BACTERIOLOGICAL ASPECTS OF THE MENINGOCOCCUS CARRIER PROBLEM[1].

By A. EASTWOOD, M.D.

Introduction.

During the year 1915, Drs F. Griffith, W. M. Scott and I have been investigating the meningococcus in the Board's Pathological Laboratory with the object of providing information which may be of service to the Board, particularly with reference to the carrier problem.

Before entering into technical details I wish to state in general terms the problems which present themselves and the difficulties which are to be overcome.

There is an obvious difference between diagnosing the meningococcus in cerebro-spinal fluid withdrawn from a patient with symptoms of meningitis and identifying this organism in a culture from the naso-pharynx of a person not clinically affected with the disease. Assuming the two organisms to be alike in cultural characters, with the former the confirmatory evidence of specific pathogenicity is supplied by the condition of the patient; with the latter organism, this confirmatory proof is lacking. In the case of such organisms as the bacilli of plague, tuberculosis, or anthrax, the proof could readily be provided by animal experiment; but for the meningococcus, as for the typhoid bacillus, there is no laboratory animal available for routine demonstration of specific pathogenicity. It is therefore necessary to be content with indirect evidence that an organism isolated from the naso-pharynx is capable of producing cerebro-spinal fever.

The question then arises whether morphological and cultural characters alone, if worked out with sufficient care, can be accepted as giving complete identification. These characters, including fermentation tests,

[1] Reprinted by permission of H.M. Stationery Office, from *Reports to the Local Government Board on Public Health and Medical Subjects*, n. s. No. 110 (1916). Ed.

https://doi.org/10.1017/S0022172400006343 Published online by Cambridge University Press

are certainly of very great aid to diagnosis, and there is practically complete agreement about what cultural characteristics are to be regarded as typical of the true meningococcus. As, however, some little differences of opinion have been expressed regarding the fermentation of sugars, this question will be treated in this report as a problem calling for consideration.

But, assuming this question of fermentation reactions to be settled, it must still be admitted that more confidence would be felt in the fermentation tests if they could be confirmed by other tests of a more specific nature. This naturally suggests an appeal to serological reactions. If, for example, strains from the naso-pharynx agreed with undoubted meningococci, not only in cultural and fermentation tests, but also in agglutination with a specific serum, their identity might be admitted; and, if the correspondence between fermentation and agglutination tests was a regular occurrence, the question might be raised whether resort to the latter might, except under exceptional circumstances, be considered unnecessary for routine diagnosis. But here a preliminary and very serious difficulty arises owing to the fact that different strains of undoubted meningococci differ among themselves in their serological reactions; and no serum is available which can be guaranteed to agglutinate every strain of meningococcus without fail. Obviously, here is a problem where research is needed.

It will probably be a long time before a complete scientific explanation is accepted for the serological reactions of all strains of meningococci isolated from cerebro-spinal fever; but, in the meantime, this consideration is no bar to comparison, with such sera as are available, between the agglutination of undoubted meningococci and that of strains isolated from the naso-pharynx. It is clearly a matter of immediate interest to see if identity can be established between the former and the latter.

Difficulties in serological work with the meningococcus, though still very considerable, are not now, and never have been, of such a nature as to render such investigations useless. Ten years ago identity in serum reactions had been established between certain strains from cerebro-spinal fluid and certain strains from the naso-pharynx. These latter strains had been mainly derived from contacts, but they had also, though in much smaller numbers, been obtained from non-contacts.

This last fact raises a question which demands urgent consideration at the present time. What practical advantage is to be gained by culturing swabs from the naso-pharynx as a routine aid to prophylactic

measures? It cannot be taken for granted, without adequate bacteriological evidence, that the meningococcus is so frequently present in the normal, or merely catarrhal, throat as the pneumococcus, or, possibly, the influenza bacillus. But still it may come under the same stigma of the bacteriological class of "common lodger," which, though more frequently innocuous, or relatively so, is capable, under favourable conditions of reduced resistance, of producing its specific disease. If that is the case, the meningococcus carrier must obviously be placed in a different class from, for example, the typhoid carrier. So the practical question is, Is it true that in this country and at the present time, when precautions have to be taken against the spread of cerebro-spinal fever, the meningococcus is to be found widely distributed in the throats of persons not known to have been in contact with the disease?

This is the main problem which has been engaging the attention of the Board's Laboratory. The investigation has involved research in the directions I have already indicated, viz.:—(1) a comparative study of the fermentation and serological reactions of various strains of meningococci derived from the cerebro-spinal fluid of patients affected with meningitis; (2) a similar study of various meningococcus-like organisms obtained from the throats of non-contacts; (3) a comparison between the cerebro-spinal strains and the throat strains.

Prior to the outbreak of the epidemic in this country towards the end of 1914, many investigators have worked on the meningococcus and have recorded their results in various scientific publications. As much of their laboratory data has an important bearing on the present enquiry, I have collected and reviewed the matter which seems to me of main importance in connection with the work done in the Board's Laboratory.

HISTORICAL SURVEY.

It is not necessary to revert to the earlier literature, written when the diplococcus of Weichselbaum was on its trial. I commence with the year 1906, when the causal relationship of this organism to cerebro-spinal fever was fully established, and its cultural characters had been thoroughly worked out. It will be convenient to dispose of fermentation tests before entering into the more complicated subject of serum reactions.

Fermentation Tests.

Lingelsheim (1906)[1] made a thorough investigation of the use of fermentation reactions as a means of distinguishing meningococci from other Gram-negative diplococci found in the naso-pharynx. He used solid media (ascitic agar) containing 1 % of the substance to be tested. This had previously been sterilised in 10 % solution with Kubel-Tiemann litmus and was added to the ascitic agar just before pouring the medium. He found that meningococci produced an acid reaction in the media containing dextrose and maltose, but gave no evidence of the formation of free acid in media containing laevulose, galactose, mannite, dulcite, saccharose, lactose, or inulin. These results he confirmed with 83 strains of meningococci; they all behaved in the same way, and the fermentation with dextrose and maltose was invariably well marked.

Buchanan (1907)[2] used for his fermentation tests a solidified medium composed of 3 parts ox-blood serum, 1 part bouillon, with 1 % of glucose, galactose, maltose, and saccharose, respectively, 1 in 10,000 of neutral red being added as indicator. He examined the throats of contacts during the Glasgow epidemic and obtained 81 positive cases (26·3 %). Cultures from each of these were tested on the sugar media and gave, in every case, an acid formation with glucose and maltose, but never with saccharose. On several occasions a slight amount of acid production was observed in the galactose tube, but, he states, "this reaction is so trivial and so seldom met with that the meningococcus may be held as giving a negative reaction with galactose in this medium." He adds that in a fluid medium, containing galactose and ascitic fluid, an acid reaction has been found after an interval of several days. He noted that in his solid glucose and maltose media the meningococcus turned the water of condensation a bright yellow colour with a greenish fluorescence and a yellow, pus-like deposit at the bottom of the tube. These features he regarded as characteristic. In his table comparing the meningococcus with other varieties of Gram-negative diplococci found in the naso-pharynx, one of the latter (No. 4) is stated to agree with the meningococcus in fermenting glucose and maltose alone and in not growing at 23°–25° C., but to differ in the following respects:—(1) on glucose and maltose tubes, "growth at first resembles meningococcus, but in 48 hours the medium becomes

[1] *Klin. Jahrb.* xv.

[2] *Trans. Internat. Congr. on Hygiene and Demography, Berlin*, September, 1907, iv.

reddened throughout"; (2) in plate culture, "colonies on Petri plate are smaller than meningococcus and become intensely red in centre."

Gordon (1907)[1] recommended, for the study of the fermentation reactions, a Lemco-peptone medium containing 1 % of the required carbohydrate; to this, after sterilisation, a little sterile raw ascitic fluid was added before inoculation. The meningococcus, he found, was characterised by producing an acid reaction with glucose, galactose, and maltose, but not with saccharose.

Arkwright (1907)[2] employed liquid media for the purpose of fermentation tests and found that the characteristics of the meningococcus were "the production of acid from maltose and usually from glucose, galactose, and laevulose, but not from cane sugar."

Shennan and W. T. Ritchie (1908)[3] studied the fermentation reaction of 19 strains of meningococci obtained from cerebro-spinal fluid, using solid media prepared after the method of Lingelsheim. Acid production was always observed with glucose and maltose and, in five instances, with dextrin; the reactions were negative with galactose, laevulose, cane sugar, lactose, dulcite, mannite, inulin, and raffinose. The authors found that the alkalinity of their media should be very slight, as slight increase of alkalinity might prevent the appearance of an acid reaction. Two of their strains, though giving distinct reactions with maltose, gave comparatively slight reactions with glucose. Comparing liquid with solid media (parovarian broth with parovarian agar), they found that when galactose was added, seven of their strains produced acid in the liquid medium, but not in the solid.

Mayer (1909)[4] used Lingelsheim's solid media for his fermentation tests of a large number of naso-pharyngeal and cerebro-spinal strains which he investigated during the occurrence of cerebro-spinal fever at Würzburg. All his strains of undoubted meningococci agreed in fermenting glucose and maltose only; saccharose, lactose, laevulose, and galactose being negative. They produced a well marked reddening of the medium with maltose, but only a slight reddening with glucose. Three strains, not classed as meningococci, are of interest in that the reddening with glucose was well marked, while in all other cultural respects they were practically identical with meningococci. Apparently they are excluded from this class because one of them was only

[1] Report to the Local Government Board on the Micrococcus of Cerebro-Spinal Meningitis and its Identification.

[2] *Journ. of Hyg.* VII, 145.

[3] *Journ. of Path. and Bact.* XII, 456.

[4] *Centrbl. f. Bakteriol.* Orig. XLIX.

agglutinated up to 1 : 250 with the Höchst meningococcus serum, and the other two were not agglutinated above 1 : 100; whereas the undoubted meningococci were agglutinated up to 1 : 500. A fourth strain also bore a close cultural resemblance to the meningococcus, but differed in that with maltose, as well as with glucose, the reddening of the medium was only slight. This strain gave an agglutination with the Höchst serum in 1 : 100.

When, instead of using Lingelsheim's medium (ascitic agar), he added the litmus-sugar solution to Kutscher's agar medium, which is made with broth prepared from human placenta and contains ox serum in place of ascitic fluid, he obtained the most confusing results. Testing five strains which gave what he regarded as the typical reactions of the meningococcus on Lingelsheim's media, he found that one gave no evidence of acid formation with any of the four sugars—glucose, maltose, galactose, lactose; one produced acid with maltose only; the remaining three produced slight acid with all four sugars. He thought this difference might be attributable to the presence of dissolved red blood corpuscles in the medium.

