THE MENINGOCOCCUS OF WEICHELBAUM.

BY

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The Teaching of Recent Experimental Research.

NOTWITHSTANDING the results of recent experimental research, it is still taught, and it is in consequence widely believed, that the meningococcus of Weichselbaum is itself the direct causal agent of that form of epidemic cerebro-spinal fever which is sometimes spoken of as meningococcal meningitis.

It is also still taught, and the teaching is accepted without question, that the meningococcus, as one of the "lower" bacteria, is an organism with an extremely simple life-cycle, which begins and ends with binary fission into two equal parts. A meningococcus—so runs the legend—always arises directly from a meningococcus, and in no other way.

As regards the first of these propositions—that the meningococcus is itself the direct cause of the disease which bears its name—I showed by experiment in 1915, and this, in conjunction with Captain A. H. Caulfeild, I amply confirmed in the following year:

1. That cultures of the meningococcus in cerebro-spinal fluid or in suitable laboratory media frequently contain a minute filterable virus, which is just as pathogenic to monkeys (though not necessarily productive of meningitis) as are unfiltered cultures.

2. That the meningococcus itself appears to be inert, in so far as the production of systemic infection is concerned, whether the cultures be of the so-called epidemic strains, or of admittedly harmless types of carrier strains.

As a result of these experiments, it became clear that the pathogenic results obtained in the past by injection into animals of living cultures of the meningococcus, believed at the time of injection to be pure cultures, are in reality to be ascribed to the injection of impure cultures, the "impurity" consisting in the minute filterable virus described.

It became manifest, in short, as a result of these experiments, that there is no reliable evidence that the meningococcus as such is itself responsible for the disease, mere constancy of presence of the organism, even when combined with agglutinability, being of little value as proof of direct etiological relationship.

Demonstration of the presence of this filterable virus in meningococcal cultures had, moreover, this important result, that it threw grave doubt on the validity of the second proposition to which I have referred, namely, that the reputed causal organism of the disease is a bacterium with a simple life-cycle, which begins and ends with equal binary fission. It unmistakably suggested, in fact, that the meningococcus represents one phase only—the filterable virus representing a second phase—in the life-cycle of an organism which has not yet been described, though, owing to the fact that the experimental method of investigation was mainly employed, it was not then possible to prove by ocular demonstration that this is indeed the case.

Studies in the Morphology of Meningococcal Cultures.

To-day, however, as a result of direct morphological studies of cultures of the meningococcus—studies which are capable of ready verification by the adoption of an extremely simple technique—I am able to confirm and amplify the results obtained already by the experimental method. I am, in short, now able to produce direct morphological evidence that the meningococcus is not a bacterium at all, and that both it and the filterable virus referred to do actually represent different phases in the life-cycle of an ascomycetic organism which has hitherto been believed to be an involution form of the meningococcus. Before, however, presenting the evidence on which these statements are based, I wish to make it perfectly clear that even now the complete life-cycle, in the human host, of the causal organism (of which the meningococcus only represents a saprophytic phase) of cerebro-spinal fever has not been worked out, and that much work on this subject still remains to be done.

This communication deals mainly with the morphological characters of the meningococcus and of its so-called giant forms. It is necessary, therefore, briefly to summarize what is at present known of their chief morphological characteristics.

The Present State of Knowledge of the Morphology of the Meningococcus.

The normal meningococcus of Weichselbaum, say the textbooks, is a Gram-negative coccus, with an average diameter of about 1μ , which may be seen either alone, or in pairs, the latter often showing flattening of the opposed surfaces. When occurring as single cocci it is generally assumed that each living organism will presently undergo equal binary fission, and in the case of many single individuals this, of course, often happens. The meningococcus appears also to be sometimes grouped in triad formation, and sometimes in tetrads. It is said never to appear in chain formation, except as the result of accidental apposition.

