza end hos andre; dog kan den ogsaa forekomme med meget betydelig Udbredning hos sidstnævnte.

Af Punkt 1, 7 og 9 sluttes, at dens Udbredning ved Influenza sandsynligvis er sekundær i Forhold til Influenza'en, og at dens Udbredning hos sunde Personer sandsynligvis er sekundæi i Forhold til dens Forekomst ved Influenza.

10. Ved Podning fra Mundhulen af 60 Marsvin fandtes Pfeiffers Bacil hos 5. Derimod kunde den ikke paavises hos Heste og Mus.

11. Fra de forskellige nævnte Kilder er ialt dyrket ca. 800 Stammer; hos alle disse (til Dels dog med uvæsentlige Undtagelser) er de nedennævnte Forhold a, d, g, h samt enten e eller f paavist; de øvrige anførte Punkter er konstaterede ved Undersøgelse af et mindre Antal, i de fleste Tilfælde ca. 150 Stammer. Da disse i det væsentlige er at betragte som tilfældigt udtagne Prøver af det hele Materiale, er det berettiget at regne med, at ogsaa de Egenskaber, som kun er paavist hos disse Stammer, dog har Gyldighed for det hele Materiale og for den samlede Flora af Pfeiffer-Baciller her i Landet.

a) Ved mikroskopisk Undersøgelse er alle de paagældende Mikrober Stave, som dog kan være af meget forskellig Skikkelse. Den samlede Formkreds for hele Gruppen er meget stor (se Tavle 3), men dog ikke ubegrænset. Det vigtigste Fællestræk er en ringe Tykkelse af Stavene, i det mindste hos en Del af Individerne i en Kultur.

Den enkelte Stammes Formkreds udgør ofte en betydelig Del af den samlede Gruppes Formkreds.

b) De er væsentlig ubevægelige.

c) Sporer kunde aldrig paavises.

d) De er udpræget Gram-negative.

e) De kan i Renkultur ikke vokse paa almindelig Bouillon-Pepton-Agar.

f) I Renkultur kan de sædvanlig heller ikke vokse paa blodfri Ascitesagar; dog er ganske undtagelsesvis en svag Vækst iagttaget.

g) Til rigelig Vækst kræves Indhold i Substratet af røde Blodlegemer eller visse Omdannelsesprodukter af saadanne. Alle

Stammerne synes at reagere paa samme Maade overfor den Form, i hvilken man byder dem „Hæmoglobinet". Saaledes vokser de alle svagt paa Blodagar med uskadte Blodlegemer og rigeligt paa Agar med ophedet eller pepsinfordøjet Blod. Forskellige Præparater af hæmolyseret Blod kunde give Vækst af meget ulige Intensitet. Men samtlige Stammer følges ad, saaledes at naar f. Eks. en vilkaarlig Stamme paa et eller andet Substrat gav en (for denne Stamme) særlig stærk Vækst, var det samme Tilfældet med alle andre samtidig udsaaede Stammer.

Den mindste Hæmoglobinkoncentration, som endnu muliggjorde Vækst, var nogenlunde ens for alle de undersøgte Stammer.

Det er altsaa ikke blot Afhængigheden af „Hæmoglobin", men ogsaa den mere specielle Art af denne Afhængighed, der er fælles for Gruppen.

Denne Egenskab holder sig væsentlig uforandret selv ved langvarig Dyrkning.

h) Paa egnede Næringssubstrater foregaar Væksten hurtigt, saa at enkeltstaaende Kolonier altid er synlige efter 1 Døgns Henstand ved 37°.

i) Alle Stammerne har (i det mindste ved den anvendte Forsøgsanordning) vist sig udpræget aërobe.

j) Der kunde ingen Forskel paavises med Hensyn til den for Væksten optimale Brintionkoncentration i Substratet.

k) Den minimale Vækst-Temperatur ligger mellem 20° og 25°.

l) Kulturerne dræbtes ved Indtørring i 24 Timer (under nærmere angivne Forsøgsbetingelser).

m) Overfladekulturer dræbtes ved 5 Ugers Henstand i Iskælder uden Omsaaning.

n) Paa Blodagar (selv med Tilsætning af pepsinfordøjet Blod, hvorved Væksten forøges betydeligt) fremkommer ikke Hæmolyse ved en Uges Henstand i Termostat.

o) Ingen væsentlig Gæringsevne kunde paavises i det benyttede glykoseholdige, flydende Substrat.

p) Arbutin spaltes ikke.

q) Der fandtes ingen proteolytisk Evne (overfor Gelatine og stivnet Serum).

