EPIDEMIC CEREBRO-SPINAL FEVER:

THE PLACE OF THE MENINGOCOCCUS IN
ITS ETIOLOGY.*

By EDWARD C. HORT, F.R.C.P. EDIN.,

HONORARY PHYSICIAN TO AND DIRECTOR OF BACTERIOLOGICAL
LABORATORY OF THE ADDINGTON PARK WAR HOSPITAL.

THE theory that the meningococcus of Weichselbaum is itself the primary infective agent in epidemic cerebro-spinal fever is one, as I have recently shown,¹ that rests on the slenderest evidence. Indeed, the only evidence worthy of the name is derived from the facts that the organism in question occurs in large numbers of acute cases of the disease and in primary and secondary contacts, that it sometimes will agglutinate with the human specific serum, and that recovery may follow the injection of animal antisera. Mere constancy of presence of an organism in any given disease or in contacts is, however, worth little as evidence of direct etiological relationship, as is universally admitted—for example, in the case of the *Streptococcus conglomeratus* of scarlet fever, of the *B. exanthematicus* of typhus fever, and of the bacillus of Hoffmann in convalescing diphtheritis. And agglutinability of an organism by a patient's serum does not sustain a thesis of etiological relationship of a direct kind, but only of pathogenicity, which may apply to secondary invaders as well as to primary. And, finally, the evidential value of the fact that recovery may follow injection of a specific antiserum is gravely impaired by the number of recoveries that take place without such injection, by the absence of control injections of normal serum, and by the repeated failures of specific serums such as were experienced in the late epidemic in this country. It is true that the failure of an antimeningococcal serum may be due to lack of identity of the strain of meningococcus supposed to be causing the individual case with the strain used in the preparation of the serum. There is no question but that there are numerous strains of genuine meningococci. But until better evidence can be produced in other directions that any single strain is capable of reproducing the disease in man, multiplicity of strains offers an unsatisfactory refuge for those who uphold the etiological claims of the meningococcus of Weichselbaum. And in this connexion it is not a little surprising that the disease has not yet been recorded to have been reproduced in any animal by injection of any strain, the production of meningitis in monkeys by intraspinal injection being of course worthless as evidence that the meningococcus is the cause of a disease in which meningitis is only an inconstant event.

Critical analysis, therefore, of the evidence—bacteriological, serological, and experimental—would appear to justify the statement that the primary cause of the disease epidemic cerebro-spinal fever is still unknown, the claims of the meningococcus not having been yet made good, and no rival having been yet established. This is the conclusion I came to in previous publications on the subject, and as a result of further research since then I am more than ever convinced of the accuracy of the statement referred to.

In thus exposing, however, the fragility of the claims of the meningococcus, I was careful only to state that its case was not proven. I did not assert that the meningococcus is not the cause of the disease, because I was not then in possession of experimental data which would justify so dogmatic a statement. Still less did I assert that the meningococcus does not bear an intimate, albeit unknown, relationship to the true infective agent. On the contrary, I insisted that the search for carriers of this organism should in no wise be abandoned, since whatever its relationship to the true infective agent ultimately proved to be the fact of the frequent presence of the meningococcus in acute cases, and in primary and secondary contacts, is as yet our main guide to efforts at epidemiological control.

To-day, however, as a result of an extensive series of

observations in monkeys, I am in a position to adduce experimental evidence which strongly suggests that the meningococcus is not itself the primary infective agent in epidemic cerebro-spinal fever.