In addition to the normal meningococcus, a few of the textbooks—notably that of Heiman and Feldstein—describe the so-called giant forms. These are said to attain to from four to five times the size of the normal coccus, and notwithstanding the fact that they are believed to disappear from solid subcultures in from twenty-four to forty-eight hours, they are usually looked upon as involution forms, or swollen decadent meningococci. These giant forms stain, say Heiman and Feldstein, deeply with the counter-stain employed in Gram's method, as do also some of the single normal-sized "meningococci," in contrast to others of the single normal cocci, as well as the diplococcal forms, both of which take the counter-stain but lightly.

In addition to the normal-sized meningococci, with an average diameter of about 1μ , it has long been recognized that sometimes considerably smaller forms may be seen, with an average diameter of about 0.5μ , and intermediate forms, above the normal organism in size, but below the giant forms, have also been described.

This, then, completes the textbook accounts of the morphology of the meningococcus. It is, however, almost exclusively based on observations of stained fixed films from cultures on solid media, practically no observations having been recorded of the morphology of the organism in stained fixed films from liquid cultures, and none at all of its morphology in wet stained or unstained preparations. And, lastly, no extensive observations appear to have been undertaken of the development of the organism on the warm stage, whether in liquid or on solid media.

In other words, apart from the usual perfunctory examination of the organism in fresh cerebro-spinal fluid, its claim to be considered as a bacterium at all finds its sole justification in the results obtained by the examination of stained dead individuals direct from cultures on solid media.

Now, all deductions as to the botanical position of micro-organisms drawn from the study of stained fixed films are notoriously dangerous unless controlled by direct examination of the living unstained organism, and by observation of development on the warm stage from a single individual. If we add to this statement the reflection that, however convenient artificial solid media may be for the identification of species (by the subsequent application of cultural, fermentative, and serological tests), artificial solid media do not occur in nature, it is not surprising that the application of more scientific methods of study should reveal the fact that the meningococcus is not what it appears.

The Morphological Characteristics of the Meningococcus as Studied in Liquid Cultures.

As I have stated, it is essential, in order satisfactorily to study the morphology of the meningococcus and to determine its real nature, to employ liquid cultures, and to examine therein—

1. Unstained and stained living organisms at room temperature.
2. Development of single individuals on the warm stage.

This should be supplemented, and not replaced, by examination of stained and of unstained fixed films, using for the latter Benians's Congo-red method, or other suitable adsorption method.

Examination of Unstained Living Organisms at Room Temperature.

The method of observation I have found to give the most satisfactory results, allowing of photography with the Pointolite nitrogen lamp, is to make a liquid cell containing a liquid culture under firm pressure between a glass slide and a cover-slip, sealed, to prevent drying and escape, with paraffin. This allows the liquid medium employed to remain liquid at room temperature for at least forty-eight hours. Slide and cover-slip are soaked for twenty-four hours in fuming nitric acid, and are then placed in absolute alcohol till ready for use, when they are finally flamed. The culture is centrifuged before filling the cell in order to enable the observer rapidly to find the various types of organism present in pure cultures of the meningococcus in serum broth or other suitable liquid medium, such as Naegeli's medium containing 2 per cent. glucose and a trace of albumin.

Prolonged examination of, for example, Gordon's strain No. 4 in serum broth, controlled by examination of the same strain (the same sample) in Naegeli's medium, revealed the following types in a liquid cell prepared as above described:

1. Meningococci in diplococcal form of normal size.
2. Giant meningococci in large numbers.
3. Triad groups.
4. Tetrad groups.
5. Short streptococcal groups, the members of the chain being usually unequal in size.
6. Very minute organisms, many being approximately 0.1 to 0.2μ in diameter, or even less.
7. Intermediate single cocci of every conceivable size between about 0.1μ and about 4 to 5μ .
8. One or two bacillary and filamentous forms.

More detailed description of these types is for the moment deferred.

Examination of Stained Fixed Films.