Alle disse Egenskaber er fælles for alle de Stammer, der er undersøgt i de paagældende Retninger.

Følgende Forhold er fælles for det langt overvejende Flertal:

r) Vækstforøgelsen omkring Kolonier af andre Bakteriearter. Hos nogle faa Stammer manglede denne Egenskab eller var meget lidt udtalt.

s) Drab ved ½ Times Opvarmning til ca. 50°. Enkelte Stammer taalte dog en lidt højere Temperatur.

Da alle de dyrkede Stammer, saa vidt det er undersøgt, har et saa betydeligt Antal Egenskaber fælles, bør de — ligesom hidtil — henregnes til samme Art. Dette begrundes yderligere ved Paavisningen af følgende udprægede Forskelligheder mellem disse Baciller og nærstaaende Arter:

12. De hæmolytiske, hæmoglobinofile Baciller adskiller sig fra Pfeifferbacillerne a) ved Hæmolysen, b) ved kun at optræde som Saprofyter, c) ved en mindre absolut Afhængighed af Hæmoglobin samt som Regel ogsaa: d) ved en grovere Morfologi og e) en ringere Holdbarhed af Kulturerne.

13. Bacillus hæmoglobinophilus canis afviger fra Pfeiffers Bacil a) ved rigeligere Vækst paa Blodagar, b) ved at dens Vækst ikke forøges af andre Mikrober; c) ved selv at virke fremmende paa Væksten af Pfeiffers Bacil; d) ved større Resistens mod Indtørring; e) ved stærkt udviklet Gæringsevne.

I øvrigt ligner disse to Arter Pfeiffers Bacil paa saa mange Punkter, at de naturligt betragtes som nærstaaende Former.

14. Mere afvigende skønnes Bordets Kighostebacil at være. Der er her hovedsagelig lagt Vægt paa at vise, hvor konstante og let konstaterbare en Række af de Egenskaber er, som adskiller denne Art fra Pfeiffers Bacil. Disse Egenskaber er:

a) Kulturens Udseende paa Blodagar.

b) Hæmolyse af bestemt Karakter paa dette Substrat.

c) Den langsomme Vækst.

d) Vækst paa blodfri Ascitesagar.

e) Ingen eller ringe Vækst paa de Substrater, som er særlig egnede til Pfeiffers Bacil.

f) Væksten fremmes ikke af andre Mikrober.

g) Den har selv vækstfremmende Evne overfor Pfeiffers Bacil.

h) Kulturens større Holdbarhed.

i) Specifik Agglutination og Komplementbinding.

De fleste af disse Punkter er undersøgt paa et Materiale af 75 Stammer.

15. Bortset fra en vis Forandring af Vækstforholdene ved langvarig Dyrkning forholdt samtlige undersøgte Stammer af denne Mikrob sig saa godt som fuldstændig ens indbyrdes paa ethvert af de nævnte Punkter samt ved Mangel paa Vækst paa almindelig Agar, hvori de stemmer overens med Pfeiffers Bacil.

16. I Modsætning til denne store Ensartethed indenfor Arten Bordets Bacil (Bac. tussis convulsivæ) findes der indenfor Arten Pfeiffers Bacil (Bac. influenzæ) — trods det store Antal fælles Egenskaber — en Mangfoldighed af individuelle Forskelligheder mellem Stammerne.

De vigtigste Omraader, paa hvilke disse Forskelligheder iagttages, er:

a) Adskillelsen i Indoldannere og Ikke-Indoldannere.

b) Kulturens makroskopiske Udseende og Konsistens; dette gælder saavel for Massekultur paa forskellige Substrater som for enkelte Kolonier.

c) Agglutination og Komplementbinding.

d) Temperaturminimum for Vækst.

16. Adskillelsen i Indoldannere og Ikke-Indoldannere er meget skarp; den har, maaske med enkelte Undtagelser, vist sig uafhængig af langvarig Dyrkning og vide Variationer af Forsøgsbetingelserne.

Der kan skelnes mellem stærke og svage Indoldannere; de sidste udgør kun et ringe Mindretal.