This evidence is partly derived from the fact that of four monkeys injected intraperitoneally with living cultures of meningococci not a single animal appeared to be affected by the procedure. The largest counted dose given amounted to 9,200,000,000 organisms. In previously recorded animal experiments with living cultures of the meningococcus via other routes than the meningeal it appears that cultures on synthetic laboratory media have been employed. Owing to the possibility of rapid loss of pathogenicity on such media the absence of pathogenic response in the animals injected is not conclusive evidence that the meningococcus is not pathogenic as it occurs in the blood or cerebro-spinal fluid. In the experiments here cited, therefore, loss of pathogenicity was as far as possible excluded by cultivation of the organism in the cerebro-spinal fluid itself, care being also taken to inject the cultures in the fluid within the few hours after collection necessary to ensuring—by incubation—a sufficient degree of multiplication. As already stated, the results of injection of these four animals were completely negative, and this fact, coupled with the precautions taken only to inject the cultures under conditions approximating as closely as possible to those of cultivation in the body, appear to suggest the conclusion that the meningococcus, when injected intraperitoneally, is not pathogenic to monkeys. And the consequent deduction that this organism is not itself the primary cause in man of the disease cerebro-spinal fever is also suggested by the additional fact that intraperitoneal injection of the fresh filtrate (Chamberland F) from the cerebro-spinal fluid of two acute human cases of the disease produced in one of two further monkeys injected a continued fever lasting for several weeks, accompanied by a herpetic rash on the face, and in the second animal death forty-eight hours after injection, preceded by coma and well-marked retraction of the head. As will be seen in the protocols of the experiments here quoted, which are being published in full elsewhere,² conclusive evidence was not obtained that the disease had been reproduced in these two animals by injection of fresh cerebro-spinal fluid filtrate. But the results obtained were sufficiently striking to justify further experiments with fresh filtrates, and in the meanwhile to make it clear that the cerebro-spinal fluid of acute cases may contain a highly infective virus which is certainly not, in view of the first four experiments quoted, the meningococcus.

Trial was then made of the pathogenic effect in monkeys of the intraperitoneal injection of incubated filtrate (Chamberland F) of cerebro-spinal fluid, incubation being allowed to proceed for one night after filtration. A series of four monkeys were injected with incubated filtrates from four different cases, one of the animals receiving an injection of the same filtered cerebro-spinal fluid as caused before incubation continued fever in the animal referred to above. In all four animals in this series the filtrate injected was turbid with the organisms described below, but in no single case was the slightest pathogenic effect observed. These results suggest that the infectivity of freshly filtered cerebro-spinal fluid may be entirely destroyed by incubation at body temperature for a few hours, a fact which, as will be seen, may in the future prove to have a direct bearing on the question of metamorphosis, and on the problem of determining the true etiology of this disease.

The stage which we have so far reached in our inquiry may therefore be thus defined:

1. There is no good evidence that the meningococcus is the primary infective agent in the disease epidemic cerebro-spinal fever.
2. On the contrary, I have produced experimental evidence which suggests distinctly that it is not.
3. And I have also shown that the cerebro-spinal fluid of acute cases, when filtered through a Chamberland F bougie, may contain, when injected fresh, a highly infective virus.
4. This infective virus appears to be present as such only in the fresh cerebro-spinal fluid, incubation, on the other hand, appearing to destroy its infectivity.

If the meningococcus is not the primary infective agent it must, according to modern bacteriological conceptions,

* This paper is, to some extent, an abstract of a fuller paper on the same subject which will appear in the *Journal of the Royal Army Medical Corps*. For the compilation of this abstract and for the fuller paper, as well as for the filtration and animal experiments referred to, and for the hitherto unpublished experiments in typhus fever, I am alone responsible. For the strictly bacteriological observations referred to I share the responsibility with my colleagues, Dr. C. E. Lakin and Dr. T. H. C. Benians.

be either a secondary invader, or its presence must be symbiotically necessary to the true infective agent, or it must represent merely a phase in the life-history of the true infective agent. Apart from the further possibility that it may be a contaminant—a suggestion which can be summarily dismissed—it is difficult to imagine any other possible explanation of the rôle of the meningococcus of Weichselbaum. In order, therefore, to present a clear idea of the nature of the problem presented by the etiology of cerebro-spinal fever, it is necessary, before summarizing the results of our bacteriological studies up to date, briefly to discuss two of these rival theories, the third theory—that of symbiotic necessity—being merely speculative.

THE THEORY OF SECONDARY INVASION.