Examination of several hundred Gram-stained films from 46 consecutive acute cases of cerebro-spinal fever in which the meningococcus was isolated and identified, and cultivated in serum broth, gave in every case the same essential results as those detailed in the last paragraph (1 to 8). In all the cases the normal-sized meningococci took up the counter-stain, neutral red or carbol-fuchsin, relatively lightly. In all the cases the giant forms, the triads, the tetrads, and large numbers of the single undivided cocci from about 0.2μ upwards all took the counter-stain deeply. The occasional bacillary and filamentous forms seen in the majority of the cultures did not as a rule take the counter-stain deeply.

In all cases the outline of the giant forms when stained by Gram's method was relatively blurred as compared with their distinct appearance as living unstained organisms. This also applied to all films stained with Leishman's stain, Jenner's stain, carbol-thionin, methylene blue, Löffler's stain, eosin, carbol-fuchsin, neutral red, or safranin. Previous treatment of the films with iodine tetrachloride vapour, or with saturated solutions of iodine in potassium iodide solution, also failed to render distinct the outline of the giant forms when subsequently stained. Staining of all the kinds tried, in fact, appeared to give an entirely fallacious idea of the true form and structure of these giant forms.

Examination of Unstained Fixed Films: Congo-Red Method.

This method requires some months' practice in order to obtain familiarity with its fallacies. Once mastered, however, it gives invaluable results, provided always that—as here—these results are controlled by careful examination of unstained living organisms, both at room temperature and on the warm stage. The results obtained are summarized in the accompanying chart of selected giant meningococci from a large number of fields, and are self-explanatory. Endospore formation and gemination, particularly the latter, are well seen. A large number of excellent photographs of giant meningococci undergoing well-marked endospore formation have been taken for me by Mr. Martin Duncan, as well as numerous autochromes. Owing to exigencies of space and paper these photographs cannot be reproduced here.

Examination of Living Organisms at Room Temperature and on the Warm Stage.

It is now necessary to describe in fuller detail what may be observed by examination at room temperature of unstained living organisms from liquid cultures of the meningococcus, as well as what may be learnt by observation of development on the warm stage.

To avoid repetition, these may be taken together.

In examining pure cultures of the meningococcus in liquid media by the method of direct observation of unstained living organisms under pressure, the most striking feature—once the attention has been called thereto—is the presence of spheroidal bodies of all sizes up to about 5μ , with a different refractive index to that possessed by genuine meningococci. To the trained observer this difference in refractive index is alone sufficient to enable a distinction to be drawn between genuine and spurious meningococci, and this applies to the smallest organisms present as well as to the largest. Many of these spheroidal bodies, on careful watching, are seen, in favourable circumstances, obviously to be undergoing unequal binary fission or gemination. Others, again, are clearly undergoing endosegmentation, with or without accompanying gemination. Multiple endospore formation is in many cases readily to be made out, as also is rapid Brownian movement of what appear to be metachromatic granules, which as a rule can easily be distinguished from the true endospores. Most of the young spheroidal bodies when undergoing gemination, or endospore formation, or both, have a clearly defined peripheral rim, which serves readily to distinguish them from genuine meningococci (with the ill-defined periphery characteristic of their appearance) when viewed with a good oil-immersion fluorite lens under optimum conditions of illumination. That these spheroidal bodies are genuine asci there can be little doubt, the larger forms being identical with the giant meningococci, as they have hitherto been called.

That the giant meningococcus is not a bacterium is, of course, proved by the facts of gemination and of endospore formation, the former of which appears to be, from warm stage observation, the main method of its reproduction in young cultures. It is, however, not the exclusive method, as some of the asci divide, radiating from a central space, into three equal segments, and others into four equal segments, the segmentation being holocytous instead of merocytous,* as in the case of the endospore production. This holocytous segmentation of asci into groups of triads and tetrads, and possibly into pairs, though the latter I cannot yet vouch for, is reminiscent of the equal fission observed in the schizogonic saccharomycetes. In the case of meningococcal cultures, however, it appears to be combined in one species with gemination and endospore formation. The new individuals formed by triad and tetrad formation may be either spheroidal or pyramidal in shape, the latter form apparently being only temporary, and later appearing to assume a spheroidal form.