Paa Substrat af passende Sammensætning danner de stærke Indoldannere en lignende Mængde Indol som Colibaciller.

17. Paa Grundlag af Kulturens makroskopiske Udseende og Konsistens foreslaas en Inddeling af Pfeifferbacillerne i to, ganske vist ikke skarpt afgrænsede Hovedgrupper: „typiske“ og „atypiske“ (Skema S. 166). Særlig tydeligt træder denne Forskel frem ved den samme Forsøgsanordning, som tjener til Paavisning af „Symbiosefænomenet“.

18. Til Paavisning af dette Fænomen er en bestemt Fremgangsmaade angivet og begrundet ved Undersøgelse af en Række forskellige Forhold, af hvilke særlig følgende kan anføres (19—21):

19. Substratet skal indeholde en ringe Mængde „Hæmoglobin“; det er nærmere angivet, i hvilken Form dette skal forefindes.

20. Kulturen af den vækstfremmende Mikrob og af Pfeiffers Bacil maa ikke anbringes i umiddelbar Berøring.

21. Man kan regne med, at de forskellige Bakteriearter danner det samme vækstfremmende Stof, blot i forskellig Mængde.

22. Der gives Stammer, som maa henregnes til Pfeiffers Bacil til Trods for, at Symbiosefænomenet er lidet udtalt eller endog helt mangler.

23. Paa Grundlag af Undersøgelserne over Agglutinationsforholdene kan det siges, at to tilfældigt valgte Stammer af Pfeiffers Bacil i de fleste Tilfælde vilde vise sig grundforskellige ved Undersøgelse ved simpel Agglutination og Agglutininabsorption.

Af 6 Stammer af Pfeiffers Bacil, dyrkede fra Sektion af Influenzapneumoni under samme Epidemi, viste kun to sig agglutinatorisk identiske; de øvrige var væsentlig forskellige saavel fra disse som indbyrdes.

Foruden de to omtalte Stammer er ogsaa i andre Tilfælde fundet Grupper paa to eller flere Stammer, som ved Agglutination og Agglutininabsorption viste sig indbyrdes identiske.

24. Der kunde ingen Sammenhæng paavises mellem Agglutinationsforholdene og Inddelingen i Indol-positive og Indol-negative Stammer.

25. Derimod er det overvejende Antal typiske Stammer Indoldannere, det overvejende Antal atypiske Stammer Ikke-Indoldannere.

26. Med Hensyn til mikroskopisk Morfologi findes der efter al Sandsynlighed virkelige individuelle Forskelligheder mellem Stammer af Pfeiffers Bacil, men paa Grund af den store Variabilitet hos de enkelte Stammer og de flydende Overgange mellem de forskellige iagttagne Billeder har det ikke været muligt at præcisere disse Forhold nøjere.

Som en fælles Egenskab for samtlige Pfeifferbaciller er paavist Forekomsten af rundagtige Legemer, som iøvrigt frembyder en stor Variationsbredde med Hensyn til Størrelse og Farvelighed.

Nogle af disse Legemer farves sorte saavel ved Gramfarvning som ved Farvning med Fuksin eller en anden simpel Farvning.

27. Der er givet en sammenfattende Oversigt over den Maade,

hvorpaa de hæmoglobinofile Bakterier formentlig bør klassificeres. Kun tre Arter betragtes som henhørende til denne Gruppe, nemlig: Pfeiffers Bacil, de hæmolytiske, hæmoglobinofile Bakterier og Bac. hæmoglobinophilus canis.

Af disse er endda kun den første Art at betragte som hæmoglobinofil i strengeste Forstand.

Foruden disse tre vel karakteriserede Arter har man beskrevet enkelte Stammer, som muligvis er at henregne til samme Gruppe.

28. Der er givet en Oversigt over og Vurdering af de Næringssubstrater, som har været anvendt til Dyrkning af Pfeiffers Bacil.

29. Paa Grundlag af egne og andres Erfaringer hævdes, at Pfeiffers Bacil efter al Sandsynlighed ikke er at betragte som Influenza'ens specifikke primære Virus. I øvrigt er anstillet en Række Betragtninger over de Forhold, der gør sig gældende ved dens Forekomst hos Sunde og Syge.

Vor Viden om de hæmoglobinofile Bakteriers Biologi trænger endnu paa adskillige Punkter til at udvides og uddybes.