Arkwright (1909)[1] preferred fluid to solid media for fermentation tests, as he found that the change with the latter, though usually more rapid, was in many instances of a very transient character. Testing 36 strains in weak broth with the addition of serum and one or other of the sugars—glucose, maltose, laevulose, and saccharose, he found that 17 fermented the first three, 15 the first two only, 1 glucose and laevulose, 1 maltose only, and 2 fermented none of the sugars.

Symmers and Wilson (1909)[2] applied fermentation tests to strains of meningococci obtained during the Belfast epidemic, using the fluid medium recommended by Gordon. They found that glucose, maltose and dextrin always produced acid and that all the other substances employed, including galactose and laevulose, failed to do so. 53 strains were tested with galactose and 43 with laevulose.

Elser and Huntoon (1909)[3] went very fully into the fermentation reactions of meningococci. They used Kahlbaum's and Merck's guaranteed pure products of dextrose, galactose, laevulose, lactose, maltose, saccharose, mannite, dulcite, inulin, and dextrin. These were sterilised in 10 % solution and then added in requisite quantity to the media. Various liquid media were tried, but though these furnished "reliable data concerning the fermentative capacities," they

[1] *Journ. of Hyg.* IX, 104.
[2] *Ibid.* p. 9.
[3] *Journ. of Med. Research*, XX, 377.

were not recommended, because failure to grow was common. They therefore adopted for routine use Lingelsheim's method of using solid media, with a basis of ascitic agar, but tubed their medium instead of plating it. Two hundred strains of meningococci were tested and were found to agree in fermenting dextrose and maltose only. They were aware that some observers, using liquid media, found that, in addition to these two sugars, one or other of the products, laevulose, galactose, and dextrin might also give rise to an acid reaction. Laevulose and galactose, they showed by experiment, are particularly liable to be altered by sterilisation, and they quote Maquenne, who found that so-called chemically pure dextrin contains maltose, iso-maltose, or glucose in small quantities.

With liquid media the acid reaction produced by glucose and maltose usually required 48 hours to develop, and sometimes 72 hours, but with solid media it was usually apparent within 24 hours. Some quantitative determinations of acid production with these two substances were made and it was found that "the general average acid production is greater in maltose media than in dextrose media, a fact which was also observed in connection with the qualitative tests." But with a few of their strains dextrose produced more acid than maltose.

Blair Martin (1910)[1] compared the fermentation reactions of the meningococcus and the gonococcus, using solid culture media. He found that all his strains of meningococci, 31 in number, agreed in producing acid with maltose and dextrose, and in failing to do so with laevulose and saccharose. He noted that some of his strains of meningococci fermented dextrose less rapidly than maltose.

Conclusions as to Fermentation Tests.

Lingelsheim deserves the credit of having shown that litmus-ascitic-agar is a thoroughly reliable medium as the basis for these tests, and that, on this medium, the meningococcus produces free acid with both glucose and maltose, but not with laevulose, galactose, mannite, dulcite, saccharose, lactose, or inulin. These results have now been corroborated on a large scale by many independent observers and must be accepted as accurate. Care must, of course, be taken in the preparation of the medium; the meningococcus is a somewhat feeble acid producer and if the medium be too alkaline the reaction may be masked.

[1] *Journ. of Path. and Bact.* xv, 76.

Another point to bear in mind is that occasionally, though rarely, a strain is found which does not give these reactions until it has been in subculture for some time.

As regards the comparative intensities of the reactions in the glucose and the maltose media, it is interesting to note that some observers have found the reaction equally well marked with both; others that more acid is liberated with maltose than with glucose; and others, again, that with the majority of strains maltose gives more acid than glucose, but that with some the reverse is true.

Several of the observers who have placed reliance on solid media have shown that when liquid media are used some differences may be found, the most noteworthy being the production of acid with galactose. Elser and Huntoon, in particular, have done valuable service in clearing up apparent discrepancies between the results obtained with liquid and with solid media by demonstrating that certain of the sugars, especially laevulose and galactose, are, when sterilised in liquid media, especially liable to modifications which enable the meningococcus to form acid from them.

As regards Buchanan's solid medium, in the report which I have quoted he apparently excludes certain strains from the class of genuine meningococci for the one reason that they produce with glucose and maltose a more intensely acid reaction than the genuine meningococcus. This is an interesting observation, but the exclusion obviously requires confirmation by more specific tests.

Serum Reactions.

Lingelsheim (1906)[1] tested the agglutinability of a large number of strains of meningococci which he had collected during the epidemic in Upper Silesia in the winter of 1904–5.

His strains were obtained partly from cerebro-spinal fluid and partly from pharyngeal swabs. He employed the macroscopic method, using 1 c.c. of fluid, in which one normal loopful of culture was emulsified, and incubated his dilutions for 20 hours at 37° C. In the course of his work he found that several circumstances affected the agglutinability of the same strain with the same serum. When a culture was emulsified and tested at once, without preliminary treatment of any kind, it showed much less agglutinability than it did when the emulsion had been kept, at room temperature, for some time. This effect of storage

[1] *Klin. Jahrb.* xv.

was not confined to a particular strain, but was found to be a general rule. During the first three to four weeks of storage, the agglutinability was unstable, but after the fourth week it became constant and then remained unchanged to the end of the sixth month. Compared with its agglutinability when tested in the fresh condition, the agglutinability of a culture emulsion which had been kept until it became constant was increased about five times. The emulsions were made from ascitic agar cultures with ·9 % saline solution, ·1 c.c. of formalin being added to 40 c.c. of saline. Lingelsheim also investigated the influence of heat on the agglutinability of fresh cultures and found that exposure for ½–1 hour to temperatures of 50° C., 60° C., and 70° C. increased agglutinability but 80° C. had the reverse effect.

He prepared two rabbit sera. The first, produced with a strain of meningococci obtained by lumbar puncture, gave a titre of 1 : 400. Sixty-three strains were tested with it, the majority being cultures from the pharynx; they were all agglutinated in dilutions of from 1 : 200 to 1 : 400, whilst none were agglutinated in 1 : 10 by normal rabbit serum. The second serum, produced by another strain of meningococci obtained by lumbar puncture, gave a titre of 1 : 800 and was tested on 47 strains. These agglutinated between 1 : 400 and 1 : 800, but were not agglutinated by normal rabbit serum in 1 : 10.

With regard to the identification of Gram-negative diplococci obtained from the naso-pharynx, Lingelsheim remarked that resort to agglutination was not usually necessary, provided that careful attention was paid to cultural and fermentation tests, but he added that *flavus* No. 3 might give trouble, if only slightly pigmented, and then might have to be differentiated by agglutination[1].

In a footnote, however, he appended a further qualification to this general statement. He said he was aware that from the throats of healthy persons strains were sometimes obtained which, though corresponding in all other respects with meningococci, failed to give a specific agglutination reaction. Whether such strains should be regarded as "pseudo-meningococci" must, he considered, be left for future research to determine.

Kutscher (1906)[2], in view of the discovery of the meningococcus in the throats of persons in contact with cerebro-spinal fever, thought

[1] In a later article (1908, *Zeitschr. f. Hyg.* LIX) he said that all three varieties of *flavus* were distinguishable from meningococci by fermenting laevulose in addition to glucose and maltose.

[2] *Deutsche med. Wochenschr.* 1906, Nr. 27, p. 1071.

it desirable to make control investigations upon the throats of non-contacts. At Berlin, during May and June, 1905, he examined swabs from the naso-pharynx of 104 persons, about half of whom were children. They had not been in contact with the disease and were examined at a time when cerebro-spinal fever was not present in Berlin in epidemic form. All of these failed to yield cultures of the meningococcus, though at the same time the specific organism was obtained from the throats of two persons who had been in contact with sporadic cases of the disease. As these negative results were obtained at a season of the year unfavourable for the production of catarrhal conditions, there was the possibility that the bacterial flora of the naso-pharynx might be different in the winter. So he continued his investigation of non-contacts in December, 1905, and January, 1906, when Berlin had remained completely free from both epidemic and sporadic cerebro-spinal fever for a period of six months. He swabbed 56 patients suffering from slight catarrhal affections of the upper respiratory tract, and found that 52 were negative, but cultures from four were indistinguishable from the meningococcus "morphologically, culturally, and in their immunity reactions."

For his agglutination tests he employed a powerful anti-meningococcus horse serum, using the macroscopic method. The dilutions were incubated at 37° C. for 24 hours, and controls were made with normal horse serum and with saline. His four throat strains and five cerebro-spinal fluid strains obtained from the epidemic in Upper Silesia were tested, with the result that well-marked agglutination was obtained with all, the throat strains being as highly agglutinable as the strains of undoubted meningococci. As regards the significance of these agglutination tests, he remarked that, in considering results with meningococci, as with other species of cocci, agglutination in low dilution only must be taken with reserve, but agglutination in higher dilutions, such as he obtained in this case (1 : 200, 1 : 500, and sometimes 1 : 1,000), must be regarded as "a link in the chain of proof." It must also be remembered, he added, that some strains of meningococci, like some strains of staphylococci, are only agglutinated with difficulty or are almost inagglutinable.

He also tested the above throat and cerebro-spinal strains as regards absorption of agglutinin. To 10 c.c. of a 1 : 20 dilution of the serum he added three cultures (24 hours' growth) and incubated at 37° C. for one hour, the absorption being facilitated by shaking. The clear liquid obtained by centrifuging was then tested, controls being made

with unabsorbed serum, saline, and normal horse serum. He found that different strains behaved differently. When the serum was absorbed with a cerebro-spinal strain, agglutinin was almost completely lost for some cerebro-spinal and some throat strains, but remained effective, even in high dilution, for one throat and two cerebro-spinal strains. When saturation was made with a throat strain, there was again loss of agglutinin for some cerebro-spinal and throat strains and retention of it for others, but as regards the action on individual strains, the changes effected were not identical with those produced in the former experiment. So specific absorption experiments afforded no evidence that his throat strains were distinguishable from meningococci. Meningococci, he remarked, resembled the organisms of cholera, typhoid and paratyphoid, in that different strains, when tested with the same serum under identical conditions, exhibited different degrees of capacity for combination with agglutinin.