The term "secondary invader" is one that in diseases of uncertain etiology should be applied with the greatest care, however suitable it may be in the description of such organisms as are liable to invade the blood stream in grave systemic infections primarily caused by other organisms whose etiological claims are beyond dispute. The chief characteristic of these genuine secondary invaders is their diversity in, and inconstancy to, any given disease in which they appear, as all who constantly make blood cultures will agree. In the case, however, of certain infections—such as typhus fever, scarlet fever, and, perhaps, cerebro-spinal fever, to mention only three—the essential characteristics of the so-called secondary invader in each of these diseases is not their diversity or inconstancy, but their remarkable constancy. We have, for example, constantly occurring in all parts of the world the *Bacillus exanthematicus* in typhus fever, the *Streptococcus conglomeratus* in scarlet fever, and the meningococcus of Weichselbaum in cerebro-spinal meningitis. And, be it noted, it is not only the constancy with which, in these diseases, the individual organism occurs (in the individual disease) that arrests the attention, but it is also the restriction of this organism to that disease and to no other. For example, the *Bacillus exanthematicus* of typhus fever has never been described in scarlet fever, nor the meningococcus in typhus, nor the *Streptococcus conglomeratus* in cerebro-spinal fever. None the less, the names of these organisms and of the respective diseases in which they constantly occur are as household words. It has already been insisted that mere constancy of presence of one type of organism in any given disease is not adequate evidence that such organism is itself the primary infective agent in that disease. But this constancy of presence of the so-called secondary invader in the three diseases mentioned is too striking to be a matter of accident. It is therefore not unreasonable to insist that there may be both genuine and spurious secondary invaders, and that spurious secondary invaders may prove to be modified primary invaders. The assumption that an organism is a genuine secondary invader is, in fact, in the present state of our knowledge of the etiology of certain diseases, often no more legitimate than the assumption—which we have seen in the case of the meningococcus is not justified—that it is the primary invader. Since we have not sufficiently reliable evidence that the meningococcus is either the primary invader or a genuine secondary invader, formulation of the theory that this organism merely represents a phase in the life-history of the true causal agent of the disease, at present unknown, requires no apology. On the contrary, the facts of the constancy of presence of this organism in cerebro-spinal fever and its apparent inability to reproduce the disease in monkeys, or, indeed, to exert any pathogenic effect whatever when injected by the intraperitoneal route, establish an *a priori* claim to necessity for formulation of the theory and investigation of its truth.

THE THEORY THAT THE MENINGOCOCCUS MERELY REPRESENTS A PHASE IN THE LIFE-HISTORY OF THE UNKNOWN CAUSAL AGENT OF THE DISEASE.

The theory—that certain bacteria may possess a more complicated life-history than their study under the artificial conditions of laboratory culture might lead one to suppose—was formulated by Hort³ in 1914 as a result of prolonged morphological and cultural study of the infective agent in typhus fever.

That certain protistic organisms, to use Professor Herbert Henry's apt word,⁴ exhibit multiple phases in the

course of their development, phases essentially characterized by morphological dissimilarity, has long been known in the protozoa. And in the intermediate zone between the bacteria and the protozoa evidence is gradually accumulating that, in the case, for example, of the infective granule-shedding spirochaetes, the same principle of diversity of developmental form is at work. There is therefore nothing unreasonable in my thesis that the meningococcus, which itself appears to be relatively innocuous, represents merely a phase in the life-history of an unknown organism which is only capable of reproducing the disease in its earliest phases. And provisional acceptance of this theoretical possibility is to some extent favoured by certain considerations which have never yet been satisfactorily explained.

Here, for example, are three facts which are quite unintelligible if we accept the Tentonic concept that the meningococcus of Weichselbaum is itself the cause of the disease.

1. That the incidence of the meningococcus in carriers varies inversely as the incidence of the disease in such carriers.

2. That the organism in acute cases is a constant inhabitant—in this disease and in no other—of a fluid which, as we have shown, appears to be only infective when the presence of no meningococci can be demonstrated.

3. That a relatively large non-motile organism, such as the meningococcus, with an average diameter of about 1 micron, must be itself capable of freely traversing the meningeal filter-bed. This fact is not disposed of by the suggestion that the meningococcus is carried to the spinal canal by leucocytes, since we have no proof that this is so, and the meningococcus is frequently found to be extra-cellular in freshly drawn cerebro-spinal fluid.

If, on the other hand, the meningococcus is merely a modified primary invader, a small but dangerous ancestor of unknown morphology having been replaced by a relatively large but innocuous descendant of known morphology, all these difficulties disappear. And in the case of such carriers as do fall victims to the disease one has only to suppose that virulent ancestors and harmless descendants exist side by side.