Literature.

All the names of the persons mentioned in the text will be found in the index of literature with only a few exceptions where it will be clear that no literary reference can be expected.

The following abbreviations are used:

B. kl. W. = Berliner klinische Wochenschrift.
B. M. J. = British Medical Journal.
C. f. B. = Centralblatt für Bakteriologie, I. Abt., from vol. 31: Originale.
C. f. B. Ref. = Centralblatt für Bakteriologie, I. Abt., Referate.
C. f. B. II. = „ „ „ , II. Abt.
D. m. W. = Deutsche medizinische Wochenschrift.
J. A. M. A. = Journal of the American Medical Association.
J. E. M. = Journal of Experimental Medicine.
J. I. D. = Journal of Infectious Diseases.
J. P. B. = Journal of Pathology & Bacteriology.
M. m. W. = Münchener medizinische Wochenschrift.
W. m. W. = Wiener medizinische Wochenschrift.
W. kl. W. = Wiener klinische Wochenschrift.
Z. f. H. = Zeitschrift für Hygiene.

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Notes on the Plates.

Plate 1 is described on pp. 124 and 127.

Plate 2 is described on pp. 140—143.

On plate 3 small sections of the microscopic appearance of fuchsin-stained dry preparations are reproduced from pencil sketches, at a magnification of 1500 in each case. Two colonies are also shown diagrammatically, — at a magnification of about 10, — with centrally grouped and with scattered granules respectively.

In parenthesis the „number of the generation" is given and the medium employed, B = blood agar, BB = brown blood agar („chocolate" agar), H = haemoglobin agar, F = FILDES agar, A = ascitic agar (with a minute normal content of haemoglobin), S = „symbiosis" plates. 1 H therefore signifies that the preparation is from a colony on the original plate which consisted of haemoglobin agar; 2 B denotes a culture on blood agar derived from an inoculation from a colony on the original plate.

The illustrations of the same strain, even when they depict different cultures, are arranged in close proximity (e. g. the 4 illustrations of I 14). Illustrations of different parts of the same preparation are furnished with a common subscript (e. g. the illustrations of I 14 (2 B)).

All the illustrations surrounded by a continuous line exhibit the elements in their actual position in the preparation but in the illustrations enclosed by an interrupted line the assembled elements are chosen from different parts of the same preparation. The figures which are collected under the common heading „Various" are however taken from preparations of a number of different strains.

In I 28, * signifies a culture grown 6 days later than the original culture; ** cultures which were grown 6 days later than those marked with *.

In I 65, S* signifies a preparation from the luxurious portion of the growth in a symbiosis test, S**, a preparation from the weak growth outside the effective zone of the foreign bacterial colony in the same culture.

The main object of this plate is to give an idea of the whole series of forms of the species Pfeiffer's bacillus which may be

observed, and to indicate in what manner the individual strain is most liable to vary.