In the above description I have said that there are minute forms of the "giant meningococcus" as well as large forms, and that this is undoubtedly the case is shown by the fact that even very minute forms may be seen to be undergoing gemination, or endospore formation, or both, apart from the evidence afforded by differences in refractive index and peripheral outline, as compared with genuine meningococci. We are therefore now in a position to explain the true nature of the numerous single cocci which may be seen in any liquid meningococcal culture. Many of these are undoubtedly meningococci about to undergo equal binary fission; others, on the contrary, will never undergo equal binary fission, but are, in point of fact, young asci, which are capable of undergoing, and often do undergo, gemination and endospore formation before attaining to their full size. Others, again, while still minute, will split into groups of threes or fours without presenting any evidence of accompanying gemination or endospore formation. Some asci undergo secondary gemination in rapid succession, the daughter and granddaughter cells still remaining attached, each to its mother cell, and in this way a streptococcal grouping is sometimes formed which is not the result of accidental apposition.

As in the case of recognized ascomycetes, the "giant

* Holocytous signifies cleavage through the entire depth of the cell, as opposed to merocytous, cleavage through a portion of the cell, as in sporulation.

Camera lucida drawing of "giant meningococci." Photograph $\times 1,500$.

WARM STAGE STUDIES IN DEVELOPMENT OF "GIANT MENINGOCOCCI."
(Freehand Drawing.)

CULTURE 1.

A¹ 3.30 P.M. A² 4 P.M. A³ 4.30 P.M. A⁴ 5 P.M. A⁵ 5.20 P.M.

CULTURE 2.

B¹ 3.10 P.M. B² 3.55 P.M. B³ 5.45 P.M.

CULTURE 3.

C¹ 1.45 P.M. C² 3.10 P.M.

CULTURE 4.

D¹ 11.30 A.M. D² 1.10 P.M. D³ 1.40 P.M. D⁴ 2.25 P.M. D⁵ 3.30 P.M. D⁶ 6.50 P.M. D⁷ 8.50 P.M. D⁸ 10.20 P.M.

Magnification approximately 3,000.

meningococci," whether large or small, are all potential asci, with the possible exception of those which undergo schizogonic segmentation, and as such all these spurious meningococci, with the exception stated, are potentially capable of undergoing in their turn gemination and endosporulation. From prolonged warm-stage observation, there is little doubt that genuine meningococci arise by multiple endosporulation from spurious meningococci, or asci, but there is as yet no evidence that the true meningococcus develops again directly into an ascus, as, on the contrary, undoubtedly do the ascus buds. For a time, at least, on solid laboratory media, equal binary fission of the meningococcus becomes stable and fixed, though I have been able to obtain evidence, at present incomplete, that some meningococci (endospores), either before or shortly after escape from the ascus, will show mycelial sprouting in suitable liquid media and so complete the cycle. Considering, however, the tendency to suppression of the mycelial phase in many of the ascomycetes, it is perhaps not to be wondered at that the mycelial aspect is much more difficult to study in laboratory cultures of the meningococcus than is the ascus aspect.

It has been stated above that in serum-broth cultures—and this also applies to cultures of the meningococcus in Naegeli's synthetic medium—very minute organisms can frequently be made out, which I have elsewhere shown to be capable of passing through Chamberland filters. These minute organisms, close to the vanishing point of vision, may, under favourable conditions, be seen in well-developed asci (giant meningococci) and in some cases may be watched in the process of extrusion therefrom. I have not been able to satisfy myself that these will subsequently develop into meningococci, though they do undoubtedly in some cases develop into unmistakable organisms, as seen with monochromatic light, of a globoid nature. Exactly similar bodies, Gram-negative and globoid in form, are often to be seen in incubated meningococcal filtrates (Chamberland F.), though their relationship to genuine meningococci is still a matter of uncertainty. They appear, however, to form a connecting link between invisible filterable virus and Weichselbaum's organism.