Attractive, however, as such a theory is, and unsatisfactory as the present theory upheld by the numerous followers of Weichselbaum has proved itself to be, the onus of proof rests on those who believe that certain bacteria do in fact exhibit different morphological phases of development associated with different phases of pathogenic activity. And in this connexion it is only fair to point out that I only applied it to the study of the etiology of cerebro-spinal fever after showing that it appeared to have a strong foundation in fact in typhus fever, though I have since found additional evidence in the case of scarlet fever and in streptococcal infections associated with cardiac disease. Before, therefore, summarizing such bacteriological evidence as I and my colleagues, Dr. Lakin and Dr. Benians, have been able to collect, which would appear to justify further study of the theory as applied to cerebro-spinal fever, it is necessary briefly to recapitulate the facts which originally justified formulation of the theory in the case of typhus fever. Such digression, though not at first sight germane to discussion of the problems presented by the etiology of cerebro-spinal fever, is really necessary in order to make intelligible the general line of inquiry we have pursued in the latter disease. And the fact that some of the ensuing observations on the bacteriology of typhus fever have not yet been published seems to justify their appearance here for the same reason.

COMPARISON OF THE PROBLEMS PRESENTED BY THE ETIOLOGY OF TYPHUS FEVER AND OF EPIDEMIC CEREBRO-SPINAL FEVER.

In typhus fever a pleomorphic organism, known as the *Bacillus exanthematicus*, can frequently be recovered from the body fluids of acute cases. In some epidemics, indeed, the organism has occurred so frequently as to suggest to many observers in the past that it was the true causal agent of the disease; and this belief was strengthened by the facts that it would often agglutinate, and even deviate

complement, in the presence of the specific human serum; that it was found in typhus fever and in no other disease, and that it was more or less pathogenic to guinea-pigs and mice. Unaccountably, however, the claims of this organism to be regarded as the primary infective agent never found general acceptance even by those who upheld the claims of the meningococcus in cerebro-spinal fever on even less satisfactory grounds. The *Bacillus exanthematicus*, in consequence, came to be looked upon as a genuine secondary invader, though no explanation was forthcoming as to why the organism occurred in typhus fever and in this disease only; and up to the year 1914 the general view of the etiology of the disease remained in this somewhat unsatisfactory condition.

In that year, however, it was shown by Hort and Ingram⁵ that the *Bacillus exanthematicus* had little or no claim to being considered either the primary infective agent or a genuine secondary invader, inasmuch as they found by injection of the organism in monkeys that it was incapable of producing pathogenic effects in these animals. Their cultures were obtained by incubating human citrated blood from acute cases of the disease, the specimens of blood injected containing large numbers of the organism in question. On the other hand, injection of unincubated specimens of blood from acute cases of the disease produced in several animals the typical disease which is generally accepted as the analogue of human typhus in monkeys. They had, therefore, in this last set of experiments, an excellent control which is at present lacking in cerebro-spinal fever. For example, I recently injected intraperitoneally each of three monkeys with 6 c.cm. of a 1 in 8 suspension in citrate solution of blood taken from three acute cases of cerebro-spinal fever without provoking any material reaction, and the same negative result followed the injection of three further monkeys with the same quantities of citrated blood after incubation.

This demonstration of the total absence of recognizable pathogenic effect after injection of monkeys with the *Bacillus exanthematicus* naturally raises the question—If this organism is neither the primary invader in typhus fever nor a genuine secondary invader, what is it? To answer that it is a contaminant is no more justifiable than would be the assertion that the meningococcus of Weichselbaum is a contaminant in cultures from cases of cerebro-spinal fever. If, however, the *Bacillus exanthematicus* is itself neither the primary invader nor a genuine secondary invader, nor yet a contaminant, the problem appears insoluble. And at the time the experiments cited were undertaken no explanation suggested itself. However, after prolonged morphological study of the bacterial content of typhus body fluids in the fresh condition, supplemented by morphological study of their content after incubation, followed by plating, some interesting facts emerged which offered a reasonable solution of the mystery, though, as will be seen, absolute proof of the correctness of this solution is a matter of great technical difficulty, and has not yet been obtained.