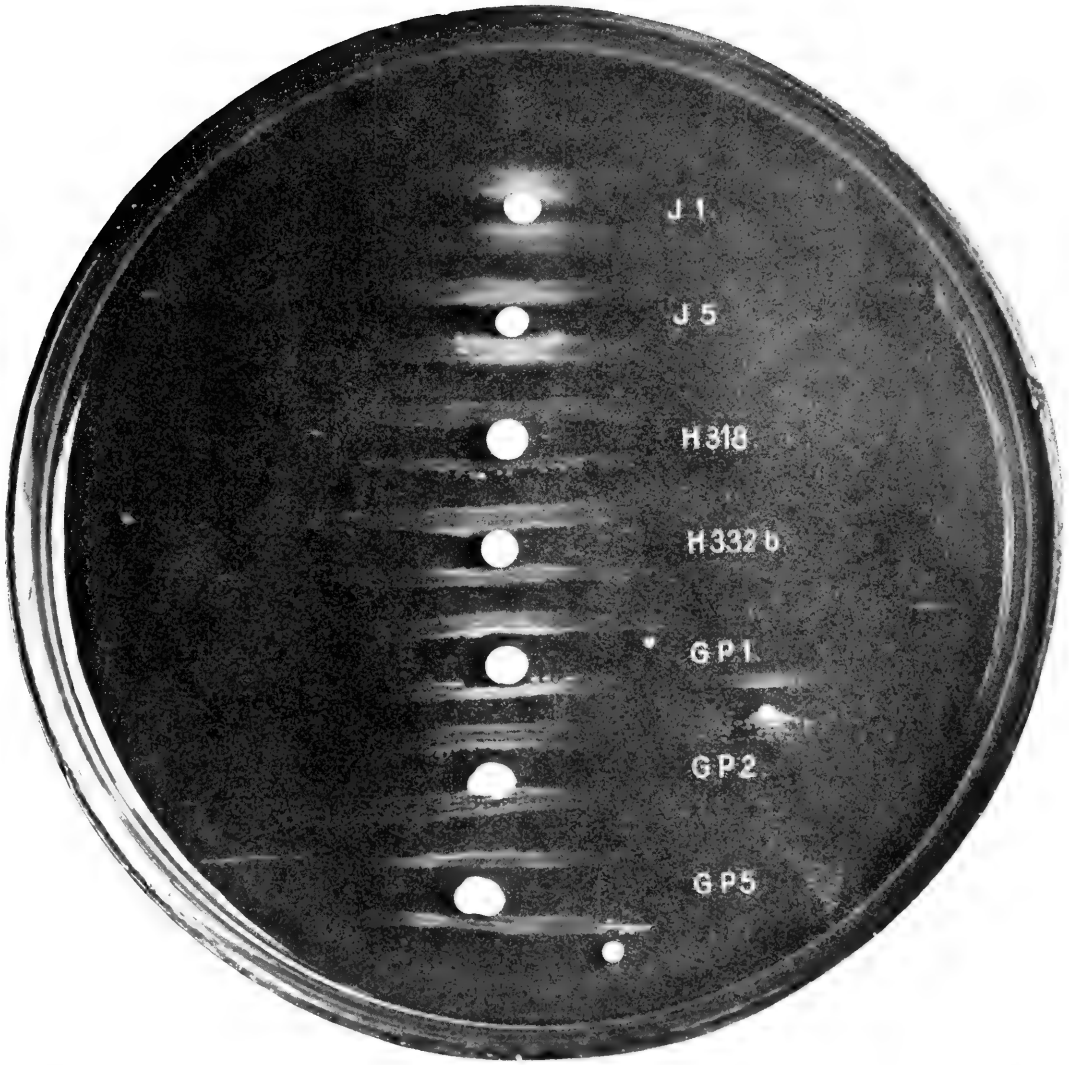
A few explanatory remarks on some of the illustrations will not be out of place. In the illustrations of I 14 it will be noticed first that while a colony on the haemoglobin agar plate consisted of relatively long elements, those on the simultaneously inoculated blood agar plates consisted for the most part of considerably shorter ones and the preparation as a whole showed a more polymorphic picture than in the first case. The two illustrations on the right show a fresh culture on blood agar inoculated from one of these colonies. We see here all gradations from quite short rods to fairly long threadshaped elements of different thickness but on the whole, rather slender. Similar appearances are observed in several of the other strains illustrated; for instance in I 43 the picture on the left shows the appearance usually described as typical, while in the next culture on the same medium it has already assumed an entirely different aspect. The length of the individual elements is thus an extremely labile character among Pfeiffer's bacilli.

As a characteristic example of an „atypical" microscopic morphology I 28 may be cited which from the macroscopic appearance also was classed as atypical. The characteristics are the great heterogeneity in the size of the elements and, — by no means least — the staining. Side by side in the same preparation are to be seen palestained and very intensely stained elements the latter often being rather larger than the typical forms. The same thing is seen in preparations of bacilli from guinea-pigs which were also all atypical macroscopically.

Four portions of a preparation of B. haemoglobinophilus canis are also seen. The great variation in the size and staining of the elements is to be noted. On the whole the microscopic picture of this bacterial species corresponded best to the microscopically atypical Pfeiffer's bacilli.

In the bottommost row preparations of 4 haemolytic strains are shown. Note the very coarse morphology even in cultures which do not exhibit any marked polymorphism.

The reader is also referred to the remarks on pp. 145—149 on the morphological characters.