After his experiments had reached this stage, two of his throat strains were accidentally allowed to die out. With the two remaining, further investigations were conducted. Tested by Lingelsheim's recently published (1906) method of using carbohydrate media for differential diagnosis, they conformed with the author's criterion for the meningococcus. Deviation of complement experiments made with these two strains and with strains of undoubted meningococci showed that the former prevented haemolysis in as high a degree as the latter. Rabbit sera prepared with the two throat strains agglutinated a cerebro-spinal strain of meningococci, but he had not had sufficient time, when this report was issued, to prepare very strong sera.

His conclusion was that the identity of his throat strains with the meningococcus had been definitely established in the case of the last two, and had been brought to a high degree of probability in the case of the two which died out before the work was concluded.

Hübener and Kutscher (1907)[1] following up Kutscher's work on the "normal carrier," examined 400 soldiers in a regiment which was free from cerebro-spinal fever, and in the throats of eight found cocci which were identical with the meningococcus in every bacteriological respect, including immunity reactions. The eight men had not been in individual contact with each other. Several months previously their regiment had been quartered, elsewhere, with a regiment in which cerebro-spinal fever had occurred.

[1] *Deutsche militärärztl. Ztschr.* 1907, No. 15. Quoted in *Centralbl. f. Bakteriol.* Ref. XLII.

Eberle (1908)[1] tested the agglutinability of 18 strains of meningococci, all of which had been obtained from cerebro-spinal fluid during life. He used five sera, viz., the Berne, Berlin, and Merck horse sera, and two monovalent rabbit sera. He adopted the macroscopic method and made his suspensions with stored emulsions to which phenol had been added. After making parallel experiments at room temperature, 37° C., and 56° C., he gave up the first, because he found that the reactions were slower, more uncertain, and did not attain to such high dilutions as with the higher temperatures. Investigating the time requisite for the reactions, he found that two hours was useless, and that incubation overnight was necessary; eventually he decided to take his readings at the end of two days, as he noted that 24 hours was not long enough; the maximum agglutination required 38–42 hours to develop.

Marked differences were found between the agglutinability of different strains when tested with the same serum, some strains reaching up to 1 : 1000 and others only up to 1 : 20 or 1 : 50. It was also noted that individual strains did not behave uniformly towards different sera. His 18 strains could roughly be divided into good, rather poor, and poor agglutinators. The last, which for the most part had been recently recovered from patients, did not agglutinate with any of the sera higher than 1 : 50. Good agglutinators would attain reactions in dilutions varying from 1 : 1000 with one serum to 1 : 100 with other sera. When the tests with the same strain and serum were repeated at different times a general conformity of results was observed. The monovalent rabbit sera did not show a distinctive difference in agglutinability as between the homologous and the heterologous strains; the more readily agglutinated strains were agglutinated as highly as, or even more highly than, the homologous strain.

Eberle concludes by saying that the identification of meningococci from the throat is difficult, and not made easier by agglutination tests.

Arkwright (1909)[2] studied the serum reactions of several strains of meningococci obtained from cases of epidemic and sporadic meningitis. In his report he designates his epidemic strains by the letter E and his sporadic strains by the letter L.

All his agglutination experiments were made by the microscopic method, at room temperature, and the observations were completed at the end of two hours.

He has recorded his tests of 25 strains with serum I., obtained from a horse which, for a period of 12 months, had received repeated

[1] *Arch. f. Hyg.* LXIV. [2] *Journ. of Hyg.* IX, 104.

injections, partly subcutaneous and partly intravenous, of the strain L 1. This strain was completely agglutinated up to 1 : 100 and partially up to 1 : 500, whilst normal horse serum gave no agglutination even in 1 : 5. Strains L 7 and L 12 were also completely agglutinated up to 1 : 100; L 7 was slightly agglutinated in 1 : 500 and L 12 was partially agglutinated in 1 : 500 and slightly in 1 : 2000. Of the remaining 22 strains, one, L 5, gave slight agglutination in 1 : 100 and also in 1 : 500; none of the others gave any agglutination in 1 : 100.

Eleven of the above 25 strains were tested with serum II. This was obtained from the same horse when, after the withdrawal of serum I., the animal had received for 10 months injections of E 12, a strain which yielded no more than partial agglutination in 1 : 5 to serum I. The tabulated results show that serum II. agglutinated L 1 slightly better than the former serum had done; but with its second homologous strain, E 12, there was no more than slight agglutination in 1 : 25. Of the remaining nine strains tested, L 7 and L 12 behaved like L 1; L 5 was now agglutinated in as high dilution as these; and another strain, E 4, now became agglutinated completely in 1 : 100 and slightly in higher dilutions. None of the rest gave as much as complete agglutination in 1 : 25.

At a later date, but without having received fresh treatment, the horse was bled again, yielding serum III. This serum differed from serum II. in that it agglutinated (complete up to 1 : 50) the homologous strain E 12. It also agglutinated, in equal degree, E 19, a strain negative to serum I., but not recorded as tested with serum II. In other respects the behaviour of serum III. towards the 12 strains tested showed only slight differences from sera I. and II.

Arkwright also mentioned briefly that he tested 22 of his strains with Kolle's anti-meningococcus serum and obtained agglutination with six in dilutions as high as 1 : 100 or 1 : 200.

In a series of absorption experiments serum I. was tested with four strains which it agglutinated relatively well, viz., L 1, L 5, L 7, L 12, and with two which were not themselves agglutinated, viz., E 5 and E 7. It was found that L 1 and L 5 absorbed agglutinin for themselves and for each other, but only slightly absorbed agglutinin for L 7 and L 12. L 7 and L 12 absorbed to a certain extent their own and each other's agglutinin, but hardly any for L 1 and L 5. E 5 and E 7 absorbed no agglutinin for any of these four strains.

These results may be compared with fixation of complement experiments with serum I. Out of the four strains mentioned above,

which agglutinated relatively well, fixation was complete or nearly complete with L 1, L 7, and L 12; with the fourth, L 5, it sometimes occurred and sometimes failed. Out of six strains which were recorded as only slightly agglutinable (1 : 5 or 1 : 20), one brought about complete, or nearly complete, deviation; the others failed.

Incidentally, on comparing Arkwright's tables, there is evidence that when the same strain was tested with serum I. on different occasions the results did not always correspond precisely. For example, out of seven tests with L 12, the result on two occasions was complete agglutination up to 1 : 200 and slight up to 1 : 500; but on another occasion the agglutination was only slight in 1 : 100 and only partial in 1 : 20 and 1 : 50.

In his "conclusions" Arkwright states that "the variations observed in the sugar and serum reactions were not such as to indicate a specific difference between the epidemic and sporadic strains, for the differences between individual members of each group were as great as any found between the two groups."

Elser and Huntoon (1909)[1] worked for the commission appointed by the Health Board of New York in March, 1905, to investigate cerebro-spinal meningitis. After presenting an epitome of their work in January, 1906, they proceeded to further study of serum reactions and issued their final report in 1909. The majority of the strains of meningococci which they examined were obtained from cerebro-spinal fluid or meningeal exudate; they also report on other cocci of interest, which were isolated from the respiratory passages in cases of meningitis, or from persons who had come in more or less close contact with such cases.

They prepared agglutinating sera by inoculating rabbits intravenously with weekly doses of meningococci suspended in saline solution and killed by heating at 65° C. for 30 minutes. The initial dose was usually ·002 gm.; this was gradually increased to ·008 gm. They relied entirely on the macroscopic method of examination, as preliminary tests with the microscopic method had been found less satisfactory. Their dilutions received, per c.c., ·004 gm. of moist cocci, measured with a standard platinum loop. The dilutions were incubated for two hours and then kept in the cold (9° C.) for 22 hours. The final reading was taken at 24 hours; preliminary readings were also usually taken at 1, 2, 3, and 4 hours. Occasionally, no appreciable change was noticed after several hours, although complete clarification was found in 24 hours.

[1] *Journ. of Med. Research*, xx.

When the agglutination was not progressive with increase of time, the tests were repeated. They recorded a reaction as positive when the great majority of the cocci were clumped and deposited at the bottom of the tube, whilst the supernatant fluid presented a slight degree of turbidity when viewed obliquely in a good light. Control tests were made with normal rabbit serum. The cultures used for testing agglutinability were grown on media which did not contain serum or ascitic fluid.

In discussing their reasons for adopting the above technique, they stated that they had made a comparative study of the influence of incubator, room, and ice-box temperature on agglutination and found that, though incubator temperature hastened the reactions, the end results were not markedly influenced. They did not agree with Kutscher that agglutinability was brought to a higher level by exposure to 55° C., though it might be accelerated. In support of these statements they have published two tables. Table X. shows agglutination tests with seven strains of meningococci at both 37° C. and 55° C., readings being taken at 1, 2, 4, and 24 hours. It is noteworthy that three of the strains were practically unaffected by the serum employed and that each of the remaining four showed more agglutination at 24 hours than at 2 hours, when incubated at 37° C. Table XI. records agglutination tests with nine strains of meningococci at temperatures of 10° C., 30° C., and 37° C., the readings being taken at 24 hours. Three of the strains were practically unaffected by the serum employed; of the remaining six, three showed most agglutination at 37° C., the remaining three were equally agglutinated at all three temperatures.

They considered it necessary to use normal rabbit serum as a control, as they found that some strains of meningococci were agglutinated by the normal serum in 1 : 50 and, rarely, in 1 : 250.

In their experience, growth on media containing serum or ascitic fluid had an unfavourable influence on agglutinability; so they waited until their cultures could be accustomed to grow without the aid of these ingredients before testing for agglutination, glucose agar being generally adopted as the most suitable medium.

They stated that "one of the great difficulties encountered in connection with this work is the very pronounced instability of the agglutinable properties of the meningococcus." When the same strains were tested on different occasions with the same sera, the results were not found to be constant. Lingelsheim, they remark, suggested that killed cultures gave better results, and in this view they agreed with him;

but they have not stated to what extent or with what degree of success they followed Lingelsheim's technique. Apparently they were unable to overcome the difficulty completely, as they have again stated, in the summary of their results, that "the unusual instability of the agglutinable properties of the meningococcus" was a serious obstacle. They tried to increase agglutinability by growing their strains in glucose broth, and reported that "while several strains reacted promptly to this treatment, others were not materially altered, even after prolonged growth, and in a few there was reduction of agglutinability."