It is now necessary very briefly to deal with certain criticisms which might, perhaps not unreasonably, suggest themselves to students who are not familiar with the appearances of meningococci in liquid media when examined in the living state. Both forms of reproduction—gemination and endosporulation—I have repeatedly watched on the warm stage. Any suggestion therefore that the asci and ascospores are of the nature of artefacts, as from a study of dried preparations alone a casual observer might be pardoned for suggesting, can be summarily dismissed. This applies also to the suggestion that the appearance of gemination under isotonic conditions has been simulated by plasmolytic changes such as may be observed in red and white blood cells under anisotonic conditions. The further suggestion that the "giant meningococci" (asci) represent involution forms is practically disproved by the facts that they can be sometimes seen in small numbers in cerebro-spinal fluid collected within a few hours of the onset of the disease; that they may be seen in small numbers in 12-hour old primary colonies on solid media if they be diligently searched for, and that they may grow and bud freely in enormous numbers in young liquid cultures, such as serum broth and in subcultures therefrom. And finally, as already stated, quite small young budding forms can be frequently seen undergoing endosporulation, especially after subcultivation from liquid cultures to serum-agar slopes. The term "involution form," in fact, is one that has in the past been much too freely applied to organisms the true nature of which escapes us, and under the optimum cultural conditions obtaining throughout its application here would be worse than unreasonable.

The same criticism applies to the facile suggestion that the organisms here described are contaminants. In connexion with the question of contamination, it is well to remember that it is just as dangerous—provided a good technique has been employed—to assume that a given organism is a contaminant as to assume that it is not. As the appended detailed account of the technique here employed will show, every device known to modern bacteriological science has been employed to prevent contamination. Any doubt remaining as to the adequacy of these devices should be dispelled by the fact that

these organisms were found and cultivated in 46 consecutive cases of the disease, an achievement which, if the organism were a contaminant not deliberately introduced, it would pass the wit of man to reproduce. And, finally, the observations here recorded have only to be repeated for the impartial student to be convinced of their accuracy. In every one of these 46 cases the meningococcus has been isolated and fully identified by its staining reactions, its morphology, its sugar reactions, and its serological reactions, as well as by well-defined cultural reactions. I have not in primary serum-broth cultures found asci in any case apart from the meningococcus, and in no case has the meningococcus been found without asci being also found. I have never been able to cultivate them in serum-broth culture apart from the meningococcus, and in every apparently pure primary culture of the meningococcus the organism has always been found if diligently searched for, and in none of the control culture tubes has any growth been found. The best and most certain method of demonstrating the presence of asci has been, in my hands, by subculture from single colonies of meningococci on serum-agar, itself inoculated with incubated cerebro-spinal fluid. This subculture, however, must be into liquid media, an excellent medium being equal parts of tested horse serum and broth, and there is little doubt but that the real nature of the "giant meningococcus" would have been noted and recorded long ago if the routine use of solid media had not been generally required for purposes of identification of the normal meningococcus.

Details of Technique Employed.

The technique I have mainly employed for detection and cultivation of the asci described is as follows:

1. Cerebro-spinal fluid from a suspected case of the disease is discharged direct into a sterile glass flask with a specially ground glass stopper provided with a sterile rubber cap sufficiently large to come well down over the neck of the flask. In all but six of the cases only samples of fluid withdrawn at first lumbar punctures have been employed.
2. On arrival at the laboratory the centrifuged deposit from a small quantity of the fluid is examined.
3. The remainder of the fluid is then incubated at 37° C. for a period varying from twenty-four to forty-eight hours.
4. A further volume of the fluid is now withdrawn from the flask and is centrifuged and examined.
5. Serum-agar slopes—and sometimes in addition serum-agar plates—are now inoculated, the plates in series, with considerable volumes of the incubated cerebro-spinal fluid.
6. On the following day likely single colonies are picked off and are individually spread on to fresh serum-agar slopes to provide a free confluent growth.
7. On the following day the entire growth of each slope is inoculated into serum-broth tubes, which are incubated for forty-eight hours.
8. From the serum-broth tubes fresh serum-agar and agar slopes are inoculated for serological tests, and lactose, saccharose, glucose, mannite, and dulcitate tubes are also inoculated from the same source.
9. Samples of the serum-broth tubes are at the same time, and subsequently every day, centrifuged and the deposits examined.
10. The filtered serum employed in each case is thoroughly tested, as regards sterility, by complete bacteriological and experimental tests, monkeys being employed for the latter. By taking these precautions and by replating at each stage and retesting from single colonies, both with fermentation and serological tests, as absolute a guarantee as it possible to obtain by observation of cultures from single colonies is assured.