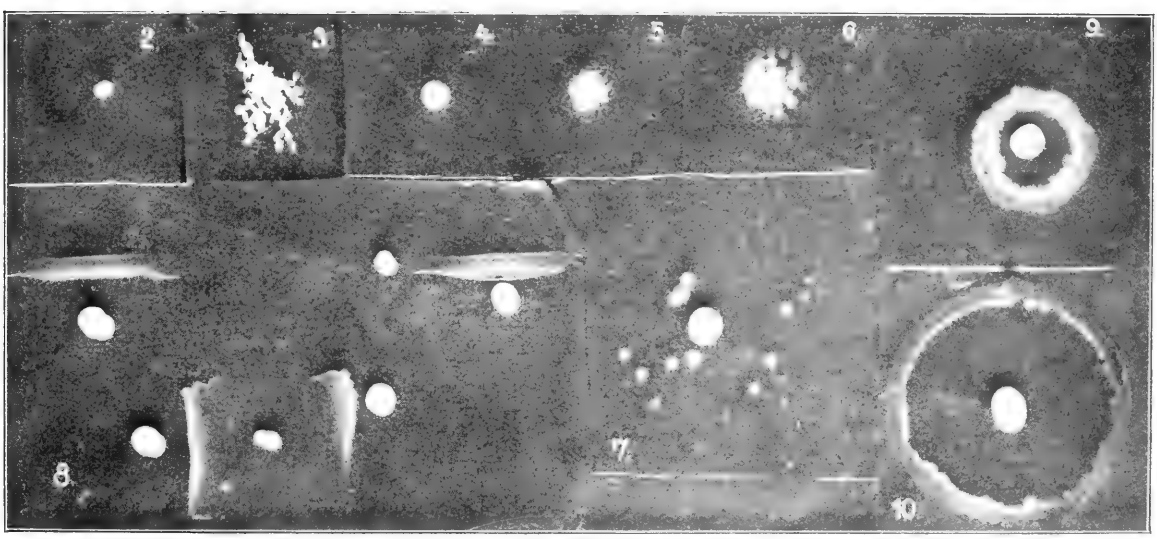
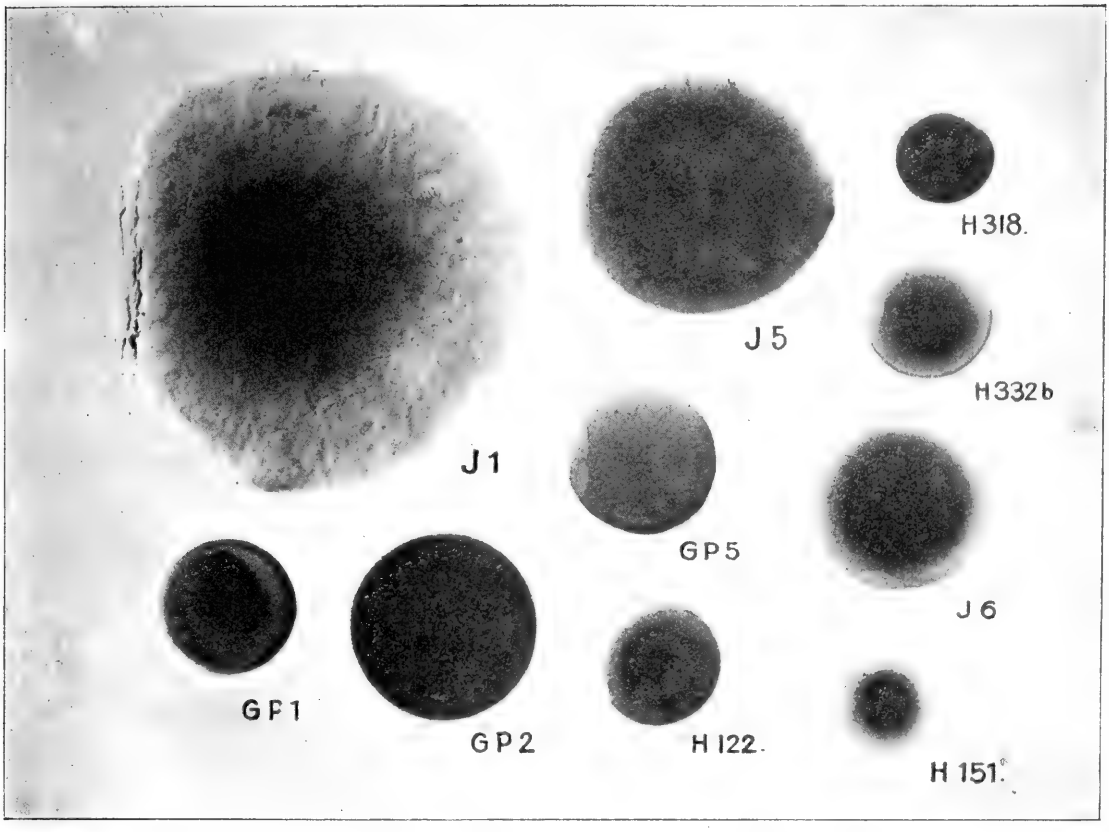