The facts referred to were these: In many cases it was found that in the fresh fluids the pleomorphic *Bacillus exanthematicus* was present in considerable numbers, especially in the urine, side by side with very minute organisms, many of which were near the vanishing point of vision. In other cases the fresh fluids, especially the urine and the cerebro-spinal fluid, were found to contain these minute organisms in what appeared to be pure culture. On the other hand, it was found that those fluids which contained, or appeared to contain, when fresh, these minute organisms in pure culture contained after incubation only the large pleomorphic organisms. Obviously, therefore, these small organisms, the discovery of which has since been amply confirmed by W. W. C. Topley,⁶ working in Serbia, had either disappeared or else had developed into the *Bacillus exanthematicus*. Attempts were therefore made to determine which was the correct explanation by filtration of the fresh fluids containing mixtures of the small and large organisms, in the hope that the small organisms would alone traverse the Berkefeld and Chamberland bougies employed. These experiments were successful to the extent that the clear filtrates on two occasions showed on centrifuging small numbers of the minute organisms alone, whilst incubation of the same filtrates produced in a few days marked turbidity due to the presence of large numbers of the *B. exanthematicus*. And pure cultures of these in some cases appeared on plating.

Unfortunately the minute organisms referred to would never survive subculture on plates beyond the first culture, so that biochemical identification was impossible. But single colonies, which appeared by film examination to be pure, on further subculture always developed the pleomorphic *Bacillus exanthematicus*. It was, however, impracticable to attempt to prove metamorphosis by growth from a single organism, either by Barber's method, or by the use of small fragments of coverslips, because in picking up—by the latter method—one minute organism near the vanishing point of vision it could not be guaranteed that another organism beyond the range of vision would not at the same time be taken up. Moreover, owing to the fact that in its earliest forms these minute organisms often appeared to have become detached from the periphery of zoogloic masses of organisms so small as to be of quite indeterminate morphology the application of serological tests for purposes of identification with the *Bacillus exanthematicus* was also out of the question. Recourse was therefore had to injection of monkeys with pure first cultures of the minute organisms on serum-agar. The procedure was, in all the cases tested, followed by the appearance, after an incubation period of a few days, of a disease accompanied by continued fever. This disease, beyond being of a somewhat shorter duration, suggesting that full pathogenicity had been to some extent lost by artificial cultivation, had as much claim to be considered genuine typhus fever as the disease produced in monkeys by the injection of fresh human typhus blood. From two of the monkeys injected with fresh human blood minute organisms morphologically identical with the minute organisms described were again recovered, as well as the *Bacillus exanthematicus*. Two of the monkeys injected with the serum-agar cultures were—after recovery from the disease following injection—several months later injected with fresh human typhus blood, but neither animal responded, though one of the unimmunized controls exhibited a typical fever curve. These two animals were, therefore, perhaps immunized. A convenient opportunity of repeating this observation has, however, not yet occurred. A further interesting experiment bearing on the question of metamorphosis was, however, made. A series of four fresh specimens of human typhus blood collected before the crisis were injected into four healthy monkeys, and in three of the animals the so-called "typhus classique" appeared and ran its course. Another series of four specimens of human typhus blood in citrate, three of which were collected also before the crisis, was then injected into another series of four monkeys after the blood had been kept in the incubator at blood temperature for periods varying from several hours to several days, but in no case was any reaction whatever observed. It will be necessary to repeat this experiment, when opportunity allows, by injecting monkeys with samples of the same specimens of human typhus blood before and after incubation, in order to be certain that insusceptibility of the individual animal recording a negative result does not come into play. But, even as carried out, the experiment suggests that, as in the case of the cerebro-spinal fluid in cerebro-spinal fever referred to earlier in this abstract, it is only the fresh body-fluid which is infective. And in view of the other experiments referred to it is legitimate to suggest that the results of this experiment lend some support to the view that the *Bacillus exanthematicus* represents a harmless phase in the life-history of the minute pleomorphic organism described by Hort and Ingram in 1914. Before leaving the subject of typhus it is necessary briefly to refer to the *Bacillus exanthematicus* of Plötz, recent accounts of which appear at first sight to affect the validity of what has been said. The organism of Plötz, which has lately been exploited as the true primary infective agent in typhus, mainly differs from the *Bacillus exanthematicus* in the fact that it is said to be only possible to grow it anaerobically. Its claim to be the primary infective agent appears to be mainly based on the statement that it may produce fever when injected into animals, and that immunization can in this way be obtained against the fever-producing effect of injections of human typhus blood. It was with some interest, therefore, that I recently examined a heated emulsion of Plötz's *Bacillus exanthematicus*, large quantities of which have been tested as prophylactic vaccines in Serbia. The specimen received was contained in a sealed capsule, and