Apart from variations in the agglutinability of individual strains, they found great differences in the degree of agglutinability exhibited by different strains in the presence of the same immune serum. They tested 65 strains with a serum, prepared from their strain M 30, which gave a titre of 1 : 1000. Twenty-five of these strains either failed to react at all or gave no more than incomplete reactions in 1 : 50. Tests have also been recorded of sera prepared from certain other strains of meningococci, and with these sera again the results showed that some strains were not agglutinated or were only agglutinated to a slight degree. Enquiring further into some of the strains which failed to agglutinate, they have reported that injection of them into rabbits produced sera which were ineffective towards the homologous strains, but were of moderate potency in agglutinating strains already found agglutinable with the sera previously mentioned. Hence they have termed the strains in question "agglutinogenic" but "inagglutinable." They admitted that this latter term was not absolutely accurate, because, though some of the strains failed to agglutinate even in 1 : 10, others were agglutinable up to 1 : 50; but they found it convenient to define as "inagglutinable" all strains which failed to react to powerful sera in 1 : 100. They remarked that they were unable to render an inagglutinable strain more sensitive by conducting the experiment at a temperature of 55° C.

Proceeding to absorption experiments, Elser and Huntoon called special attention to their technique. "Instead of exhausting a serum with large and variable quantities of bacteria, each serum was absorbed with small and constant amounts in the hope of detecting slight differences in the absorptive capacities of different strains belonging to the same species." The suspension of cocci was ·004 gm. per c.c., and was mixed with equal parts of diluted serum. Two control tubes received the same amount of diluted serum and equal parts of saline. The tubes

were incubated for 2 hours. Preliminary tests showed that the maximum absorption was attained within this time; no more was obtained in 24 hours. Their attention was called to the condition of the contents of the tubes after centrifuging. The agglutinable homologous cultures were firmly packed at the bottom; the fluid was perfectly clear, and vigorous shaking failed to re-establish a perfect suspension. But the heterologous strains and the inagglutinable homologous strains were less firmly packed; the supernatant fluid was not quite clear; and vigorous shaking produced a perfect suspension.

They found that the inagglutinable strains were capable of absorption, to a certain extent, and, in general, that the binding capacity of these corresponded with their agglutinogenic capacity. There was, however, one exception. A certain inagglutinable strain was found to have no power of absorption with the serum produced from it, though this serum was capable of agglutinating one strain up to 1 : 1000. No definite relationship could be established between absorptive capacities and agglutinability. They have quoted, for example, an instance where an inagglutinable strain absorbed as much as an agglutinable one.

In summary of the above, a large number of strains, though undoubtedly meningococci, were more or less inagglutinable by the sera with which they were tested. The authors have made no definite statement as to the number of these strains which produced sera capable of agglutinating other meningococci but incapable of agglutinating the homologous strain.

They have recorded, however, an experiment with five strains—two "agglutinable" and three "inagglutinable." With regard to two of the three latter, they have noted that they "were inagglutinable when first selected for the present purpose, but subsequently reacted to certain immune sera in dilutions of 1 : 100." With each of the five strains one rabbit was immunised. The sera from the five rabbits were tested against 18 strains, the result being that all five sera "affected agglutinable meningococcus strains in fairly high dilutions." Taking the results which did not attain to a positive reaction in 1 : 100—with the serum prepared from one of the agglutinable strains, only two of the 18 strains failed to reach this standard; with the serum prepared from the second agglutinable strain, 11 strains failed to reach the same standard; with the sera produced by the three "inagglutinable" strains, the numbers of strains which fell short of this standard were, respectively, 8, 11, and 12.

In this same experiment with the sera of five rabbits, each immunised

with a different strain, the maximum reactions of the three "inagglutinable" strains towards these five sera were:

	Sera prepared with the				
	Agglutinable strains		Inagglutinable strains		
Cultures	M 24	M 83	M 101	M 175	M 61
M 101	0	1 : 100	1 : 25	0	0
M 175	1 : 25	1 : 100	1 : 25	1 : 100	1 : 50
M 61	0	1 : 50	0	0	0

The above are all the experimental data which I can find in support of the generalised statement as regards the "agglutinogenic" capacities of their "inagglutinable" strains, which the authors make in their "conclusions." This statement is that "towards the homologous inagglutinable strains they were ineffective."

With regard to their Gram-negative diplococci, other than those regarded by them as meningococci, they found that all except six strains could be differentiated from the meningococcus by means of cultural and fermentation tests. These six, which they term "pseudo-meningococci," agreed with the meningococcus in their cultural and fermentation tests, but differed in their serum reactions. They were not agglutinated with a meningococcus serum, and were found incapable of abstracting specific agglutinin from it. They found their pseudo-meningococci more toxic for rabbits than meningococci; many succumbed to very small doses. They succeeded, however, in producing a serum (titre 1 : 500) with one strain. This serum did not agglutinate any of the other five strains of pseudo-meningococci; nor did it agglutinate strains of meningococci in higher dilution than did normal serum. Normal rabbit serum agglutinated the strain used for producing the immune serum up to 1 : 50, and a strain of meningococci (M 250) up to 1 : 100. In a series of absorption experiments with its own strain this "pseudo-meningococcus" serum lost the agglutinin to its homologous strain, but not to M 250.

Mayer (1909)[1] tested, with the Höchst serum, the agglutinating properties of various strains, including undoubted meningococci and organisms, obtained from the naso-pharynx, which presented certain resemblances to these. No statement is made as to how this serum was prepared. Mayer used both the macroscopic and the microscopic methods of examination. His dilutions were kept for 12 hours at 37·5° C. before the final reading. He attached particular importance to exposure to this temperature for at least 12 hours, and has recorded

[1] *Centralbl. f. Bacteriol.* Orig. XLIX.

experiments showing that, when the readings were taken successively at 1, 2, 3, 6, and 12 hours, standard strains did not give definite agglutination in high dilution (1 : 500) before the 12 hours' period was reached.

His standard strains of undoubted meningococci were four in number. One was obtained from Ruppel and one from Weichselbaum; the other two, designated M. 1 and W. 1, were cultured by Mayer from lumbar puncture fluid. These four all gave definite agglutination with the Höchst serum up to 1 : 500; and they were all identical morphologically, in their mode of growth with various media, and in fermentation tests.

With these four strains he compared his throat strains, which he had collected in the course of an examination of 251 persons.

Of these throat strains, thirteen, all derived from the throats of infected persons or carriers, agreed in every respect with his standard strains. Fifteen other throat strains corresponded with his standard strains in all respects except agglutination; with his Höchst serum they were either not definitely agglutinated in any dilution or at least not in a dilution as high as 1 : 500. These strains he has called "pseudo-meningococci." The remaining throat strains could be excluded from the class of meningococci without resort to agglutination tests.

He stated that amongst normal individuals, who had not been in contact with cerebro-spinal fever and were examined at a time when this disease was not prevalent, cocci might be found in the throat which could only be distinguished from meningococci by the agglutination test. He maintained that such strains could not be regarded as meningococci unless they gave a positive reaction up to 1 : 500.

Friese and Müller (1909)[1] found cocci closely resembling meningococci in the throats of persons who had not been associated with cases of cerebro-spinal fever. They were led to this discovery whilst investigating, at Beuthen, an outbreak amongst the troops which was regarded as influenza. These cocci were found in 28 out of 36 soldiers affected with this epidemic. Then 60 normal soldiers were taken as controls, and similar cocci were found in the throats of 28 of these. In some cases the cocci were abundant, in others only moderately numerous, and, in others, scanty. By microscopic methods and cultural tests, including fermentation tests, they were unable to distinguish them from meningococci. They therefore resorted to agglutination tests and, on the strength of the results obtained by this method, designated these cocci as pseudo-meningococci or "S. cocci."

In their agglutination work they employed the macroscopic methods

[1] *Klin. Jahrb.* xx.

and used 48 hours' cultures grown on slightly alkaline ascitic agar. They used cultures of this age because Lingelsheim had observed that such cultures agglutinated better than 24 hours' growths. The serum used was Merck's polyvalent horse serum, giving a titre of 1 : 600 with good agglutinating strains of undoubted meningococci. Their dilutions were treated in one of two ways:—(1) incubation at 37° C., preliminary reading at 24 hours, final reading at 48 hours; (2) incubation at 55° C., final reading at 24 hours.

They found that the latter method gave sharper distinctions between their "S. cocci" and undoubted meningococci. Out of 21 "S." strains tested at 55° C., only one gave a complete reaction in 1 : 100; whilst, out of 16 strains of true meningococci, 12 gave a complete and 4 a partial reaction in 1 : 200.

They also made comparative tests with a rabbit serum prepared from "S. cocci." This had been prepared with a single strain, and the fact that it was not polyvalent they found to be a disadvantage. With this serum, again, a temperature of 55° C. brought out sharper differences than 37° C. Twenty-three "S." strains were tested at 55° C.; six gave complete reactions up to 1 : 400, and with four others the reaction was complete up to 1 : 200. On making parallel tests with 13 strains of true meningococci it was found that none gave complete reactions in 1 : 200 and only three were complete in 1 : 100.

The above data illustrate the point on which Friese and Müller have laid chief emphasis, viz., the differences between their "S. cocci" and the strains of undoubted meningococci with which these were compared. At the same time they admitted that there was a relationship between "S. cocci" and true meningococci, and they considered that this relationship was shown more strongly by some strains than by others. They also found that the agglutination reactions both of their "S. cocci" and their true meningococci were liable to some degree of variation.

The following statements in their report require mention, as they have a bearing on the question whether the authors' distinction between "S. cocci" and true meningococci can be accepted without qualification.

(1) They have reported that their earliest strains of "S. cocci" agglutinated better than all the later ones and at first seemed undoubted meningococci. But unfortunately these strains died out, and as they could not be included in their comparative tests, the data as regards their agglutinability could not be ascertained.

(2) A large number of their strains, both "S. cocci" and undoubted meningococci, were tested on two or three different occasions against

the same serum. Frequently the results obtained on different occasions with the same strain did not correspond closely; and sometimes the difference was great.

For example, on June 1st, an "S." strain gave a completely negative result with Merck's anti-meningococcus serum; but on July 10th, though the control test with normal serum was again completely negative, a complete agglutination up to 1 : 100 was obtained, and there was a slight reaction in 1 : 200. These variations in agglutinability they were unable to explain. They found them to be greater with "S. cocci" than with undoubted meningococci.

(3) Several of the "S." strains which, at 37° C., gave complete agglutination in 1 : 100 with Merck's anti-meningococcus serum, also gave some agglutination with normal horse serum.

(4) Although the majority of their meningococci agglutinated well with the Merck serum, a few did not.

(5) In the course of their endeavours to prepare an "S." serum in rabbits, they found that, though the homologous strain was only agglutinated to a slight degree, several strains of meningococci were agglutinated more strongly.