However useful for purposes of identification and for ensuring the absence of contaminants, cultivation from single colonies nevertheless cannot give an absolute guarantee of purity of culture. To obtain this only two methods are open to us. The first is to pick off single meningococci from a solid culture and to place individual organisms into a separate tube containing a suitable liquid medium in the hope of successful cultivation therefrom.

This, of course, is the classical method, and in theory it is ideal. In practice, however, it cannot be relied on, and for this reason: As I have previously shown, the presence

of the minute filterable virus frequently to be found in meningococcal cultures makes it impossible to be certain that in picking up a normal sized meningococcus one is not at the same time picking up some of this filterable virus. And this objection also applies to the presence of the very minute organisms described above. Now the smallest of these forms—and this is a point I have repeatedly tested—are quite invisible as organisms under a dry lens and the highest compensating ocular that will allow of good illumination. Even with a magnification of 3,000 obtained with a 1.5 mm. apochromatic oil-immersion lens and the appropriate tube length and compensating ocular it is still impossible to be certain that one organism, and one only, has been picked up. The photograph of the camera lucida drawings will illustrate the point of this objection, which applies both to Barber's method and to the fragmented cover-slip method. The only alternative, therefore, is actually to watch on the warm stage growth from a single organism, and even here—on account of these extremely minute forms—the problem is one of great difficulty, which is accentuated by the fact that growth and gemmation of asci is unsatisfactory on solid media, necessary as solid media may be for observing endosporeulation. It became necessary, therefore, to use methods of observation of individual organisms in liquid cultures other than the hanging drop method, which is, of course, useless for fine work owing to the curve and depth of drops.

The method I have adopted involves, as stated, the use of shallow cells covered with sterile slips, and the method ensures relative immobilization of the organisms under observation. On the whole it gives satisfactory results.

These results, however, require the greatest care in interpretation, owing to the presence, first, of the filterable virus and of the almost invisible organisms referred to, early gemmation and even endosporeulation being often exactly simulated by minute organisms brought into apposition by slight streaming movements still persisting; and owing, secondly, to slow rotatory and dipping movements. These movements lead to error, unless careful and prolonged watch is made, because an early bud, or a minute endospore, under observation may either temporarily vanish from view, or without having been previously seen may come into view for the first time, and simulate either a fresh bud or an endospore.

In all cases, therefore, most tedious and prolonged observation is necessary, which has to be repeated again and again with fresh cultures, as disappointment is frequent owing to death or arrest of growth of an individual organism under observation.

ILLUSTRATIVE FERMENTATION AND SEROLOGICAL TESTS OF THE SERUM-BROTH CULTURES EMPLOYED IN THE ABOVE OBSERVATIONS.

Fermentation Reactions.

In the case of all the fourteen cultures, details of which are here recorded, no reaction was observed in lactose, saccharose, mannite, or dulcitol.

In twelve of the cultures acid was produced quite definitely in glucose, in the remaining two cultures no acid being produced in glucose.

In every case incubation at 37° C. was continued for eight days.

Serum Reactions.

Dilutions:	1:4.	1:8.	1:16.	1:32.	1:64.	1:128.	1:256.	Control.
Culture 1 ...	C	C	C	C	C	C	C	NIL.
Culture 2 ...	C	C	C	C	C	P	NIL	NIL.
Culture 3 ...	C	C	C	C	P	NIL	NIL	NIL.
Culture 4 ...	C	C	C	C	C	NIL	NIL	NIL.

C = Complete agglutination. P = Partial agglutination.