Plate 1


J 1
J 5
H 318.
H 332b
GP 5
J 6
GP 1
GP 2
H 122.
H 151.

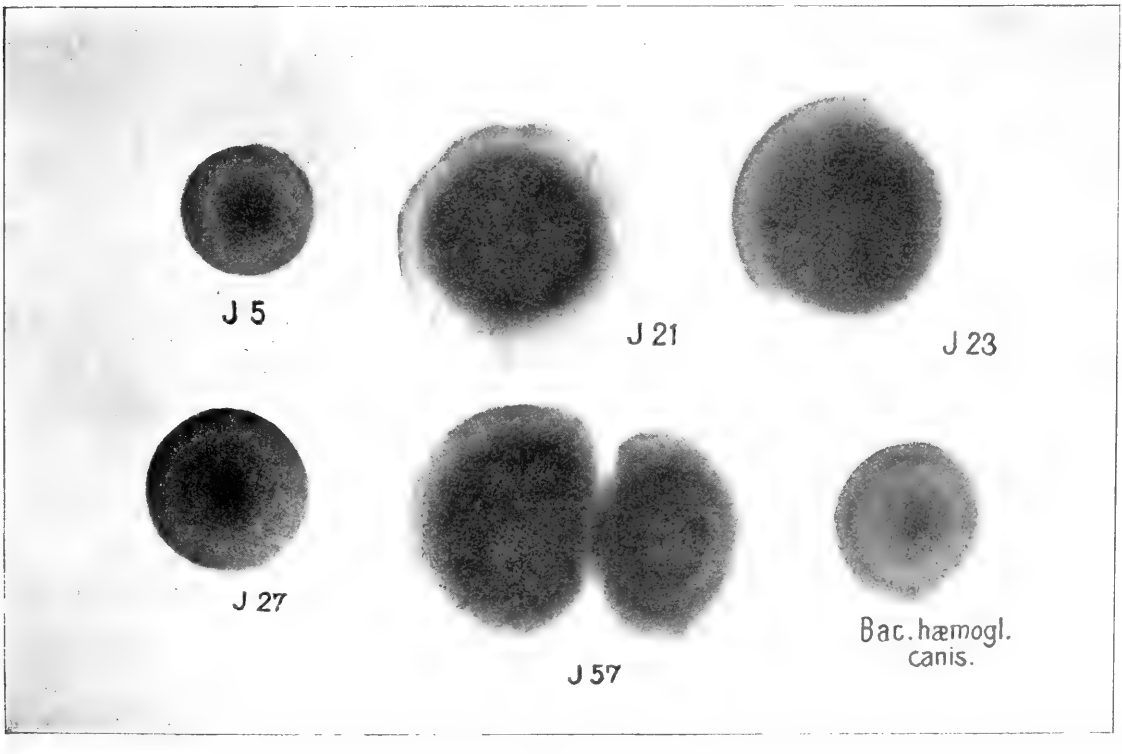

J 5
J 21
J 23
J 27
J 57
Bac. hæmogl. canis.