(6) Reference has already been made to agglutination tests at 55° C. with a rabbit serum prepared from a strain of "S. cocci." This serum was also tested at 37° C. It agglutinated the homologous strain completely up to 1 : 800. But, out of 19 other "S." strains which were tested, only one gave complete agglutination up to 1 : 200; whereas, in parallel tests with 11 strains of undoubted meningococci, three gave complete reactions in 1 : 200. Of these three, two agglutinated well with Merck's anti-meningococcus serum, but one was completely negative.

(7) Absorption experiments at 37° C. (48 hours' reading) with three "S." strains and three strains of meningococci. Merck's serum, after saturation with one of the latter strains, lost all agglutinin, beyond 1 : 50, for these three strains; but when the serum was saturated with an "S." strain the same three strains of meningococci gave complete reactions up to 1 : 200 and partial up to 1 : 400. An "S." serum, after saturation with a strain of meningococci, gave only slight agglutination with the meningococci and the "S." strains; when the serum had been saturated with an "S." strain, it gave rather more agglutination with strains of meningococci, but none with "S." strains.

(8) Absorption experiments at 55° C. (24 hours' reading) with five strains of meningococci and five strains of "S." cocci. Saturation of

"S." serum with meningococci removed agglutinin for meningococci, but not for "S." strains; whilst saturation of the serum with "S." cocci removed agglutinin for both types.

Lieberknecht (1909)[1] examined at Posen the throats of 150 healthy school children, not known to have been in contact with cases of meningitis, and found meningococcus-like colonies in cultures from 8 per cent. In culture tests, including fermentation tests, for which he used Lingelsheim's media, they behaved like meningococci. He then proceeded to examine the serum reactions of these cocci, and to compare them with the reactions of undoubted meningococci. He used cultures not over 24 hours old, and adopted the macroscopic method. He incubated one sample of each dilution at 37° C., and a second at 55° C. In each case he took the final readings at 24 hours.

Thirteen throat strains were tested with a Berlin meningococcus serum (titre 1 : 1000). Except with two strains, the reactions, when positive at all, were better at 55° C. than at 37° C. Taking the reactions at 55° C. only:—In 1 : 100, 6 strains were negative, 3 gave slight, 2 partial, and 2 complete reactions; at 1 : 200, 8 were negative, 3 gave slight, 1 a partial, and 1 a complete reaction. None of the strains were agglutinated by normal rabbit serum in 1 : 10.

He made similar tests with the same serum applied to 12 strains of undoubted meningococci. Here again, with slight exceptions, the reactions were rather better at 55° C. than at 37° C. Taking the reactions at 55° C. only:—In 1 : 100, 1 strain was negative, 2 gave slight, 1 a partial, and 8 complete reactions; at 1 : 200, a second strain also was negative, 4 gave slight, 3 partial, and 3 complete reactions. These last 3 also gave complete reactions in 1 : 400.

Lieberknecht prepared a rabbit serum with one of his throat cultures, giving repeated subcutaneous doses of living cocci in increasing amount. He has tabulated the effects of this serum on the homologous strain and 10 other throat strains at 37° C. (reading at 24 hours). The homologous strain was completely agglutinated in 1 : 200, and partially or slightly up to 1 : 600. Two other strains behaved similarly; the 8 remaining strains were negative in 1 : 100.

He tested the same serum with 10 strains of undoubted meningococci, and has tabulated, in this case, the 24 hours' readings at both 37° C. and 55° C. Six strains, he noted, were agglutinated better at 55° C. than at 37° C. Taking only the reactions recorded as complete:—At 1 : 600, one strain was complete at 55° C., but not at 37° C.; at 1 : 400,

[1] *Arch. f. Hyg.* LXVIII.

the same strain was complete at both temperatures; at 1 : 200, a second strain was complete at 55° C. but not at 37° C., and a third strain was complete at 37° C. but not at 55° C.; at 1 : 100, this third strain was again complete at 37° C., but not at 55° C., the second strain was complete at both temperatures, a fourth and a fifth strain were complete at 55° C., but not at 37° C. Taking evidence of agglutination, whether complete or incomplete, 1 strain showed evidence up to 1 : 100, 3 up to 1 : 200, 2 up to 1 : 400, and 2 up to 1 : 600.

Lieberknecht has also made brief mention of some absorption experiments. Taking a specific meningococcus serum and saturating this for one hour at 37° C., with one loopful of culture to 1 c.c. of 1 : 20 dilution of serum, he found that a genuine meningococcus culture removed specific agglutinin, but all his throat cultures failed to do so. He has called particular attention to the fact that the throat strain which produced the rabbit serum, mentioned above, did not absorb the agglutinin for meningococci present in his meningococcus serum.

Dopter (1909)[1] found certain organisms which he called "para-meningococci" in the naso-pharynx of persons who had been in contact with cases of cerebro-spinal fever. Though agreeing with true meningococci in ordinary cultural and fermentation tests, he excluded them from this group owing to certain differences brought out by serological tests. They did not give specific agglutination with a meningococcus serum and failed to respond to the absorption test for specific precipitins. They agreed with meningococci, however, in fixation of complement tests. Though calling them "para-meningococci," he considered them to be nearly allied to meningococci, and advised that, from the practical point of view, persons in whose throats they were found should be treated as carriers of the meningococcus.

Mayer, Waldmann, Fürst and Gruber (1910)[2] have stated that, at a time when cases of cerebro-spinal fever were found, they examined, in various garrisons, the throats of 1911 healthy persons, and found 47 (2·46 %) to be carriers. None of these developed the disease. They controlled these results by examining garrisons at Munich in 1910, when there was no cerebro-spinal fever. They swabbed the throats of 9111 men, and found 158 carriers (1·73 %). None of these developed cerebro-spinal fever.

They concluded that the meningococcus must be regarded, for practical purposes, as ubiquitous, and that bacteriological examination

[1] *C. R. Soc. de Biol.*, July, 1909.

[2] *München. med. Wochenschr.*, July 26th, 1910.

of throats was impracticable as a useful aid to prophylaxis in time of epidemic.

Sachs-Müke (1911)[1] enquired at Beuthen, in the winter months of the year, into the occurrence of meningococci and pseudo-meningococci in the naso-pharynx of normal persons. Out of 202 soldiers, none of whom were associated with cerebro-spinal fever, he found no carriers of true meningococci, but 15 carriers of pseudo-meningococci or "S." cocci. There was no evidence that these carriers had infected each other, as they were widely distributed in different quarters. The "S." coccus was obtained also from the throat of 1 out of 28 civilians, not associated with cerebro-spinal infection. He also took the opportunity, at the same time of the year, of examining the throats of a batch of recruits newly arrived from other parts of the country. In none of these did he find either genuine meningococci or "S." cocci.

The " S." cocci fermented dextrose and maltose, but never laevulose; and in the other routine cultural tests they were indistinguishable from true meningococci, though perhaps the outline of the primary colonies was less sharply circular, and there were fewer tetrads. Differentiation was effected by serological reactions, upon which he proceeded to report.

His agglutination tests may be summarised as follows:—

I. (*a*) Agglutination tests at 37° C.; reading at 48 hours. Sixteen "S." strains tested with Merck's polyvalent meningococcus serum (titre 1 : 600). The total number of tests was 35, many strains being tested twice or oftener. The results of different tests with the same strain did not always coincide precisely. In all the 35 tests some reaction was obtained in 1 : 100, the controls being all negative, and in all except 6 tests the reaction was complete in 1 : 100. At 1 : 200, only six of the 35 tests were negative; and, in this dilution, six strains were completely positive on at least one occasion. At 1 : 400 no test gave more than a partial reaction; and, in this dilution, 14 of the tests were completely negative.

I. (*b*) Agglutination tests at 55° C.; reading at 48 hours. The same strains and the same serum as in I (*a*). At 1 : 100, none of the tests was completely positive, but 11 out of the total 36 tests gave a partial reaction. At 1 : 200 all the tests were negative.

II. (*a*) Agglutination tests at 37° C.; reading at 48 hours. Twenty strains of meningococci tested with Merck's polyvalent meningococcus serum. The total number of tests was 48. At 1 : 100, only one test

[1] *Klin. Jahrb.* XXIV, 225, 451.

was negative; this was with strain 13; this strain was tested twice; on the second occasion it gave a complete reaction in 1 : 100. At 1 : 200 all the tests were completely positive, except 3, viz., the two tests with strain 13, which were completely negative, and one of the two tests with another strain, which gave a partial reaction. At 1 : 400 there were 10 completely negative and two partial results, all the remaining tests being completely positive.

II. (*b*) Agglutination tests at 55° C.; reading at 24 hours. The same strains and the same serum as in II (*a*). Strain 13 was negative as before (three tests). All the other tests gave completely positive reactions in 1 : 400.

III. (*a*) Agglutination tests at 37° C.; reading at 48 hours. Seventeen "S." strains tested with the Beuthen Institute's polyvalent "S." serum. Total number of tests, 30. At 1 : 100 every test gave some reaction, and all except four were complete; each strain gave at least one complete reaction. At 1 : 200 every test gave some reaction, but six were incomplete, viz., the four which were incomplete at 1 : 100 and two others. At 1 : 400, 16 tests were completely positive, five completely negative, and nine partial. Reviewing the tests as a whole, there was no consistently weak agglutination, but some little irregularity.

III. (*b*) Agglutination tests at 55° C.; reading at 24 hours. The same strains and the same serum as in III (*a*). At 1 : 100 five of the 17 strains failed completely; each of the remaining 12 gave at least one completely positive reaction. At 1 : 200 only six strains gave, each on one occasion, a completely positive reaction. At 1 : 400 there were only four completely positive reactions; and at 1 : 600 there was only one.

IV. (*a*) Agglutination tests at 37° C.; reading at 48 hours. Twenty strains of meningococci tested with the Institute's polyvalent "S." serum. Each strain tested twice, except one, which was tested once, and one which was tested three times. Strain 13 (see previous tests) was completely blank in both tests. The strain which was only tested once was also completely blank. One strain was completely blank in one test, but positive up to 1 : 100 in the second test. One strain, also completely negative in one test, was positive up to 1 : 400 in the second test. In summary:—At 1 : 200 there were 28 completely positive results, belonging to 15 strains; at 1 : 400 there were 17 completely positive results, belonging to 11 strains.

IV. (*b*) Agglutination tests at 55° C.; reading at 24 hours. The same strains and the same serum as in IV (*a*). Strain 13 was again

completely blank in both tests. Eight other strains were completely blank in one test, but gave some agglutination in a second; in one of these instances the agglutination was complete up to 1 : 400. In summary:—At 1 : 200 there were 22 completely positive results, belonging to 13 strains; at 1 : 400 there were 10 completely positive results, belonging to six strains.