presumably it was looked upon as a pure culture of Plötz's Gram-positive pleomorphic organism, which is morphologically indistinguishable from a strain grown aerobically in this country. The contents of the capsule were centrifuged, and a film from the deposit was stained with Gram and counterstained with dilute fuchsin. Examination of the film revealed, however, that it was not a pure culture, and that mixed with the *Bacillus exanthematicus* were considerable numbers of the minute Gram-negative organisms morphologically similar to those described by Hort and Ingram. The Gram-positive bacillus measured in its greatest diameters from 0.5 to 1.5 microns, whilst the minute organisms varied from about 0.2 to 0.6 micron, though organisms nearer to the vanishing point of vision were also seen. It is, therefore, not an unfair inference that the infectivity of Plötz's organism, and its reputed immunizing properties, on which the claims on behalf of this organism have been largely based, are in reality due to the presence of the minute cocco-bacillary organisms described, and the value of the serological reactions reported is also impaired for the same reason. Final determination of these points can, of course, only be effected by examination of living cultures, which will be carried out as soon as opportunity for so doing occurs. But, in the meanwhile, the original observations carried out by Hort and Ingram in this country hold good.

With these observations in mind as regards typhus fever, the following notes on the bacterial content of the body fluids in cases of cerebro-spinal fluid now become intelligible.

THE TRUE BACTERIAL CONTENT OF THE INFECTED BODY FLUIDS IN ACUTE CASES OF CEREBRO-SPINAL FEVER.

The necessity of determining whether the meningococcus is or is not usually the sole inhabitant of importance of the infected body fluids in cerebro-spinal fever is, of course, an essential preliminary to attempting to determine if this organism is or is not biologically related to organisms other than the meningococcus. In other words, demonstration that the body fluids in this disease are commonly sterile—apart from the meningococcus—would go far towards disproving the theory that the meningococcus represents only a phase in the life-history of the true causal organism. On the other hand, demonstration that the constantly occurring meningococcus is only one of two or more types commonly infecting the body fluids in this disease would constitute a good case for inquiry, provided that the meningococcus was itself shown to be inert—as regards reproduction of the disease—and that the fluids originally containing it were in the fresh condition possessed of pathogenic properties. And both of these provisos we have shown to be fulfilled.

Before, however, recording the results of examination of the body fluids in cerebro-spinal fever it is necessary briefly to note that in order to determine the true bacterial content of any body fluid the ordinary method of judging of sterility by recording the results of inoculating laboratory media with these fluids in the fresh state or with their centrifuged deposits is often inaccurate. For example, as has recently been shown by Hort,⁷ neglect to incubate normal urine, before inoculation of the laboratory medium selected as an indicator of sterility or infection, often leads to serious fallacies. In the case of normal male urine, hitherto believed to have been proved to be sterile because in many cases no growth occurred on solid laboratory media inoculated with the fresh urine or its deposits, it was shown that if the specimens were first incubated not one out of 128 samples derived from several healthy individuals was in reality sterile. And control experiments done in parallel, in a small number of cases, completed the exposure of the fallacy involved by trusting to readings of the results obtained by inoculation of laboratory media with fresh urine. And it has since been shown by Hort and Ingram⁸ that in the case of the urine of subjects of systemic infections—such as lobar pneumonia, influenza, scarlet fever, mumps, measles, cerebro-spinal fever, infective endocarditis, typhus fever—and in convalescing urinary carriers of the enteric group, as shown by Hort,⁹ the same principle holds good. Similarly, in attempting to estimate the true bacterial content of the blood in any systemic infection, one is not justified in declaring the blood as sterile until examination has been made of laboratory media inoculated with