In the case of these four cultures the dilutions were made from undiluted meningococcal serum supplied by the Lister Institute: Titre 1 in 250; No. cl.; date of tubing, February 9th, 1916; dates of agglutination, March 8th, 1916; March 4th, 1916; April 29th, 1916; April 29th, 1916.

Incubation at 37° C. was allowed for twenty-four hours before reading, plus one hour at room temperature.

The agglutination tests of the remaining ten cultures were kindly carried out by Dr. Lawes, using Gordon's method for identification of "strains."

The agglutination tubes and their contents were incubated at 56° C. for twenty-four hours before being read.

Serum Reactions.

	Normal.	I.			II.			III.			IV.		
		A	B	C	A	B	C	A	B	C	A	B	C
Culture 5 ...	-	++	++	++	-	-	-	+	-	-	-	-	-
Culture 6 ...	-	-	-	-	++	++	++	-	-	-	-	-	-
Culture 7 ...	-	-	-	-	-	-	-	-	-	-	-	-	-
Culture 8 ...	-	++	++	++	-	-	-	-	-	-	-	-	-
Culture 9 ...	-	++	++	+	-	-	-	-	-	-	-	-	-
Culture 10 ...	-	-	-	-	++	+	-	-	-	-	++	++	+
Culture 11 ...	-	-	-	-	++	++	+	-	-	-	-	-	-
Culture 12 ...	-	-	-	-	++	++	++	++	(+)	-	-	-	-
Culture 13 ...	-	-	-	-	++	++	++	-	-	-	-	-	-
Culture 14 ...	-	-	-	-	++	++	++	-	-	-	-	-	-

Cultures 8 and 9, 11 and 12, 13 and 14, were from three cases. Cultures 1 to 7, as well as culture 10, being from eight separate cases. A, B, C represent respectively dilutions 1 in 100, 1 in 200, 1 in 400.

Conclusions.

1. The so-called giant meningococcus is not a bacterium. It represents an ascual stage in the life-cycle of an organism allied to the ascomycetes.*

The ascus may be either large or small, or of any intermediate size (0.2 μ to 5 μ).

2. The meningococcus of Weichselbaum is not a bacterium. It is an ascospore, derived from the "giant meningococcus" by a process of endosporeulation.

3. The filterable meningococcal virus of my earlier papers probably represents a stage in the life-cycle of the ascomycetic organism described.

4. The true nature of the meningococcus and its alleged giant forms has hitherto been missed because of the practically exclusive use of solid media for purposes of identification, and because direct examination of unstained living organisms, including warm-stage development in liquid cultures, has not been carried out.

An Experimental Investigation

OF

THE SUITABILITY OF THE MORE SOLUBLE SALTS OF QUININE AND CINCHONINE FOR INTRAVENOUS INJECTION.

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THE most soluble salt of quinine—namely, the bi-hydrochloride—has for many years been given intravenously in malaria, although only, as a rule, in very severe infections with cerebral symptoms. The method has gained a reputation for being dangerous, although I have long thought this to be due to the extreme class of cases in which it is generally used rather than to the method itself. Nevertheless, the intravenous route is apparently the most efficacious, as shown by the fact that some cerebral cases of malaria can be saved by it when quinine by the mouth or even subcutaneously would be too slow in its action to be trustworthy. The following experience helped to convince me of this fact. In a case with high continued fever, leading to a suspicion of typhoid, I found very numerous malignant tertian parasites in every field of the microscope, and, recognizing a very dangerous infection, 10 grains of bi-hydrochloride of quinine was at once given intravenously, with the result that the temperature fell finally to normal the next morning.† Some years later I found equally dangerous malignant tertian infections on

* The mycelial or "bacillary" phase is still the subject of study, and will be dealt with separately. Owing to the variable number of ascospores (meningospores) which the ascus (meningogascus) contains, and owing to the absence of perithecia, the organism here described is provisionally placed amongst the hemiascomycetes, instead of amongst the ascomycetes proper.

† See Chart 50, page 227 of my *Fever in the Tropics*.