Sachs-Müke also made some absorption experiments, using Merck's polyvalent meningococcus serum (titre 1 : 600) and the Beuthen Institute polyvalent "S." serum (titre 1 : 400) against eight strains of meningococci and seven of "S." cocci. He added a 48 hours' ascitic agar culture to 5 c.c. of a 1 : 20 serum dilution. His tables show the following results:—

I. (*a*) Meningococcus serum saturated with meningococci. "S." cocci showed some incomplete agglutination at 37° C., but were completely negative at 55° C.

I. (*b*) The same serum saturated with "S." cocci. Meningococci agglutinated.

II. (*a*) "S." coccus serum saturated with "S." cocci. Meningococci agglutinated.

II. (*b*) The same serum saturated with meningococci. Agglutinins for "S." cocci not affected at 37° C., but completely removed at 55° C.

Arkwright (1911)[1] recorded further work on serum reactions, which he employed to compare the meningococcus with the gonococcus by the complement fixation test. In so far as the comparative reactions of different strains of meningococci are concerned, the following points in his article may be quoted.

Referring to his previous work on agglutination, he states that, "when working with a monovalent meningococcal serum, the number of strains agglutinated was very limited, and, even when a polyvalent serum of a titre of 1 : 1000 made by injecting twelve different strains of meningococcus was employed, strains of meningococcus were easily found which were not agglutinated more highly by the specific serum than by normal serum."

In his fixation experiments he used extracts of meningococci. An emulsion of the growth on ascitic agar was made with 10 c.c. of saline. "This was centrifuged and the deposit made up to its original volume with salt solution. After adding a few drops of chloroform and shaking, the emulsion was left at room temperature for three or four days. The extract was then centrifuged before use. It was

[1] *Journ. of Hyg.* XI, 515.

found that if the deposit from the last centrifuging was again made up to the original volume with salt solution and left for a further period of two to three days, a second extract as good as the first could be obtained, and by again repeating the same process, a third and even a fourth extract could sometimes be obtained of almost undiminished value for complement fixation experiments."

The following is a summary of his experiments with meningococcal sera and extracts:—

(1) A monovalent serum was prepared from a horse by inoculation with M 12, a culture isolated from the meninges of a sporadic case of meningitis. This serum was tested with three strains, M 119, isolated from the spinal cord of a case of acute epidemic meningitis, and M 141 and M 162, each isolated from cerebro-spinal fluid of sporadic cases of meningitis. A positive complement fixation reaction was obtained with M 141; M 119 was negative; M 162 was negative on one occasion, and in a second test it gave only a very slight reaction.

(2) A polyvalent serum was prepared from a horse by inoculation with 20 strains of meningococci. This serum was tested with the above three strains. It was positive with M 141 and M 162, but negative with M 119.

(3) A monovalent serum was prepared from a rabbit by inoculation with M 141. This was tested with the homologous strain and also with M 162, M 135, M 164, and M 165. M 135 and M 164 were isolated from cerebro-spinal fluid of sporadic cases of meningitis; M 165 was isolated from cerebro-spinal fluid of a case of meningitis occurring in an epidemic area. On one occasion the serum gave positive reactions with all five strains; on a second occasion the reactions with M 162 and M 164 were very slight.

(4) A monovalent serum was prepared from a rabbit by inoculation with M 162. This was tested with the homologous strain and also with M 135 and M 164. It gave positive reactions with all three.

Dopter (1911)[1] reported the discovery of his "para-meningococcus" in seven sporadic cases of cerebro-spinal fever. These cases were all typical, clinically, and all ended fatally.

Dopter (1912)[2] reported that, up to date, he had collected 12 cases of para-meningococcal cerebro-spinal fever. Clinically the cases were typical of cerebro-spinal fever, but the organisms were not agglutinated with anti-meningococcus serum, and such serum was of no therapeutic

[1] *Bull. Soc. Méd. des Hôp. de Paris*, xxxi, 590.
[2] *Ibid.* June 14th, 1912, p. 828.

benefit. Success in treatment was, however, attained in cases where anti-para-meningococcal serum was employed.

Darré and Dumas (13, VI. 1914)[1] reported on cultures which they obtained from two cases of cerebro-spinal fever, one an adult and the other a child. In cultural and fermentation reactions these organisms were identical with meningococci. When tested with a meningococcus serum, both were agglutinated, one up to 1 : 100 and the other up to 1 : 20. This they regarded, after making absorption tests, as due to group agglutination. When the serum was saturated with their organism no agglutinin was removed for an undoubted meningococcus; but when the serum was saturated with the meningococcus it was incapable of agglutinating their new strains. Testing these two strains with Dopter's para-meningococcus serum, they found that both were agglutinated, one up to 1 : 500 and the other up to 1 : 150. When this serum was saturated with one of their strains they found it lost its property of agglutinating the para-meningococcus of Dopter. They prepared a serum from a rabbit by inoculations with the strain which only agglutinated up to 1 : 150 with Dopter's para-meningococcus serum. This rabbit serum gave a titre of 1 : 400. It failed to agglutinate undoubted meningococci, but agglutinated Dopter's para-meningococci.

From these experiments they concluded that their two new strains were para-meningococci, but of a variety different from those previously described by Dopter, which were not agglutinated by meningococcus serum.

Dopter and Pauron (20, VI. 1914)[2] reported that they had made further investigations on para-meningococci, with a view to differentiating them from true meningococci by means of absorption tests. They found that some strains of para-meningococci were agglutinated by meningococcus serum, though the majority were not. Para-meningococcus serum very frequently agglutinated meningococci as well as para-meningococci. But the differences between these two groups of organisms became clear when absorption tests were employed. Para-meningococcus serum, saturated with meningococci, lost its agglutinin for meningococci, but retained its agglutinin for para-meningococci; the same serum, saturated with para-meningococci, lost its agglutinin for para-meningococci, but retained its agglutinin for meningococci. Meningococcus serum, saturated with meningococci, lost its agglutinin for meningococci, but retained its agglutinin for para-meningococci.

[1] *C. R. Soc. Biol.* LXXVII, 106.
[2] *Ibid.* LXXVII, 157.

These statements were supported by a table showing tests, after absorption, with a meningococcus and a para-meningococcus serum upon selected strains of meningococci and para-meningococci. It was found that, before absorption, each of the strains was agglutinated by both sera. The number of strains was seven, viz., 4 meningococci and 3 para-meningococci. After absorption, all the tests were either completely negative or definitely positive up to 1 : 600. Thus, the para-meningococcus serum, when saturated with meningococci, gave 0 with the 4 meningococci and + with the 3 para-meningococci; when saturated with para-meningococci it gave exactly the converse. And precisely similar results, *mutatis mutandis*, were obtained in the saturation experiments with the meningococcus serum.

Dopter and Pauron (27, VI. 1914)[1], in a subsequent paper, divided their para-meningococci into groups by means of saturation tests. They dealt with 7 strains of para-meningococci, designated S, W, H, B, M, L, Z.

A horse was immunised, at first with S alone. The serum agglutinated S and 3 others, but failed with the other 3. Then the horse was inoculated with the strains which had not agglutinated. The result was a serum which agglutinated all 7, up to 1 : 400 or 1 : 600. With this serum saturation experiments were performed.

(1) After saturation with S, all agglutinin was removed for S, W, H, and B, but agglutinins remained (up to 1 : 400) for M, L, and Z. The results were the same when W, H, and B were used for saturation.

(2) After saturation with M, all agglutinin was removed for M and L, but agglutinins remained (up to 1 : 400 or 1 : 600) for the other 5 strains. Saturation with L gave the same result.

(3) Saturation with Z removed agglutinin for Z only, the other six being positive up to 1 : 400 or 1 : 600.

The above demarcation into three groups was confirmed by cross-agglutinations with the serum of rabbits vaccinated against each of the 7 strains. Thus: (1) the sera of rabbits vaccinated with S, W, H, and B, agglutinated these 4, but failed with the remaining 3; (2) sera from M and L agglutinated M and L only; (3) the serum from Z agglutinated Z only.

They proposed to call the above three groups the α, β, and γ varieties of the para-meningococcus, and suggested that perhaps additional varieties might subsequently be found. They stated that, of the 3 varieties, α was the commonest and γ the rarest; but I was unable

[1] *C. R. Soc. Biol.* LXXVII, 231.

to find figures giving the total numbers of each which they have identified. Comparing minor differences as regards cultural growth in these 3 groups, they declared that α was apparently characterised by a growth like that of the meningococcus, as well as by its group-agglutinability by anti-meningococcus serum; β appeared to differentiate itself from the two others by producing a very viscid growth, heaped up above the surface of the medium, and it also appeared to be more resistant; γ gave a drier type of culture and its colonies appeared flatter.

Discussion of Literature on Serum Reactions.

The most important feature of the literature summarised above is that it gives evidence of substantial progress in a definite direction.

At first it was thought, despite the recognised trouble of agglutination work with other important cocci, that the agglutination test was going to be a simple matter, providing a definite and unequivocal answer to the question whether any given Gram-negative diplococcus was or was not a meningococcus.

Then a wave of scepticism supervened, because it was found that some strains of undoubted meningococci were not agglutinated by the standard serum employed. Investigation of this difficulty served at first to bring to light further complications, rather than to clear the ground, and, indeed, it induced some observers to take not merely a sceptical but a pessimistic view of the value of agglutination work. But their laboratory data, as a frank statement of difficulties encountered, have aided progress, because they have shown (1) that, with so delicate an organism as the meningococcus, relatively slight differences in technique or condition of the culture may produce apparently wide discrepancies in results; (2) that the idiosyncrasies of individual strains of undoubted meningococci must be thoroughly worked out with monovalent sera; (3) that, until this is done, the resort to polyvalent sera, obviously actuated by the laudable desire for practical expediency, tends to mask the main scientific problem rather than to eliminate difficulties.

More or less concurrently with this output of work by observers whose conclusions practically amounted to the opinion that, in their experience, agglutination tests were unreliable, there was another trend of research, made by men who adhered, as an article of faith, to the doctrine that a really good and, preferably, polyvalent serum

must be infallible, and that any meningococcus-like organism which failed to show specific agglutination with this serum must be stigmatised with some such prefix as "pseudo," "S.," or "para." In its inception, this idea, or working hypothesis, was prompted by the thoroughly sound scientific principle that a meningococcus-like organism found in the naso-pharynx, particularly if found in the naso-pharynx of a non-contact, must be put through its paces very scrupulously before it should be accepted into the order of true meningococci, capable of producing cerebro-spinal fever. But, in the course of further work on the serum reactions of these doubtful organisms, the differences of some of them from undoubted meningococci were not sharp or decisive; evidence of relationship had also to be conceded, and finally it had to be admitted that certain "para" organisms were identical in every respect with strains proved to have been the cause of cerebro-spinal fever.