incubated blood, for which purpose suspension in solution of citrate or oxalate is better than defibrination, since loss may occur by entanglement in the fibrinous mesh produced by this procedure. And in all pathological cases, whether of urine or blood, it is always advisable to examine the fluids themselves after incubation as well as the laboratory media inoculated therefrom, as it sometimes happens that organisms will grow freely in the natural medium, but will resolutely decline to adapt themselves to the alien environment of a synthetic medium, even when a liquid medium is inoculated. And, finally, by application of this principle to the cerebro-spinal fluid in acute cases of cerebro-spinal fever, my colleagues and I found, as recorded in April, 1915, that not only may the meningococcus be missed if the ordinary routine be adopted, but also that it is in many cases impossible, otherwise than by incubation, to estimate the true bacterial content of specimens of cerebro-spinal fluid in this disease as regards organisms other than the meningococcus. These results, in so far as they concern the meningococcus, have since been amply confirmed by Captain Gaskell,¹⁰ working in Cambridge. It is, indeed, apparently the absence of this precaution of preliminary incubation which appears to be responsible for the number of specimens reported to be innocent of the meningococcus when in reality they are often not so, and, on the other hand, for the excessive attention which has in the past been paid to this organism as the reputed causal agent of the disease. In all cases, therefore, both for purposes of demonstration of the presence of the meningococcus and of determining what other organisms are likewise present in a given sample of this fluid, it is wise to make preliminary incubation a routine procedure—in parallel if desired—with the ordinary method.

SUMMARY OF BACTERIOLOGICAL EXAMINATION OF INFECTED BODY FLUIDS IN ACUTE CASES OF CEREBRO-SPINAL FEVER.

Full details of the results obtained by bacteriological examination of the specimens of infected body fluids we were able to obtain previous to April, 1915, are given in the two papers already referred to.

In the notes recorded in these papers reference is made to the examination of 10 specimens of cerebro-spinal fluid, 17 specimens of citrated blood, and 19 specimens of urine, several of which were catheter specimens.

Filtrates of these specimens were examined in five instances, the cerebro-spinal fluid supplying 1 filtrate, the blood 2 filtrates, and the urine 2 filtrates. In the case of the cerebro-spinal fluid, and in the case of one specimen of urine, two new Chamberland F bougies were employed. In the case of the two specimens of blood, and of the remaining specimen of urine, three new Berkefeld V bougies were employed. In numerous specimens of the unfiltered fluids submitted to complete examination the following organisms were described as occurring in the same specimens:

- (a) The meningococcus of Weichselbaum.
- (b) The meningobacillus (previously referred to by us as the "biscuit" bacillus).
- (c) Clusters of very minute organisms, sometimes diplococcal or diplobacillary in form, at other times of quite indeterminable morphology.
- (d) Jaeger's diplococcus.
- (e) Involution forms, diphteroid forms, and large bacillary forms.

The biochemical and cultural characteristics of the meningobacillus, which ferments the same sugars as the meningococcus, and of Jaeger's diplococcus are given in full in the protocols already published. In the case of the filtrates, whether the filter employed was a Berkefeld bougie or a Chamberland bougie, the results were the same, with the exception that the organisms which morphologically resembled the meningococcus were not submitted to identification tests.

These findings, in filtered and in unfiltered body fluids from acute cases of cerebro-spinal fever, were subsequently confirmed in their morphological details by five sets of workers independently of each other and of ourselves, as judged by their published papers.^{11 12 13 14 15}

From the occurrence of these several different types of organism in filtered and in unfiltered fluids, in many of

which from the application in parallel of the ordinary methods of examination without preliminary incubation the existence of pure cultures of the meningococcus was suggested, it was at first difficult to resist the conclusion that contamination must have occurred. And the presence of the involution forms, and of organisms of a diphtheroidal form, made this conclusion doubly difficult to resist. Increasing experience, however, of the value of preliminary incubation of the body fluids in cerebro-spinal fever before inoculation of laboratory media—in attempting to determine their true bacterial content—soon enabled us to refute the frequently made suggestion of contamination as being merely the outcome of inexperience of the method. And this conviction was greatly strengthened by finding that the same types of organisms simultaneously occurred again and again in increasing numbers of cases, and this largely irrespective of whether the fluid examined was cerebro-spinal fluid, blood, or urine. Moreover, the apparent existence of pleomorphism, although, as we have said, not absolutely proved by culture from single individuals, showed itself so constantly in subculturing from single colonies as practically to exclude the possibility of perpetually recurring contamination with the same organisms.