Plate 2

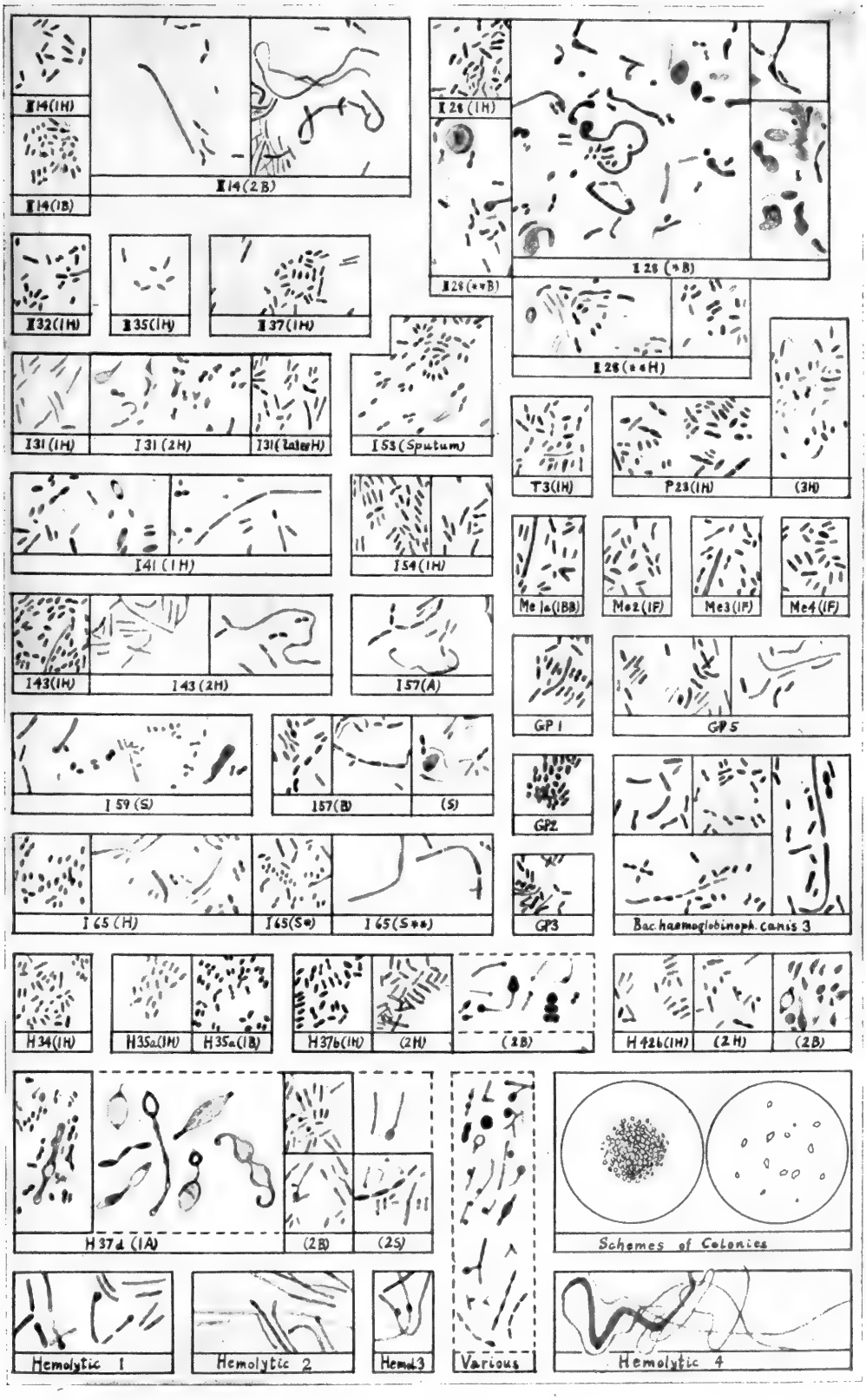

I 14 (1H)
I 14 (2B)
I 14 (1B)
I 28 (1H)
I 28 (+B)
I 28 (++B)
I 28 (++H)
I 32 (1H)
I 35 (1H)
I 37 (1H)
I 31 (1H)
I 31 (2H)
I 31 (later H)
I 53 (Sputum)
T 3 (1H)
P 23 (1H)
(3H)
I 41 (1H)
I 54 (1H)
Me 1a (1BB)
Me 2 (1F)
Me 3 (1F)
Me 4 (1F)
I 43 (1H)
I 43 (2H)
I 57 (A)
GP 1
GP 5
I 59 (S)
I 57 (B)
(S)
GP 2
I 65 (H)
I 65 (S+)
I 65 (S++)
GP 3
Bac. haemoglobinoph. canis 3
H 34 (1H)
H 35a (1H)
H 35a (1B)
H 37b (1H)
(2H)
(2B)
H 42b (1H)
(2H)
(2B)
H 37d (1A)
(2B)
(2S)
Schemes of Colonies
Hemolytic 1
Hemolytic 2
Hemol 3
Various
Hemolytic 4

Plate 3