These facts have cleared the ground by showing that there is a way of reconciliation between these latter results and the laboratory data of the investigators who were sceptical about the reliability of agglutination tests even with meningococci from cerebro-spinal fluid. It has become evident that serological tests have, at least provisionally, placed meningococci into a variety of groups, not, as yet, defined in respect either of number or inter-relationship; and that the criterion for a *doubtful organism isolated from the naso-pharynx must be widened* accordingly, so as to embrace (1) conformity with one or other of these groups, or (2) failure to conform with any of them.

Taking in detail the laboratory data furnished by individual investigations:

The technique employed by Lingelsheim in 1906 is, in its two essentials (adoption of the macroscopic method and incubation of the dilutions overnight), identical with that which has been followed by the majority of subsequent observers. The two monovalent sera which he prepared appear to have been almost equally satisfactory in agglutinating all the strains tested with them; so there is no evidence from his work that the outbreak in Upper Silesia from which they were derived was due to more than one type of meningococcus. As neither his cerebro-spinal strains nor his strains from the throats of contacts appear to have given any indication that sera such as he employed might not serve as a universal criterion for the diagnosis of the meningococcus, a tribute must be paid to the shrewdness of his remark that, in the case of meningococcus-like organisms obtained from non-contacts,

it must be left for future research to determine whether failure to agglutinate with the particular serum employed justifies the labelling of such organisms as "pseudo."

Kutscher's work at Berlin in 1906 is particularly important from an epidemiological point of view in that he demonstrated the meningococcus in the throats of persons who had not been in contact with the disease. This work was done during the winter 1905–6, at a time when Berlin had remained completely free from the disease during a period of six months. The laboratory details of his work show that it is thoroughly reliable and that the identity of his throat organisms with true meningococci was confirmed by serological tests.

His work with Hübener in 1907 strengthens the importance of these observations, and the significance of the above results is further increased, particularly from the epidemiological aspect, by the findings, based on a much larger number of cases, which were published by Mayer and others in 1910 (p. 427).

The laboratory data published by Eberle in 1908 are of great value in showing (1) the importance of prolonged incubation of the suspensions used in agglutination tests; (2) the fact that an individual cerebro-spinal strain shows different degrees of agglutinability with different specific sera; (3) the fact that different cerebro-spinal strains show different degrees of agglutinability with the same serum. Obviously, these difficulties required further investigation before a throat coccus could be excluded from the group of meningococci because it failed to agglutinate with a particular anti-meningococcus serum.

Arkwright's technique (1909) differs from that of Lingelsheim, Kutscher, and the majority of other observers. He employed the microscopic method, at room temperature, and completed his observations at the end of two hours. Hence it is impossible to make exact comparison between his results and those obtained with the more usual technique. The difference in technique is particularly important because several investigators have shown that a period of two hours, even with incubation at 37° C., is not enough to bring out to the full the specific agglutinative capacities of the serum tested. This difficulty, however, does not obscure a very important feature of Arkwright's work; he has shown clearly that different strains of meningococci obtained in this country differed very markedly in their agglutinability with the sera with which they were tested.

His experiments on complement fixation, in 1911, also illustrate differences between different strains of meningococci.

Elser and Huntoon (1909) differ from many other observers in their method of performing agglutination tests. They incubated their suspensions for two hours at 37° C., and then kept them in the cold (9° C.) for 22 hours, the final reading being taken 24 hours from the start. In support of this method they refer to their comparative experiments on the influence of incubator, room, and ice-box temperature. These experiments (pp. 418–422) fail, in my opinion, to justify a general statement that their method brings out agglutinability as well as incubating for 24 hours, or overnight, at 37° C. or 55° C.

Other observers have found that, when working with such a delicate organism as the meningococcus, results sometimes fail to tally on testing the same strain with the same serum upon different occasions. But Elser and Huntoon go much further; they state that the instability of the meningococcus is very pronounced and constitutes one of the great obstacles to the work. It must be left an open question whether these difficulties would have remained so great if the technique of other observers had been followed.

In their "conclusions" they make the general statement that "approximately 40 % of the meningococcus strains studied were relatively inagglutinable"; and they explain elsewhere that they mean by "inagglutinable" failure to react to powerful sera in 1 : 100.

Turning to their laboratory data for recorded evidence in support of this general statement, one finds that a serum prepared from their strain M 30 was tested with 65 strains and failed to give specific agglutination with 25 of these. This experiment is interesting and important, but insufficient, *per se*, to justify the above broad generalisation. A strain of meningococci may not be agglutinated by one serum, but that does not prove that it is insusceptible to specific agglutination by any other serum.

Seeking further evidence, one finds that experiments were made with monovalent sera prepared from other strains than M 30, and that with these sera, again, some strains were agglutinated but not others.

Whilst recognising that this is corroborative evidence, it must be pointed out that the serological experiments which they have recorded, taken *in toto*, do not suffice to provide definite answers to the three questions: (1) Is it proved that a strain found to be agglutinated by a serum prepared against one meningococcus will necessarily be agglutinated by other sera of high titre? (2) Is it proved that a strain inagglutinable by one serum will necessarily be found inagglutinable by other sera? (3) Is there any ground for supposing that a meningococcus

when tested with two different anti-meningococcic sera, each of high potency with its homologous strain, may give good agglutination with the one, but fail to agglutinate with the other? Elser and Huntoon's data, in so far as they go, would indicate that the answers to questions (1) and (2) are to be "yes," and the answer to question (3) is to be "no." Other observers, working, admittedly, with different strains, have answered questions (1) and (2) by "no" and question (3) by "yes."

Elser and Huntoon make the observation, confirmed by other bacteriologists, that a meningococcus may produce a serum which is a relatively feeble agglutinator for the homologous strain, but produces higher, and undoubtedly specific, agglutination with other strains. But whilst nearly all other observers treat this as no more than a relatively infrequent idiosyncrasy of the meningococcus, Elser and Huntoon regard it as very frequent and highly important. In fact they give the reader the impression that 40 % of their strains belong to the group which are "inagglutinable" but "agglutinogenic." On searching for their recorded laboratory data in support of this view, I find (p. 422) that they are extremely scanty and by no means justify such a sweeping generalisation. In fact the number of strains shown to be agglutinogenic as well as inagglutinable is so small that this apparent discrepancy between Elser and Huntoon's results and those of other observers disappears.

Their experiments with six strains of "pseudo-meningococci," whilst showing differences between these and some of their meningococci, have not been carried far enough to justify the exclusion of the former from the class of meningococci as a whole.

Mayer (1909) maintained that an organism isolated from the nasopharynx could not be regarded as a true meningococcus unless, in addition to conformity in cultural and fermentation tests, it was agglutinated by a good serum, such as the Höchst, up to 1 : 500. This criterion errs on the side of exclusiveness; no one serum, whether monovalent or polyvalent, has been found which will agglutinate without fail all strains of undoubted meningococci derived from cerebro-spinal fluid.

The work of Friese and Müller (1909), Lieberknecht (1909), and Sachs-Müke (1911) is of great importance in its bearing on the question whether persons not known to have been in contact with the disease may be carriers of the meningococcus. They all found what they called "pseudo-meningococci" or "S." cocci; Friese and Müller found them in the throats of soldiers during the prevalence of an epidemic regarded

as influenza; Lieberknecht, in normal school children; and Sachs-Müke, in normal soldiers. From the laboratory data of their work which I have given, it is evident that they did their best to prove that these organisms were not true meningococci, but that they were compelled to admit a close kinship. They have not succeeded in proving non-identity, because the criteria on which they relied would also exclude some strains of meningococci which have been proved to be the cause of cerebro-spinal fever. I refer in particular to their assumptions (1) that one particular anti-meningococcic serum, if polyvalent and of high titre, must be infallible; (2) that a strain is not a meningococcus if its behaviour in serological reactions is not identical with one or two examples of undoubted meningococci; and (3) that there is a specific difference between true meningococci as a class and "pseudo-" meningococci as a class in respect of their comparative agglutinability at 55° C. and 37° C. As regards this last point, it must be pointed out that Kutscher, who was the first to show the advantages of incubation at 55° C. in enhancing the agglutinability of certain strains, denies (1912)[1] that specific differences are brought out between true and "pseudo-" meningococci by comparing agglutinability at 37° C. and 55° C. He concludes his criticisms of the efforts of the above authors to distinguish their throat strains from true meningococci with a verdict of "not proven."

When Dopter, in 1909, found organisms in the naso-pharynx which differed from his strains of undoubted meningococci only in respect of certain serological reactions, he was too sound a pathologist to stigmatise these as "pseudo" (*i.e.*, not specifically pathogenic for man); he explicitly stated that he thought they might be capable of producing cerebro-spinal fever. But to distinguish them from his fully accredited meningococci he gave them the prefix of "para." In 1911 he was able to give his para-meningococci their full credentials, by producing instances where cerebro-spinal fever had been caused by them; and in 1912 he reported that the number of such cases which he had collected amounted to twelve.

The great interest and importance of this work is that it goes far beyond the stage when, though it was recognised, willingly or unwillingly, that strains of meningococci did not all agree in serological reactions, these differences were treated merely as inconvenient idiosyncrasies which should be neutralised, as far as possible, by the production of polyvalent sera. Dopter showed that serological tests

[1] Kolle und Wassermann, *Handbuch der path. Mikroorganismen*, 2nd ed., IV.

brought out what appeared to be group distinctions between different strains of Weichselbaum's diplococcus, the smaller of the two groups to which he called special attention being distinguished from the larger by the somewhat irksome prefix "para."

In 1914 this "para" group received further attention at the hands of Darré and Dumas, and Dopter and Pauron; and some differences were found between individual members of it. Perhaps the sub-division of the group, by Dopter and Pauron, into α, β, and γ varieties should be regarded as merely provisional. If the authors devoted equal endeavour to the discovery of possible differences in their larger group, which I may call "ortho" as a convenient distinction from "para," they might again find distinction between individual members, tempting to another sub-division into varieties; or they might even find a strain which could not properly be called either "ortho" or "para," but demanded a third group. In short, it seems to me that it would be premature to take the division into "ortho" and "para" as completely comprehensive and final, and then try to force any and every meningococcus into one or other of these groups. The meningococci are a large family and many strains must be worked out, each on its own merits and irrespective of any preconceived idea of grouping, before it can be settled what are the distinctive features, each common to several members and confined to these members, and whether it is possible to establish a system of grouping without overlapping or cross-division. Before this is done, sub-division into α, β, and γ varieties is likely to lead to confusion.