And, finally, the suggestion of contamination we have again found to be quite inadequate to explain the results obtained by repeating the observations with a fresh series of 18 specimens of cerebro-spinal fluid in the summer of 1915. Of these 18 specimens 6 were submitted to filtration, a new Chamberland bougie being in each case employed, and in the majority of the specimens, filtered and unfiltered, submitted to complete examination the same essential results were obtained, and may be seen by reference to the full paper now in the press. The total number of specimens examined amounts, therefore, to 64, of which 11 were filtrates, a Chamberland F filter being employed in 8.

The stage, therefore, we have now reached is this—that the true bacterial content of the body fluids in this disease is by no means adequately represented by the meningococcus. On the contrary—provided that these fluids are thoroughly examined by (1) study of the morphology of their deposits in fresh and in incubated specimens, both filtered and unfiltered; (2) supplemented, after plating, by cultural and biochemical study in, or on, laboratory media inoculated with incubated material, filtered and unfiltered; (3) injection of monkeys with the fresh filtrates—ample justification will be found for directly attacking the problem of the true ancestry of the meningococcus. And I therefore propose to make this the next stage in the investigation of the etiology of the disease side by side with further experimental observations in monkeys.

CONCLUSIONS.

1. There is at present available no adequate evidence to show that the meningococcus of Weichselbaum is itself the causal agent of the disease cerebro-spinal fever.
2. On the contrary, the experimental evidence here recorded distinctly suggests that it is not.
3. The fresh filtrate of cerebro-spinal fluid in the two cases examined contained a virus which was highly infective to monkeys.
4. The true bacterial content of the body fluids in this disease can only, as a routine procedure, be satisfactorily determined by, in the first instance, using the fluids themselves as the optimum medium of growth, combined with injection into suitable animals in the fresh condition.
5. Incubation of the body fluids, infective to monkeys in the unincubated state, appears to destroy their infectivity and, in so doing, to throw some light on the ancestry of the meningococcus.

INFLUENCE OF CONCLUSIONS ON THERAPEUTIC AND EPIDEMIOLOGICAL CONTROL OF THE DISEASE.

1. Although the meningococcus appears itself to be incapable of reproducing the disease, the search for and isolation of carriers of this organism should, for the present, be vigorously pursued as heretofore, since its presence is a valuable danger signal that the true infective agent is, or has been, present.

2. Attempts to prepare antisera to the meningococcus, whether multiple strains are used or not, do not

appear likely to lead to effective therapeutic control of the disease.

3. Confirmation of the infectivity of fresh cerebro-spinal fluid would justify the preparation and trial of effective antisera thereto in the treatment of the disease.

4. Attempts to destroy the meningococcus in the naso-pharynx of contact carriers, as a means of epidemiological control, would appear to be less valuable than an attempt to determine what the true infective agent of the disease really is.

5. In order to discover the true infective agent, whether biologically related to the meningococcus or not, further research is imperative, attention being particularly directed to filtrable organisms in the naso-pharynx and the cerebro-spinal fluid of acute cases.

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STUDIES IN ANTISEPTICS (II):

ON CHLORAMINE: ITS PREPARATION, PROPERTIES, AND USE.*

BY

H. D. DAKIN, D.Sc., J. B. COHEN, F.R.S.,

AND

J. KENYON, D.Sc.,

THE UNIVERSITY OF LEEDS.

REFERENCE was made in a paper on the use of certain antiseptic substances for the treatment of infected wounds, recently published in this *JOURNAL*,¹ to a number of compounds containing chlorine linked to nitrogen and possessing strong germicidal properties. Substances containing this type of linkage were studied because analogous compounds were believed to be formed by the action of the actively germicidal hypochlorites upon proteins and some other nitrogenous cell constituents. After studying a very large number of substances of the type referred to, a selection has been made of the one which on the whole seemed to possess the greatest number of desirable properties and which experience showed to be practically useful. The substance chosen is para-toluenesodium-sulphochloramide, and possesses the following structure:

The systematic name of this substance is somewhat unsuitable for general use, so that it has been decided to assign the more convenient name "chloramine" to the compound, thus indicating its membership in the group of substances containing the NCl linking. If, in future, other members of the group should come into use, it is suggested that the present compound should be known as chloramine-T to indicate its relationship with toluene.

Properties.

Chloramine, the preparation of which is described in a later section of this paper, is a white solid, crystallizing in prisms containing three molecules of water of crystallization. The solid has a very faint chlorous odour, while solutions of it are without smell. It is freely soluble in water, a saturated solution at room temperature containing

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