The Investigations in the Board's Laboratory.

(1) *Meningococci from Cerebro-spinal Fluid.*

The Board's Laboratory has received for diagnosis specimens of cerebro-spinal fluid withdrawn by lumbar puncture from patients, in various parts of England and Wales, whose disease was suspected to be cerebro-spinal fever. Cultures from 16 of these cases have been utilised by Dr Griffith and me in the present investigation. We are also indebted to Dr Nabarro who has supplied us with 7 additional strains (5 from Great Ormond Street Hospital and 2 from private cases); to Dr Forbes, of the London County Council, who has sent us 7 strains; and to Dr Caiger, of the South-Western Fever Hospital, who has supplied us with 4 strains, through Dr W. M. Scott.

The details of Dr Scott's work on cerebro-spinal strains are given in his report (pp. 464–484).

As the cultural characters of the meningococcus are well known, Dr Griffith and I consider that a brief description of our observations and technique will suffice. We used for plating our material Kutscher's serum-agar medium, the special features of which are that the serum is fresh ox serum and the agar is made with broth from human placentas. The day after inoculation the colonies appear as translucent, shiny, bluish grey, raised, convex discs, of circular outline and from about $\frac{1}{2}$ to $1\frac{1}{2}$ mm. in diameter. Under low magnification they appear homogeneous, or only very finely granular, and have a clear periphery. When touched with a platinum needle, they are found to be slightly viscid, not coherent and not friable. They emulsify readily. On the second day the colonies are larger (1 to $2\frac{1}{2}$ mm.), semi-opaque, with a narrow, clear periphery. The colonies are not pigmented, but the central portion of the older colonies may appear creamy or yellowish in contrast to the grey colour of the thinner peripheral zone. Microscopically, the organisms are Gram-negative diplococci, many of which are flattened along their apposed surfaces; their size is variable, some being conspicuously larger than the rest and more deeply stained; tetrads are usually found. On plain agar slants, sub-cultures from a primary colony usually give no growth, but if the tube be thickly inoculated a few colonies may develop; from later generations a fairly good growth may be obtained. At 22° C., there is no growth on gelatine; on nutrose- (or glucose-) ascitic agar there is usually no growth, but if the tube be richly inoculated there is sometimes a scanty growth, in the form of discrete colonies; on Kutscher's medium growth may fail, but a scanty growth is more frequently obtained; on egg it is the rule to obtain a growth at this temperature, usually in the form of a thin, shiny layer. The coccus rapidly dies out in unsealed tubes of Kutscher's medium or ascitic agar, but in sealed egg tubes it remains alive for several weeks.

For the fermentation tests we used Lingelsheim's solid media (ascitic agar containing 1 % of the sugar and coloured with litmus). We used five sugars, viz., glucose, maltose, galactose, laevulose, and saccharose. We never obtained indication of acid formation with any of the last three; but there was always acid formation with both glucose and maltose. Some strains produced stronger fermentation with one of these sugars than with the other.

Further observations on the fermentation tests and also the work on

the serological relations of our cerebro-spinal strains are recorded in Dr Griffith's report (pp. 446–463).

(2) *Examination of Naso-pharyngeal Swabs from Non-contacts.*

(*a*) *Patients at St Bartholomew's Hospital.*

For this investigation the Board obtained the assistance of Mr C. E. West, F.R.C.S., Aural Surgeon to St Bartholomew's Hospital, who made arrangements for the examination of patients not known to have been in contact with any cases of cerebro-spinal fever.

The majority of the persons swabbed were out-patients attending the Aural Department; the rest were from other departments of the Hospital, some being wounded soldiers and the remainder civilians under treatment for one or other of a variety of medical and surgical diseases not affecting the naso-pharynx.

The persons examined were taken as general examples of hospital patients, without any selection according to their clinical condition. When pharyngitis was observed, a note to this effect was recorded.

Freshly poured plates were sent from the Board's Laboratory to the Hospital, where Mr West, or in some cases his house surgeon, took the swabs and immediately inoculated a plate with each. Within from one to three hours after the swabbing the plates were received in the Laboratory, together with notes of each case.

Dr F. Griffith and I then proceeded with the bacteriological investigation. The plates (Kutscher's medium) were spread at once with a bent glass rod, which was transferred to a second plate, and, when much mucus was present, from a second to a third. The time elapsing between swabbing the patient and incubating the plates did not exceed three hours. Colonies resembling meningococci macroscopically, microscopically, and in readiness of emulsification were put through the same cultural and fermentation tests as the cerebro-spinal strains.

Cultures of 502 swabs from the naso-pharynx were examined. Of these, the following are excluded in the appended tabulation of results:—8 which rapidly become overgrown; 10 which were repeats from patients previously cultured and found positive; 1 (positive) where it was found that the patient had been in contact with a case of cerebro-spinal fever; 1 (positive) where the patient gave a vague history of an illness, 3 months previously, which might have been meningitis; 2 where, on sub-culture, the cultural and fermentation reactions appeared typical at first, but yellow pigmentation subsequently

developed. Of the remaining 480, 49 yielded cultures which resembled meningococci microscopically, culturally, and in fermentation tests.

Cultural Tests of Naso-Pharyngeal Swabs.

Age period	Males		Females		Totals (male and female)	
	Positive	Negative	Positive	Negative	Positive	Negative
0– 5 years	0	13	1	8	1	21
5–10 „	4	38	0	15	4	53
10–20 „	8	62	3	69	11	131
20–40 „	17	78	6	79	23	157
Over 40 „	7	42	3	27	10	69
Totals ...	36	233	13	198	49	431
	13·4 % positive		6·2 % positive		10·2 % positive	

With reference to the interpretation of these statistics, attention must be called to the following special circumstances:—(1) The persons investigated, being hospital patients, were not representative of the normal, healthy population. (2) The swabs were taken by an aural surgeon who was expert in obtaining naso-pharyngeal swabs free from contamination by mouth organisms; so the risk of any meningococcus-like organisms present being overgrown was reduced to a minimum. (3) The medium (Kutscher's) on which the swabs were plated is especially favourable for the development of meningococcus-like organisms and for their identification. (4) In examining the plates we were more fortunately placed than bacteriologists who are required to diagnose as many cases as possible in as brief a time as possible. We never received more than 20 cases in one day, or more than about 50 in a week, and had time to make minute and repeated examination of each plate inoculated.

For the above reasons, our percentages of positives are, perhaps, above the average.

The collection of the above 480 samples commenced on March 29th, 1915, and terminated on July 22nd of the same year. The first 100 (completed April 19th) gave 20 positives; the second 100 (completed May 6th) gave 7; the third 100 (completed June 7th) gave 6; the fourth 100 (completed June 24th) gave 7; the last 80 (completed July 22nd) gave 9.

The "repeats" of previous positives were too few to justify any general conclusions. Two out of the ten were positive, one after an interval of 28 days from the first swabbing and the other after an interval of 61 days.

On comparing the positive and negative results with the clinical notes, it is not found that a conspicuously large number of the positive cases showed pharyngitis when the swab was taken.

Further work on the identification of these meningococcus-like organisms, with special reference to their serological reactions, was undertaken by Dr F. Griffith and is recorded in his report (pp. 446–463).

(*b*) *Investigations by Dr Scott.*

Dr W. M. Scott, working in the Board's Laboratory independently, but on parallel lines and employing the same technique and culture media, examined naso-pharyngeal swabs from 194 non-contacts, consisting of 138 persons attending the out-patient department of the Lambeth Infirmary, and 56 children attending a rural school in Kent. His results are recorded in his report (pp. 464–484).

Conclusions.

It has been definitely established that organisms indistinguishable from meningococci by microscopic, cultural, and fermentation tests occur in the naso-pharynx of some persons who, so far as is known, have not been in contact with cases of cerebro-spinal fever.

The question whether this apparent identity can be confirmed by serological tests raises the wider problem:—what are the serological criteria to which strains of undoubted meningococci conform? This problem is far from being completely elucidated. It has, however, already been made clear that differences exist between the reactions of different strains, and that these differences appear to separate out the majority of the strains into different groups. But it is still, in my opinion, an open question to what extent and under what principles a permanent grouping can be established.

In considering the principles of classification, one must endeavour to determine distinctive group characteristics and, at the same time, to discriminate between such characteristics and minor distinctions which might, perhaps, offer a basis for sub-grouping. Taking the experimental work in the order of laboratory procedure, there are, at once, three important characteristics which require consideration. The first step is to immunise an animal with a particular culture; the second to see what strains are agglutinated by the serum prepared; and the third, when necessary, to ascertain by absorption tests if the

agglutination is specific. Correspondingly, the three characteristics to be considered as criteria are:—(1) agglutinogenic capacity; (2) agglutinability; (3) absorptive capacity. If each individual strain of meningococci behaved consistently in respect of these three criteria, there would be a substantial basis for the grouping of different strains. But if individual strains differ in the above respect, grouping under these three criteria would lead to cross-division. Such cross-division could only be avoided by making one of these criteria absolute, and relegating the other two to the minor function of criteria for sub-grouping. In my opinion, enough work has not yet been done to justify the establishment of criteria for the demarcation into hard-and-fast groups; and, in view of this consideration, I think it would be premature to adopt a particular standard and then attempt to dispose of discrepancies from it by elastic expansion into sub-groups.

It is likely to be a long time before the question of classification is finally settled. In the meantime, comparative serological tests are available between individual cerebro-spinal strains and strains of naso-pharyngeal origin. It has been found that some of the latter, derived from non-contacts, coincide with certain cerebro-spinal strains, and that other non-contact strains, though not coinciding precisely, show serological relationship to cerebro-spinal strains.

How far do the above considerations provide an answer to the question: is a naso-pharyngeal strain, found identical with true meningococci in cultural and fermentation tests, to be regarded, *ipso facto*, as capable of producing cerebro-spinal fever in a suitable soil? It is impossible to reply by a categorical "Yes" or "No," because the evidence is incomplete. The balance of available evidence is on the side of "Yes." Is future work likely to reverse that balance? It might, if it can be shown that serum reactions or animal experiments place the great majority of non-contact strains into a distinct class. But this evidence is not yet forthcoming. If it is provided in the future, it will be necessary for me to reconsider my present opinion, which is that all naso-pharyngeal strains, as defined above, should be regarded as possibly capable of producing cerebro-spinal fever.